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LABORATORY MANUAL
FOR PRACTICAL BIOCHEMISTRY

Tutorial

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L11

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Biochemistry is a constantly changing and evolving academic discipline that requires continuous study to keep one's knowledge current. The practical book is written in accordance with the Federal State Educational Standard of Higher Professional Education for students studying in the basic vocational and educational programs – specialty programs: "General Medicine" and "Pediatrics".

There are laboratory works in each unit, which should be carried out by students duringtheir practical lessons. The methods presented in the tutorial are used in clinical diagnostic laboratories. They assist the physician to diagnose the disease and to monitor the efficacy of treatment. Each unit is divided into several parts: questions for self-study, test tasks and case studies for testing the knowledge gained.

The tutorial contributes to the formation of professional competencies, as well as the identification of the patient's basic pathologies, clinical signs, disease syndromes, nosologies.

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UNIT 1
STRUCTURE, FEATURES AND FUNCTIONS of PROTEINs

THEME 1.1. AMINO ACID STRUCTURE AND CLASSIFICATION. PROTEIN STRUCTURE

INTRODUCTION

In fact, proteins are known to be the the main members of human metabolism. They regulate all biochemical processes, act as enzymes, hormones, receptors, antibodies and are required for the structural integrity of cells. The plethora of protein functions and structures is associated with amino acid variety. Amino acids are involved in the formation of biogenic amines and nitrogenous bases of mononucleotides, neurotransmitter, etc. Therefore, protein-based therapies are highly successful in clinical practice.

THE AIM OF THE PRACTICAL IS TO:

Obtain simplified knowledge about amino acid structure.

Practically apply this knowledge by performing amino acid color and protein precipitation reactions.

SELF-STUDY QUESTIONS

1. Amino acid classifications.
2. Amino acids differ according to:
- their biological role (essential and non-essential);
- their physical and chemical features (neutral, acidic, basic, hydrophilic, hydrophobic);
- their chemical structure (with aliphatic radicals, with additional functional group, with aromatic and heterocyclic group, imino acids);
- their solubility in water (non-polar, polar positive charged, polar negative charged).

3. The structure and formula of proteinogenic amino acids.
4. Physical and chemical properties of amino acids, their functional group role.
5. The iso-electic point of amino acids and proteins, its dependence on different factors.
6. The influence of pH solution on amino acid charge.
7. The peptide bond, its formation. Peptide bond properties.
8. Influence of pH changes on protein charge and solubility.
9. Color qualitative amino acid reactions. Their principles and practical use.

Practical

COLOR QUALITATIVE PROTEIN AND AMINO ACID REACTIONS

Reagents

1) 1% albumin solution 2) 0.5% ninhydrine, 3) 30% NaOH solution, 4) 10% NaOH solution, 5) 5% $Pb(CH_3COO)_2$ solution, 6) 5% sodium nitroprusside solution, 7) concentrated solution of HNO_3, 8) 5% $CuSO_4$ solution

Material of the investigation

The object of investigation is 1% albumin solution with a complex set of amino acids.

BIURET TEST (PEPTIDE BOND)

Biuret test is specific for proteins – to differentiate between proteins and amino acids.

Principle:

The Biuret reagent (copper sulfate in a strong base) reacts with peptide bonds in proteins to form a violet complex known as the "Biuret complex". Two peptide bonds are at least required for the formation of this complex, this is why amino acids give negative results with Biuret test.

Procedure and observation:

Add 1.0 ml of 10% sodium hydroxide solution and 2-3 drops of 1% copper sulfate solution to 1.0 ml of protein solution in a test tube. Mix well; a violet color is obtained.

NINHYDRIN TEST

Ninhydrin is specific for amino acids and proteins – to differentiate between carbohydrates and amino acid andproteins.

Principle

When heating ninhydrin reacts with α-amino acids ($–NH_2$) in proteins giving a purple colored complex, except for proline and hydroxy proline gives yellow color(no $–NH_2$).

Procedure and observation:

Add 2-3 drops of ninhydrin reagent to 1.0 ml amino acid solution in a test tube. Put it into a boiling water bath and observe the formation of a purple color.

XANTHOPROTEIC ACID TEST

Aromatic amino acids, such as phenyl alanine, tyrosine and tryptophan, respond to this test.

Principle

In the presence of concentrated nitric acid, the aromatic phenyl ring is nitrated to give yellow colored nitro-derivatives. At alkaline pH, the color changes to orange due to the ionization of the phenolic group.

Procedure & observation:

Add 2-3 drops of concentrated nitric acid to 1.0 ml amino acid solution in a test tube. Put in a boiling water bath and observe the formation of a yellow color. When

the solution doesn’t become yellow, add 1-2 drops of concentrated nitric acid. At presence of 30% sodium hydroxide the color in test tube could change to orange.

TESTS FOR SULPHUR CONTAINING AMINO ACIDS AND PROTEINS

Principle

Sulphur containing amino acids, such as cysteine and cystine upon basic hydrolysis, yield sodium sulphide. This reaction occurs due to partial conversion of the organic sulphur to inorganic sulphide.

- **Lead sulphide test (Fole test).** Sodium disulfide is detected by precipitating it to lead sulphide, using lead acetate solution
- **Nitroprusside’s test.** Sodium disulfide reacts with nitroprussideand andgives a red-purple colour called "Morner test".

Procedure & observation

Add 5 drops of 30% NaOH solution to 1.0 ml of protein solution containing Cysteine/Cystine in a test tube and boil the test tube. Divide the receivedsolution in two parts for carring out a) and b) reactions.

a) **Lead sulphide test (Fole test)**

after boiling add 1 drop of lead acetate solution to 5 drops of received protein solution and heat it till brown or black residual matter appears.

b) **Nitroprusside’s test**

after boiling add 3 drops of a 5% solution of sodium nitroprusside to 5 drops of received protein solution. Mix well and add few drops of ammonia solution, a deep red-purple color appears; called also Morner test.

Practical use of color reactions

Color reactions can beuniversal (Biuret and ninhydrine reactions) and specific (lead sulfide test, xanthoprotein reaction). They allow to estimate and identificate the protein and amino acid content. For instance, ninhydrin iscommonly used as a forensic chemical to detect "fingerprints", as amines left over from proteins sloughed off in fingerprints react with ninhydrin giving a characteristic purple color. Principles of these reactions are also used for quantitative determination of amino acids and proteins in biological fluids.

Laboratory exercise:

Using the provided solutions of albumin perform the tests in the table below and write down your observations. The color intensity is denoted as follows: "–" color absence; "+" weak color; "++"intensive color; "+++" very intensive color.

object of study	Tests				
	Biuret test	**Ninhydrin reaction**	**Xanthoprotein reaction**	**Lead sulfide test (Fole test)**	**Reaction with nitroprusside**
Albumin solution					

In conclusion the possibility of peptide bond detection and presence of specific amino acids using color tests shoud be noted.

THEME 1.2. STRUCTURE, PHYSICAL AND CHEMICAL PROPERTIES OF PROTEINS

INTRODUCTION

The protein isolation as well as salting out, denaturation or precipitation are used in modern medical practice. Theirmain roles are the disease diagnostic, experimental investigation, production and purification of proteins as medicine.

THE AIM OF THE PRACTICAL IS TO:

Study the physical and chemical properties of proteins (molecular weight, shape, ionization, hydration, solubility) and the basic types and structures of protein molecules.

Studythe protein precipitation reaction of proteins by different reagents and their use in clinical practice.

SELF-STUDY QUESTIONS

1. The structure of proteinogenic amino acids. The peptide bond formation, difference between peptides and proteins.
2. The organization of protein molecules (primary, secondary, tertiary, quaternary structures).
3. Bonds, involved in the formation of the protein structure. Functional groups of amino acids responsible for the formation of these relations.
4. The quaternary structure of proteins. The complementarity of protomers. The cooperative protomers conformation changes.
5. Properties of proteins: amphoteric ionization (charge), hydration, solubility. What is the isoelectric point?
6. The molecular weight peptides and proteins. Methods of its determining (ultracentrifugation, gel filtration).
7. Properties of protein solutions. Factors that stabilize the protein molecule in the solution. The colloidal properties of proteins.
8. Denaturation of proteins. Factors that cause the proteins denaturation (physical, chemical, biological). Properties of denatured proteins.
9. Protein renaturation, its mechanism.
10. Protein removal methods. Precipitation reactions by acids, heavy metals, organic solvents in solution. The principle of protein precipitation reactions.
11. Protein purification methods from impurities in solutions (salting out, gel filtration, dialysis). Their principles.

TOPICS FOR REPORTS

1. Chromatography of proteins, its types and the practical application.

2. First aid and medical aid in case of poisoning by organic and inorganic acids, salts of heavy metals. Biochemical basics.

3. The dialysis and plasmapheresis application in clinical practice: their principles, efficacy and application.

Practical 1

PRECIPITATION REACTIONS OF PROTEINS (SALTING OUT)

Salting out is the protein precipitation reaction due to the action of alkali and neutral salts. This process is reversible. Native protein features are preserved.

Reagents

1) Saturated ammonium sulfate solution $(NH_4)_2SO_4$, 2) ammonium sulfate crystals $(NH_4)_2SO_4$, 3) 10% NaOH solution, 4) 1% $CuSO_4$ solution.

Material of investigation

Serum.

Principle

Protein molecules contain both hydrophilic and hydrophobic amino acids. In aqueous medium, hydrophobic amino acids form protected areas while hydrophilic amino acids form hydrogen bonds with surrounding water molecules (solvation layer). When proteins are present in salt solutions (e.g. ammonium sulfate), some of the water molecules in the solvation layer are attracted by salt ions. When salt concentration gradually increases, the number of water molecules in the solvation layer gradually decreases until protein molecules coagulate forming precipitates; this is known as "salting out". As different proteins have different compositions of amino acids, different proteins precipitate at different concentrations of salt solution.

Procedure and observation

Add equal amount of saturated ammonium sulfate solutionto 2.0 ml of serum Mix well and note the globulin is precipitated in the resulting half of saturated solution of ammonium sulfate.

Wait 5 minute and separate globulin by centrifugation or filtration and recover the clear supernatant. The presence of proteins in the precipitate or on the filter is proved by Biuret reaction (UNIT 1.1.)

Add Biuret reactive to 10 drops of supernatant or flowthrough and perform the color qualitative test for protein presence. Add ammonium sulfate crystals gradually to the remaining part of supernatant until full saturation occurs; another precipitate (albumin) is obtained. Separate albumin by centrifugation.

Clinical and diagnostic significance

Salting out is the method for the separation of serum albumin and globulin, the albumin/globulin ratio (A/G) determination, which is previously used in clinical laboratory practice. The normal A/G ratio is 1.2-1.8. It could be changed at many pathological situations. The A/G ratio can be decreased in response to a low albumin or to elevated globulins. Total globulins may be increased in some chronic inflammatory diseases (tuberculosis, syphilis), multiple myeloma, collagen disease, and rheu-

matoid arthritis. Decreased levels are seen in case of hepatic dysfunction, renal disease and various neoplasms.

Design of laboratory work

Note the principle, laboratory procedure, results of analysis and conclusion. The possibilities of albumin and globulin separation due the use of this method have to be précisedin it.

Practical 2

PROTEIN DENATURATION

Denaturation is a process when proteins lose thequaternary structure, tertiary structure and secondary structure which is present in their native state and change physical, chemical and biological properties. Application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent (chemical factors), radiation, utrasound or heat (physical factors) cause it.

Reagents

1) Acetone, 2) 10% trichloroacetic acid 3) concentrated HNO_3, 4) 1% $CuSO_4$, 5) concentrated H_2SO_4, 6) 5% lead acetate ($Pb(CH_3COO)_2$), 7) tannin, 8) 20% sulfosalicylic acid.

Material forthe investigation

1% Albumin solution.

Principle

Denaturation reduces the protein charge and hydration shells and leads to thechange in dissolving features in water and stability.

Chemical denaturation.

Procedure and observation

Put 5 drops of albumin solution in 5 test tubes and add the reagents, which are included in the following table. Signs "+" and "–" indicate the results of observation. The intensity of denaturation indicates the number of "+" sign.

Reversibility and irreversibility of protein precipitation is proved by adding 20-30 drops of water to the pellet.

Probe number	Reagents	Number of drops	Mechanism and reaction features	Result
Denaturation by heavy metal salts				
1 2	Coopersulfate Lead acetate	2 2	The metal ions bind with charged amino groups, thereby change of the protein spatial structure is observed	
Denaturation by concentrated mineral acids				
3 4	Nitric acid Sulfuric acid	2 2	Concentrated acids cause the protein denaturation by changing its charge. Cationicgroups areneutralized.	
5 6	Nitric acid Sulfuric acid	10 10	The disappearance of the protein precipitate by adding an excess of sulfuric acid group is the result of ionic recharging	
Denaturation by organic acids				
7 8	Trichloroacetic acid Sulfosalicylic acid	2 2	Protein neutralizes the acid charge system, destroy shydrogen bonds and forms complexes with the protein	
Denaturation by tannin				
9	Tannin	2	It is formed insoluble salt-like compound with basic amino groups of amino acids	
Denaturationby organic solvents				
10	Acetone	5	It destroys hydrophobic interactions in the protein molecule	

Practical significance

Chemical denaturation reaction is used to precipitate the protein in biological material with the following definition of low molecular weight substances in the filtrate. This test is performed to detect the presence of the protein in various body fluids and its quantitative analysis.

In medical practice they are used in the treatment and prevention of poisoning of heavy metal salts athome and at work. Their next significant functions arethe disposal of waste in sanitary practices, disinfection of skin and mucous membrane.

Design of laboratory work

Note the principle, laboratory procedures, and results of analysis inthetable. Make a conclusion about the most effective protein precipitation methods.

THEME 1.3. PROTEIN CLASSIFICATION. PROTEIN STRUCTURE AND FUNCTIONS IN HUMAN BODY. COMPLEX PROTEINS

INTRODUCTION

Peptides and proteins have multiple specific functions in the body. They are structural, transport, hormonal, and enzymatic.

The change in the protein structure may underlie the development of pathological processes like sickle cell anemia, or thalassemia. At the same time, many diseases entail changes and ratios in protein level, particularly in blood proteins, which has a diagnostic value.

Protein and the specific reactions of prosthetic group allow us to understand the complex protein composition, as well as to use the data in research of the protein content carrying out the protein-specific reactions.

THE AIM OF THIS PRACTICAL SESSION IS TO:

Study the complex protein structure: phosphoprotein, nucleoproteins, glycoproteins, chromoproteins (hemo- and flavoproteins), metalloproteins, lipoproteins.

Learn how to allocate complex proteins of different objects and perform qualitative reactions to complex protein components.

SELF-STUDY QUESTIONS

1. Structures of proteinogenic amino acids.
2. Protein classification according to their functional characteristics (safety, structural, transport, contractile, hormonal, enzyme). Protein examples for each class.
3. The classes of proteins based on their structure: simple and complex, monomers and polymers, globular and fibrillar. Protein examples of each class.
4. Characterization of simple proteins (albumin, globulins, histones, protamines). Note the features of their structure and function.
5. Characteristics and features of the complex proteins structure classes:

- **Nucleoproteins.** Structure and properties of DNA and RNA. Differences between DNA and RNA. The structures of AMP, ADP, ATP, cAMP nucleotides. Types of histones and their role in the formation of DNA and nucleosomes laying.
- **Chromoproteins** (hemoproteins, flavoproteins, retinal proteins). The chemical structure concept, their examples and functions. Representation of the hemoglobin molecule structure. The structure of heme.
- **Glycoproteins.** Structure, function in the body. Representation of the carbohydrate moiety structure. Proteoglycans, the structure, functions in the body. The chemical structure of hyaluronic acid and chondroitin sulfates.
- **Lipoproteins.** The structure of the lipoprotein particles. The main transport forms of plasma lipids – chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL), their functions.
- **Metalloproteins**, representation of the structure, main examples. Metalloenzymes, definition, their examples.

• **Phosphoprotein.** How does phosphate group join a protein? The main examples.

6. Analysis of the complex proteins chemical composition – glycoproteins and phosphoproteins. The principle of methods.

TOPICS FOR REPORTS

1. The structural proteins: keratin, collagen, elastin. Features of the structure. Functions.

2. Contractile muscle proteins: actin and myosin. Features of the structure. Function.

Practical

ISOLATION AND ANALYSIS OF PHOSPHOPROTEINS AND GLYCOPROTEINS CHEMICAL COMPOSITION

Qualitative test for non-protein components used for the detection of complex proteins in various objects.

Reagents

1) 1% thymol solution in ethanol, 2) 10% solution NaOH, 3) molybdenum reagent, 4) concentrated H_2SO_4, 5) 1% $CuSO_4$ solution, 6) 10% CH_3COOH solution, 7) 10% trichloroacetic acid.

Analysis of phosphoprotein chemical composition

Material of the investigation

Milk.

Procedure and observation

Take two test tubes and poure 1.0 ml of milk into them. Then add equal amountof distilled water and mix. Perform Molybdenum and Biuret tests according to the following instructions.

Molybdenum test for phosphoric acid	*Principle* Phosphoric acid presented in the precipitate interacts with ammonium molybdate in nitric acid, forms painted in lemon-yellow color ammonium phosphomolybdate complex compound. *Procedure* Take a half of the precipitate from the filter and put it into the test tube. Add 20 drops of a molybdenum reagent in it and the ammonium phosphomolybdate precipitates.
Biuret test	*Principle* A pink-violet or blue-violet color complex compound is formed in alkaline solution at the protein present. *Procedure* Add 10 drops of 10% solution of NaOH and 1 drop of 1% solution of $CuSO_4$ to the precipitate on the filter.

Analysis of glycoprotein chemical composition

Material for the investigation

Saliva, collected after rinsing the mouth with water.

Procedure and observation

Collect 2.0 ml of saliva in 2 test tubes; add 10% trichloroacetic acid drop by drop till the precipitate appears.

Molisch's test (for the presence of carbohydrates)	*Principle* The hydroxymethyl sulfuric acid is formed after pentoses dehydration. Its condensation with thymol hydroxymethyl furfural is associated with the development of red color, and pink rings appear in vitro. *Procedure* Remove the liquid from the 1st test-tube and add 2-3 drops of thymol solution to the clot. Mix gently and add the concentrated H_2SO_4.
Biuret test	*Principle* A pink-violet or blue-violet color complex compound is formed in the alkaline solution with protein. *Procedure* Add 10 drops of 10% solution of NaOH in the second test tube for the acid neutralization. Then put 10 drops of 10% solution of NaOH and 1 drop of 1% solution of $CuSO_4$.

Design of practical

Note the principle, laboratory procedures, and results of analysis in the table. Make a conclusion about complex protein composition by component detection reactions.

Object	Complex proteins	Component	Coloring	Conclusion
Saliva	Glycoproteins	Protein Carbohydrates		
Milk	Phosphoproteins	Protein Phosphoric acid		

TESTS

Choose one or more correct answers.

1. AN ESSENTIAL AMINO ACID IS
   1) glycine
   2) valine
   3) tyrosine
   4) serine

2. AT PH 7.4 POSITIVELY CHARGED AMINO ACIDS IS
   1) proline
   2) oxyproline
   3) arginine
   4) aspartate

3. SIMPLE PROTEIN IS
    1) hemoglobin
    2) somatotropin
    3) albumin
    4) ceruloplasmin

4. ALPHA HELIX IS STABILIZED BY
    1) disulfide bonds
    2) hydrophobic interactions
    3) cooperative binding
    4) peptide bonds
    5) hydrogen bonds

5. THE TERTIARY STRUCTURE OF PROTEINS IS STABILIZED BY
    1) amino acids radical electrostatic interactions
    2) hydrogen bonds
    3) hydrophobic interactions
    4) disulfide bonds
    5) interaction with the prosthetic group

6. RECOGNIZING PROTEIN-LIGAND BINDING SITE IS
    1) protein and non-protein cofactor binding site
    2) "niche" of the peptide molecule
    3) fragment of hydrophilic peptide
    4) a region of the protein that is complementary to a specific molecule or ion (ligand)

7. AT AN ELECTRIC FIELD AND AT PH OF 6.8 PEPTIDE ASP-LEU-GLU-GLY WILL
    1) move to the anode
    2) stay put
    3) move to the cathode

8. LIGAND BINDING SITE IS MADE OF
    1) primary structure
    2) tertiary structure
    3) secondary structure
    4) quaternary structure

9. THE SPECIFIC PROTEIN PROPERTY IS USED IN THE "ARTIFICIAL KIDNEY"
    1) the failure to penetrate through a semipermeable membrane (dialysis)
    2) the ability to connect the polar molecules

3) the formation of oncotic pressure
4) low rate of diffusion

10. COOPERATIVE INTERACTIONS IN PROTEIN ARE PERFORMED AT THE LEVEL OF
    1) primary structure
    2) tertiary structure
    3) secondary structure
    4) quaternary structure

CASE STUDIES

1. Partial hydrolysis of insulin (B chain) is detected be recovering the tetrapeptide Glu-Ala-Glu-Leu.

Note the direction of the peptide in an electric field at a pH of 3.0 and 10.5.

2. The electrophoretic mobility of proteins is studied.

Note the direction of following proteins in the electric field: ovalbumin at pH 5.0 (isoelectric point pH 4.6); lactoglobulin at pH 5.0 and 7.0 (the isoelectric point pH 5.2); chymotrypsinogen at pH 5.0; 9.5 and 11.0 (isoelectric point pH 9.5).

3. Two peptides are obtained in partial hydrolysis and protein fractionation:

a) Gly-Ala-Val-Leu-Ile;

b) Thr-Asp-Lys-Tyr-Glu.

Indicate a compound that is more similar in properties to hydroxyl carbon.

Select a compound which is more soluble in nonaqueous fat-like medium.

Explain the features of each of these compounds in the procedure of Biuret, ninhydrin reactions, lead sulfide test and xanthoprotein reaction.

Indicate a compound capable to form the salt bridges.

UNIT 2
VITAMIN STRUCTURE, THEIR CLASSIFICATION AND ROLE

THEME 2.1. FAT SOLUBLE VITAMINS

INTRODUCTION

Fat soluble vitamins are hydrophobic organic substances. They cannot be synthesized and are essential food factors. The vitamin D is an exception, it could be synthesized in the skin, but in insufficient quantities. The lack and insufficient intake of vitamins in the body develops severe condition, leading to metabolic disorders. The biological role of fat soluble vitamins is associated with the regulation of metabolic processes. Additionally, these vitamins affect the synthesis of different structural proteins and enzymes, especially in children.

Knowledge about vitamins, as well as practical skills in the qualitative determination of these substances in food are of great importance. They are an effective method of the hypo-and avitaminosis prevention and used in the treatment of different diseases (pathologies of skin, liver, muscle, and bone).

THE AIM OF THIS PRACTICAL SESSION IS TO:

Study chemical structure, features, classification and biological role of fat soluble vitamins. Describe the clinical signs of avitaminosis.

Perform fat soluble quantitative reactions of vitamins with standardized solutions.

SELF-STUDY QUESTIONS

1. General characteristics of vitamins, their role. Classification and nomenclature of vitamins.

2. Characteristics of hypo-and avitaminosis, hypervitaminosis, their exogenous and endogenous causes. Causes of hypovitaminosis in children.

3. Provitamins – beta carotene, ergosterol, 7-dehydrocholesterol. Conversion of provitamins in the vitamin, beta carotene is an example. The concept of carotenoids and their roles in the body.

4. The concept of antivitamin. Antivitamin as medicines. Dicumarol, mechanism of its action.

5. Characteristics of the individual fat soluble vitamins under the plan:

- the structure of vitamins A, E, K, D_2 and D_3, F,
- the structure of the vitamin A and D active forms,
- food sources,
- the minimum daily requirement,
- biochemical functions, examples of reactions and / or processes that takes vitamin participation,
- clinical signs of hypo-, avitaminosis, hypervitaminosis.

6. Biochemical manifestations of vitamin D deficiency, vitamin D-dependent and vitamin D-resistant rickets. The role of liver and kidney diseases in the development of clinical signs of hypovitaminosis.

7. Qualitative reaction for retinol, tocopherol, menadione, cholecalciferol. Operating principles of methods.

8. Make a table for the fat soluble vitamins.

The name of a vitamin (letter, chemical, physiological)	Chemical structure	Daily requirement	Biological role	Clinical signs of hypo- hyper- and avitaminosis	Food sources	Medicines

TOPICS FOR REPORTS

1. History and discovery of vitamins. Works of Russian and foreign scientists.

2. Rickets – types, biochemical causes, prevention, treatment. Rickets-like conditions.

3. Vitamin A, its active forms: retinal and retinoic acid. Participation in metabolism and photochemistry of visual process?

4. Vitamin D, its active form. Participation in metabolism.

Practical 1
QUALITATIVE RECTIONS OF FAT SOLUBLE VITAMINS

RETINOL QUALIITATIVE REACTION

Principle

The method is based on the ability of concentrated sulfuric acid to take water from retinol with the formation of colored products.

Reagents

1) Concentrated H_2SO_4, 2) butanol.

Material for investigation

VitaminA, 3.44% oil solution.

Procedure and observation

Add 2 drops of vitamin A solution and 5 drops of butanol in tube. Leave for 1 minute, shaking occasionally. Then add 5-7 drops of conc. H_2SO_4, and a blue color turns into purple, then red-brown appears.

CHOLECALCIFEROL QUALITATIVE REACTION

Principle

A red-violet color appears as a result of the interaction of vitamin D_3 with hydroxymethylfurfural formed from sucrose due to the action of concentrated sulfuric acid.

Reagents

1) Concentrated H_2SO_4, 2) butanol, 3) 20% sucrose solution.

Material for investigation

Vitamin D_3, oil solution, 15 thousand IU/ml.

Procedure and observation

Take 3 drops of vitamin D_3 and 5 drops of butanol, add 3 drops of sucrose solution and 5-7 drops of concentrated H_2SO_4. A red-violet color turns into black and then converts into white.

TOCOFEROL QUALITATIVE REACTION

Principle

A compound of quinoid structure of red or yellowish-red color is formed as a result of the reaction of tocopherol with concentrated nitric acid.

Reagents

Concentrated HNO_3.

Material for investigation

Vitamin E, 30% oil solution.

Procedure and observation

Take 2 drops of vitamin E in a dry tube, and then add 10 drops of concentrated HNO_3. Shake the tube and observe the appearance of a red color. To accelerate the reaction, the tube can be placed in a boiling water bath for 3 minutes.

VITAMIN K QUALITATIVE REACTION

Principle

Menadione (synthetic analogue of vitamin K_1) in the presence of cysteine in a basic medium turns to lemon yellow color.

Reagents

1) 0.025% cysteine solution, 2) 10% solution of sodium hydroxide.

Material for investigation

0.05% Menadione solution.

Procedure and observation

Add 5 drops of cysteine and 1 drop of 10% NaOH solution to 5 drops of menadione. Yellow lemon color appears.

Clinical and diagnostic significance

Vitamin qualitative reaction allows us to establish the authenticity (confidence) of vitamin drugs, as well as their use for the detection and quantification of vitamins in food and medical plants.

Design of laboratory work

Note the principle, laboratory procedures, and results of analysis in the table. Make a conclusion about the ability of vitamin detection.

Vitamins	Method and reagents	Result

THEME 2.2. WATER SOLUBLE VITAMINS

INTRODUCTION

Water soluble vitamins are organic compounds of different chemical nature with low molecular weight. They are the regulators of body's metabolism, which cannot be synthesized in the body and belong to the essential food supplements. Being synthesized in the liver vitamin PP is the exception. The insufficient intake of vitamins leads to the development of severe metabolic disorder – hypo- and avitaminosis. The biological role of water soluble vitamins is associated with the regulation of metabolic processes in the body. Most of them are a part of coenzymes and prosthetic groups of enzymes.

Knowledge about vitamins, acquiring the practical skills in the determination of these substances is of great importance. They are used as an effective method of hypo-and avitaminosis prevention. Vitamins are therapeutic agents in a non-specific treatment for a variety of diseases.

THE AIM OF THIS PRACTICAL SESSION IS TO:

Study the properties, chemical structure, classification, biological role of vitamins, clinical sign of deficiency diseases (avitaminosis).

Master practical skills for conducting qualitative analysis of vitamins.

SELF-STUDY QUESTIONS

1. Characteristics of water soluble vitamins according to the following plan:

- Chemical structure of B_1 (thiamine), B_2 (riboflavin), B_3 (PP, niacin, niacin amide), B_6 (pyridoxine), C (ascorbic acid), H (biotin). The structure of vitamin B_5 (pantothenic acid), B_9 (folic acid), B_{12} (cobalamin),
- food sources,
- vitamin’s daily requirement,
- structure of coenzymes (TPP, FMN and FAD, NAD^+ and $NADP^+$, PLP),
- biochemical functions, examples of reactions and / or processes in which coenzyme takes part,
- the possible causes of hypo- and avitaminosis and their clinical signs.

2. The mechanism of antibacterial activity of sulfonamides.

3. Antivitamin – isoniazid, avidin, pteridines. The mechanism of their action. Application of antivitamin as medicaments.

4. Qualitative reactions of riboflavin, niacin, pyridoxine, cobalamin, ascorbic acid. Operating principles of methods.

TOPICS FOR REPORT

1. Vitamin-like compounds, general characteristics, types, their role in metabolism.
2. Vitamin P (bioflavonoids), its importance and the need for the children's body.
3. The mono and multivitamin preparations for nonspecific therapy. Advantages and disadvantages of the use of these drugs in everyday life.
4. Hereditary metabolic disorders and functions of vitamin B_{12}.

Practical

QUALITATIVE REACTIONS OF WATER SOLUBLE VITAMINS

Reagents

1) Concentrated HCl, 2) 1% solution of $FeCl_3$, 3) 10 % thiourea solution, 4) 10% CH_3COOH solution, 5) 5% solution of $Cu(CH_3COO)_2$, 6) metal zinc, 7) 0.01% methylene blue solution.

Equipment

Water bath.

Material for investigation

1% pyridoxine hydrochloride solution, dry nicotinic acid, 0.025% riboflavin solution, 1% vitamin B_{12} solution, 1% ascorbic acid solution.

RIBOFLAVIN QUALITATIVE REACTION

Material for investigation

0.025% riboflavin solution, 1:5 dilutions.

Reducing reaction

Principle

The method is based on the reduction of riboflavin hydrogen released during the addition of metallic zinc to concentrated HCl. The pink color product, riboflavin, appears which then transforms to colorless leucoform of the product.

Procedure and observation

Take 10 drops of riboflavin solution and add 5 drops of concentrated HCl and the granule of metal zinc. The liquid gets pink and then becomes colorless.

NICOTINIC ACID QUALITATIVE REACTION

Principle

Heating vitamin PP solution with acetic acid copper is associated with blue precipitation sparingly soluble copper salt of nicotinic acid development.

Material for investigation

Nicotinamide powder.

Procedure and observation

Take 10.5 mg (pinch) of nicotinic acid, place it into tube with 10 drops of 10% acetic acid solution, and dissolve the precipitate by heating. Then add equal amount

of acetic acid solution of copper ($Cu(CH_3COO)_2$) to the obtained solution. The liquid becomes turbid.

PYRIDOXINE QUALITATUVEREACTION

Principle

Reaction of Vitamin B_6 with $FeCl_3$ is led to red color complex salt development.

Material for investigation

1% vitamin B_6 solution.

Procedure and observation

Take 5 drops of 1% vitamin B_6 solution and add equal quantity of 1% $FeCl_3$ solution. Then the red color appears.

COBALAMIN QUALITATIVE REACTION

Principle

Cobalt ions contained in a vitamin are interacted with thiourea. Then the obtained solution is heated, and a green color of cobalt thiocyanate appears.

Material for investigation

1% vitamin B_{12} solution.

Procedure and observation

2-3 drops thiourea is applied on ashless filter, dried in hot air over the tiles. Then add 1-2 drops of vitamin $B_{12}$to the filter. The filter is dried again in hot air.

In the filter, on the edges the green color spots are appears, indicating the presence of cobalt.

ASCORBIC ACID QUALITATIVE REACTION

Material for investigation

1% ascorbic acid solution.

Principle

Ascorbic acid has the ability to restore the methylene blue. It is oxidized at the same time to dehydroascorbic acid. Blue methylene during the restoring becomes colorless.

Procedure and observation

Take 5 drops of 1% ascorbic acid solution to the first tube. Add 5 drops of distillated water to the second tube. Put 1 drop of methylene blue to both tubes and place them to the water bath at 40°C. The decoloration of liquid with vitamin is observed.

Design of laboratory work

Note the principle, laboratory procedures, and results of analysis in the table. Make a conclusion about ability of vitamin detection.

Vitamins	**Methods and reagents**	**Result**

TESTS

Choose one or more correct answers.

1. THE MAIN FUNCTION OF WATER-SOLUBLE VITAMINSIS
    1) precursors of hormones
    2) protection of biological membranes
    3) precursors of coenzymes
    4) carbohydrate precursors

2. THE COENZYME FORM OF VITAMIN B_1 IS CALLED
    1) pyridoxal phosphate
    2) flavin mononucleotide
    3) thiamindiphosphate
    4) nicotinamide

3. THE COENZYME FORM OF VITAMIN B_2 IS CALLED
    1) pyridoxal phosphate
    2) flavin mononucleotide
    3) tetrahydrofolate
    4) coenzyme A

4. THE PANTHOTHENIC ACID IS THE COMPONENT OF the COENZYME
    1) coenzyme A
    2) tetrahydrofolate acid
    3) thiamin pyrophosphate
    4) flavinmononucleotide

5. THE HYPOVITAMINOSIS B_6 DURING THE LONG INTAKE OF ANTIBIOTICS AND SULFANILAMIDES IS ASSOCIATED WITH
    1) suppression of intestinal microflora
    2) binding of drugs with vitamin
    3) the influence of drugs on the synthesis of coenzyme form
    4) inhibition of pyridoxine-dependent enzymes

6. THE SCURVY IS A DISEASE CAUSED BY A DEFICIENCY OF VITAMIN C CHARACTERIZED BY
    1) oxidation of SH-groups of enzymes
    2) altered collagen synthesis
    3) altered albumin synthesis
    4) oxidation of the connective tissue cells lipid membrane

7. THERHODOPSIN PROSTHETIC GROUP, THE RECEPTOR PROTEIN OF THE RETINA IS
    1) riboflavin
    2) calciferol
    3) retinal
    4) tocopherol

8. THEVITAMIN F CONSISTS OF FATTY ACIDS
   1) oleic
   2) linoleic
   3) linolenic
   4) stearic acid
   5) arachidonic

9. THEVITAMIN A DEFICEINCY IS CHARACTERIZED BY
   1) hyperkeratosis
   2) reduction in blood concentration of rhodopsin
   3) bleeding
   4) osteomalacia

10. THE MAIN ROLE OF VITAMIN K
   1) is an antioxidant
   2) increases platelet production
   3) participates in the coagulation factors synthesis
   4) participates in blood coagulation reactions

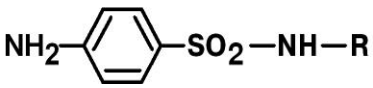
CASE STUDIES

1. *Lactobacillus casei* can grow in a simple culture medium containing vitamins and riboflavin pyridoxine and 4 amino acids. If in the culture medium a complete set of amino acids and riboflavin is added, pyridoxine amount needed for optimal growth of bacteria will be reduced in 90%.

Explain the fact.

2. Sulfonamides were first used as antibacterial medicines with wide spectrum. They have a similar structure to para-aminobenzoic acid.

Justify the use of sulfonamides. Give further guidance in the application of these drugs.



Sulfanilamides structure

3. Patient will have a pre-operational treatment.

Consider the application of required vitamins.

UNIT 3
ENZYMOLOGY

THEME 3.1. ENZYME STRUCTURE AND PROPERTIES. APPLICATION IN MEDICINE

INTRODUCTION

Enzymes are protein molecules, which serve as biocatalysts in metabolic processes. The study of their structure and functions are necessary for understanding the biochemical features in variety of tissues and the ways of its possible regulation. The pathogenesis of different diseases is found to be accompanied by the altered enzyme function.

THE AIM OF PRACTICAL IS

to study the enzyme structure, properties and features;
to observe the influence of different factors on enzyme activity in vitro;
to obtain the practical skills for enzyme specificity investigation.

SELF-STUDY QUESTIONS

1. Simple and complex protein structure.
2. Coenzyme forms of vitamins B_1 (TPP), B_2 (FMN and FAD), PP (NAD^+ and $NADP^+$), B_6 (PLP).
3. The biological role of enzymes. The concept of an energy level and the activation energy of the reaction.
4. Stages of enzymatic catalysis.
5. Characteristics of the structural and functional enzymes organization according to plan:
   - simple enzymes,
   - complex enzymes: term holoenzyme, apoenzyme, a cofactor, coenzyme, prosthetic group,
   - active center (contact and catalytic sites),
   - allosteric center.
6. Acid-base catalysis and covalent mechanisms.
7. The similarities and differences in the action of enzymes and inorganic catalysts.
8. The general principles of quantifying enzyme activity. Units of enzyme activity.
9. A multi-complex structure, the principles of self-assembly, the role. Examples.
10. Isozyme, especially their structure on the example of creatine kinase and lactate dehydrogenase.
11. The main properties of enzymes. The graphs of enzymatic reaction rate depend on:
    - temperature,

- the pH of the environment,
- the substrate concentration,
- the enzyme concentration.

12. Specificity, types. Mechanisms of specificity (Fisher's theory and the theory of Koshland).

13. Practical uses of enzymes in medicine: diagnostic and enzyme replacement therapy. Examples.

14. Enzymopathies, primary and secondary forms. Examples. The role of the lack of co-enzymes in enzymopathy development.

15. The study of enzyme action specificity on the example of amylase and urease. The principle of the method.

16. The dependence of the enzymatic reaction on temperature. Salivary amylase as an example. The principle of the method.

TOPIC FOR REPORTS

1. The role of the essential microelements in the functioning and regulation of enzyme activity.

Practical 1

DEPENDENCE OF ENZYME ACTIVITY ON TEMPERATURE

Investigation of influence of temperature on salivary amylase activity

Reagents

1) 1% starch solution, 2) Lughole's solution.

Material of investigation

Saliva, diluted in 1:10 (source of α-amylase).

Principle

To investigate the dependence of enzymatic reaction activity on temperature in the hydrolysis of starch using saliva amylase (diastase, 1.4-α-D-glucose hydrolase, EC 3.2.1.1.). During the incubation of the substrate mixture (starch) and enzyme (amylase of saliva) under different temperature conditions enzyme will hydrolyze different amounts of substrate.

Starch hydrolysis by amylase passes through the stage of dextrin formation and then disaccharide maltose formation.

$$\text{Starch} + H_2O \xrightarrow{\alpha\text{-amylase}} \text{Maltose} + \text{Dextrins}$$

The amount of split starch is evaluated by color reaction with iodine. Undigested starch with iodine gives a blue color. The products of hydrolysis of starch (dextrin), depending on the size of the molecules give iodine staining: ● amylodextrins – purple, ● erythrodextrins – red-brown, and maltose ● achrodextrins – no color reaction, yellow color corresponds to the color of an aqueous solution of iodine.

Procedure and observation.

Preparation of saliva (the student on duty performs the saliva for the whole group).

Collect 1.0 ml of saliva into a measuring tube and dilute with distilled water to 10.0 ml, mix well (not shake!).

1. Add 10 drops of starch to the four tubes (1, 2, 3, 4). Bottle the starch solution before use! Add10 drops of saliva diluted (solution of α-amylase) to the following four tubes (5, 6, 7, 8). Divide the tubes into pairs – 1-5, 2-6, 3-7, 4-8.

2. To eliminate the enzymatic reaction after reaching the required temperature, saliva and starch solutions should be initially heated separately:

Place 1st pair of tubes in an ice bath (0°C). Leave 2nd pair at room temperature (20°C). Maintain the third pair at a temperature of 38-40°C. Place the 4th pair of tubes in a boiling water bath (100°C) (water bath).

3. Wait for 3 minutes, and then combine the content of each pair of tubes, mix and place immediately for 10 minutes in the same conditions.

4. Check the progress of the reaction. To do this, take 3 drops of mixture from the third tube (38-40°C) and put it on the slide, then add 1 drop of Lughole's reagent:

- the color of mixture is blue. It indicates a lower rate of starch hydrolysis. In this case it is necessary to extend the incubation time.
- the appearance of red or yellow color indicates the completion of amylase starch hydrolysis (you can proceed to step 5.).

5. After the hydrolysis of starch in the third sample add 2 drops of Lughole's reagent in all tubes at the same time and compare the color in all tubes.

Design of laboratory work

Note the principle, laboratory procedures, and results of analysis in the table. Make a conclusion about the optimal temperature of enzyme action.

N tube	**Temperature of incubation**	**Color**	**Relative rate of enzymatic reaction**
1	0°C		
2	20°C		
3	38°C		
4	100°C		

Practical 2

INVESTIGATION OF ENZYME SPECIFICITY

Reagents

1) 1% urea solution, 2) 1% thiourea solution, 3) 0.5% phenolphthalein alcohol solution, 4) 1% starch solution, 5) 1% sucrose solution, 6) Felling reagents: Felling I and Felling II.

Material for investigation

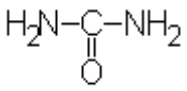
Urease solution made from the seeds of watermelon

Saliva, diluted in 1:10 (source of α-amylase).

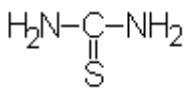
Detection of urease absolute specificity

Principle

The method is based on a comparison of the possibility of similar substrates – urea and thiourea to be hydrolyzed by urease (EC 3.5.1.5.).

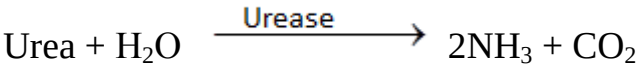


Urea



Thiourea

The enzyme action is detected by a phenolphthalein color change in an alkaline medium, which results in ammonia release by urea hydrolysis.



$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

Procedure

Urease preparation:

Grind clear 3-4 watermelon seed, corn in a mortar with 1 ml of distilled water. Add 10.0 ml of water and filter the emulsion. Use it as urease.

	Sample 1, Drops	**Sample 2, Drops**
1% urea solution	10	—
1% thiourea solution	—	10
Urease	10	10
Phenolphthalein solution	1-2	1-2
	Mix well. Leave for 3-5 minutes. Observe the pink color appearance in one of the tubes.	

Design of laboratory work

Note the principle, laboratory procedures, and results of analysis in the table. Make a conclusion about the reason of color absence in one of the tubes and specificity of an enzyme.

Samples	**Substrate of reaction**	**Color**	**Presence of color**
Sample 1 Sample 2			

Detection of salivary amylase specificity

Principle

The method is based on a comparative study of the enzyme amylase ability to hydrolyze different substrates carbohydrate – starch polysaccharide and the disaccharide sucrose (EC 3.2.1.1.)

$$\text{Substrate} + H_2O \xrightarrow{\alpha\text{-amylase}} \text{Product (maltose, dextrins)}$$

The enzyme action on the substrate is detected using qualitative response of carbohydrate to free aldehyde group (Trommer reaction).

Trommer reaction may be positive (red-orange color) only by splitting substrates reducing sugars (maltose, glucose, etc.), which have a free aldehyde group and have reducing properties. Reaction substrates (starches and sucrose) do not have free aldehyde group, so do not give a positive reaction to Trommer's test.

Procedure and observation

Preparation of saliva (the student on duty performs the saliva for the whole group).

Collect 1.0 ml of saliva into a measuring tube and dilute with distilled water to 10.0 ml, mix well (not shake!).

	Sample 1, Drops	**Sample 2, Drops**
1% starch solution	—	10
1% sucrose solution	10	—
Saliva solution	5	5
	Mix well. Incubate for 10 min at 37°C.	
Felling I reagent	3	3
Felling II reagent	3	3
	Mix well. Incubate in a boiling water bath at a temperature of 100°C until the appearance of yellow-orange or reddish color in one of the tubes.	

Design of laboratory work

Note the principle, laboratory procedures, and results of analysis in the table. Make a conclusion about the reason for color absence in one of the tubes and specificity of an enzyme.

Samples	**Substrate of reaction**	**Color**	**Presence of color**
Sample 1 Sample 2			

THEME 3.2. ENZYME ACTIVITY REGULATION

INTRODUCTION

A plethora of drugs affects the enzymes activity in the body. Enzymes and affecting their activity drugs can be used in medicine as therapeutic agents.

The rapidly expanding knowledge of enzyme activity regulation is providing new targets for disease characterization, and early diagnosis.

THE AIM OF THE PRACTICAL IS

To know the features of enzymatic catalysis and to study the enzyme activity regulation in the cell.

To introduce the methods of enzymes detection in tissues and biological fluids. To determine the amylase activity in serum and urine.

SELF-STUDY QUESTIONS

1. Ways of enzymatic reactions regulation in the cell (in vivo):

- compartmentalization,
- change in the enzyme amount – the example: the effect of glucocorticoids on gluconeogenesis,
- substrate availability change on an example of oxaloacetate and the citric acid cycle,
- proenzymes and their limited proteolysis by the example of the enzymes in the gastrointestinal tract,
- protein-protein interactions, for example, adenylate cyclase activation (join of regulatory proteins) and the protein kinase A (dissociation of protein protomers). Scheme of processes,
- allosteric regulation mechanisms of enzymes: a) changes in the scheme of enzyme activity when exposed to effector b) the role of allosteric regulation of metabolism by the example of phosphofructokinase,
- covalent modification of the enzyme by the example of enzyme glycogen synthase and glycogen phosphorylase. Mechanism of regulation.

2. Characteristics of enzyme inhibition. Competitive and non-competitive inhibition. Reversible and irreversible inhibition. Examples.

3. The use of enzymes inhibitors as drugs. Examples.

4. Determination of amylase activity in blood serum and urine. The principle of the method. Clinical and diagnostic significance and reference values.

Practical

DETERMINATION OF AMYLASE ACTIVITY IN SERUM AND URINE

Principle

α-Amylase (diastase, 1.4-α-D-glycan hydrolase, EC 3.2.1.1.) catalyzes the hydrolysis of starch and glycogen α-1.4 glycosidic bonds to maltose and dextrin.

$$\text{Starch} + H_2O \xrightarrow{\alpha\text{-amylase}} \text{Maltose} + \text{Dextrin}$$

The amount of remaining starch proportional to the catalytic activity of the enzyme is determined by the color reaction with iodine.

Reagents

1) Substrate, 0.04% starch solution in distilled water, 2) working solution of iodine, 0.01 M.

Material for investigation

Serum, urine.

Procedure

	Test 1, ml	**Test 2**, ml	**Control**, ml
Starch solution	1.0	1.0	1.0
	Incubate 5 minutes at 37°C		
Serum Urine	0.02 —	— 0.02	— —
	Incubate 5 minutes at 37°C		
0,01 Mworking solution of iodine Cold distilled water	1.0 8.0	1.0 8.0	1.0 8.0
	Mix well. Measure the optical density of the test and control solutions against the water at a 670 nm wavelength (red filter).		

Calculation:

$$\text{Amylase activity, g/l·h} = \frac{E_{control} - E_{test}}{E_{control}} \times 240$$

Note: $E_{control}$ and E_{test} – optical density of control and test samples, 240 – coefficient of calculation.

Normal values

Serum	16-30 g/l·h
Urine	28-160 g/l·h

Clinical and diagnostic significance

There are two amylase isozymes in human blood: pancreatic – P type (30%) and salivary – S (70%), which are released into the bloodstream as a result of natural aging of salivary glands and pancreas cells. The enzyme has a relatively low molecular weight (about 48.000 Da). It is filtered in the glomerulus and is contained in urine. The ratio of isozymes in urine differed from the blood: P type – 70%, S type – 30%.

Serum and urine

The increased enzyme activity in the serum and urine occurs in the pancreatic lesions. The enzyme activity in the blood reaches a maximum in 12-24 hours after the disease onset and increases in 10 to 30 times in acute pancreatitis. The treatment of lesions could lead to enzyme activity normalization during 2-6 days. The activity of

the enzyme is moderate in chronic pancreatitis. The increase in enzyme activity is detected in lesions of salivary glands, cholecystitis, inflammation of biliary tract diseases, during the pregnancy, renal failure, bowel obstruction, and diabetic ketoacidosis, in some tumors as well as lung and ovarian cancers.

The reduced amylase activity is rarely detected in clinical practice. It usually doesn't have a diagnostic value. Sometimes it is seen in patients with liver disease (cirrhosis), cancers, hypothyroidism, cachexia, with toxemia of pregnancy.

Design of laboratory work

Note the principle, laboratory procedures, and results of analysis, its clinical and diagnostic significance. Make a conclusion about the pathologies associated with changed amylase activity.

THEME 3.3. ENZYME CLASSIFICATION AND NOMENCLATURE (SEMINAR)

INTRODUCTION

Enzymes are protein molecules which serve as biocatalysts in cells. General principles of enzyme classification are found to be necessary for understanding the role of biocatalysts in the metabolism.

THE AIM OF THE SEMINAR

To introduce the enzyme classification and to study specific reactions for each enzyme class.

SELF-STUDY QUESTIONS

1. Role of enzymes and coenzymes in catalysis.
2. Coenzyme forms of vitamins (TPP, FMN and FAD, NAD^+ and $NADP^+$, PLP).
3. The principles of the modern classification and nomenclature of enzymes: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases (synthetases) (Supplement 1).
4. Characteristics of each class of enzymes according to the plan:

- the name and number of the class,
- biochemical role,
- basic subclasses (subclasses 1-3),
- basic coenzymes,
- the rules of enzyme systematic names,
- examples of biochemical reactions (1-3 responses).

TOPIC FOR REPORTS

1. The application of enzymes and therapeutic enzyme inhibitors.
2. The application of enzymes in industry, in biochemical and immunological investigations.

TESTS

Chose the correct answer.

1. ENZYMES DIFFER FROM NON-PROTEIN CATALYSTS BY
   1) the reduction the activation energy
   2) the devoicing the consumption in the reaction
   3) the absence of irreversible changes
   4) the high specificity

2. A TRANSFER OF GROUPS WITHIN THE MOLECULE CATALYZES
   1) isomerase
   2) transferase
   3) lyase
   4) hydrolase

3. A REVERSIBLE REACTION OF SYNTHESIS AND BREAKDOWN WITH FORMING A DOUBLE BONDBETWEEN GROUPSIS CATALYZED BY
   1) isomerase
   2) ligase
   3) lyase
   4) transferase

4. A ALCOHOLDEHYDROGENASE IS A_____.
   1) oxidoreductase
   2) hydrolase
   3) ligase
   4) transferase

5. THE ENZYME FROM THE CLASS OF HYDROLASES IS
   1) catalase
   2) alcohol dehydrogenase
   3) pepsin
   4) hemoglobin

6. THE DECREASE IN ENZYME ACTIVITY AT 50°C ISPOSSIBLEDUE
   1) the denaturation of apoenzyme
   2) the denaturation of coenzyme
   3) the breakdown of the holoenzyme
   4) the hydrolysis

7. ENZYME SUBSTRATE SPECIFICITY EXISTS DUE TO
   1) a set of determined functional groups in the active center
   2) the compliance of the active chemical substrate center
   3) the presence of coenzyme
   4) the spatial conformity of the substrate active center
   5) the complementarity of the substrate active center

8. THE ACTION OF COMPETITIVE INHIBITORS CAN BE REMOVED BY
   1) the increase the inhibitor concentration
   2) the increase the substrate concentration
   3) the reducing the enzyme concentration
   4) the reaction conditions change: pH and temperature

9. THE COVALENT MODIFICATIONS DURING THE REGULATION OF THE ENZYME ACTIVITY IS
   1) the linkage of any chemical group
   2) the intramolecular rearrangement of the enzyme structure
   3) the addition or removal of a small portion of the substrate
   4) the addition or removal of a small fragment of the enzyme

10. ENZYMES HAVE FOLLOWING PROPERTIES THAT DIFFER THEM FROM THE INORGANIC CATALYSTS
   1) enzymes accelerate the onset of the reaction
   2) enzymes are adjustable
   3) enzymes are consumed in the reaction process
   4) enzymes do not catalyze the reaction impossible energetically

CASE STUDIES

1. If an allosteric enzyme aspartate carbamoyl-transferase (molecule consists of 12 protomers) is incubated for 60 minutes at 4°C, it loses its sensitivity to allosteric inhibitor (CTP). But its enzymatic activity is preserved. All allosteric enzymes have similar properties.

Identify possible mechanisms of an enzyme activity modification.

2. Explain the biochemical rules for enzymes storage and application in enzyme replacement therapy:

- a dry drug dissolution must be performed by distilled water at room temperature,
- the drug must be stirred gently during the dissolving,
- the storage of the drug solution must be at a low temperature,
- a long-term storage of the drug is possible after its drying and sealing in evacuated vials.

3. Lipase is an enzyme of adipose tissue, provides neutral lipids metabolism. It can be in two different forms: in the form of a simple protein and in a form of phosphoprotein.

Explain why the transition from one form to another is accompanied by a change of its activity.

Indicate the condition when the lipase is active. It is known adrenaline induces a cascade of reactions leading to phosphorylation of intracellular proteins during physical activities.

CHECKLIST FOR FINAL LESSON (UNITS 1, 2, 3)

1. Amino acids classification according to their biological role, chemical structure, physical and chemical properties, their solubility in water.

2. The structure of proteinogenic amino acids. Physical and chemical properties of amino acids. The concept of an isoelectric point.

3. The peptide bond formation. The properties of the peptide bond.

4. The biological role of proteins. Classification of proteins according to the function and structure. Physical and chemical properties of proteins and protein solutions. Protein molecule stabilizing factors in the solution. The colloidal properties of proteins.

5. pH changes and amino acids, proteins properties. Charge of amino acids and proteins. Factors causing the precipitation of proteins. The properties of denatured protein. Specific features of denaturation and renaturation.

6. Levels of protein structural organization. The types of bonds stabilizing the protein structure. Amino acids forming these bonds.

7. Simple proteins (albumins, globulins, histones, protamines), their examples, a role in the body.

8. Complex proteins: phosphoproteins, nucleoproteins, glycoproteins and proteoglycans, chromoproteins, metalloproteins, lipoproteins. Structure of nucleotides AMP, ADP, ATP, cAMP. Scheme of heme, hyaluronic acid and chondroitin sulfates.

9. The principle of protein and amino acids color qualitative reactions. Possibility of their use in practice.

10. Removing proteins from the solution and purification of protein solutions from impurities. Mechanisms of reactions. Their use in biochemistry and medicine.

11. Methods of protein precipitations, that could be used for the production of proteins and enzymes in their native state.

12. Composition of random tetrapeptide with determined properties, the ability to call them, the charge and solubility definition, the possible pH of their isoelectric points.

13. Determination of phosphoprotein and glycoprotein composition components.

14. Note the properties of vitamins, their classes. Provitamins and antivitamins, their examples. Common reasons of hypo-and avitaminosis development. Hypervitaminosis.

15. Characteristics of the fat-soluble vitamins A, D, E, K, F: physiological name, chemical structure of vitamins A, D_2, D_3, E, K, F, active forms of vitamins A and D, daily requirement, dietary sources. Biochemical functions and processes of vitamins. Possible reasons and clinical features of hyper-, hypo- and avitaminosis. What are carotenoids? Note their role in the body.

16. Characteristics of water-soluble vitamins B_1, B_2, B_3 (nicotinic acid), B_5 (pantothenic acid), B_6, B_9, B_{12}, C, H: scientific and chemical names, chemical structure (except vitamin B_{12}, folic and pantothenic acid), the daily requirement, dietary sources. Biochemical functions and reactions of vitamins. The structural formulas of

coenzymes (for B_1, B_2, B_3, B_6). Possible reasons and clinical features of hypo- and avitaminosis development. The role of vitamins in child growth and development.

17. The mechanism of sulfonamides antibacterial activity.

18. Qualitative reaction of vitamins A, E, K, D_3, B_2, B_3, B_6, B_{12}, C. Principles of methods, the procedure of determination, practical significance.

19. Enzymes and their role in biochemical reactions. Comparison of inorganic catalysts and enzymes.

20. Enzymes, structural and functional organization (the level of structure, simple and complex enzymes). Holoenzyme, apoenzyme, cofactor, coenzyme, prosthetic group, active and allosteric centers. Apoenzyme and coenzyme role in catalysis. The structure of cell multienzyme complexes.

21. Isozymes, structural features. Characteristics and examples of isozymes.

22. Classification of enzymes. The main sub-classes. The enzyme nomenclature. What is the classification number? Examples of biochemical reactions of and enzymes.

23. Stages of enzymatic catalysis. Features of covalent and acid-base catalysis.

24. Quantitative determination of enzyme activity in biological objects. Units of enzyme activity.

25. The basic properties of the enzymes, dependence of enzyme activity on various factors. The enzyme specificity, its types. Specificity mechanisms (theory of Fisher and Koshland).

26. The methods of metabolic activity regulation in the cell: compartmentalization, change of enzyme concentration, substrate concentration change, the presence of isozymes, mechanisms of enzyme allosteric regulation, covalent modification of enzymes, proenzymes and their limited proteolysis, protein-protein interactions.

27. The main types of enzyme inhibition: competitive and non-competitive, reversible and irreversible. Examples.

28. The use of enzymes in medicine. Enzyme and diagnostic. The use of enzyme inhibitors as drugs. Examples.

29. The difference between primary and secondary forms of enzymopathy. Examples.

30. The effect of temperature on enzyme activity, salivary amylase as an example. The principle of the method and the procedure.

31. The investigation of the enzyme action specificity on the example of salivary amylase and urease. The principle of the method and the procedure.

32. The principle of the method and the procedure of amylase activity determination in serum and urine. Reference values, clinical and diagnostic significance.

UNIT 4
BIOLOGICAL OXIDATION

THEME 4.1. MAIN CATABOLIC PATHWAYS: PYRUVATE OXIDATIVE DECARBOXYLATION. TRICARBONIC ACID CYCLE. ENZYMES OF RESPIRATORY CHAIN. OXIDATIVE PHOSPHORYLATION (SEMINAR)

INTRODUCTION

The catabolism occurs in all living cells of the body in the form of oxidation. This results in multiple transfer of protons and electrons or only electrons from a donor to an acceptor. The final products of this oxidation process are water and carbon dioxide (CO_2 and H_2O). The main function of biological oxidation is to provide the body with energy for life processes. The form of energy in human body is adenosine triphosphate (ATP).

Some substances, such as drugs (barbiturate), and toxic (cyanides, carbon monoxide) are able to inhibit the oxidative phosphorylation and ATP synthesis.

THE AIM OF PRACTIC IS

The study of pyruvate dehydrogenase complex reactions and citric acid cycle, the structure of the respiratory chain and mechanisms of mitochondrial oxidative phosphorylation.

SELF-STUDY QUESTIONS

1. Plastic (anabolism) and energy (catabolism) metabolic functions.
2. Stages of catabolic reactions in the body related to the free energy release. What is the release and storage of energy at each stage?
3. The structure and function of mitochondria.
4. ATP chemical formula, the role of ATP? Significance of cycles ATP-ADP and NADPH-$NADP^+$.
5. The main energy-rich substances in cell: ATP, 1.3-diphosphoglycerate, phosphoenolpyruvate, creatine, phosphate, acetyl-S CoA. Substrate phosphorylation.
6. Sources of key metabolic products – acetyl-S CoA and pyruvic acid. Further fate of substances.
7. The structure of the multienzyme pyruvate dehydrogenase complex, its enzymes and coenzymes. The overall reaction of oxidative decarboxylation of pyruvic acid. The chemical reactions of five separate reactions. Its regulation.
8. Reaction tricarboxylic acid cycle (Krebs cycle, citric acid cycle). The mechanism of the acetyl group oxidation. Enzymes and coenzymes participation in this process. The biological significance of the TCA cycle. Role of oxaloacetate, and NADH of TCA cycle in metabolic rate regulation.
9. Connection of TCA with catabolism of carbohydrates, lipids, proteins.

10. Characterize of the oxidative phosphorylation following the plan:

- molecular organization and sequence of the electron transport chain enzyme complexes, draw a scheme of the respiratory chain enzymes,
- transport of electrons in the respiratory chain complexes, the role of coenzyme (FMN, FeS-proteins, the Q coenzyme, cytochrome heme group),
- the role of oxygen – the final electron acceptor substrates recovered biological oxidation,
- pumping protons from the matrix of the mitochondria – areas of transmembrane transport (area of coupling oxidation and phosphorylation), the electrochemical gradient formation,
- ATP synthase structure, the role of an electrochemical gradient in the ATP synthase.

11. The ratio of phosphorylation – P/O. Its significance for NADH and $FADH_2$ forming. Calculation of ATP produced by oxidation of several substrates (alanine, aspartic and glutamic acid).

12. Complexes of respiratory enzymes, which could act as inhibitors. How is the process of oxidative phosphorylation being inhibited?

13. Uncoupling of oxidation and phosphorylation. The mechanism of this phenomenon. Uncouplers.

14. Brown adipose tissue: its function, localization. The function of the protein thermogenin. Its role in thermogenesis.

15. Causes of hypo energetic states.

16. The oxidative phosphorylation regulation. Respiratory control. Role of ATP and ADP ratio in the regulation of the respiratory chain.

17. Examples of the nucleotides application as medicines (ATP, ADP, AMP, FMN).

TESTS

Choose the correct answer.

1. THE PYRUVATE DEHYDROGENASE REACTION RATE IS INHIBITED BY
    1) ATP, calcium, NADH
    2) calcium, acetyl-S CoA, NAD^+
    3) ADP, $FADH_2$, NADH
    4) acetyl-S CoA, NADH, ATP

2. $FADH_2$ MOLECULE IS FORMED IN THE CITRIC ACID CYCLE BY ACTION OF
    1) malate dehydrogenase
    2) isocitrate
    3) succinate
    4) α-ketoglutarate dehydrogenase

3. THE RATE OF THE CITRIC ACID CYCLE IS DETERMINED BY
    1) α-ketoglutarate
    2) oxaloacetate
    3) succinic acid
    4) citrate

4. THE ELECTRONS FLOW THROUGH THE RESPIRATORY ENZYMES CHAIN IS DEPENDENT ON
    1) the energy of ATP breakdown
    2) the pumping the hydrogen protons through the membrane
    3) the work of iron-sulfur centers
    4) a different electronegativity of carriers

5. THERE IS A DEPENDENCE OF ELECTRONS FLOW RATE THROUGH THE RESPIRATORY CHAIN ON
    1) the ratio of ADP and ATP concentration
    2) the NADH concentration
    3) the amount of consumed oxygen
    4) the ATP synthase activity

6. THE INCREASE OF A ELECTROCHEMICAL GRADIENT IS LEAD TO
    1) an increase the protons pumping speed
    2) an acceleration of ATP synthesis
    3) an improvement the electron transfer rate
    4) an increased CO_2 and H_2O release

7. ENERGY RELEASED BY ELECTRON TRANSFER ALONG THE RESPIRATORY ENZYMES CHAIN IS USED FOR
    1) the pumping of H^+ ions through the membrane
    2) the oxidation of the iron-sulfur centers
    3) the formation of water molecules
    4) the ATP synthesis

8. THE FORMING OF A PROTON GRADIENT ON THE MITOCHONDRIAL MEMBRANE IS DETERMINED BY
    1) the ATP breakdown
    2) the oxidation of NADH
    3) the flow of electrons
    4) the pumping out of H^+ ions in exchange for Na^+

9. THE UNCOUPLERING AGENTS LEAD TO
    1) the reduction of NADH oxidation
    2) the activation of ATP synthesis
    3) the reduction of respiratory chain electron transport
    4) an increase of the proton gradient

10. REDUCED EQUIVALENTS FORMED IN THE CITRIC ACID CYCLE ARE USED
    1) in the respiratory chain enzymes
    2) in the synthesis reactions of glucose, fatty acids, etc.
    3) for the ATP synthase
    4) for the acetyl-S CoA synthesis

CASE STUDIES

1. The intake of uncoupling agents leads to sweating and increased body temperature. Give an explanation of this fact and describe the molecular mechanism.

Explain the change in the ratio P/O in the presence of uncoupling agents.

2. The brown adipose tissue is well developed in some animals hibernate or adapted to living in cold areas. The ATP yield is less than one molecule per 1 atom of oxygen. It is produced 2-3 ATP molecules per 1 atom of oxygen in other tissues.

Identify physiological function of thee low P/O ratio in brown adipose tissue of babies. Mark a possible mechanism of a low P/O ratio in mitochondria of brown adipose tissue.

UNIT 5
AMINO ACIDS AND PROTEINS METABOLISM

THEME 5.1. EXTERNALMETABOLISM OF PROTEINS. PROTEINSDIGESTION AND ABSORPTION

INTRODUCTION

Food sources of proteins are animal and vegetable products. The modification of digestive juice composition or the appearance of its pathological components leads to development of digestive diseases. Abnormalities of protein digestion and amino acids absorption benefit in a lack of protein synthesis in the body and in a metabolism disturbance development.

The measuring of the gastric juice composition is of great importance, especially the investigation of its digestive ability in normal state and in pathologies.

THE AIM OF THE PRACTICAL CLASS IS

To study the enzymes and mechanisms of protein digestion in the stomach and intestine.

To learn the techniques of gastric juice qualitative analysis for measuring the stomach secretory function in normal state and pathologies.

SELF-STUDY QUESTIONS

1. Structure of amino acids and proteins, the peptide bond role in the organization of the protein molecules.
2. Characteristics of the enzyme class of "hydrolases".
3. The term "nitrogen balance" and the reasons for its change (balance of positive and negative nitrogen balance). Features of nitrogen balance in children.
4. Dietary sources of protein. The daily protein requirements for children according to their age and for adults.
5. The biological value of proteins. The concept of the reference protein. Clinical manifestations of protein lack in children. "Kwashiorkor" disease.
6. The mechanism of the hydrochloric acid synthesis in gastric juice and its biological role. "Hyperchlorhydria", "hypochlorhydria", "achlorhydria", "achylia".
7. Digestion of proteins in the stomach and intestine. Characterize gastric enzymes (pepsin, gastrixin, chymosin (rennin)), pancreatic juice (trypsin, chymotrypsin, elastase, carboxypeptidase) and intestinal juice (aminopeptidase, dipeptidase) according to the plan:
   - place of synthesis,
   - mechanism of activation,
   - optimal conditions for work,
   - substrate specificity.
8. Secondary active transport of amino acids through cell membranes.

9. Age characteristics of protein digestion and amino acid absorption in children. Causes of the normal digestion and absorption in children and the relationship of these disorders with the development of allergic reactions. Causes and clinical features of "celiac disease".

10. General characteristics of the "protein putrefaction" in the large intestine. The causes and consequences of this process. Substances formed due to the protein decay.

11. Reactions of amino acid conversion by the enzymes of the intestinal microflora:

- formation reaction of phenol and cresol,
- formation reaction of scatole and indole,
- formation reaction of cadaverine and putrescine,
- sources of methyl mercaptan and hydrogen sulfide.

12. Disposal of toxic products in the liver: microsomal oxidation and conjugation system. Enzymes involved in the oxidation of microsomal. The structure of UDP-glucuronic acid (UDPGA) and phosphoadenosine phosphoric acid (PAPA). Reactions of indicant formation.

13. Qualitative reaction of free hydrochloric acid. The principle of methods. The normal pH of gastric juice, clinical and diagnostic significance of pH determination in gastric juice.

14. Qualitative reaction of lactic acid in the gastric juice. The principle of the method and normal values. Clinical and diagnostic significance.

15. Hemoglobin detection in blood and in gastric juice. The principle of the method. Normal values. Clinical and diagnostic significance.

16. Tubeless method of determining the gastric juice acidity (acidotest). Clinical and diagnostic significance.

TOPICS FOR REPORTS

1. Diseases of the gastrointestinal tract, accompanied by disturbances of protein digestion and amino acid absorption.

2. Specific changes in protein deficiency and in "kwashiorkor"disease.

Practical 1

QUALITATIVE REACTION OF FREE HYDROCHLORIC ACID IN GASTRIC JUICE

Gastric acid analysis methods are used for diagnosis and monitoring of diseases treatment in clinical practice.

Material of investigation

Test samples of gastric juice N 1, 2, 3 with different activity.

With Congo red

Principle

In the presence of free hydrochloric acid in the gastric juice Congo red changes color to blue. At weakly acidic, neutral or alkaline medium the color of stain remains red (transition zone 5.2 pH 3.0).

Reagents

Indicator paper "Congo red".

Procedure and observation

Apply 1 drop of gastric juice samples on the test paper strip with a glass rod.

With methyl orange

Principle

Methyl orange indicator in the presence of free hydrochloric acid is red, in the alkaline medium – orange-yellow (the transition zone pH 3.1-4.4).

Reagents

Indicator methyl orange.

Procedure and observation

Take 10 drops of gastric juice in test tubes. Then add 2 drops of methyl orange.

Normal values

Gastric juice	pH 1.5-1.8

Clinical and diagnostic significance

High acidity of gastric juice is observed in duodenal ulcer, and in some cases of stomach ulcers. It is known the stress development is associated with vagus-mediated enhancement of acid secretion.

Reducing the acidity of gastric juice is found in atrophic gastritis, pernicious anemia, gastric carcinoma. At the pathological conditions the acidity of gastric juice may be zero, increased or decreased.

Hyperchlorhydria (the increased free HCl and total acidity content) occurs in case of hyper acidic gastritis and often is accompanied by gastric ulcer and duodenal ulcer.

Hypochlorhydria (low acidity) occurs in hypo acidic gastritis, sometimes in stomach ulcers. As a consequence, in this case the absorption of B vitamins, iron absorption reduces and iron deficiency anemia develops. Then processes of proteins putrefaction are activated in the intestine.

Achlorhydria (complete absence of hydrochloric acid) and is observed in a case of a significant reduction of total acidity. This condition is found in atrophic gastritis, pernicious anemia, gastric carcinoma. Achlorhydria is diagnosed only after the test with stimulation of secretion.

Since in the absence of hydrochloric acid in the stomach under the influence of microbial fermentation processes develop, achlorhydria is accompanied by the appearance in the stomach fermentation products – dairy, oil, acetic acid, as a result, patients may be bad breath.

Achylia (lack of hydrochloric acid and pepsin) is associated with cancer of the stomach, pernicious anemia.

Design of laboratory work

Note the principle, laboratory procedures, and results of analysis, its clinical and diagnostic significance. Make a conclusion about the presence of pathologies presence.

Sample of gastric juice	Changes of indicator color		pH value
	Congo red	Methyl orange	
1 2 3			

Practical 2

QUALITATIVE REACTION OF LACTIC ACID PRESENCE IN GASTRIC JUICE

Principle

Lactic acid converts phenolate iron (III) of violet color in iron lactate salt of yellow-green color.

Reagents

1) 1% phenol solution, 2) 1% $FeCl_3$ solution, 3) 40% lactic acid solution.

Material of investigation

Normal gastric juice and gastric juice with lactic acid.

Procedure and observation

Prepare the solution of iron phenolate (III). Mix the solution of iron with 2.0 ml of 1% phenol solution, then add 3 drops of 1% $FeCl_3$ solution.

Pour the mixture into 4 tubes:

- take a lactic acid solution in first tube dropwise;
- take a sample of normal gastric juice and gastric juice with lactic acid in other tubes.

In the presence of lactic acid, the violet color of solution is changed to a yellow-green.

Normal values

Lactic acid — Absence

Clinical and diagnostic significance

The accumulation of lactic, butyric, acetic acid in the stomach is the result of hypo- or achlorhydria and achylia due to lack of hydrochloric acid protective function. It develops in cases of chyme stagnation and bacterial fermentation of food components.

Design of laboratory work

Note the principle, laboratory procedures, results of analysis, its clinical and diagnostic significance. Make a conclusion about the presence of pathologies.

	Solution color	Presence of lactic acid
Lactic acid solution Normal gastric juice Gastric juice with lactate		

Practical 3

DETECTION OF BLOOD AND HEMOGLOBIN IN GASTRIC JUICE BY DIAGNOSTIC STRIPS "HEMOPHAN"

Principle

The "Hemophan" test strip contains stable organic hydroperoxide, an acid buffer and a chromogen. There is an enzymatic activity in the blood presence in the sample. The peroxidase catalyzes the oxidation of chromogen hydroperoxide to form a blue colored product. In the presence of free (dissolved) hemoglobin band zone turns to blue color. In presence of red blood cells in gastric juice bright blue dots on a light background are revealed.

Material for investigation

Normal gastric juice and gastric juice with blood.

Procedure and observation

In each test tube dip an indicative gastric band of diagnostic strip, then take out rapid and compare with the color scale on the label after 30 seconds. On a scale of comparison on the packaging test strip the concentration of hemoglobin and red blood cells is determined.

Normal values

Blood Absence

Clinical and diagnostic significance

Bleeding in the stomach cavity is observed in gastritis, ulceration of the stomach wall, in malignant tumors. Thus, under the action of hydrochloric acid the blood is converted to dark brown color hematin reminding coffee grounds. It is detected either by probing or coloring feces black. Bleeding gums may also give a positive result.

Design of laboratory work

Note the principle, laboratory procedures, results of analysis, its clinical and diagnostic significance. Make a conclusion about the presence of pathologies.

Practical 4 (in theory)

TUBELESS METHOD OF ACIDITY DETECTION IN GASTRIC JUICE (TEST OF ACIDITY)

The tubeless method of the gastric acidity detection is convenient, reliable, sparing the patient. It is recommended for patients with contraindications to fractional probing (hypertension, neuropathy), as well as for young children.

Principle

Inputted dye per os (2.4-diamino-4-etoksiazobenzol) is exempt from pellets and absorbed in the stomach due to hydrochloric acid action (pH<3). The amount of dye is detected in 1.5 hours in the urine after its intake. The hydrochloride salt of a red color is formed in the case of the urine acidifying to 25% level. The degree of urine

coloration (amount of dye) is directly proportional to the acidity of gastric juice. The comparison of the color intensity with the scale is a quantitative measurement of acidity.

Reagents

1) 25% hydrochloric acid, 2) dragee of 2.4-diamino-4-ethoxybenzol dye, 3) tablets of sodium caffeine benzoate.

Material for investigation

"Control" urine portion and urine sample collected in 1.5 hours after the dye intake.

Procedure and observation

Patient preparation: patient intakes sodium caffeine benzoate (in 100 ml of water) after starvation for 8 hours and bladder empting. This medicine stimulates gastric secretion and diuresis.

The "control" urine is collected in 1 hour after sodium caffeine benzoate intake. The patient swallows dye pellets not chewing and in1.5 hours after its intake urine is collected again.

The analysis is performed simultaneously with both urine portions: adjust to 200.0 ml with water, take 5.0 ml of dilute urine and add 5.0 ml of a 25% solution HCl and compare with the scale.

Design of laboratory work

Note the principle, laboratory procedures, clinical and diagnostic significance.

THEME 5.2. INTRACELLULAR AMINO ACID METABOLISM

INTRODUCTION

Proteins perform a number of unique functions, maintaining a dynamic state between the organism and the environment. There are over 20 amino acids, some of them are essential and are included both in the general and in specific metabolism that explains the specific features in amino acid metabolism.

A variety of amino acid metabolism disorders are described in medicine.

THE AIM OF THE PRACTICAL CLASS IS

To study the general amino acid metabolism and amino acid transport system through the cellular membrane.

To study the amino acids basic reactions in intracellular metabolism (deamination, transamination, decarboxylation).

To learn the method of transaminases activity determination in serum.

SELF-STUDY QUESTIONS

1. Transport of amino acids through cell membranes.

2. Sources and ways of amino acid transformations in the tissues (amino acid metabolism). Metabolism of glucogenic and ketogenic amino acids.

3. Types of amino acid deamination (reductive, hydrolytic, intramolecular, oxidative).

4. Oxidative deamination. The difference between direct and indirect oxidative deamination.

5. Reaction of direct oxidative glutamic acid deamination.

6. Indirect oxidative deamination – trans deamination.

7. The mechanism of transamination reactions. The role of vitamin B_6. The vitamin B_6 structure and coenzyme forms.

8. The significance of transamination reactions. Characteristics of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Reactions catalyzed by these enzymes.

9. Features of indirect deamination in muscle tissue (a IMP-AMP cycle).

10. The fate of the α-keto acid formed in the process of deamination by the example of pyruvate, oxaloacetate, α-ketoglutarate.

11. The reactions of the biogenic amine synthesis (γ-amino butyric acid, histamine, serotonin, dopamine). The role of these biogenic amines.

12. Methods of biogenic amine disposal. Reactions involved in deamination of monoamine oxidase (MAO) and methylation reactions.

13. Anabolic role of amino acids, formation of creatine as an example. The structure of creatine and creatine phosphate, the reaction of their synthesis, the process of localization. The biological role of creatine phosphate. The cause of physiological creatinurea in children?

14. Determination of AST and ALT activity in serum. The principle of the method, clinical and diagnostic significance. Normal values.

TOPICS FOR REPORTS

1. Anabolic processes in which amino acids are involved. Application of amino acids in the medical practice.

2. Aminoaciduria, its types, etiology and pathogenesis, clinical manifestations, treatment bases.

Practical

DETERMINATION OF AMINOTRANSFERASES ACTIVITY IN SERUM

Principle

The aspartic acid and alanine are formed in transamination reactions of 2-oxoglutarate and pyruvate. The enzymes are aminotransferase (AST, L-aspartate : 2-oxoglutarate aminotransferase, EC 2.6.1.1. and alanine aminotransferase (ALT, L-alanine : 2-oxoglutarate aminotransferase, EC 2.6.1.2). The 2-oxoglutarate, undergoing spontaneous decarboxylation, is converted into pyruvate. Adding 2.4-dinitrophenylhydrazine in enzymatic reaction leads to reaction stoppage with formation of hydrazine of pyruvic acid. This product gives brown color in alkaline medium. The color intensity is proportional to the amount of formed pyruvic acid.

Reagents

1) AST substrate solution: mixture of α-ketoglutarate and aspartic acid, 2) ALT substrate solution: mixture of α-ketoglutarate and alanine, 3) 2.4-dinitrophenylhydrasine in 1.0 M HCl, 4) 0.4 M NaOH solution.

Standardized pyruvic acid solution, 0.1 mmol/l.

Material of investigation

Serum.

Procedure and observation

	Tube 1, standard, ml	**Tube 2, test for ALT, ml**	**Tube 3, test for AST, ml**
ALT substrate solution	0.25	0.25	—
AST substrate solution	—	—	0.25
Standardized pyruvic acid solution	0.05	—	—
Serum	—	0.05	0.05
	Incubate 30 min at 37°C		
2.4-dinitrophenylhydrasine	0.25	0.25	0.25
	Incubate 20 min at room temperature		
NaOH	2.5	2.5	2.5
	Incubate 10 min at room temperature. Measure the optical density of standard and test tubes at 540 nm against the water (green filter).		

Calculation

$$\text{ALT activity, mmol/l·h} = \frac{E_{test2}}{E_{standard}} \times C_{standard} \times 2$$

$$\text{AST activity, mmol/l·h} = \frac{E_{test3}}{E_{standard}} \times C_{standard} \times 2,$$

$E_{standard}$, E_{test2}, E_{test3} – optical density of standard and test samples for ALT and AST activity measurement, $C_{standard}$ – concentration of standard solution, 2 – coefficient of 30 min in 1 hour conversion.

Normal values

Serum	ALT activity	0.10-0.68 mmol/l·h
	AST activity	0.10-0.45 mmol/l·h
The de Ritis ratio	$\frac{\text{AST activity}}{\text{ALT activity}}$	1.33±0.40

Clinical and diagnostic significance

The definition of AST and ALT activity is used usually in clinical practice to identify the pathological processes in the myocardium and liver.

The **myocardium** has a high activity of AST than ALT. Increased activity in the blood of both enzymes, AST especially, is a marker in acute myocardial infarction and is available in 95% of cases. AST activity reaches a peak after 24-36 hours (usually increased by 4-5 times) and in a case of adequate treatment is reduced in 3 or 7 day. The activity of enzymes in blood varies slightly in stenocardia (angina).

Lesions of liver (toxic, infectious hepatitis) lead to increase in both enzymes activity. ALT level is more expressed than AST in this case. The enzyme activity increases before the appearance of jaundice in infectious hepatitis. In half of the cases of cirrhosis a AST activity is higher than ALT activity.

The de Ritis ratio (the ratio of AST/ALT) is significantly increased in myocardial infarction, decreased in hepatitis.

Design of laboratory work

Note the principle, laboratory procedures, normal values, its clinical and diagnostic significance and make a conclusion of possible pathological processes.

THEME 5.3. AMMONIA METABOLISM AND ITS DISPOSAL

INTRODUCTION

The formation of ammonia in the body determines the need for its detoxification and disposal. Inherited and acquired disorders of ammonia disposal processes cause serious clinical complications. The knowledge of these processes is necessary for the treatment of liver and kidney diseases.

THE AIM OF THE PRACTICAL CLASS IS

To study the basic ways of ammonia disposal with the formation of the protein metabolism end products.

To learn the method of urea and creatinine determination in serum and urine.

SELF-STUDY QUESTIONS

1. The main sources of ammonia in the tissues. Reactions of biogenic amine disposal, the direct deamination of glutamic acid.

2. The main ways of ammonia formation in the cells:

- reductive amination reaction (reamination), an enzyme, and the significance of reactions,
- amide formation reaction of glutamic and aspartic acid, mark their biological importance, describe organs where these reactions occur,
- carbamoyl phosphate synthesis.

3. The ammonia transport form in blood (glutamine, asparagine, alanine). Glucose-alanine cycle.

4. The role of the liver, kidney and intestines in the formation and disposal of ammonia.

5. Ornithine urea cycle reactions, its localization, enzymes, significance. Connection of the ornithine cycle with the TCA cycle.

6. Presentation of hyperammonemia, their causes and consequences. Normal and maximum permissible levels of ammonia concentration in blood. Causes of ammonia toxicity.

7. Ammoniagenesis, reactions, location, significance.

8. Creatine and phosphocreatine synthesis reaction. The biological role of creatine phosphate.

9. Creatinine, formation reaction, excretion.

10. Quantitative determination of urea in blood serum and urine. The principle of the method, its clinical and diagnostic significance, normal values.

11. Quantitative determination of creatinine concentration in the serum and urine. The principle of the method, its clinical and diagnostic significance, normal values.

TOPICS FOR REPORT

1. Hyperammonemia: causes, pathogenesis, clinical manifestations, treatment. Neonatal hyperammonemia. Molecular mechanisms of ammonia toxicity.

Practical 1

DETERMINATION OF UREA CONTENT IN SERUM AND URINE

Principle

Urea by the action of urease hydrolyzes to ammonia and CO_2. Ammonium ions in an alkaline medium react with the nitroprusside salicylate hypochlorite reagent to form a green color complex of indophenol. The color intensity is proportional to the urea amount.

Reagents

1) Urease stabilized solution, 2) nitroprusside salicylate reagent, 3) hypochlorite, 4) urea standardized solution (8.33 mmol/l).

Material of investigation

Serum, Urine (diluted in 1:100).

Procedure and observation

	Test 1, ml	**Test 2**, ml	**Standard**, ml
Urease stabilized solution	0.1	0.1	0.1
Serum Urine (diluted in 1:100) Urea standardized solution	0.01 — —	— 0.01 —	— — 0.01
	Mix well and incubate 5 min at room temperature		
Nitroprusside salicylate reagent	1.0	1.0	1.0
Hypochlorite	1.0	1.0	1.0
	Mix well and incubate 5 min at 37°C. Measure the optical density at 620nm against the water (red filter).		

Calculation

$$\text{Serum urea concentration, mmol/l} = \frac{E_{test}}{E_{standard}} \times C_{standard}$$

$$\text{Urine urea concentration, mmol/day} = \frac{E_{test}}{E_{standard}} \times C_{standard} \times 100 \times D,$$

E_{test} and $E_{standard}$ – optical density test and standard samples, $C_{standard}$ – urea concentration in standard sample, 100 – urine dilution, D – diuresis amount (daily urea formation) (1.3-1.5 l/day).

Normal values

Serum	Children	1.8-6.4 mmol/l
	Adults	2.5-8.3 mmol/l
Urine		330-580 mmol/day

Clinical and diagnostic significance

The urea level in serum and urine is dependent on its synthesis rate in the liver and on its excretion by kidneys, on the protein metabolism.

Serum

The increased serum urea levels are observed in diseases of the kidneys (disorders of kidney excretory functions), modifications of renal perfusion (congestive heart failure), depletion of water in the body by vomiting, diarrhea (relative increase in concentration), in cases of increased protein catabolism (fever, starvation) and at a diet with high protein consumption.

The reduced urea concentrations are found in cases of a diet with low protein consumption, in increased protein metabolism in tissues (children, pregnancy), severe liver disease associated with impaired urea synthesis (parenchymal jaundice, hepatitis, cirrhosis).

Urine

The determination of urea in urine allows monitoring processes of body proteins anabolism and catabolism (nitrogen balance).

The increasing concentration of urea in the urine is observed in cases of negative nitrogen balance, in the excess protein in diet in the postoperative period, in hyperthyroidism, in fevers and starvation.

The reducing urea excretion is a sign of a positive nitrogen balance, and could be observed during pregnancy, during the growth.

Design of laboratory work

Note the principle, laboratory procedures, normal values, clinical and diagnostic significance and make a conclusion of possible pathological processes.

Practical 2

DETERMINATION OF CREATININE CONTENT IN SERUM AND URINE

Principle

The creatinine in an alkaline medium reacts with picric acid and forms creatinine picrate of orange color. The color intensity is proportional to the solution concentration of creatinine in a biological fluid.

Reagents

1) 10% NaOH solution, 2) saturated solution of picric acid, 3) 10% trichloroacetic acid solution (TCA), 5) standardized creatinine solution, 177 μmol/l.

Material of investigation

Serum, urine (dilution in 1:50).

Procedure and observation

	Test 1, ml	**Test 2**, ml	**Standard,** ml
Serum	0.5	—	—
Distilled water	1.0	—	—
10% TCA solution	0.5	—	—
	Mix well, then centrifuge at 1500 RPM (revolutions per minute) or filtrate through pre-moistened with distilled water filter		
Filtrate	1.0	—	—
Distilled water	—	0.5	0.5
Urine (dilution in 1:50)	—	0.5	—
Standardized creatinine solution	—	—	0.5
10% NaOH solution	0.5	0.5	0.5
Saturated solution of picric acid	0.5	0.5	0.5
	Mix and after 20 min measure the optical density at 540 nm against water (green filter).		

Calculation

$$\text{Serum creatinine concentration, μmol/l} = \frac{E_{test}}{E_{standard}} \times C_{standard} \times 2,$$

$$\text{Urine creatinine concentration, mmol/day} = \frac{E_{test}}{E_{standard} \times 1000} \times C_{standard} \times 50 \times D, \text{ where}$$

E_{test} and $E_{standard}$ – optical density test and standard samples, 1000 – coefficient of calculation micromol in mmol; $C_{standard}$ – creatinine concentration in standard sample, 2 – dilution of serum, 50 – dilution of urine, D – diuresis amount (daily urea formation) (1.3-1.5 l/day).

Normal values

Serum	Children up to 1 year	18-35 μmol/l
	Children from 1 year to 12 years	27-62 μmol/l
	Women	44-97 μmol/l
	Men	52-132 μmol/l
Urine		4.4-17.7 mmol/day

Clinical and diagnostic significance

Serum

The concentration of creatinine in the blood of healthy people is relatively constant and depends on the muscles weight.

The increase of serum creatinine level in 2-7 times is observed in an acute renal failure, in most severe cases in 15-25 times. In addition, the creatinine level may be not dependent on muscle word. Its concentration is found to be elevated in hyperthyroidism, diabetes, muscular dystrophy, extensive burns, fevers, frequent intramuscular injections.

The decrease of creatinine in the blood does not have a diagnostic value.

Urine

The increase of creatinine concentration in urine is observed in persons with increased physical activity, in fevers. It is found to be elevated in liver diseases, in diabetes mellitus and diabetes insipidus, with crush syndrome, acute infections.

The decrease of creatinine in urine is found in chronic nephritis and other kidney diseases, muscle atrophy, leukemia and starvation.

Design of laboratory work

Note the principle, laboratory procedures, normal values, clinical and diagnostic significance and make a conclusion of possible pathological processes.

THEME 5.4. METABOLISM OF SOME AMINO ACIDS. THEIR FEATURES AND DISORDERS (SEMINAR)

INTRODUCTION

Apart from common reactions of amino acid metabolism, there are specific ones, which are associated with unique amino acid function. The derivatives of amino acid metabolism could play an important and sometimes key role in metabolic processes and determine the physiological state of the body. There are more than 100 diseases caused by inherited defects of amino acid metabolism.

THE AIM OF THE PRACTICAL CLASS IS

To study the metabolism of glycine, serine, cysteine, methionine, phenylalanine, tyrosine, tryptophan, and dicarboxylic amino acids and their disorders.

SELF-STUDY QUESTIONS

1. The structure of proteinogenic amino acids.

2. Sources and common pathways of amino acid metabolism in tissues.

3. Ways of dicarboxylic amino acids and their amide use (glutamate and aspartate) in metabolic reactions. Connection between the metabolism of dicarboxylic amino acids with citric acid cycle.

4. The synthesis of glucose from serine, alanine, aspartic and glutamic acids

5. Ways of cysteine and sulphur use. Reactions of taurine synthesis. Characteristics of "cystinosis", its cause, the clinical features. Cystinuria and its causes.

6. The use of glycine and serine in the body. Reactions of interconversion of glycine and serine, the role of tetrahydrofolic acid.

7. The correlation of glycine, serine, methionine and cysteine metabolism:

- S-adenosylmethionine synthesis reaction from S-adenosylhomocysteine, its role in transmethylation processes and the synthesis of definite substances,
- the reaction of homocysteine formation and pathways of its further transformation,
- participation of vitamin B_9 (folic acid), vitamin B_6 (pyridoxine) and B_{12} (cyanocobalamin.

8. Causes of homocysteinemia and homocystinuria. Comorbidities. Treatment.

9. Ways of phenylalanine and tyrosine use. Anabolic and catabolic pathways of tyrosine transformations. The reaction of converting phenylalanine to tyrosine.

10. Characteristics of phenylketonuria type I (classical) phenylketonuria disease and type II (variant). Defective enzymes, clinical manifestations, treatment bases

11. Reactions of tyrosine catabolism. Enzymes, a defect which lead to the characteristic features of the disease. The basis of treatment.

12. Changes of tyrosine anabolic function – albinism and Parkinson’s disease. Molecular mechanism, special features and the basis of treatment.

TOPICS FOR REPORT

1. A method of substances separating. Chromatography, its types. Application of separating substances in medicine.

2. The artificial kidney. Hemodialysis. The principle of blood purification and its use in the correction of pathological conditions.

3. Phenylketonuria and its types (classical type I, II and III variant types). Maternal phenylketonuria. The molecular cause of disease pathogenesis, clinical manifestations, treatment bases.

4. Glycine catabolism to ammonia, carbon dioxide, or low molecular weight organic acids (formic acid, oxalic acid). Glycosylate cycle. Modifications of catabolism – hyperglycinemia, hyperoxalateuria.

5. The catabolism of amino acids with branched radical (leucine, isoleucine, valine). Diseases "with the urine odor of maple syrup" and isovaleric acidemia. Clinical features, basis of treatment.

6. Tryptophan metabolism. Causes of pellagra in tryptophan metabolism disorders. Hartnus’a disease. Clinical features, the basis of treatment.

TESTS

Choose the correct answer.

1. BIOLOGICAL SIGNIFICANCE OF PROTEINS IS DETERMINED BY
   1) the order of amino acids in the protein molecule interlace
   2) the amino acid composition
   3) the molecular weight of proteins
   4) the charge of the protein molecule

2. TO NEUTRALIZE TOXIC SUBSTANCES FORMED IN THE INTESTINE, WHICH ENZYME IS USED IN THE LIVER
   1) hexokinase
   2) aminotransferase
   3) glucuronyl transferase
   4) sucrase

3. ENTEROPEPTIDASE IS AN ACTIVATOR OF ENZYME
   1) pepsinogen
   2) trypsinogen
   3) chymotrypsinogen
   4) proelastase

4. AN ACTIVE ENZYME GLUTAMATE DEHYDROGENASE CONTAINS OF
   1) nicotinamide adenine dinucleotide (NAD)
   2) flavin adenine dinucleotide (FAD)
   3) flavin mononucleotide (FMN)
   4) pyridoxal phosphate (PLP)

5. THE SEROTONIN IS FORMED IN THE DECARBOXYLATION REACTION OF
   1) cysteine
   2) tryptophan
   3) tyrosine
   4) 5-hydroxytryptophan

6. THEFORMATION OF γ-AMINOBUTYRIC ACIDS IS CATALYZED BY
   1) histidine decarboxylase
   2) tyrosine monooxygenase
   3) glutamate decarboxylase
   4) ornithine

7. THE COENZYME OF AMINOTRANSFERASES IS
   1) nicotinamide
   2) flavin adenine dinucleotide
   3) thiamine diphosphate
   4) pyridoxal phosphate

8. THE HYDROCHLORIC ACID SYNTHESIS IS STIMULATED BY _____ IN THE STOMACH
   1) tyramine
   2) histamine
   3) dopamine
   4) tryptophan

9. THE HYPOVITAMINOSIS C IS ASSOCIATED WITH DISORDERS OF _____ METABOLISM
   1) tyrosine
   2) leucine
   3) methionine
   4) cysteine

10. THE BINDING OF AMMONIA OCCURS IN
   1) synthesis of glutamate from 2-oxoglutarate
   2) synthesis of creatine
   3) transamination of alanine
   4) synthesis of serotonin

CASE STUDIES

1. The patient complains of a pain in the stomach, belching smelly "rotten eggs", rumbling and flatulence.

Identify the processes that may cause these symptoms. Give recommendations for the digestion improvement.

2. It is found that the addition of the glutamic acid in solution has a positive impact on the physiological function of the heart muscle, particularly under low oxygen supply.

Explain the mechanism of the amino acid positive impact on the heart activity.

3. The urea content in blood is about 2 mmol/l. About 180 mmol of urea is excreted with urine per day.

Identify the impaired body function. Name the enzymes that should be investigated to verify the diagnosis.

UNIT 6
STRUCTURE AND METABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES

THEME 6.1. STRUCTURE AND METABOLISM OF PURINE AND PYRIMIDINE NUCLEOTIDES

INTRODUCTION

Purine and pyrimidine nucleotides perform several important functions in the cell, one of which is the nucleic acid synthesis. Nucleic acids are not essential nutritional factors, and therefore the majority of cells in the body is capable for nucleotide synthesis. It determines the nucleotide metabolism rate.

The disorders of purine nucleotide metabolism are gout, kidney disease and urolithiasis (formation of uric acid stones), Lesch-Nyhan syndrome. The orotate aciduria is a disease associated with pyrimidine nucleotide metabolic disorders.

Nucleotides are also medicines which could be used in sport medicine and as the therapeutic agents (for example, methyluracil, potassium orotate).

THE AIM OF THE PRACTICAL CLASS IS

The study of biosynthesis and catabolism of purine and pyrimidine nucleotides, introduction to metabolic disorders associated with these processes.

Acquiring the skills for determining the uric acid concentration in the serum and urine.

SELF-STUDY QUESTIONS

1. The digestion of nucleoproteins in the gastrointestinal tract, enzymes. The further fate of purine and pyrimidine nucleotides and bases.

2. The synthesis of purine nucleotides:

- reaction of 5-phosphoribosylamine formation,
- sources of carbon and nitrogen atoms of the purine ring,
- synthesis of AMP and GMP from IMP,
- AMP conversion reaction into ATP and GTP conversion reaction into GMP.

3. The regulation of the purine nucleotide synthesis according to the negative feedback mechanism. Its cross positive regulation with the participation of ATP and GTP.

4. Catabolism of purine nucleotides:

- the reaction of AMP decay,
- the reaction of GMP decay,
- the reaction of uric acid formation from hypoxanthine and xanthine, the role of xanthine oxidase.

5. Primary and secondary hyperuricemia:

- urolithiasis, its causes, basis of treatment,

• gout, its causes, clinical manifestation, basis of treatment. The mechanism of allopurinol action in the gout treatment.

6. Lesch-Nyhan syndrome, its causes, basis of treatment and prognosis.

7. Synthesis of pyrimidine nucleotides:

• reaction of UMP and UTP synthesis,

• reaction of CTP synthesis from UTP.

8. Regulation of the pyrimidine nucleotides synthesis by the mechanism of negative feedback.

9. Synthesis of deoxyribonucleotides. Role of NADPH and thioredoxin.

10. Synthesis of dTMF. The role of tetrahydrofolic acid. The cause of megaloblastic anemia with folate deficiency. The mechanism of sulfonamides antibacterial activity.

11. The catabolism of pyrimidine nucleotides. The end products of the process.

12. Diseases associated with disorders of pyrimidine metabolism. Orotatate aciduria, causes, clinical manifestation, basis of treatment.

13. Application of synthesis inhibitors of purine and pyrimidine nucleotides in medicine. Methotrexate, 5-fluorouracil, azidothymidine.

14. Quantitative determination of the uric acid concentration in serum and urine. The principle of the method, clinical and diagnostic significance, normal values.

TOPICS FOR REPORTS

1. Gout: etiology, molecular mechanisms of development, clinical manifestations, treatment.

2. Urolithiasis: etiology, types of urinary stones, diagnosis and clinical manifestations, treatment.

3. Enzymes of ribonucleotides or deoxyribonucleotides synthesis as targets for antiviral and anticancer drugs.

Practical

DETERMINATION OF URIC ACID CONCENTRATION IN SERUM AND URINE

Principle

The uric acid is cleaved by the enzyme uricase to allantoin with simultaneous formation of hydrogen peroxide. The last reacts with dihydroxybenzol sulfate and 4-aminoantipyrinedue to the action of peroxidase forming products of pink color. The color intensity is proportional to the amount of uric acid.

Reagents

1) Working reagent with phenol, uricase, peroxidase, dihydroxybenzol sulfate and 4-aminoantipyrine in potassium phosphate buffer, 2) standardized solution of uric acid, 500 μmol/l.

Material of investigation

Serum. Urine (dilution 1:5).

Procedure and observation

	Test 1, ml	**Test 2**, ml	**Standard**, ml
Serum	0.025	—	—
Urine (dilution 1:5)	—	0.025	—
Standardized solution of uric acid	—	—	0.025
Working reagent	1.0	1.0	1.0
	Wait 10 minutes at 37ºC. Measure the optical density against water at 540 nm (green filter).		

Calculation

$$\text{Serum uric acid concentration, mmol/l} = \frac{E_{test}}{E_{standard}} \times C_{standard},$$

$$\text{Urine uric acid concentration, mmol/ day} = \frac{E_{test} \times 5 \times D}{E_{standard} \times 1000} \times C_{standard},$$

E_{test} and $E_{standard}$ – optical density test and standard samples, $C_{standard}$ – uric acid concentration in standard sample, 50 – dilution of urine, 1000 – coefficient of calculation micromol in mmol; D – diuresis amount (daily urea formation) (1.3-1.5 l/day).

Normal values

Serum	children	0.12-0.32 mmol/l
	Adult	0.16-0.45 mmol/l
Urine		1.46-4.43 mmol/day

Clinical and diagnostic significance

Serum

Blood urate (monosodium salt in combination with the protein) is determined by the intensity of uric acid synthesis, and the rate of its removal in the body. Serum urate stabilizes proteins, but at lower pH urate crystallizes in tissues.

Primary hyperuricemia divides into metabolic and renal one. Metabolic type is the result of increased purine nucleotides synthesis, such as increased activity of ribose-phosphate diphosphokinase (or phosphoribosyl pyrophosphate synthetase) or insufficient activity of hypoxantine-guaninephosphoribosyl tranferase. Renal type may be conditioned at genetic disorders associated with reducing excretion of uric acid by the kidneys.

Secondary hyperuricemia is observed in all conditions associated with enhanced decay of nucleoproteins: leukemia, treatment with cytostatics, irradiation, extensive psoriasis, pernicious anemia, hemolytic anemia. The most common cause of kidney failure is an altered filtration rate and tubular secretion of uric acid. The slowing urate excretion rate in the body is found in myxedema, hyperparathyroidism, diabetes mellitus, preeclampsia.

Detection of **hypouricemia** is diagnostically insignificant, and sometimes is observed in anemia, after in taking salicylates, in an excess of corticotropin.

Urine

Increased uric acid in the urine is observed in hyperuricemia of nonrenal origin. Salicylates, lithium salt also increase the excretion of urate.

The concentration of uric acid is reduced in cases of alcohol abuse, poisoning by salts of heavy metals and in kidney disease.

In gout uric acid is deposited in the tissues, joint capsules, cartilage, tendons, its daily amount may sometimes decrease in urine.

Design of laboratory work

Note the principle, laboratory procedures, normal values, its clinical and diagnostic significance and make a conclusion of possible pathological processes.

TESTS

Choose the correct answer.

1. THE DONOR OF NITROGEN IN THE SYNTHESIS OF PYRIMIDINE NUCLEOTIDES IS
    1) glycine
    2) asparagine
    3) aspartate
    4) glutamate

2. THE DONOR OF CARBON IN THE SYNTHESIS OF PURINE NUCLEOTIDE IS
    1) aspartic acid
    2) formyl-THFA
    3) glutamine
    4) tryptophan

3. THE REGULATORY ENZYME IN THE SYNTHESIS OF PYRIMIDINE NUCLEOTIDES IS
    1) xanthine oxidase
    2) carbamoyl phosphate synthase II
    3) dihydroorotase
    4) carbamoyl phosphate synthase I

4. ALLOPURINOL IS AN ANTI-GOUT DRUG. ITS EFFICANCY IS ASSOCIATED WITH
    1) competitive inhibition of xanthine oxidase
    2) reduction hypoxanthine concentration in urine
    3) increase of uric acid rate excretion by kidneys
    4) decrease of purine bases formation

5. ANTIVITAMINS OF FOLIC ACID BLOCK REACTION
    1) formation of dTMP from dUMP
    2) formation of orotic acid
    3) synthesis of carbamoyl phosphate
    4) formation of CTP from UTP

6. THE INHIBITOR OF CARBAMOYL PHOSPHATE SYNTHASE IS
   1) CTP
   2) GMP
   3) dATP
   4) 5-phophoribosyl-1-pyrophosphate

7. THE OROTATE ACIDURIA DEVELOPMENT IS ASSOCIATED WITH ENZYME DISORDER
   1) carbamoyl phosphate synthase I
   2) carbamoyl phosphate synthase II
   3) xanthine oxidase
   4) OMP-decarboxylase

8. THE END PRODUCT OF PURINE NUCLEOTIDES DEGRADATION IS
   1) urea
   2) uric acid
   3) lactic acid
   4) malonic acid

9. DURING THE DECAY OF PYRIMIDINE NUCLEOTIDES IS FORMED
   1) acetyl-SCoA
   2) sodium urate
   3) xanthine
   4) β-aminoisobutyric acid

10. PATIENTS WITH LESCH-NYHAN SYNDROME HAVE THE GENETIC DEFECT OF
   1) xanthine oxidase
   2) hypoxanthine-guanine phosphoribosyl transferase
   3) galactose-1-phosphate uridylyl transferase
   4) phosphoribosyl diphosphate synthase

CASE STUDIES

1. 1-year-old child was admitted to the emergency room with symptoms of physical and mental retardation. It was revealed a high concentration of uric acid in the urine.

Identify the source of uric acid in the urine.

2. A lack of folic acid in the diet leads to megaloblastic anemia development, leukopenia, impaired state of the mucous membranes and skin.

Indicate the biochemical cause of described disturbances.

UNIT 7
BIOSYNTHESIS OF NUCLEIC ACIDS AND PROTEINS

THEME 7.1. NUCLEIC ACIDS SYNTHESIS AND ITS REGULATION

INTRODUCTION

Nucleic acids are responsible for the storage and transferring the genetic information. Errors that occur during DNA replication and repair, protein biosynthesis lead to the appearance of the abnormal products and disruption of biochemical processes in the cell. The development of many diseases is caused by the presence of such hereditary or acquired errors.

THE AIM OF THE PRACTICAL CLASS IS

To study the main stages of nucleic acids synthesis.

To learn the extraction of yeast nucleoprotein components and to perform the qualitative reactions for their detection.

SELF-STUDY QUESTIONS

1. The structure of nucleic acids DNA and RNA. The structure of the nucleoprotein. Types of histones, features of their structure and their role. Non-histone proteins and their function.
2. The structure of ribosomes, their role in the cell.
3. DNA biosynthesis (replication) in eukaryotes according to the following plan:
- the total equation,
- connections with phases of the cell cycle,
- the location of process,
- components of DNA synthesizing system,
- main stages, the sequence of reactions, substrates and enzymes,
- end products,
- energy sources for the DNA synthesis,
- scheme of replication fork, specify the location of the Okazaki fragments and each replication enzyme in view of its function.

4. The DNA repair process, its significance.
5. The biosynthesis of RNA (transcription) in eukaryotes according to the following plan:
- the total equation,
- connections with phases of the cell cycle,
- the location of process,
- components of the RNA-synthesizing system,
- main stages, the sequence of reactions, substrates and enzymes,
- end products,

• energy sources for the biosynthesis,

• scheme of transcriptional folk, select the position of the promoter, the TATA box, and the terminator of RNA polymerase.

6. The regulation of transcription in prokaryotes by synthesis induction (Jacob-Monod scheme), the lactose operon as an example, and by synthesis repression, the tryptophan operon as an example.

7. Main methods of transcription regulation in eukaryotes.

8. The processing of messenger RNA: splicing, capping, attaching poly A sequence.

9. The secondary transfer of RNA structure, the concept of tRNA processing. Localization of tRNA and the role of modified nucleotides (pseudouridine, dihydrouriine). An adapter role of tRNA.

10. The concept of ribosomal RNA processing. Types of rRNA in eukaryotes. Function rRNA.

11. The application of DNA and RNA biosynthesis inhibitors as drugs. Doxorubicin, melphalan, novobiocin, rifamycin. What is the mechanism of their action?

12. The analysis of the complex protein chemical composition (nucleoproteins). The principle of the methods.

TOPICS FOR REPORT

1. The gene engineering, the principle of method and its significance. The application of gene engineering for the medication production.

2. Genetically modified foods. Prospects, challenges and solutions.

3. Cloning, the principle of method and significance. The potential application in medicine.

4. The hybridization of nucleic acids, principles and significance. The application in medicine and biology.

5. Ribozymes: structure, classification, properties. Ribozymes as medicaments.

Practical

ANALYSIS OF CHEMICAL COMPOSITION OF NUCLEOPROTEINS

Nucleoproteins comprise a protein part, purine or pyrimidine bases, ribose and deoxyribose carbohydrates, and phosphoric acid. All these components are determined during the practical lesson.

Reagents

1) 1% thymol solution in ethanol, 2) 10% NaOH solution, 3) concentrated NH_4OH (ammonium), 4) ammonium molybdate acidic, 5) concentrated H_2SO_4, 6) 1% $CuSO_4$ solution, 7) 1% $AgNO_3$ ammonia solution.

Material for investigation

Yeast hydrolysate.

Procedure and observation

Biuret Test	*Principle* The Biuret reagent (copper sulfate in a strong base) reacts with peptide bonds in proteins to form a violet complex known as the "Biuret complex". *Procedure and observation:* Add 10 drops of 10% sodium hydroxide solution and 1 drop of 1% copper sulfate solution to 5 drops of yeast hydrolysate.
Silver test for purine bases	*Principle* *Purine bases (adenine and guanine) react with silver nitrate in 5-10 minutes to form a light fluffy brown precipitate of silver salts.* *Procedure and observation:* Add 10 drops of concentrated ammonium solution, 10 drops of 1% of ammonium silver nitrate solution to 5 drops of yeast hydrolysate. After waiting specific precipitate is formed.
Molisch's test (for the presence of carbohydrates– β-D-ribose)	*Principle* After dehydration of pentoses the hydroxymethyl sulfuric acid is formed. Its condensation with thymol hydroxymethyl furfural is associated with the development of red color, and pink rings appear in vitro. *Procedure and observation:* Add 2-3 drops of thymol solution to 10 drops of yeast hydrolysate. Mix gently and add concentrated H_2SO_4.
Molybdenum test for phosphoric acid	*Principle* Phosphoric acid presented in the precipitate interacts with ammonium molybdate in nitric acid, forms a lemon-yellow color ammonium phosphomolybdate complex compound. *Procedure and observation* Add 20 drops of a molybdenum reagent to 10 drops of yeast hydrolysate. Heat the tube in water bath. Ammonium phosphomolybdate precipitates after cooling.

Design of laboratory work

Note results of work and fulfil the table. Make a conclusion of chemical composition of nucleoproteins:

Object of investigation	**Complex proteins**	**The revealed component**	**Color**	**Conclusion**
Yeast	Nucleoproteins	Protein		
		Purine bases		
		Pentoses		
		Phosphoric acid		

THEME 7.2. PROTEIN BIOSYNTHESIS AND ITS REGULATION

INTRODUCTION

Proteins, as well as other cellular components are in a state of dynamic equilibrium, that is continuously being updated. Knowledge of the mechanism of protein biosynthesis and principles of its regulation are necessary for understanding the molecular basis and for the rational application of therapeutically agents in practical medicine.

THE AIM OF THE PRACTICALCLASS IS

To study the main stages of protein biosynthesis and mechanisms of its regulation.

To introduce the method of protein determination in the serum.

SELF-STUDY QUESTIONS

1. The structure of proteinogenic amino acids, DNA and RNA nucleic acids. The structure of ribosomes, their role in the cell.
2. The genetic code and its properties.
3. The transfer RNA adapter role. The synthesis of aminoacyl-tRNA, the aminoacyl-tRNA synthetase.
4. Characteristics of protein biosynthesis according to the following plan:
   - the total equation,
   - connections with phases of the cell cycle,
   - the location of the process,
   - components of the protein-synthesizing system,
   - main stages, the sequence of reactions and enzymes,
   - end products,
   - energy sources for biosynthesis.
5. The post-translational modification of protein molecules. Examples of proteins involved in these processes. What is a folding, what is the role of the chaperone?
6. Medicines as protein biosynthesis inhibitors. The mechanism of action (tetracycline, chloramphenicol, erythromycin, streptomycin).
7. The quantitative determination of protein in the blood serum. Biuret test. The principle of the method, clinical and diagnostic significance, normal values.

TOPICS FOR REPORTS

1. Chaperones. Their types, structure, participation in maturation and stabilization of the protein molecule. Protein folding.
2. Prions. Their origins and properties. Prion diseases.
3. Medicines as inhibitors of matrix biosynthesis of RNA, DNA and protein. The mechanism of action.

Practical

DETERMINATION OF PROTEIN CONCENTRATION IN SERUM

Biuret test

Principle

The peptide bond in an alkaline medium forms a complex compound with copper (Biuret reaction). The intensity of development of a blue-violet color is proportional to the protein content.

Material for investigation

Serum.

Reagents

1) Biuret reagent: mix of $CuSO_4$ and NaOH, 2) 0.9% NaCl solution.

Standardized albumin solution, 70 g/l.

Procedure

	Test, ml	**Standard**, ml
Serum	0.04	—
Standardized albumin solution	—	0.04
Biuret reagent	3.0	3.0
	Wait for 15 minutes. Measure the optical density of tubes against the water at 540 nm (green filter).	

Calculation

$$\text{Protein concentration, g/l} = \frac{E_{test}}{E_{standard}} \times C_{standard},$$

E_{test} and $E_{standard}$ – optical density of test and standard tubes, $C_{standard}$ – protein concentration in a standard tube.

Normal values

Serum	Children from 1 year to 3 years	54-85 g/l
	Children from 4 to 18 years Adults	65-85 g/l

Clinical and diagnostic significance

Changes in the total protein concentration in the blood can be either absolute or relative. The absolute changes are the result of protein content modification in the blood. Relative changes depend on the volume of blood that is observed in dehydration or hyperhydration.

Hyperproteinemia

Absolute increase in protein concentration in the blood is most often associated with an excess of globulin fractions**.** It occurs in acute infections (increased synthesis of acute phase proteins), in chronic infections (γ-globulinemia), in multiple myeloma, lymphogranulomatisis, sarcoidosis.

Relative hyperalbuminemia is caused by the loss of intravascular fluid as a result of profuse diarrhea (cholera), sweating, vomiting, diabetes insipidus, severe and extensive burns, generalized peritonitis.

Hypoproteinemia

Reducing the protein concentration in the blood is most often associated with a decrease in albumin fraction in the blood.

The absolute hypoproteinemia is connected with:

• insufficient protein intake with food – gastrointestinal disease, narrowing of the esophagus by tumors, total or partial starvation;

• with a decrease in protein synthesis– the unbalanced amino acid composition of food, chronic parenchymal hepatitis, toxic, cancer, treatment with corticosteroids;

• with an enhanced dissolution of proteins – cachexia, severe infections, prolonged inflammation, fevers, hyperthyroidism;

• protein loss– disorders of the permeability of the capillary walls, bleeding, burns, acute and chronic bleeding, nephrotic syndrome.

Relative hypoproteinemia is associated with the changes in water balance – hyperhydration with hyperaldosteronism, renal failure with decreased excretion of salts, when sea water with inadequate infusions of saline solutions was used for drinking.

Design of laboratory work

Note the principle, laboratory procedures, normal values, its clinical and diagnostic significance and make a conclusion about possible pathological processes.

TESTS

Choose one or more correct answers.

1. PROPERTIES OF GENETIC CODE COULD BE CHARACTERIZED BY THE FOLLOWING STATEMENT
    1) each codon corresponds to three amino acids
    2) one amino acid can encode several triplets
    3) each amino acid corresponds to only one codon
    4) The mRNA codons are read in a direction from the 3' end 5'

2. MATRIX FOR TRANSCRIPTION PROCESS IS
    1) DNA
    2) mRNA
    3) tRNA
    4) rRNA

3. IT IS NECESSARY FOR TRANSLATION PROCESS
    1) lysosomes
    2) RNA-polymerase
    3) mRNA
    4) ATP

4. THE FORMATION OF PEPTIDE BOND IS DEPENDENT ON
    1) aminoacyl-tRNA synthase
    2) peptidyl transferase
    3) translocase
    4) carboxypeptidase

5. POST-TRANSLATIONAL CHANGES OF PROTEINS ARE
    1) partial proteolysis
    2) polyadenylation
    3) covalent attachment of a prosthetic group
    4) carboxylation

6. THE PROTEIN BIOSYNTHESIS IS STIMULATED BY THE HORMONE
    1) insulin
    2) glucagon
    3) adrenalin
    4) vasopressin

7. THE REASON OF PHENOTYPIC DIFFERENCES OF ORGANS AND TISSUES IN MULTICELLULAR ORGANISMS IS
    1) persistent repression of individual genes
    2) the allosteric inhibition of various enzymes
    3) the differences in the set of DNA
    4) The differences in posttranslational modification of proteins

8. THE PROTEIN SYNTHESIS IN THE PROMOTION STAGE IS INHIBITED BY
    1) penicillin
    2) streptomycin
    3) erythromycin
    4) chloramphenicol

9. THEPEPTIDYLTRANSFERASE REACTION IS INHIBITED BY
    1) chloramphenicol (levomycetin)
    2) ampicillin
    3) rifampicin
    4) erythromycin

10. THETRANSLOCASE REACTION IS INHIBITED BY
    1) erythromycin
    2) tetracycline
    3) ampicillin
    4) chloramphenicol

CASE STUDIES

1. It is found that substitution of last adenine for uridine in the mRNA codon 5'-GAA-3' leads to the impaired formation of β-polypeptide chain and to the disturbances of hemoglobin synthesis.

Identify the reason for the substitution, and name the disease.

2. The antitumor medicine, cisplatin, is an inhibitor of topoisomerase enzymes.

Describe the state of tumor cells by its action.

3. There are molecules of lipoproteins, which are used for the transfer of lipoproteins in the blood. Lipoproteins contain proteins called apoB-48 and apoB-100. It is known that these proteins are encoded by one gene. But the molecular weight of proteins is differed approximately in 2-fold from one another (apoB-48 – 241 kDa, apoB-100 – 512 kDa).

Consider the reason for the difference.

CHECKLIST FOR THE FINAL LESSON (UNIT 5, 6, 7)

1. The nitrogen balance in the body. The concept of nitrogen equilibrium. The biological significance of peptides. Essential and non-essential amino acids. Normal values of protein intake in children and adults. Protein food sources. What is the reference protein? Features of protein deficiency.

2. The protein digestion in gastro-intestinal tract. Hydrochloric acid formation, its role. Regulation of hydrochloric acid secretion. Gastro-intestinal enzymes, exon and endopeptidases, their location, mechanism of enzyme activation, their pH optimum and specifics. The mechanism of amino acid absorption.

3. Features of protein digestion and absorption in children of different age. Reasons for protein digestion and absorption disorders in children. The connection of these disorders with the development of allergic reactions. What is a celiac disease? Identify the causes and clinical signs of the disease.

4. Disposal of toxic products in the liver: microsomal oxidation and conjugation system. What enzymes are involved in the microsomal oxidation? The structure of UDP-glucuronic acid (UDPGA) and phosphoadenosine phosphoric acid (PAPA). Reactions of indicant formation.

5. Qualitative reaction of free hydrochloric acid in gastric juice.

6. Detection of lactic acid in gastric juice. Principle of method, procedure, normal values and clinical and diagnostic significance.

7. Detection of blood and hemoglobin in gastric juice. Principle of method, procedure, normal values and clinical and diagnostic significance.

8. Tubeless method of acidity detection in gastric juice. Principle of method.

9. Sources of amino acids in tissues. What is the principle of amino acid division into on glucogenic and ketogenic? Application of amino acids in the medical practice.

10. Types of amino acid deamination reactions. Feature of an oxidative deamination. Feature of trans deamination– the mechanism of reactions, enzymes, coenzymes, the location process. The significance of transamination reactions. The role of the cycle IMP-AMP, its reactions.

11. Characteristics of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), their reactions. The method of quantitative ALT AST activity determination in the serum. Note their clinical and diagnostic significance in the blood, normal values.

12. Glutamate dehydrogenase: location, structure, role, activity regulation. The fate of nitrogen and α-keto acids formed in the deamination process.

13. The significance of the amino acid decarboxylation. Role of biogenic amines – histamine, serotonin, γ-aminobutyric acid dopamine. The reactions of synthesis of biogenic amines – chemistry, enzymes, coenzymes, products, process location. Reactions of biogenic amine inactivation.

14. Reactions of formation and binding of ammonia in tissues (scheme). The role of the liver, kidney and intestines in the removal of ammonia. What is the acceptable level of ammonia concentration in the blood? The main causes of ammonia toxicity. Hyperammonemia, note their causes and consequences. The glucose-alanine cycle, its significance.

15. The urea synthesis reaction, its location, significance. Changes in urea synthesis. Quantitative determination of urea in the blood serum and urine. The principle of the method, the procedure, normal values, clinical and diagnostic values.

16. Ammoniagenesis, reactions, their location, value.

17. Synthesis of creatine and phosphocreatine, reaction. The biological role of creatine phosphate. Physiological creatinurea in children.

18. Synthesis of creatinine, reaction, location. Determination of the creatinine concentration in the serum and the urine. The principle of the method, the procedure, normal values, clinical and diagnostic significance.

19. Metabolism of glutamic and aspartic acid (scheme). Glutamic and aspartic metabolic reactions. Connection of amino acid metabolism with the citric acid cycle.

20. Metabolism of cysteine and its sulfur fate (scheme). Reactions of taurine synthesis. Causes and consequences of disorders in cystinosis and cystinuria.

21. Metabolism of serine and glycine (scheme). Reactions of glycine and serine interconversion, glycine catabolism reaction. The role of tetrahydrofolic acid.

22. Reactions reflecting exchange relationship between glycine, serine, methionine, and cysteine. Participation of folic acid and vitamin B_{12}. Role of adenosylmethionine in transmethylation processes. Homocysteinemia and homocystinuria, its causes and consequences.

23. Substances in the synthesis reactions involving THFA (dTMP, serine, methionine). The mechanism of the sulfonamides antibacterial activity.

24. Metabolism of tyrosine and phenylalanine. Ways of tyrosine use (Scheme). Reaction of tyrosine synthesis and phenylalanine catabolism.

25. Phenylketonuria, types I and II: causes, clinical features, basis of treatment.

26. Tyrosinemia, types I, II and III, homogentisuria, parkinsonism, albinism: causes, characteristics of diseases, the basis of treatment.

27. Nucleoprotein structure: proteins, nucleic acids. The structures of nitrogenous bases, nucleosides, nucleotides. Enzymes of nucleoproteins digestion in the stomach. The metabolism fate of the purines and pyrimidines.

28. Purines structure, sources of carbon and nitrogen atoms in the purine ring. The first two reactions of purine nucleotide synthesis, the synthesis reaction of AMP and GMP, AMP conversion reaction into ATP, GTP conversion reaction into GMP. Regulation of the purine nucleotide synthesis.

29. Purine catabolism, uric acid. Reutilization of guanine and hypoxanthine.

30.Disorders of the purine catabolism:

- hyperuricemia, its causes, types and consequences, basis of treatment,
- urolithiasis, its causes, types and consequences, basis of treatment,
- gout, its causes, types and consequences, basis of treatment,
- Lesch-Nyhan syndrome, its causes, types and consequences, basis of treatment.

31. Synthesis of UTP and CTP pyrimidine nucleotides, reactions, location, its regulation. Orotate aciduria.

32. Synthesis of deoxyribonucleotides. Role of NADPH and thioredoxin. Reactions of dTMP synthesis, participation of methylene THFA.

33. Degradation of pyrimidine nucleotides to the carbon dioxide, ammonia and water.

34. Medicines – inhibitors of purine and pyrimidine nucleotide synthesis. The mechanism of their action.

35. Features of the structure and the differences between RNA and DNA primary and secondary structures. Types of RNA, their location and function. The role of histones in the formation of the tertiary structure of DNA (supercoiling).

36. Replication of eukaryotic DNA. The overall equation, DNA-synthesizing enzyme system, basic stages and features of DNA replication. Connection with the cell cycle phases. DNA repair.

37. RNA transcription, enzymes and components of RNA-synthesizing system. The concept of exons and introns. The processes of tRNA, rRNA and mRNA maturation. Regulation of transcription in prokaryotes by induction and repression. Methods of transcription regulation in eukaryotes.

38. Stages of translation, components of the protein-synthesizing system, enzymes, regulation of processes. What is the genetic code? The properties of the genetic code. An adaptive role of transfer RNA. The synthesis reaction of the aminoacyl-tRNA.

39. The post-translational modification of proteins, examples. Folding mechanism. The role of the chaperone.

40. Medicines – RNA, DNA, protein biosynthesis inhibitors. The mechanism of their action.

41. The principle and the procedure of protein determination in the serum and the urine. Biuret method. Normal values, clinical and diagnostic significance.

42. Determination of the uric acid concentration in the serum and the urine. The principle of the method, the procedure, normal values, clinical and diagnostic significance.

43. Analysis of the nucleoprotein chemical composition. The principle and the procedure of the method.

UNIT 8
STRUCTURE AND METABOLISM OF CARBOHYDRATES

THEME 8.1. STRUCTURE AND METABOLISM OF CARBOHYDRATES. GLYCOGEN METABOLISM

INTRODUCTION

Carbohydrates play a vital role in the body of human beings and animals and perform subsequent functions:

- supply of energy (to 67% of daily energy required for the body),
- building material for cells,
- precursor molecules for synthesis of lipids, proteins and nucleic acids,
- carbohydrate compounds are included in immunoglobulins and other molecules.

Diseases associated with pathology of carbohydrate metabolism include diabetes mellitus, glycogen storage diseases, mucopolisacharidosis, galactosemia, fructosemia, lactose and sucrose intollerance.

THE AIM OF THE PRACTICAL CLASS IS:

To study carbohydrate digestion in the digestive tract. Glycogen metabolism as the energy storage pool.

SELF-STUDY QUESTIONS

1. Biological role of carbohydrates. Daily needs in carbohydrates for adults and children. Dietary products rich in carbohydrates.
2. Classification of carbohydrates depending on the number of monomers in the molecule (mono-, di-, oligo-, and polysaccharides), depending on the number of carbon atoms (trioses, tetroses, pentoses, hexoses) and on the localization of a carbonyl group (aldoses and ketoses).
3. Structure and functions of carbohydrates:
   - monosaccharaides (glucose, fructose, galactose, ribose, deoxyribose, glyceraldehyde, dioxyacetone),
   - disaccharides (maltose, lactose, sucrose),
   - polysaccharides (starch, glycogen, cellulose).
4. Monosaccharide derivatives. What are the sialic acids? The chemical structure of N-acetylneuraminic acid.
5. Compound carbohydrates – glycosaminoglycans. The structure of hyaluronic acid and chondroitin acids, their biological significance. The structure and role of glycoproteins.
6. Carbohydrate digestion in the mouth and intestine. Characteristics of digestive enzymes: α-amylase of the mouth, enzymes of pancreatic juices (α-amylase, oligo-1.6-glycosidase), enzymes of small intestine responsible for carbohydrate digestion.

7. Age-dependent peculiarities of digestion and absorption of carbohydrates. Biochemical reasons for sucrose and lactose intolerance in children.

8. Reasons why cellulose can't be digested in digestive tract of humans. Role of cellulose in digestion.

9. Transport of monosaccharide through cellular membranes.

10. Pathways of glucose metabolism in the cell. Sources of glucose in the cell. Phosphorylation of glucose, the significance of the reaction.

11. Conversion of fructose into glucose. Ways of fructose metabolism. What is the role of fructose in the metabolism of the fetus and the newborn? Essential fructosuria.

12. The role of galactose in the body. Conversion of galactose to glucose. Galactosemia, molecular causes, clinical manifestations and the basics of treatment.

13. Synthesis of glycogen from glucose-6-phosphate (glycogenesis). Breakdown of glycogen to glucose-6-phosphate (glycogenolysis). Specific features of glycogen metabolism in the liver and muscles (well-fed state, fast, muscle exercises).

14. Regulation of the activity of glycogen metabolism enzymes – glycogen synthase:

- hormonal – the influence of adrenaline and glucagon (adenylyl cyclase mechanism, role of cAMP and protein kinase A); role of insulin and participation of phosphodiesterase in decreasing cAMP concentration on the cell,
- allosteric regulation of glycogen phosphorylase by AMP,
- Ca^{2+}-dependent activation of glycogen phosphorylase kinase.

15. Genetic glycogen storage diseases: liver, muscle and combined glycogen storage disease. Glycogenosis.

16. Specific properties of carbohydrate metabolism in the liver. The role of glucosidase and glucose-6-phosphatase in hepatic regulated maintenance of glucose concentration in the blood.

17. Carbohydrates are medicines (glucose, hyaluronic acid, chondroitin sulfate, dextrins, heparin, cardiac glycosides).

18. Detection of glucose in urine. Principle of method. Clinical-diagnostic significance.

TOPICS FOR REPORTS

1. Sialic acids. Glycosaminoglycans (hyaluronic acid, chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin). Their characteristics: structure, biological role. Use in medicine.

2. Fructose: its importance in fetal and neonatal exchanges. Chemistry of fructose metabolism. Disorders of fructose metabolism: essential fructosemia, hereditary intolerance to fructose.

Practical

DETECTION OF GLUCOSE IN URINE USING "GLUCOPHAN" DIAGNOSTIC STRIPS

Principle

The principle of glucose determination is based on enzymatic reaction of glucose oxidase. The display area is impregnated with solutions of the enzyme glucose oxidase, peroxidase and dye tetramethylbenzidine. Glucose using glucose oxidase is oxidized by air oxygen to gluconic acid with formation of hydrogen peroxide. Hydrogen peroxide in the presence of enzyme peroxidase oxidizes a dye, and yellow turns into green.

Normal values

Urine

Glucose	test strips "Glucophan"	test was negative
	other methods	0.1-0.8 mmol/l

Clinical and diagnostic significance

The level of glucose in the urine increases in all cases of hyperglycemia over 10 mmol/l (renal threshold).

The glycosuria can be physiologic and pathologic. Physiologic ones include alimentary glycosuria, glycosuria of pregnant and neurogenic stress glycosuria.

Pathological glycosuria occurs most often as a result of severe hyperglycemia and pathological changes in the pancreas (acute pancreatitis, diabetes mellitus), adrenal gland ("bronze" or steroid diabetes), hyperthyroidism, acromegaly, myocardial infarction, hemorrhages in internal organs, poisoning by morphine, phosphorus, in acute infections.

The defeat of the renal tubules and the absence of reabsorption of glucose also lead to glycosuria.

Design of work:

Write down the principle of the used analytical method, put all results in a clearly organized table, note the clinical-diagnostic value, and make a conclusion about the possible pathology.

The examined material	**Reaction**	**Result**
Normal urine Urine with glucose		

THEME 8.2. OXIDATION OF GLUCOSE IN AEROBIC CONDITIONS. GLUCONEOGENESIS

INTRODUCTION

Glycolysis is the main pathway of glucose catabolism. Glycolysis is the only mechanism in bodythat produces energy in anaerobic conditions. Particularly due to glycolysis the organism can survive in hypoxic conditions. In erythrocytes anaerobic

metabolism of carbohydrates is the only process that produces ATP and supports their integrity and function. Under aerobic conditions glycolysis is the initial stage of glucose breakdown, ending in the aerobic oxidation of the resulting intermediate products.

A stable glucose level during physical exercises or starvation is sustained by the reactions of gluconeogenesis. This contributes to the reduction of acidosis in these states and provides glucose for nervous tissue and erythrocytes.

THE AIM OF THE PRACTICAL CLASS IS:

To study the processes of glycolysis, gluconeogenesis, regulation of the processes of glucose breakdown and synthesis.

To obtain practical skills for the quantitative determination of glucose in urine and serum as well as lactic acid in homogenate of muscle tissue.

SELF-STUDY QUESTIONS

1. Sources and ways of glucose metabolism in the cell. Role of glucose-6-phosphate in metabolism of glucose.

2. Characteristics of glycolysis process (lactic acid fermentation):

- localization and the conditions of the process,
- the sequence of reactions and enzymes,
- the final products,
- participation of adenyl nucleotides and energy effects,
- irreversible reactions of glycolysis,
- reactions of glycolysis associated with ATP consumption,
- the reaction of substrate phosphorylation, their nature and value,
- glycolytic oxidation reduction of NAD^+ and NADH, its meaning and value.

3. Characteristics of the process of gluconeogenesis according to the plan:

- localization and conditions of a reaction,
- substrates (lactic acid, glycerol, amino acids). Where do these substances arise from?
- the sequence of reactions and enzymes,
- reactions of gluconeogenesis associated with the consumption of GTP and ATP,
- irreversible reaction of gluconeogenesis,
- biological significance during fasting and physical work,
- energy consumption for the synthesis of one molecule of glucose.

4. The role of glycolysis and gluconeogenesis in the metabolism of fetus and newborns.

5. Hormonal regulation of glycolysis and gluconeogenesis. The role of insulin, adrenaline, cortisol, glucagon. The enzymes regulated by these hormones.

6. Allosteric regulation of glycolysis and gluconeogenesis, role of ATP, ADP, AMP, citrate, fatty acids, glucose-6-phosphate, fructose-6-phosphate, fructose-1.6-diphosphate, acetyl-S CoA. Regulated enzymes.

7. Glucose-lactate cycle (Cori cycle), its value in physical work. Sources of lactic acid in the body.

8. Glucose-alanine cycle, its value in physical work and fasting.

9. Energy effect of glucose oxidation in anaerobic conditions. Comparison of the energy effect of anaerobic glucose oxidation and breakdown of glycogen to lactate.

10. Alcoholic fermentation: the localization process, the specific reaction conditions, the sequence of reactions, energy effect, the end products. The similarities and differences between alcoholic fermentation and glycolysis.

11. The metabolism of ethanol in the liver, with the participation of alcohol dehydrogenase and acetyl dihyddehydrogenase, sequence of reactions, the end products. Energy effect of the oxidation of one molecule of ethanol.

12. The influence of ethyl alcohol on the metabolism of carbohydrates in the human body. What are the causes of hyperlactatemia and hypoglycemia in alcoholic intoxication?

13. The synthesis of glucose from alanine, aspartate and glutamate. Under what conditions and where do these reactions take place? What is their biological significance?

14. Quantitative determination of glucose in serum and urine. The principle of the method. The normal levels. Clinical-diagnostic value.

15. How to detect lactic acid in the muscle tissue by reaction of Uffelman?

TOPICS FOR REPORTS

1. Molecular mechanism of alcohol intoxication development. The causes of hypersensitivity of children to alcohol. Chronic alcoholism. Clinical and laboratory diagnostic methods.

2. Alcohol dehydrogenase, catalyzed reactions, substrates. Its participation in biochemical and physiological processes. Isozymes.

3. Gluconeogenesis and its importance in the metabolism of a fetus and a newborn. Hypoglycemia of newborns, criteria, causes.

Practical 1

THE GLUCOSE OXIDASE TEST FOR THE DETERMINATION OF GLUCOSE CONCENTRATION IN SERUM AND URINE

Principle

Glucose using glucose oxidase is oxidized to gluconic acid with formation of hydrogen peroxide. Hydrogen peroxide in the presence of the chlorophenol and the enzyme peroxidase oxidizes dye 4-aminoantipyrine, transforming it in to Heinemann, painted product raspberry pink color. The color intensity is proportional to concentration of glucose and is determined by photocolorimetry.

Material of investigation

Serum. Blood.

Reagents

1) The working reagent, containing phenol, glucose oxidase, peroxidase, 4-aminoantipyrine in potassium phosphate buffer.

A standard solution of glucose, 5.5 mmol/l.

Procedure

	Experiment 1, ml	**Experiment 2,** ml	**Standard,** ml
Blood serum	0.02	—	—
Urine	—	0.02	—
A standard glucose solution	—	—	0.02
The working reagent	2.0	2.0	2.0
	Incubation for 25 minutes at 37°C. Measure the optical density against water at a wavelength of 540 nm (green light filter).		

Calculation

$$\text{Glucose concentration, mmol/l} = \frac{E_{ex}}{E_{st}} \times C_{st}, \text{ where}$$

E_{ex} and E_{st} – optical density of examined samples and standard one, C_{st} – standard solution concentration.

Normal values

Blood serum	adults	3.5-5.5 mmol/l
urine		0.06-0.83 mmol/l

Clinical and diagnostic significance

Serum

The glucose content increased in the blood is observed both in physiological and in pathological conditions.

Physiological hyperglycemia

The physiological hyperglycemia is *alimentary (nutritional)* one that arises when simultaneously the large amounts of easily digestible carbohydrates – mono- and disaccharides are absorbed, and *neurogenic one*, for example, in stressful situations through the release of large quantities of catecholamine into the blood. Physiological hyperglycemia is transient and quickly passes.

Pathological hyperglycemia

Pathological hyperglycemia is usually caused by neuroendocrine disorders:

- diabetes mellitus is associated with absolute or relative insulin insufficiency,
- pituitary disease, accompanied by increased secretion of somatotropin and corticotropin into the blood (acromegaly, a Itsenko-Cushing disease, pituitary tumors, etc.),
- medulla tumors of the adrenal glands, when formation of catecholamines is enhanced (pheochromocytoma),
- tumors of the adrenal cortex with increased production of glucocorticoids,
- hyperthyroidism, some liver diseases (infectious hepatitis, liver cirrhosis).

The glucose concentration decreased in the blood can also be physiological and pathological.

Physiological hypoglycemia

Physiological hypoglycemia includes the excess blood insulin secretion in response to nutritional hyperglycemia; hypoglycemia after severe and prolonged muscular work, at full or partial fasting. Hypoglycemia can occur in women during lactation as a result of enhanced absorption of glucose by the mammary gland. Hypoglycemia is often observed by newborns, caused by sudden stoppage of the "parent" glucose, immaturity of the liver structure and increased sensitivity of cells to insulin.

Pathological hypoglycaemia

Pathological hypoglycemia is observed in hyperinsulinism, hyperplasia β-cells of the islets of Langerhans (insulinoma, adenoma and cancer of the pancreas). The most common cause of hypoglycemia – insulin overdose. In addition, hypoglycemia causes a deficiency of the hormone cortisol by hypofunction of the adrenal cortex (Addison's disease, adrenal tumors), hypofunction of the anterior pituitary (Simmonds disease), hypothyroidism. Hypoglycemia can occur because of toxic liver damage, glycogen storage, diseases of kidneys due to the glycosuria.

Urine

(Theme 8.1.).

Design of work:

Write down the principle of the method, the experimental procedure, the normal value and the results of the study, note the clinical and diagnostic value of the index and draw conclusions on the possible pathology.

Practical 2

DETECTION OF LACTIC ACID IN MUSCLE TISSUE BY UFFELMAN'S REACTION

Principle

The method is based on the complex compound interaction of violet iron phenolate with lactic acid to form yellow-green lactate of iron.

Material for investigation

Muscle tissue.

Reagents

1) Phosphate buffer, pH 7.2, 2) 1% solution of phenol, 3) 1% solution of $FeCl_3$.

Procedure

1. Preparation of the extract of muscle tissue.

A piece of muscle tissue is triturated in a mortar with 5.0 ml of phosphate buffer solution, and then mixed. Obtained muscle slurry is filtered through 2 layers of cheesecloth or centrifuge at 1500 rpm.

2. Color reaction for lactic acid.

In the test tube the 1% $FeCl_3$ solution is added to 10 drops of 1% phenol solution until the appearance of the purple color. Then, 3 drops of extract of muscles are add-

ed to the vial and the color change is observed. In the presence of lactic acid, the violet color of the solution becomes yellow-green due to the formation of lactate of iron.

Practical significance

Lactic acid is the end product of catabolism of glucose under anaerobic conditions, a small amount of lactate in the muscle is formed under aerobic conditions. Production of lactate is activated with the muscular exercise both physiologically (physical work), and pathologically (disruption of blood flow and hypoxia of the muscles, epilepsy, tetanus, tetany).

The production of lactic acid is activated in most tissues in hypoxic conditions associated with cardiac and pulmonary failure, anemia and blood rheology disorders.

Design of work:

Describe the principle of the method, the experimental procedure and the results of the study, note the practical value of an indicator and draw conclusions about possible pathology.

THEME 8.3. AEROBIC OXIDATION OF GLUCOSE. PENTOSE PHOSPHATE PATHWAY

INTRODUCTION

Aerobic breakdown of glucose is a main pathway of its catabolism in aerobic organisms. Aerobic breakdown of glucose releases more energy than anaerobic glycolysis. Intermediate products of the oxidative catabolism of glucose are also used as precursors in the biosynthesis of amino acids, lipids and other biomolecules. The brain is the most dependent on aerobic breakdown of glucose. It consumes about 120 g of glucose per day.

The pentose phosphate pathway fulfills an anabolic function. It provides the cell with the molecules of NADPH for reductive synthesis and pentoses for synthesis of nucleotides.

THE AIM OF THE PRACTICAL CLASS IS:

To study the reactions of aerobic breakdown of glucose to carbon dioxide and water, reactions of pentose phosphate pathway, the nervous and hormonal regulation of glucose metabolism, the disorders of carbohydrate metabolism.

The acquisition of practical skills for testing glucose tolerance and building glycemic curves.

SELF-STUDY QUESTIONS

1. Sources of blood glucose. The normal concentration of glucose in the blood. Possible causes of hypo- and hyperglycemia.

2. Specific and general pathways of catabolism of glucose. The overall equation of aerobic breakdown of glucose.

3. Stages of aerobic glucose breakdown: 1 – oxidation of glucose to pyruvate, 2 – oxidative decarboxylation of pyruvate, 3 – citric acid cycle, 4 – electron transport chain and the formation of endogenous water.

4. The overall equation of oxidative decarboxylation of pyruvic acid and its individual reaction. Components of the multienzyme pyruvate-dehydrogenase complex, enzymes and coenzymes. The regulation of the process. What vitamins are involved in the work of pyruvate dehydrogenase? Their characteristics. What other enzyme complexes have similar structure?

5. The citric acid cycle, enzymes and coenzymes, biological role of the cycle. The regulation of the process.

6. Glycerol phosphate and malate-aspartate shuttle systems. What is their value?

7. Benefits of aerobic oxidation of glucose. Pasteur's effect, its biochemical mechanism.

8. Feature pentose phosphate pathway of glucose oxidation according to the plan:

- distribution and the role of pentose phosphate pathway,
- the reaction of the oxidative phase,
- the idea of non-oxidative phase (schematically),
- enzymes, coenzymes, vitamins,
- the relationship of the process with glycolysis,
- the value of pentose phosphate pathway, for example, in an adipose cell, the erythrocyte, in dividing cells.

9. The formation of ATP in aerobic and anaerobic breakdown of glucose. The role of anaerobic and aerobic breakdown of glucose during muscular work. How does the dependence of the metabolism of nervous tissue from the aerobic breakdown of glucose manifest?

10. Specific features of glucose oxidation in the erythrocyte. The role of glycolysis, pentose phosphate shunt, 2.3-diphosphoglycerate shunt.

11. Hereditary enzymopathy of glucose-6-phosphate dehydrogenase. The factors causing the manifestation of insufficiency of the enzyme. Consequences.

12. Nervous regulation of carbohydrate metabolism. The role of the sympathetic and parasympathetic systems.

13. Hormonal regulation of carbohydrate exchange. The effect of insulin, adrenaline, glucagon, cortisol on blood glucose level and intracellular processes of transformation of glucose. Endocrine-sensitive enzymes of carbohydrate metabolism.

14. Characteristics of diabetes type 1 and 2. What ways of carbohydrate exchange is broken? Biochemistry of diabetes complications.

15. Glucose tolerance test. Diagnostic value of parameters of glycemic curve – the steepness of the ascent, the magnitude of ascent, the returning time to the original values. Under what conditions does the type of the glycemic curve change?

TOPICS FOR REPORTS

1. Metabolic functions of NADPH. Reactions of the formation of NADPH formation. Anabolic reactions with NADPH participation.

2. Molecular mechanisms of diabetes mellitus 1 and 2 types. Biochemical mechanisms of fast and delayed complications of diabetes mellitus.

Practical

GLUCOSE TOLERANCE TEST

The glucose tolerance test (test with a sugar loading) is an informative test to detect diabetes at the early stages, violations of liver glycogenolysis function and to assess the function of the small intestine.

Principle

Glucose tolerance test is based on the determination of the concentration of glucose in the blood after a certain period of time after ingestion of glucose.

The concentration of glucose in the blood is determined by the glucose oxidase method (see Theme 8.2.).

The glucose tolerance test procedure

In clinical diagnostic laboratories samples of capillary blood, taken on an empty stomach and after a certain period of time after the glucose loading is examined. The test is carried out as follows:

Patient fasting blood is taken from a finger, afterwards glucose with warm water or weak tea is given to the patient. It is recommended to give children, aged 1.5 to 3 years, glucose at the rate of 2.0 g per 1 kg of body weight, from 3 to 12 years – 1.75 g/kg, after 12 years – 1.25 g/kg. Adults take glucose in the amount of 1.0-1.5 g/kg. Blood samples are taken repeatedly after 30, 60, 90 and 120 minutes after taking glucose. Next, the glucose concentration in samples is measured.

In practical class the method of the sugar loading is carried out with a model serum samples of blood taken prior to a glucose loading and after 30, 60 and 120 minutes after glucose loading. The glucose concentration in all taken samples is determined by glucose oxidase method (see Theme 8.2.).

Material for investigation

Three sets of model samples of blood serum containing normal, reduced and elevated concentrations of glucose.

Reagents

1) The working reagent, containing phenol, glucose oxidase, peroxidase, 4-aminoantipyrine in potassium phosphate buffer.

A standard solution of glucose, 5.5 mmol/l.

The glucose concentration determination

	Experimental samples, ml				**Standard sample**, ml
	Before glucose loading	Time after glucose loading			
		30 minutes	60 minutes	120 minutes	
	1	2	3	4	5
The working solution	2.0	2.0	2.0	2.0	2.0
Blood serum	0.02	0.02	0.02	0.02	—
Glucose standard	—	—	—	—	0.02

	The content of the tubes is mixed, incubated at 37°C for 15 minutes. The optical density at a wavelength of 540 nm (green filter) is measured.

Calculation

In each blood sample, the concentration of glucose is calculated according to the following formula:

$$\text{Glucose concentration, mmol/l} = \frac{E_{ex}}{E_{st}} \times C_{ST}, \text{ where}$$

E_{ex} and E_{st} – optical density of experiment and standard samples, C_{st} – concentration of standard glucose solution.

Normal values

Fasting	3.5-5.5 mmol/l	100%
After 60 minutes	5.3-9.6 mmol/l	150-175%
After 120 minutes	less than 5.3 mmol/l	approximately 100%

Evaluation of the glycemic curve

There are the following types of glycemic curves:

Type of curve	**Initial level of glucose**	**Maximum rise**	**Hypoglycemic phase**	**Glucose level by the end of the 2nd hour**
Normal	**Normal**	**In one hour**	**In two hours or missing**	**The initial level**
Hyperglycemic	Hyperglycemia	After 1.0-1.5 hours	missing	The initial level is not reached
Hypoglycemic	Hypoglycemia	One hour	missing	The initial level

In a healthy person after glucose loading the glucose level in the blood is changed as follows:

1. 30 minutes after the intake of glucose, an increase in the glucose amount in the blood is measured. The increase rate of glucose concentration during the first 30 minutes (the steepness of the curve) shows the reflex irritation strength of the sympathetic nerve endings during the contact with glucose in the digestive tract and the efficiency of glucose absorption in the intestine.

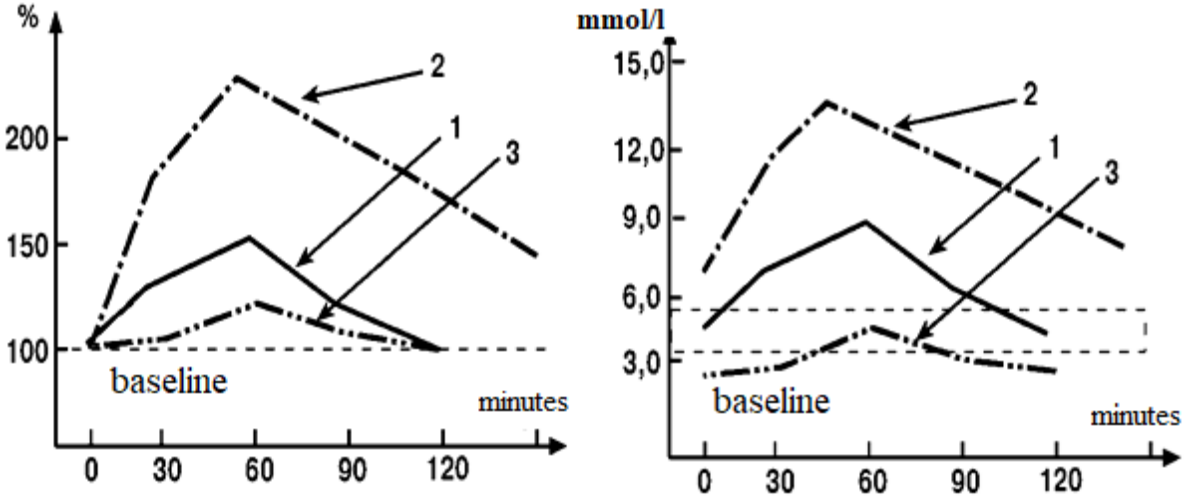
2. By 60-th minute, there is a maximum increase in the glucose concentration in the blood about 50-75% over the baseline. The interval from 30 to 60 minutes is associated with the speed of glucose absorption, and with the general state of the liver and its glycogenesis function.

3. In 90-120 minutes, glucose content in the blood returns to normal. The decrease in the level of blood glucose in this period is due to enhanced release of insulin from the pancreas. The degree of reduction reflects the functional activity of parasympathetic nervous system, the glycogenesis function of the liver, insulin sensitivity of muscle and adipose tissue. In some cases, the glucose concentration may fall below the initial value, since it is usually secreted more insulin than it is required to restore normal glucose levels in the blood, which leads to hypoglycemia.

In a healthy person the glucose loading does not cause glycosuria.

The results of the survey are usually expressed graphically and can be reflected in relative or absolute units:

1. Normal curve,
2. Hyperglycemic curve,
3. Hypoglycemic curve.



1 - Nomal curve
2 - Hyperglycemic curve
3 - Hypoglycemic curve

Clinical-diagnostic significance

Hyperglycemic curves are presented during the damage of the parenchyma of the liver, diseases of the central nervous system, hidden forms of diabetes, hyperthyroidism and adrenal cortex, infectious diseases (rheumatism, diphtheria, typhoid, dysentery, sepsis, pneumonia), pancreatitis, glycogen storage diseases.

Hypoglycemic curves are observed in the adenoma of the islets of Langerhans, hypothyroidism, Addison's disease, encephalitis, bowel disease, dysbiosis, and helminthiasis.

Design of work:

Write down the principle of formation of the glycemic curves, determine the obtained values, build on them glycemic curve in absolute and relative units.

The number of sample	**Glucose Concentration in the blood**			
	Before loading	Time after loading		
		30 minutes	60 minutes	120 minutes

Note the clinical diagnostic significance of the method. Make a conclusion about the possible causes of changes in the shape of glycemic curves.

TESTS

Choose one or more correct answers.

1. MALTASE IS SYNTHESIZED BY
    1) pancreas cells
    2) mucous cells of gastric
    3) mucous cells of small intestine
    4) mucous cells of large intestine

2. CARBOHYDRATE WITH GLUCOSE-FRUCTOSE STRUCTURE, CONNECTED WITH α-1.2-GLYCOSIDIC BIND IS
    1) lactose
    2) maltose
    3) sucrose
    4) residue of starch

3. DIGESTION IS ACCOMPANIED BY
    1) decomposition of disaccharides to CO_2 and water
    2) splitting of polysaccharides to oligo- and monosaccharides
    3) hydrolysis of cellulose
    4) decomposition of glucose with the formation of lactate

4. THE KEY ENZYME OF GLYCOGEN MOBILIZATION IS _____
    1) glycogen synthase
    2) amylase
    3) hexokinase
    4) glycogen phosphorylase

5. THE ANAEROBIC TRANSFORMATION OF GLUCOSE INTO LACTATE IS TAKEN PLACE IN
    1) cells of nerve tissue
    2) cells of the cortical layer of the kidneys
    3) erythrocytes
    4) myocarditis

6. LACTATE – THE GLYCOLYSIS FINAL PRODUCT - CAN BE RESYNTHESIZED IN GLUCOSE IN _____
    1) muscle tissue
    2) nervous tissue
    3) the liver
    4) kidney tissue

7. SHORT-TERM FASTING LEADS TO ACTIVATION OF _____
    1) glycolysis in muscles
    2) glycogenolysis in cardiac tissue
    3) glycogenolysis in the liver
    4) synthesis of glycogen in the liver

8. THE PROLONGED STARVATION LEADS TO ACTIVATION OF ___
   1) glycolysis in muscles
   2) glycogenolysis in the liver
   3) gluconeogenesis
   4) synthesis of glycogen in the liver

9. THE RATE OF GLUCONEOGENESIS INCREASES DUE TO THE ACTION OF_____
   1) cortisol
   2) increased concentration of ADP and AMP
   3) insulin
   4) high concentration of NAD and FAD

10. THE PENTHOSE PHOSPHATE PATHWAY FUNCTION IS _____
   1) the formation of glucose
   2) generation of NADPH
   3) ATP supplement of tissues
   4) formation of lactate

CASE STUDIES

1. A 7-year-old child needs to determine blood sugar to detect diabetes mellitus. The child cried before the sample in the laboratory. It was found that the child's blood sugar level is higher than normal.

Note the cause of hyperglycemia.

2. One athlete ran at a distance of 100 m, the other – 5000 m.

What is the level of lactic acid in sportsmen's blood?

3. Suggest a change in the ratio between the pentose-phosphate and glycolytic pathways of carbohydrate metabolism in the bone marrow of a patient who had bleeding.

Identify enzymes that are useful for the hypothesis evaluation.

CHECKLIST FOR FINAL LESSON (UNIT 8)

1. The role of carbohydrates in the body. Classification of carbohydrates according to their structure and functions. The structure of the main representatives of carbohydrates: monosaccharides (triose, pentoses, hexoses), di- and polysaccharides. The role and structural formula of glycosaminoglycans: neuraminic and N-acetylneuraminic, chondroitin and hyaluronic acid. Examples of the use of carbohydrates as the drugs.

2. The carbohydrates presented in the food. Where and what enzymes participate in their digestion? The mechanism of glucose absorption. The role of cellulose in digestion. The reasons for intolerance to sucrose and lactose.

3. Detection of glucose in urine. The principle of the method. Clinical-diagnostic value.

4. The role of the liver in the metabolism of carbohydrates in different situations. Specific features of the functioning of the enzyme glucokinase and glucose-6-phosphatase. The reaction of interconversion of carbohydrates: metabolism of galactose and fructose in the body.

5. Reactions of biosynthesis of glycogen and glycogenolysis, the physiological significance of the processes. The energy effect of glycogen usage in aerobic and anaerobic conditions. Regulation of activity of phosphorylase and glycogen synthase (the role of cAMP, calcium ions and calmodulin). Differences in glycogen metabolism in the liver and in the muscles. Characteristics of the glycogenoses and aglycogenoses, defects of enzyme and consequences of such defects.

6. Sources and ways of transformation of glucose in tissues (scheme). Characteristics of oxidation of glucose under anaerobic conditions: the sequence of reactions of glycolysis, the net reaction, the energy effect, regulation, method of ATP formation, the localization of the process. Subsequent fate of lactic acid. Specify the role of anaerobic breakdown of glucose in red blood cells and in the muscle tissue.

7. The sequence of reactions of alcoholic fermentation, its net reaction, energy effect, the method of ATP formation, the localization of the process. Similarities and differences of glycolysis and alcoholic fermentation

8. The metabolism of ethanol in humans. Localization of enzymes. What is the cause of hypoglycemia and lactic acidosis in alcohol poisoning?

9. The determination of glucose concentration in blood serum with glucose oxidase method. The principle of the method, definition, clinical-diagnostic value, normal values.

10. Detection of lactic acid in the muscles by the reaction of Uffelman. The principle of the method and steps of analysis, practical significance.

11. The oxidation of glucose in aerobic conditions: the sequence of reactions, energy effect. Pasteur's effect, its biochemical mechanisms. Reactions of glycerol phosphate and malate-aspartate shuttle system functioning, the source of NADH. The role of the aerobic breakdown of glucose in the brain.

12. The pentose phosphate pathway (PPP) conversion of glucose, its localization. The reaction of the oxidative phase of the pentose formation. Reactions of non-oxidative phase (scheme). The role of the 1st and 2nd stages of the PPP in adipose tissue and red blood cells, in dividing cells, its relationship to glycolysis. The regulation of the process. The consequences of glucose-6-phosphate dehydrogenase enzymopathies.

13. The sequence of reactions of gluconeogenesis, possible precursors, its value. The regulation of gluconeogenesis. Glucose-lactate cycle (Cori cycle) and glucose-alanine cycle (scheme), their role. Reaction of synthesis of glucose from amino acids on the example of alanine, aspartate and glutamate.

14. Reactions of carbohydrate metabolism, accompanied by the formation of carbon dioxide and reactions which use it.

15. What is allosteric regulation of enzymes? What enzymes are affected by intermediate metabolites of carbohydrate metabolism, NADH, ATP and AMP?

16. Regulation of glucose concentration in the blood. Sources and ways of use of blood glucose. The impact of insulin, glucagon, adrenaline and cortisol. Change in carbohydrate metabolism when fasting, during exercise and after meal.

17. Types of diabetes. What are the changes of carbohydrate metabolism in diabetes type 1 and type 2?

18. Test of tolerance to glucose. The principle of the method, of procedures. Clinical-diagnostic value of the test. Normal values of the glycemic curve. The form of normal, hypo- and hyperglycemic curves. What determines the shape of the curve?

19. The stages of metabolism and their relationship. What, besides ATP, are the high energy compounds? ATP-ADP cycle. The main methods of phosphorylation of ADP and the use of ATP. The general scheme of the catabolism of proteins, fats and carbohydrates in the body, specific and general ways of catabolism, their value.

20. NAD-dependent dehydrogenase, the reactions catalyzed by them in metabolism of carbohydrates. Structural formula of oxidized and reduced forms of NAD^+. Characteristics of vitamin that is included in NAD^+: the biological name, signs of deficiency, daily requirement, dietary sources.

21. FAD-dependent dehydrogenases, reactions catalyzed by them in metabolism of carbohydrates. Structural formula of oxidized and reduced forms of FAD. Characteristics of vitamin that is included in the structure of FAD: the biological name, signs of deficiency, daily requirement, dietary sources.

22. The sequence of oxidative decarboxylation of pyruvate, the link with the respiratory chain. The regulation of the process. Participation of vitamins in the process and their characteristics: biological name, signs of deficiency, daily requirement, dietary sources.

23. The sequence of reactions of the citric acid cycle, the link with the respiratory chain. Regulation of reactions. Participation of vitamins in the process, their characteristics, energy effect.

24. The principle of oxidative phosphorylation. Scheme of the structural organization of the respiratory chain. The coupling of oxidation with phosphorylation. The structure of the H^+-ATP synthase. The ratio P/O for NADH and $FADH_2$. The mechanism of respiratory control. How does ATP influence the oxidative phosphorylation?

25. The uncoupling of respiration and phosphorylation. What determines the function of heat-producing brown adipose tissue? Inhibitors of the respiratory chain. Causes of hypoenergetic states. The ratio P/O and the number of produced ATP molecules during complete oxidation of glucose.

UNIT 9
STRUCTURE AND METABOLISM OF LIPIDS

THEME 9.1. STRUCTURE AND EXTERNAL METABOLISM OF LIPIDS

INTRODUCTION

Lipids, low-molecular organic substances, are diverse in chemical structure and functions, insoluble in water, but soluble in organic solvents. Lipids include triacylglycerols, complex lipids (phospholipids, glycolipids, sphingolipids), cholesterol (including, as a precursor of bile acids, hormones, vitamin D). The multiplicity of lipid biological functions determines the need for their study.

THE AIM OF THE PRACTICAL CLASS IS:

To study the structure and functions of the major lipids in human tissues, processes of their digestion and absorption, as well as resynthesis in enterocytes and transport of fats.

To obtain practical skills for the determination of phosphatidyl choline composition from egg yolk.

SELF-STUDY QUESTIONS

1. Characteristics of fatty acids according to the following plan:

- classification according to the number of carbon atoms, double bonds and their position,
- sources of saturated and polyunsaturated fatty acids for the body,
- use of saturated and polyunsaturated fatty acids in the cell,
- biological role.

2. Fatty acids of ω-6 family (linoleic, γ-linolenic, arachidonic acid) and ω-3 family (α-linolenic, eicosapentaenoic, docosa hexaenoic acid). The length and position of double bonds. Biological role of polyunsaturated fatty acids.

3. Characteristics of derivatives of eicosatrienoic (ω-6), arachidonic (ω-6) and eicosapentaenoic (ω-3) acids – eicosanoids (prostaglandins, prostacyclin, leukotrienes, thromboxanes). Biological role of certain types of eicosanoids. Scheme of the initial reactions of synthesis on the example of arachidonic acid. Role of enzymes: phospholipase A_2, cyclooxygenase, lipoxygenase. What hormones and pharmaceutical substances influence on the synthesis of eicosanoids?

4. Structure of triacylglycerols, and fatty acids included in their composition. Characteristics of the class of triacylglycerols (neutral fats), their biological role and functions.

5. Chemical formula of cholesterol, its biological role and functions. Derivative of cholesterol.

6. Characteristics of complex lipids:

- glycerophospholipids (phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol), their chemical formula, biological functions,

• sphingophospholipids (sphingomyelins), their structure. Biological functions of sphingolipids,

• glycolipids (cerebrosides, gangliosides, sulfolipids), and their structure. Biological functions of glycolipids.

7. External lipid metabolism. Food sources of lipids, the daily need of children and adults in liquid and solid fats.

8. Composition of bile and its role for body in digestion of lipids. Types of bile acids, their functions, structure. Reaction of the synthesis of bile acids, for example cholic acid, vitamins involved in this process. Chemical formula of taurocholic and glycocholic acids. Causes and consequences of disorders of bile production and bile secretion.

9. What is the emulsion? What are the characteristics of emulsions? Role of bile and bile acids in the emulsification of dietary fat. Scheme of emulsified fat droplet.

10. Enzymes engaged in the digestion of triacylglycerols, phospholipids and cholesterol esters in small intestine. Place of formation and activation of these enzymes.

11. Micelle products of fat digestion. Scheme of micelle structure formed after the digestion of lipids. What is their role in the absorption of lipids?

12. Characteristic of lipid digestion in infants.

13. Possible causes of digestion and absorption disorders of dietary fat. Causes of hypovitaminosis and steatorrhea in disorders of lipiddigestion.

14. Resynthesis of lipids in enterocytes and its role. Reaction of resynthesis of triacylglycerols, cholesterol esters and phospholipids in the intestinal wall.

15. Composition of chylomicrons, and their functions. Apoproteins. Scheme of chylomicron structure. Utilization of chylomicrons in tissues. Role of lipoprotein lipase. What hormones influence on its activity?

16. Characteristics of very low density lipoproteins: their composition, the ratio of lipid fractions, their value. Apoproteins, their function. Scheme of VLDL structure. Where and when are these lipoproteins formed? Utilization of VLDL in tissues. Role of lipoprotein lipase.

17. Characteristics of triacylglycerol transport disorders in tissue – dyslipoproteinemia I and V types. Their cause and clinical implications.

18. Determination of the composition of egg-yolk phosphatidylcholine. Principle for determining the constituent elements.

TOPICS FOR REPORTS

1. The use of lipids as drugs and biologically active additives (essential, lecithin, choleic acid, ω-3 fatty acids, etc.).

2. Unsaturated and polyunsaturated fatty acids. Types, structural role, participation in metabolism and behavioral reactions.

3. Liposomes in medicine. The structure and characteristics of liposomes. Possibility of using medicines in the blood as a transport form.

4. Glycolipids, the structure of lipid and carbohydrate components. Functions of glycolipids. Disturbance of glycolipid metabolism.

Practical

THE STUDY OF PHOSPHATIDYL CHOLINE COMPOSITION

Principle

Method is based on the hydrolysis of egg-yolk phosphatidylcholine (lecithin) by heating it in NaOH solution with subsequent determination of its structural components in the extract: fatty acids, glycerol, choline, phosphoric acid.

Material for investigation

Dry egg yolk.

Reagents

1) 10% NaOH solution, 2) 10% HCl solution, 3) conc. HNO_3, 4) molybdenum reagent, 5) 1% $CuSO_4$ solution.

Procedure

1. Hydrolysis of phosphatidylcholine

Place a piece of egg yolk into a test tube, add 3.0-4.0 ml of 10% NaOH solution, boil in water bath for 15 minutes.

Detection of choline	During heating in alkaline environment choline is converted into trimethylamine $N(CH_3)_3$, which is detected at the end of hydrolysis by the smell of herring brine

2. Hydrolysate is divided into 3 tubes:

Detection of fatty acids	In the 1-st test tube: add dropwise 10% HCl solution until the appearance of flakey suspension of fatty acids.
Detection of phosphoric acid	To the 2-nd part of hydrolysate: carefully add 5-7 drops of concentrated HNO_3 and drops of molybdenum reagent until the appearance of yellow precipitate of ammonium phosphomolybdate. If it is necessary, heat in a boiling water bath. After cooling tubes with running water, phosphomolybdate ammonium precipitates.
Detection of glycerol	Add 5 drops of 10% NaOH solution and 1 drop of 1% $CuSO_4$ solution to the 3-rd part of hydrolysate, mix. Chelated copper compound with glycerol of bright blue color get formed.

Design of work:

Record results of reactions and conclusions in the form of a table:

Products of hydrolysis of phosphatidylcholine	**Result of reaction**	**Conclusions**
Choline Fatty acids Phosphoric acid Glycerol		

THEME 9.2. INTRACELLULAR METABOLISM OF FATTY ACIDS AND TRIACYLGLYCEROLS

INTRODUCTION

Triacylglycerols (neutral fats) are esters of glycerol and higher fatty acids. Knowledge of the neutral fat metabolism is necessary to understand the metabolic switch between fasting and muscular work, to study the pathogenesis of diseases, for example, obesity, diabetes, atherosclerosis.

Acquaintance with the method for determining the triacylglycerols blood content allows to use this information to detect diseases associated with lipid metabolism disorders.

THE AIM OF THE PRACTICAL CLASS IS:

To study the processes of lipogenesis, lipolysis, oxidation of fatty acids, fatty acid synthesis from glucose and synthesis of ketone bodies; regulation of lipid breakdown and synthesis.

To obtain practical skills for the quantitative determination of triacylglycerol in serum.

SELF-STUDY QUESTIONS

1. Reactions of tricarboxylic acid cycle and their connection with the respiratory chain and energy yield. Process of oxidative phosphorylation. Concept of respiratory control.

2. Characterization of fatty acid synthesis from glucose according to the following plan:

- localization and conditions of the process,
- reaction of acetyl-S CoA formation from glucose,
- role of citrate to transfer acetyl groups in cytosol, and its further transformations,
- synthesis of malonyl-S CoA,
- structure of the multienzyme complex, chemistry of reactions occurring in the complex,
- the final product of synthesis,
- participation of vitamins and coenzymes, sources of NADPH, role of "malic"-enzyme,
- regulation of the process with the participation of hormones: insulin, adrenaline, glucagon,
- influence of ATP, acyl-S CoA, malonyl-S CoA, citrate on fatty acid synthesis.

3. Synthesis reaction of glycerol-3-phosphate from glucose. Localization and role of the process.

4. Reaction of phosphatidic acid synthesis from fatty acids and glycerol-3-phosphate according the following plan:

- localization in the cell,
- sources of glycerol-3-phosphate, fatty acids and energy,

- sequence of reactions,
- relationship with the metabolism of carbohydrates,
- further use of phosphatidic acid.

5. Structure of triacylglycerols, their fatty acid composition. Reactions of triacylglycerol synthesis (lipogenesis). Conditions for the occurrence of lipogenesis in the liver and adipose tissue. Communication of triacylglycerol synthesis with carbohydrate metabolism.

6. Sources of TAG in the liver. Characteristics of very low density lipoproteins: their composition, Apoproteins, function. Scheme of VLDL structure. Conditions for the formation of these lipoproteins. Utilization of VLDL in tissues. Role of lipoprotein lipase.

7. Similarities and differences in the biosynthesis of triacylglycerol in adipose tissue and liver.

8. Characteristic reactions of lipolysis according to the following plan:

- localization and conditions of the process,
- sequence of reactions and enzymes,
- the final products,
- hormonal regulation of the process,
- transportation and utilization of free fatty acids, formed during lipolysis.

9. Hormone sensitive lipase, role of adenylate cyclase system in the regulation of its activity. Regulation of lipase by hormones: adrenaline, glucagon, cortisol and insulin. Allosteric regulation of activity of lipolysis and lipogenesis enzymes.

10. Oxidation of glycerol to pyruvate. Possible metabolic pathways of pyruvate. Energy yield of glycerol oxidation in aerobic and anaerobic conditions.

11. Oxidation of fatty acids to carbon dioxide and water:

- role of carnitine in fatty acid oxidation,
- localization and flow conditions of β-oxidation,
- sequence of β-oxidation reactions and enzymes,
- participation of vitamins and coenzymes,
- the final products,
- connection with the TCA and the respiratory chain,
- energy yield of the process,
- calculation of the energy value β-oxidation of palmitic acid.

12. Features of triacylglycerol metabolism during certain physiological conditions (food intake, starvation, muscle activity). Features of brown adipose tissue.

13. Causes of disorders of triacylglycerol metabolism – obesity, hyperlipoproteinemia type I (hyperchylomicronemia) and type V.

14. Synthesis of ketone bodies. Conditions, localization and role of the process. Reaction of ketone bodies utilization in tissues. Causes of ketoacidosis in starvation and diabetes. Role of oxaloacetate deficiency in the activation of ketogenesis.

15. Determination of the concentration of triacylglycerol in the blood serum. Clinical-diagnostic significance and normal values.

TOPICS FOR REPORT

1. White and brown adipose tissue. Adipose tissue distribution in the body and metabolic features. Its role in the body.

2. Primary and secondary acetonemic syndromes in children.

3. Modern ideas about the role of L-carnitine in the body. Participation of carnitine in the pre- and postnatal development of the child. The assumed role of carnitine in "death in the cradle" syndrome development (syndrome of sudden child death).

Practical

THE DETERMINATION OF TRIACYLGLYCEROL CONCENTRATION IN SERUM

Principle

The method is based on the use of connected enzymatic reactions, carried out:

1) lipase, catalyzing the hydrolysis of triacylglycerol to glycerol and fatty acids, 2) glycerol kinase, catalyzing the phosphorylation of glycerol, 3) glycerol phosphate oxidase, oxidizing glycerol-3-phosphate in the presence of O_2 to dioxyacetone phosphate with the formation of H_2O_2, 4) peroxidase, catalyzing oxidation of 4-aminoantipyrine by hydrogen peroxide with the formation of khinonimine, painted the product in raspberry pink color, in the presence of chlorophenol.

Material for investigation

Serum.

Reagents

Working reagent: lipase, glycerol kinase, glycerol phosphate, peroxidase, chlorophenol, 4-aminoantipyrine in a buffer solution.

A standard solution of triacylglycerol (triolein): 2.29 mmol/l.

Procedure

	Experienced test, ml	**Standard,** ml
Serum	0.03	—
Standard solution of triacylglycerols	—	0.03
Working reagent	3.0	3.0
	Incubate for 20 minutes at 20-25°C, measure the optical density of experimental and standard samples against water at a wavelength of 540 nm (green light filter).	

Calculation

Concentration of triacylglycerols, mmol/l = $E_{ex}/E_{st} \times C_{st}$

Where E_{ex} and E_{st} – optical density of experiment and standard samples, C_{st} – concentration of standard solution.

Normal values

Serum	Children	0.15-1.56 mmol/l
	Adults:	0.45-1.71 mmol/l

Clinical-diagnostic significance

Determination of triacylglycerol concentration of is the most informative for typing primary defects of its exchange – hyperlipoproteinemia.

Increase in the level of triacylglycerols is observed in obesity, diabetes, hypertension, coronary heart disease, pancreatitis, chronic renal failure and nephrotic syndrome, hypothyroidism, atherosclerosis, alcoholism.

Decrease in the concentration of triacylglycerols is observed in hyperthyroidism, chronic obstructive pulmonary disease, end-stage liver disease, malabsorption syndrome.

Design of work:

Write down the principle of the method, the experimental procedure, the normal value and the results of the study, note the clinical and diagnostic value of the index and draw conclusions on the possible pathology.

THEME 9.3. INTRACELLULAR METABOLISM OF PHOSPHOLIPIDS AND CHOLESTEROL. TRANSPORT OF LIPIDS IN BLOOD

INTRODUCTION

Phospholipids and cholesterol are the part of the cell membranes. They participate in the formation of lipoproteins. If the synthesis of phospholipids is disrupted, the normal metabolism of cells and the formation of transport lipoproteins will change.

Disorders of cholesterol metabolism cause many diseases: atherosclerosis and cholelithiasis.

The drugs applied in cardiology affect the reactions of cholesterol synthesis in tissues, as well as the low and high density lipoprotein metabolism in blood.

THE AIM OF THE PRACTICAL CLASS IS:

To study the processes of biosynthesis and catabolism of phospholipids and glycosphingolipids, synthesis of cholesterol, the role of lipoproteins in transport of free cholesterol and its esters in blood plasma.

To obtain practical skills for the quantitative determination of cholesterol concentration in blood serum.

SELF-STUDY QUESTIONS

1. Structure of phospholipids: phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol. Their biological role.

2. Catabolism of phospholipids. Enzymes, splitting phospholipids in the intestine and tissues. Role of phospholipase A_2 and C.

3. Characterization of phosphatidic acid synthesis from fatty acids and glycerol according to the following plan:

- localization in the cell,
- sources of glycerol, fatty acids and energy,
- the sequence of reactions,
- relationship with carbohydrate metabolism,
- further use of phosphatidic acid.

4. Interconnection reactions of glycine, serine and methionine, role of vitamins B_6, B_{12} and folic acid in the metabolism. Synthesis reaction of S-adenosyl methionine from S-adenosyl homocysteine, its role in the transmethylation in the synthesis of a number of substances, including phosphatidyl choline.

5. Reactions of phospholipid biosynthesis in tissues. Two pathways of phospholipid biosynthesis. Role of amino acids and vitamins in the process. Lipotropic substances. Causes of change in phospholipid synthesis. Causes and consequences of fatty liver.

6. Chemical structure and biological role of cholesterol. Food sources of cholesterol. Ways of removing cholesterol from the body.

7. Transport of free cholesterol and its esters in blood plasma. Composition and structure of lipoproteins of low and high density. Types of apoproteins and their functions. Metabolism of LDL and HDL in blood plasma. The reaction catalyzed by LCAT (lecithin cholesterol acyl transferase).

8. Localization and role of apoB-100-receptor. Importance of receptor-mediated endocytosis of LDL and pathway components after endocytosis. Role of ACAT (acyl cholesterol acyl transferase).

9. Scheme of cholesterol synthesis stages. Interconnection of cholesterol synthesis and metabolism of carbohydrates. Regulation of the synthesis. Hormonal and allosteric regulation mechanisms. Medicinal regulation of cholesterol synthesis.

10. Characterization of cholesterol metabolic disorders – hyperlipoproteinemia type IIA (family hypercholesterolemia), atherosclerosis (in stages), gallstone disease. Causes, consequences, basics of treatment.

11. Characteristics of acetyl-S CoA formation: catabolism of glucose, amino acids, fatty acids and ketone bodies. Using of acetyl-S CoA: TCA, fatty acid synthesis, cholesterol, ketone bodies.

12. Lipidoses or lipid storage diseases. Niemann-Pick's, Gaucher’s and Tay-Sachs diseases.

13. Determination of cholesterol concentration in blood serum. Principle of the method, its clinical-diagnostic value, normal levels.

TOPICS FOR REPORT

1. Atherosclerosis, causes, pathogenesis, consequences. Modern approaches to the treatment of atherosclerosis.

2. Metabolic syndrome (syndrome X). Its connection with obesity, atherosclerosis, type 2 diabetes, hypertension. Causes. Basics of treatment.

3. Disease of lipid accumulation: Niemann-Pick, Thea-Sachs, Gaucher, Schueller-Christian, Wolman. Molecular causes, pathogenesis. Basics of treatment.

Practical

THE DETERMINATION OF CHOLESTEROL CONCENTRATION IN SERUM

Principle

The method is based on conjugated enzymatic reactions. Cholesterol esterase carries out the hydrolysis of cholesterol esters; then cholesterol oxidase turns cholesterol into cholestenone with the formation of H_2O_2. Hydrogen peroxide, in the presence of phenol with the participation of peroxidase, oxidizes 4-aminoantipyrine with the formation of khinonimine, paints the product in raspberry pink color. The color intensity is proportional to the concentration of cholesterol and is determined by photo colorimetric method.

Material for investigation

Serum.

Reagents

1) Working reagent: solution of cholesterol esterase, cholesterol oxidase, peroxidase, phenol, 4-aminoantipyrine in 0.1 M potassium-phosphate buffer.

A standard solution of cholesterol: 4.65 mmol/l.

Procedure

	Test, ml	**Standard, ml**
Serum	0.02	—
Standard solution	—	0.02
Cholesterol	2.0	2.0
	Incubated for 20 minutes at 37°C. Measure the optical density of experienced and standard samples against water at a wavelength of 540 nm (green light filter).	

Calculation:

Concentration of cholesterol, mmol/l = $E_{test}/E_{st} \times C_{st}$

Where E_{test} and E_{st} – optical density of experiment and standard samples, C_{st} – concentration of standard solution.

Normal values

Serum	Children	1.2-5.2 mmol/l
	Adults	3.0-5.2 mmol/l

Clinical and diagnostic significance

Cholesterolemia, more than 5.2 mmol/l, is a high-risk factor of atherosclerosis, and coronary heart disease and stroke are its clinical complications. The high concentration of cholesterol in blood is observed in hyperlipoproteinemia IIA and IIB, III, nephrotic syndrome, diabetes mellitus, hypothyroidism, kidney damage, intra- and extrahepatic cholestasis.

Reduction of cholesterol concentration in the blood (hypocholesterolemia) is observed in starvation and malabsorption syndrome, hyperthyroidism, acute pancreatitis, liver cirrhosis, malignant tumors.

Design of practical:

Write down the principle of the method, the experimental procedure, the normal value and the results of the study, note the clinical and diagnostic value of the index and draw conclusions on the possible pathology.

TESTS

Choose one or more correct answers.

1. STEROIDS ARE
   1) bile acids
   2) gangliosides
   3) sphingomyelins
   4) pituitary hormones

2. THE NUTRITION ESSENTIAL FACTOR IS_____
   1) cholesterol
   2) phosphatidylcholine
   3) linolenic acid
   4) oleic acid

3. THE HIGH FATTY ACID METABOLISM IS_____
   1) decarboxylation
   2) glycogenolysis
   3) β-oxidation
   4) lipolysis

4. THE RESIDUE OF FATTY ACID TRANSFERS THROUGH THE MITOCHONDRIAL MEMBRANE IS THE MOLECULE OF _____
   1) carnosine
   2) carnitine
   3) creatine
   4) keratin

5. THE NADPH SOURCE FOR FATTY ACID SYNTHESIS IS _____
   1) pentose phosphate pathway
   2) catabolism of triacylglycerols
   3) oxidative decarboxylation of pyruvate
   4) the reaction of glycolysis

6. THE CHYLOMICRON CONSISTS OF _____
   1) 90% of triacylglycerols and 2% of proteins
   2) 50% cholesterol and its esters
   3) 50% of proteins and 20% of cholesterol and its esters
   4) 10% proteins and 50-55% triacylglycerols

7. _____ DOES NOT USE KETONE BODIES IN STARVATION
   1) nerve cell
   2) skeletal muscle
   3) heart
   4) liver

8. _____ IS ACTIVATED IN LONG-TERM STARVATION
   1) the lipogenesis in adipose tissue
   2) the β-oxidation in the liver
   3) the synthesis of cholesterol in nerve tissue
   4) the cycle of tricarboxylic acids in the liver

9. THE KEY ENZYME OF CHOLESTEROL SYNTHESIS IS_____
   1) hydroxymethylglutaryl-SCoA reductase
   2) acyltransferase
   3) pyruvate kinase
   4) acetoacetyl-SCoA synthase

10. CHOOSE THE HORMONES, STIMULATING THE LIPID SYNTHESIS IN ADIPOSE TISSUE
   1) adrenaline
   2) glucagon
   3) the hormone of growth
   4) insulin

CASE STUDIES

1. A delay in the outflow of bile from the gallbladder was found in patients after an endoscopy investigation.

Suggest the consequences of this pathology.

2. The concentration of triacylglycerols and phospholipids is 1.5-2.0 times higher in the cardiac muscle as compared to skeletal one.

Identify the biochemical value of this difference.

3. Parents are worried about an overweight child. Without consulting a doctor, they limited the amount of sugar in the baby's food and increased the protein content, without non-reduced fat amount. The child's health worsened in a few weeks. The vomit appeared.

Identify the metabolism disturbance and its cause.

Recommend the set of biochemical investigations for metabolic disorder detection.

CHECKLISTS FOR FINAL LESSON (UNIT 9)

1. Structural formula and characteristics of the main classes of lipids.

2. Types of fatty acids, their physic-chemical properties and biological role, food sources. Structure of palmitic, stearic and oleic acids, polyunsaturated fatty acids of ω-6 family (linoleic, γ-linolenic, arachidonic acid) and ω-3 family (α-linolenic, eicosapentaenoic, docosahexaenoic acid). Transport of fatty acids in the blood.

3. Derivatives of polyunsaturated fatty acids of ω-6 and ω-3 families, biological role of certain types of eicosanoids. The initial reaction of arachidonic acid synthesis. Role of phospholipase A_2, cyclooxygenase, lipoxygenase. What hormones and pharmaceutical substances influence on the synthesis of eicosanoids?

4. Triacylglycerols: chemical structure, fatty acids included in the composition, physic-chemical properties, biological role. Transport of triacylglycerols in the blood.

5. Phospholipids: chemical structure (phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol), fatty acids contained in phospholipids, physical and chemical properties, biological role. What is the role of phospholipids in lipid transport in the blood?

6. Chemical structure of sphingolipids (sphingomyelins) and glycolipids (cerebrosides, sulfolipids, gangliosides), their biological role and functions.

7. Structure of cholesterol and its esters, and its biological role. Transport of cholesterol in the blood.

8. Daily needs and food sources of fat. Role of enzymes and components of bile in the digestion of dietary lipids in the digestive tract. Synthesis of bile acids, role of vitamins in the process. Chemical structure of taurocholic and glycocholic acids. Resynthesis of lipids in the intestinal wall. Consequences of violations of digestion and absorption.

9. Characteristics of chylomicrons and very low density lipoproteins, their composition, scheme of structure, function, reactions of metabolism in the bloodstream. Utilization of chylomicrons and VLDL in tissues. The role of lipoprotein lipase. What hormones are activated?

10. Detection of egg yolk phosphatidyl choline. Principle of the method and course of the measurement.

11. Lipolysis, chemical reactions, the role of lipolysis. Regulation of lipolysis. In what physiological situations does it happen? Transport and utilization of fatty acids formed during lipolysis. Adenylate cyclase mechanism of TAG-lipases activation. The influence of insulin, adrenaline, glucagon on lipolysis.

12. Reaction of β-oxidation of fatty acids, connection to the TCA and the respiratory chain. Energy yield of the process on the example of palmitic, stearic and palmitoleic acids.

13. Ways of formation and use of acetyl-S CoA in the body (schema).

14. Reactions of synthesis and degradation of ketone bodies. Causes of ketonemia and ketonuria in starvation and diabetes.

15. Reactions of fatty acid synthesis from glucose. The key stage and localization of the process, role of citrate. Regulated enzymes. Composition of the multienzyme complex (fatty acid synthase).

16. Synthesis reaction of glycerol-3-phosphate from glucose and oxidation of glycerol to pyruvate. Energy yield of the process.

17. Chemistry of triacylglycerol synthesis. In what conditions and where does lipogenesis take place? Differences in biosynthesis of fats in adipose tissue and in the liver. Regulation of lipogenesis. Sources of glycerol, fatty acids and energy. Relationship of triacylglycerols synthesis with glucose metabolism.

18. Metabolism of triacylglycerols and saturated fatty acids in certain physiological conditions (food intake, hunger, muscular activity, diabetes type 1 and 2).

19. Determination of triacylglycerols concentration in the blood serum. Principle of determination, clinical-diagnostic and normal values.

20. Pathways of the synthesis of phospholipids, difference between them. Chemistry of reactions. Role of amino acids and vitamins. Energy sources for the synthesis of phospholipids. What is lipotropic substances? Their functions. Consequences of the lack of lipotropic substances.

21. Metabolism and functions of cholesterol: synthesis to mevalonic acid, the view about further stages, regulation of synthesis, interconnection of cholesterol synthesis and metabolism of carbohydrates. Ways of cholesterol excretion from the body.

22. Determination of concentration in the blood. Principle of the method, definition, clinical-diagnostic value, normal values.

23. Characterization of the lipoproteins of high and low density: role in the cholesterol metabolism and its esters, the main apoproteins of lipoproteins. Interaction of LDL and HDL in blood plasma. Role of lecithin-cholesterol-acyltransferase. Utilization of LDL in cells. Intracellular use of cholesterol and removal of its excess from the cell. Role of acyl-S CoA cholesterol acyltransferase.

24. Interconnection of lipid and carbohydrate metabolism. The conversion of glucose into fatty acids, cholesterol and triacylglycerol (scheme). Role of pentose phosphate pathway in the synthesis of fats.

25. Hormonal and allosteric regulation of lipid metabolism. The effect of insulin, glucagon, adrenaline, glucocorticoids on lipolysis and lipogenesis, synthesis and β-oxidation of fatty acids, synthesis of cholesterol.

26. Biochemical mechanism of lipid metabolism disorders: atherosclerosis, gallstone disease, obesity, fatty liver, diabetes type 2, hyperlipoproteinemia type I (chylomicronemia), type V and IIa type (family hypercholesterolemia). What is Tay-Sachs, Niemann-Pick's, Gaucher's diseases?

27. Stages of metabolism and their relationship. What other high-energy connections are there apart from ATP? A cycle of ATP ADP. The main ways of phosphorylation of ADP and ways of using ATP. The general scheme of catabolism of proteins, fats and carbohydrates in the body, specific and common ways of catabolism, their significance.

28. NAD-dependent dehydrogenases, carbohydrate metabolism reactions catalyzed by them. Structural formulas of oxidized and reduced forms of NAD^+. Characteristics of the vitamin, which is a part of NAD^+: biological name, signs of insufficiency, daily need, food sources.

29. FAD-dependent dehydrogenases, carbohydrate metabolism reactions catalyzed by them. Structural formulas of oxidized and reduced forms of FAD. Charac-

teristics of the vitamin, which is a part of the FAD: biological name, signs of insufficiency, daily need, food sources.

30. The sequence of reactions of the oxidative decarboxylation of pyruvate, the connection with the breathing chain. Regulation of the process. The participation of vitamins in the process and their characteristics: biological name, signs of insufficiency, daily need, food sources.

31. Sequence of reactions of the tricarboxylic acid cycle, connection with the respiratory chain. Regulation of reactions. The participation of vitamins in the process, their characteristics, energy effect.

32. Principle of oxidative phosphorylation. Scheme of the structural organization of the respiratory chain. Conjugation of oxidation with phosphorylation. Structure of H^+-ATP synthase. P/O ratio for NADH and $FADH_2$. The mechanism of respiratory control. How does ATP affect oxidative phosphorylation?

33. Dissociation of respiration and phosphorylation. What determines the heat-forming function of brown adipose tissue? Inhibitors of the respiratory chain. The causes of hypo energetic states. P/O ratio and the number of ATP molecules formed upon complete oxidation of palmitic acid.

UNIT 10
HORMONAL REGULATION OF METABOLISM AND FUNCTIONS IN THE HUMAN BODY

THEME 10.1. THE MECHANISMS OF HORMONAL SIGNAL TRANSDUCTION. CLASSIFICATION OF HORMONES. HORMONES OF PITUITARY GLAND. (SEMINAR)

INTRODUCTION

One of the characteristic features of living organisms is their ability to maintain the constancy of homeostasis by means of self-regulatory mechanisms in which a major role belongs to hormones. The effect of hormones on cells is carried out through special mechanisms, the violation of which leads to the failure or change of the hormonal effect. In order to correctly evaluate the causes of hormonal diseases it is necessary to know the mechanisms by which the hormonal signal is transmitted into the cell.

The changes in the endocrine regulation with insufficient or excess synthesis of hormones lead to disorders of metabolic processes in the body. In clinical practice hormones and hormonal drugs are widely used for the treatment of endocrine and no endocrine diseases.

THE AIM OF THE PRACTICAL CLASS IS:

To study the mechanisms of action of protein and peptide hormones, hormones – derivatives of amino acids, steroid hormones.

SELF-STUDY QUESTIONS

1. Principles of regulation of metabolic processes. The hierarchy of the regulatory systems of the body. The role of the hypothalamus and pituitary.
2. The mechanism of a negative feedback in the regulation of hormone production and action.
3. Common biological characteristics of hormones. Classes of hormones according to their chemical structure, biological functions and belonging to endocrine glands.
4. Characterization of three membrane mechanisms by which the hormonal signal is transmitted in the target cells:

- a receptor with enzymatic activity (schematically on the example of the insulin receptor),
- the receptor that forms an ion channel (schematically on the example of the acetylcholine receptor),
- transmission of the hormonal signal using G proteins (cAMP-mediated, calcium-phospholipid mechanism). Specify the components of the signal transmission system, note the role of activating and inhibitory α subunit of the G protein. What hormones use these mechanisms?

5. cGMP-mediated mechanism of signal transmission. General characteristics of this mechanism.

6 The structure and sources of secondary mediators of hormonal signal transmission: cAMP, cGMP, inositol triphosphate (IP_3), diacylglycerol (DAG), Ca^{2+}ions.

7. Characterization of the cytosolic mechanism of hormonal signal transmission into the target cells. Hormones which operate according to this mechanism.

8. Hormones of the hypothalamus.

9. Hormones – derivatives of proopiomelanocortin.

10. Characterization of pituitary hormones (growth hormone, vasopressin, oxytocin, lipotropic hormone, melanocyte stimulating hormone) according to the plan:

- name,
- chemical nature,
- regulation of hormone synthesis and secretion
- the target organs,
- the localization of receptors in the cell and mechanism of action,
- impact on the metabolism of carbohydrates, proteins, lipids, minerals, water – reactions and enzymes that are sensitive to the hormone action,
- hyper- and hypofunction of hormones, the reasons and main clinical manifestations.

TOPICS FOR REPORT

1. Influence of cholera and diphtheria toxins on the activity of adenylate cyclase mechanism in the affected cell.

2. Mechanisms and levels of regulation of endocrine gland functions, violation of these mechanisms, consequences.

3. Oxytocin – a hormone of interhuman relationships?

4. Guanylate cyclase, types, regulation, ways of transmission of the hormonal signal. Hormones using the guanylate cyclase mechanism.

5. The role of nitric oxide (NO) in the regulation of a cell activity under normal and pathological conditions. The participation of nitric oxide in the action of drugs.

6. Hormone-dependent induction and repression of protein synthesis. Features of the receptor and hormone receptor complex, their effect. The hormone sensitive element of DNA.

THEME 10.2. HORMONES OF HYPOTHALAMUS, PITUITARY, THYROID, PANCREAS AND PARATHYROID GLANDS

INTRODUCTION

Hormones of the hypothalamus and pituitary gland regulate the growth and function of other endocrine glands or influence of metabolic responses in target tissues. The posterior pituitary secretes hormones that regulate water balance and milk ejection from lactating mammary gland. Loss of function of the anterior pituitary (hypopituitarism) leads to the atrophy of thyroid, adrenal and sex glands.

Thyroid hormones play an important role in the regulation of metabolism, development and differentiation of tissues. The calcium homeostasis regulating hormones are produced in the parathyroid and thyroid glands and maintain the plasma calcium concentration in very narrow limits.

Insulin and glucagon, pancreatic hormones, play a major role in the blood glucose homeostasis, affecting the metabolism of carbohydrates and lipids in the liver and adipose tissue.

THE AIM OF THE PRACTICAL CLASS IS:

To study the protein and peptide hormone influence on the metabolism of carbohydrates, lipids, proteins, water and minerals.

To conduct qualitative reactions for the detection of insulin and the concentration of prolactin in serum.

SELF-STUDY QUESTIONS

1. Classes of hormones according to their chemical structure, biological functions and belonging to endocrine glands.

2. Hormones of the hypothalamus (releasing hormones).

3. Characterize the pituitary hormones – growth hormone, vasopressin, oxytocin, adrenocorticotropic, lipotropic and melanocyte stimulating hormones, lactotropic, follicular-stimulating and luteinizing hormones according to the plan:

- name,
- chemical nature,
- regulation of hormone synthesis and secretion
- the target organs,
- localization of the receptors in the cell and mechanism of action,
- impact on the metabolism of carbohydrates, proteins, lipids, minerals, water – reactions and enzymes that are sensitive to the hormone action.

4. Causes and metabolic consequences of the antidiuretic hormone hypofunction (diabetes insipidus). What clinical manifestations of a disease are observed? What is the vasopressin hyperfunction?

5. Characteristics of disorders associated with growth hormone: pituitary dwarfism, acromegaly, gigantism. What are the causes and metabolic disorders? The clinical manifestation of a disease.

6. Characteristics of thyroid hormones: tyroliberin. thyroid stimulating hormone, tri- and tetra iodothyronine. The thyroxine and triiodothyronine chemical structure, their target organs, localization of the receptors in the cell and mechanism of action. Impact on the carbohydrates, proteins, lipids metabolism. Enzymes that are sensitive to hormone. Hypo- and hyperthyroidism causes. Metabolic disturbances and clinical manifestations of the diseases.

7. Characterization of calcitonin and parathyroid hormone according to the plan:

- name,
- the chemical nature,
- the hormone synthesis and secretion regulation,

- the target organs,
- localization of the receptors in the cell and mechanism of action,
- the influence on the exchange of mineral substances.

8. How does the calcitonin and parathyroid hormone effect together with the calcitriol action (a vitamin D derivate)?

9. Characterize the pancreas hormones – glucagon and insulin according to the plan:

- the name,
- the chemical nature,
- the regulation of hormone synthesis and secretion,
- the target organs,
- localization of the receptors in the cell and mechanism of action,
- the impact on carbohydrates, proteins, lipids metabolism – hormone sensitive reactions and enzymes.

10. Types of diabetes. The causes of absolute and relative insulin deficiency. Metabolic disturbances in different types of diabetes, their clinical manifestations, basic treatment.

11. Conducting the insulin qualitative reactions.

12. Enzyme linked immunoassay for the prolactin determination in serum. The principle of the method. Normal values. Clinical-diagnostic value.

13. Make a table: hormones of pituitary gland, thyroid, parathyroid and pancreatic glands according to the following diagram:

The name and the chemical nature	Place of synthesis	Regulation of hormone action	The target organs	Receptors localization, mechanism of action	The impact on metabolism				Hypo- and hyperfunction of hormones
					Carbohydrates	Proteins	Lipids	Minerals and water	

TOPICS FOR REPORT

1. Melatonin, a place of synthesis, chemical nature, influence on metabolism under the normal and pathological conditions. The possibility of melatonin use in the clinical practice.

2. Dysfunction of the growth hormone. Molecular causes. Clinical manifestations of diseases, their diagnosis. Basics of treatment.

3. Diabetes mellitus, its types. Molecular mechanisms. Metabolic disorders. Clinical symptoms. Basics of treatment.

4. Causes of deficiency of thyroid function. Symptoms, clinical manifestations in children and adults. Basics of treatment. The role of hypothyroidism in the reproductive health of women.

Practical 1

INSULIN QUALITATIVE REACTIONS

The principle

Insulin is a simple protein and gives characteristic qualitative reactions on protein: Biuret, xanthoprotein, Fole etc. These reactions are not specific.

Material for investigation

Insulin solution.

Reagents

1) The Fole's solution containing 5% solution $Pb(CH_3COO)_2$ and 30% NaOH solution, 2) 0.5% solution of ninhydrin, 3) 30% solution NaOH, 4) 10% solution NaOH, 5) 5% solution $Pb(CH_3COO)_2$, 6) 5% solution sodium nitroprusside, 7) strong HNO_3, 8) 5% $CuSO_4$ solution.

Procedure

5 drops of insulin solution are poured in a test-tube and qualitative reactions on protein are made.

Biuret test for Proteins

The Biuret Test is done to show the presence of **peptide bonds**, which are the basis for the formation of **proteins**. These bonds will make the **blue** Biuret reagent turn **purple**.

The principle

In alkaline medium the peptide group forms with ions Cu^{2+} complex compound of violet color with a red or blue hue depending on the number of peptide bonds. The color intensity is proportional to the number of peptide groups.

Procedure

3 drops of 10% solution NaOH and 1 drop of 5% solution $CuSO_4$ are added to 5 drops of insulin solution in a test tube.

Reaction for α-amino group detection

The ninhydrin reaction is used to detect α-amino groups contained in the amino acids and the final insulin of α-amino groups.

The principle

The oxidative α-amino group cleavage and recovery of ninhydrin occur while the protein is heated with ninhydrin. The restored ninhydrin reacts with ammonia and another oxidized ninhydrin molecule to form ninhydrin complex of blue-violet color.

Procedure

5 drops of insulin solution are mixed with 5 drops of 0.5% solution of ninhydrin. The tube is heated and boiled until the appearance of blue-purple staining.

Aromatic amino acids reaction

For the detection of aromatic amino acids (phenylalanine, tyrosine, tryptophan) **xanthoprotein reaction** is used**.**

The principle

2 drops of concentrated HNO_3 are added to 5 drops of 1% insulin solution and carefully heated. Observe the appearance of yellow staining, in the absence of yellow color 1-2 more drops of conc. HNO_3 are added. While adding an excess 30% NaOH solution the color changes to orange

Sulfur-containing amino acids reactions

The principle

Insulin sulfhydryl groups are subjected to alkaline hydrolysis, resulting in the cleavage of sulfur in the form of sodium sulfide Na_2S, entering into the further reactions:

- **Fole's reaction** – Na_2S with lead acetate $Pb(CH_3COO)_2$ gives black or brown lead sulfide sediment,
- **nitroprusside reaction**– Na_2S with nitroprusside sodium gives the red-brown compound.

Procedure

5 drops of insulin solution and 5 drops of 30% NaOH solution are boiled for 1-2 minutes. The content is split into 2 parts for reactions "a" and "b".

a) Fole's reaction

1 drop of acetic acid lead is added to 5 drops of hydrolysate and heated to boiling. The appearance of brown or black sediment is determined.

b) Nitroprusside reaction

2-3 drops of 5% sodium nitroprusside solution are added to 5 drops of hydrolysate. The appearance of red-brown staining is noted.

Design of practical:

Explain the principle of methods, record the analysis results and make a conclusion about the insulin presence in the test material.

Practical 2

IMMUNOASSAY METHOD FOR PROLACTIN DETERMINATION IN SERUM

The principle

The solid-phase enzyme immunoassay is based on specific binding of monoclonal prolactin antibodies adsorbed on the wells of immunological tablet, with the subsequent formation of the conjugate.

Material for investigation

Serum.

Reagents

1) Conjugate monoclonal prolactin antibodies with horseradish peroxidase, a solution for dilution of serum, 2) phosphate-saline buffer solution with tween, 3) a solution of tetramethylbenzidine, 4) stop reagent 5) the control sample with a known prolactin content, 6) calibration samples containing a known prolactin amount.

Procedure

1. The introduction of the samples. Make in duplicate, starting from the top wells of the first two strips with 100 µl calibration samples. Add 100 µl of control sample and 100 µl of the analyzed serum samples in the rest of the wells.

2. The introduction of monoclonal antibody conjugate. The conjugate is ready to be used. 50 µl of conjugate are added into the wells.

3. Incubation. Sealed film strips are incubated at the temperature of 37°C for 60 minutes in a thermostatic shaker with a frequency of 650 rpm.

4. Washing. At the end of incubation, the sticky bar is removed and placed in a disinfectant solution container. With the help of the flushing device the tablet is washed 5 times with washing solution, alternating aspiration and immediate filling up the holes of each strip. In each well at least 350 µl of liquid are contributed in each wash cycle. After rinsing the remaining moisture from the wells is thoroughly removed by tapping the inverted tablet on the filter paper.

5. The introduction of tetramethylbenzidine (TMB). A TMB solution is ready for use. Insert 100 µl of TMB to all wells.

6. Incubation. Sealed film strips are incubated in the dark at a temperature of 37°C for 15 minutes in a thermostatic shaker with a frequency of 650 rpm.

7. The introduction of stop reagent. 100 µl of stop reagent are added into all wells. Shake the plate on a shaker for 10-15 seconds; the content of the wells turns yellow.

8. Measuring. The optical density in the wells of the tablet is measured by the spectrophotometer in dual-wave mode: when the main wavelength is 450 nm and the comparison length is in the range 620-655 nm.

Normal levels

Men's 2.5-17 ng/ml (53-360 mlU/l);

Women – follicular phase of 4.5-33 ng/ml (98-784 mlU/l), middle of a cycle 6.3-49 ng/ml (134-975 mlU/l), luteal phase 4.9-40 ng/ml (104-848 mlU/l).

Clinical-diagnostic value

The normal prolactin increase occurs during sleep, exercise, sexual intercourse. During pregnancy the hormone increases from the 8-th to the 25-th week and during lactation. Before birth there is a decrease in prolactin.

Concentration increase	**Concentration decrease**
Prolactinoma	Acute porphyria
Neurogenic and psychiatric disorders, menstrual problems	Acute and chronic physical and mental stressful situation (depression, surgery, painful periods)
Acromegaly	Hypoglycemia
Hirsutism (hyperandrogenism)	

Design of practical:

The principle of the method, the working procedure, the normal values and the results of the study are indicated, clinical and diagnostic value of the index is noted and conclusions on the possible pathology are drawn.

THEME 10.3. HORMONES OF HYPOPHYSIS, ADRENAL AND SEXUAL GLANDS

INTRODUCTION

In the human body corticotropin and adrenal hormones perform functions related to the activities of the body in a state of acute and chronic stress, providing resistance to damaging environmental influences. Hormones of reproductive organs are involved in the maintenance of sexual behavior and reproduction.

In clinical practice, glucocorticoids are used as anti-inflammatory and anti-allergic drugs. Sex hormones and their analogues are used in cancer patients, hormone replacement therapy, hormonal contraception.

THE AIM OF THE PRACTICAL CLASS IS:

To learn the structure and biological effects of hormones of the adrenal glands and the gonads.

To determine the testosterone level in serum.

SELF-STUDY QUESTIONS

1. Classes of hormones according to their chemical structure, biological functions and belonging to endocrine glands.

2. Chemical formula of adrenaline and noradrenaline. Characteristic features of adrenaline according to the plan:

- chemical nature,
- place and the chemistry of synthesis reactions,
- regulation of synthesis and secretion of the hormone,
- the target organs,
- localization of the receptors in the cell and mechanisms of action,
- impact on the metabolism of carbohydrates, proteins, lipids – reactions and enzymes that are sensitive to the hormone action,
- the concept of pheochromocytoma, clinical manifestations, basic treatment.

3. Types of adrenergic receptors and their actions. Biochemical effects of the hormone in stressful situations. What is the mechanism of therapeutic action of epinephrine during cardiac arrest, asthma attacks?

4. The characteristics of the following hormones: corticoliberin, corticotropin (ACTH), cortisol according to the plan:

- name,
- the chemical nature and structure,
- place of synthesis, transport in the blood,
- regulation of hormone synthesis and secretion,
- the target organs,
- localization of the receptors in the cell and mechanism of action,
- impact on the carbohydrates, proteins, lipids, mineral substances metabolism, reactions and enzymes that are sensitive to the hormone action,
- hypo- or hyperfunction of the hormone, metabolic disorders, symptoms.

5. Altered metabolism in adipose, muscle, lymphoid, epithelial tissue under hypo- and hypercortisolism. What does the expression "steroid diabetes" mean?

6. The main stages of the steroid hormone synthesis. The role of pregnenolone and progesterone – the key compounds in the synthesis pathway. A specific hydroxylase, determining the formation of mineralocorticoids and glucocorticoids. The role of aromatase in the synthesis of estrogens.

7. Characteristic of mineralocorticoids (aldosterone) according to the plan:

- the chemical nature and structure,
- place of synthesis, transport in blood,
- regulation of hormone synthesis and secretion,
- the target organs,
- localization of the receptors in the cell and mechanism of action,
- influence on the exchange of mineral substances and water – reactions and enzymes that are sensitive to the hormone action,
- hypo- or hyperfunction of the hormone, metabolic disorders, symptoms.

8. The role of the renin-angiotensin system in the regulation of aldosterone synthesis and secretion. The biochemical mechanism of renal hypertension development

9. Oxytocin, prolactin, follicle-stimulating and luteinizing hormones of the pituitary gland, progesterone and estradiol, testosterone. Their characteristics according to the plan:

- name,
- the chemical nature and chemical formula (for steroid hormones),
- place of synthesis,
- regulation of the hormone synthesis and secretion,
- target organs, transport in blood,
- localization of the receptors in the cell and mechanism of action,
- impact on the metabolism of carbohydrates, proteins, lipids, mineral substances; the biochemical processes that are sensitive to hormones,
- hypo- or hyperfunction of the hormone, metabolic disorders, symptoms.

10. Cyclical changes in the concentration of gonadotropins, progesterone and estrogen in a woman's body (menstrual cycles).

11. Immunoassay method for testosterone determination in serum. The principle of the method. Normal values. Clinical-diagnostic value.

12. Make a table for adrenocortical hormones and sex hormones, according to the given scheme:

The name and the chemical nature	Place of synthesis	Regulation of hormone action	Target Organs	Localization of the receptors, mechanism of action	Effect on metabolism				Hypo- and hyperfunction of hormones
					Carbohydrates	Proteins	Lipids	Minerals and water	

TOPICS FOR REPORT

1. Glucocorticoids as medicines. The mechanism of anti-inflammatory and anti-allergic action of glucocorticoids. Side effects.

2. The role of glucocorticoids in adaptive reactions under stress, in the development of the adaptation syndrome. The works of H. Selje, F. Meerson, and others.

3. Use of synthetic analogues of estrogens and progestins for hormonal contraception. Their mechanisms. Side effects.

4. Synthetic analogues of testosterone as medicaments. Use of anabolic steroids and other hormones in sports medicine. Side effects.

5. Hormonal changes in the body of a woman during pregnancy. Influence of hormonal diseases of the mother on fetal development.

Practical

IMMUNOASSAY METHOD FORTESTOSTERON DETERMINATION IN SERUM

The principle

The method based on a solid-phase enzyme immunoassay is specific binding of monoclonal antibodies to testosterone, adsorbed on the wells of immunological tablet, with the subsequent formation of the conjugate.

Material for investigation:

Serum.

Reagents

1) Conjugate of the monoclonal antibodies to testosterone with horseradish peroxidase, a solution for dilution of serum, 2) phosphate-saline buffer solution with tween, 3) a solution of tetramethylbenzidine, 4) stop reagent 5) the control sample with a known testosterone content, 6) calibration samples containing known testosterone amounts.

Procedure

1. The introduction of the samples. Make in duplicate, starting from the top wells of the first two strips with 100 µl of calibration samples. In the rest of the wells add 100 µl of a control sample and 100 µl of the analyzed serum samples.

2. The introduction of monoclonal antibody conjugate. The conjugate is ready to be used. 50 µl of conjugate are added into the wells.

3. Incubation. Sealed film strips are incubated at the temperature of 37°C for 60 minutes in a thermostatic shaker with a frequency of 650 rpm.

4. Washing. At the end of incubation, the sticky bar is removed and placed in a disinfectant solution container. With the help of the flushing device the tablet is washed 5 times with washing solution, alternating aspiration and immediate filling up the holes of each strip. In each well at least 350 µl of liquid are contributed in each wash cycle. After rinsing the remaining moisture from the wells is thoroughly removed by tapping the inverted tablet on the filter paper.

5. The introduction of tetramethylbenzidine (TMB). A TMB solution is ready for use. Insert 100 µl of TMB to all wells.

6. Incubation. Sealed film strips are incubated in the dark at a temperature of 37°C for 15 min in a thermostatic shaker with a frequency of 650 rpm.

7. The introduction of stop reagent. 100 µl of stop reagent is added into all wells. Shake the plate on a shaker for 10-15 seconds; the content of the wells turns yellow.

8. Measuring. The optical density in the wells of the tablet is measured by the spectrophotometer in dual-wave mode: when the main wavelength is 450 nm and the comparison length is in the range of 620-655 nm.

Normal levels

Men above 14 years: 5.76-28.14 nmol/l;

Women over 10 years: 0.45-3.75 nmol/l.

Clinical-diagnostic value

Insufficient secretion of testosterone causes the development of hypogonadism, wherein the clinical picture is directly related to the age of hormonal deficiency development onset. Related disorders are Klinefelter or Turner syndromes and cryptorchidism or anorchia, and climacteric period and menopause in women. Hypergonadism characterized by excessive testosterone secretion is diagnosed in women or men with androgen-producing tumor of the testicles, ovaries or adrenal cortex. Elevated testosterone levels in women are the confirmation of diseases such as hirsutism, virilization and polycystic ovaries.

The testosterone level increase is one of the factors contributing to the prostate cancer development in men older than 60 years, and is used as a treatment efficiency control in this category of patients.

Design of practical:

The principle of method, the working procedure, the normal values and the study results are indicated, the clinical and diagnostic value of the index is noted and conclusions on the possible pathology are drawn.

TESTS

Choose one or more correct answers.

1. THEEXCHANGE OF POTASSIUM, SODIUM AND CHLORINE IONS IS REGULATED BY HORMONE _____
   1) insulin
   2) aldosterone

3) glucagon
4) adrenaline

2. THECONTENT OF CALCIUM AND PHOSPHORUS IS REGULATED BY _____
   1) adrenaline
   2) thyroxine
   3) parathyroid hormone
   4) calcitonin

3. GLUCOCORTICOID SYNTHESIS IN ADRENAL GLANDS IS STIMULATED BY _____
   1) corticotropin
   2) corticoliberin
   3) cortisol
   4) calcitonin

4. THEINSULIN FUNCTION IS _____
   1) lipolysis stimulation
   2) activation of glycogenolysis
   3) increased glycemia
   4) increased lipogenesis

5. THEGLUCOCORTICOID FUNCTION IS_____
   1) enhancement of gluconeogenesis
   2) an increase in the proteins synthesis
   3) stimulation of glucose uptake by tissues
   4) activation of glycolysis

6. HYPOTHALAMUS HORMONES PROVIDE DIRECT ACTION ON
   1) the thyroid gland
   2) the pancreas
   3) the pituitary gland
   4) the adrenal glands

7. THEGLUCONEOGENESIS STRENGTHENS ACTION OF THE-HORMONE_____
   1) aldosterone
   2) insulin
   3) cortisol
   4) testosterone

8. THE EFFECT OF CALCITONIN INVOLVES
   1) a decrease in the level of Ca^{2+} ions in the blood
   2) increase in the level of Ca^{2+} ions in the blood
   3) increase in the level of K^{+} ions in the blood
   4) decrease in the level of K^{+} ions in the blood

9. THEINCREASE IN SOMATOTROPYN CONTENT LEADS TO
   1) acromegaly
   2) Itsenko-Cushing syndrome
   3) Basedov's disease
   4) diabetes mellitus

10. THEPARATHYROID HORMONE AFFECTS THE CALCIUM ION EXCHANGE DUE TO THE AFFECTON_____
   1) kidney and bone tissue
   2) pancreas and intestine
   3) liver and muscles
   4) adipose tissue and bone tissue

CASE STUDIES

1. A five-year old boy was examined by the doctor. A mental and physical retardation, a slowdown in growth, a decrease in body temperature were revealed. The child was non-active and lack in emotions. In the blood, the cholesterol content was reduced.

Specify the gland whose function was altered. Explain the cause of symptoms.

2. The patient had dry mouth, thirst, copious and frequent urination, weakness, sleep disturbance, weight loss.

Identify a disease characterized by these symptoms. Suggest a complex of biochemical research to clarify the diagnosis and evaluate the state of metabolism.

3. The patient complained of severe weakness, increased fatigue. There was often hypoglycemia. Pigmentation of the skin was intensive. There was anemia, lymphocytosis, eosinophilia, reduced reabsorption of sodium from urine.

Indicate hormones, the insufficiency of which can be assumed.

UNIT 11
BIOCHEMISTRY OF BLOOD

THEME 11.1. NITROGEN-CONTAINING SUBSTANCES OF THE BLOOD: PROTEINS, ENZYMES, FRACTIONS OF RESIDUAL NITROGEN

INTRODUCTION

There is a close relationship between blood and all tissues of the body. The study of various nitrogen-containing blood components and their role in metabolism allows diagnosing metabolic disorders in the body, monitoring the development of the pathological process and to evaluating the therapy efficacy. The ratio of nitrogen-containing compounds in the blood varies depending on the lifestyle and age of the person.

THE AIM OF THE PRACTICAL CLASS IS:

To study the composition of blood, nitrogen-containing substances of blood, definition of a total blood protein and the main protein fractions.

To obtain practical skills for the thymol test of colloid-resistance of serum proteins, as well as the determination of protein fractions of blood serum by the electrophoresis.

SELF-STUDY QUESTIONS

1. Organic and inorganic components of blood. Formed elements, plasma, serum.
2. Sources of glucose, triacylglycerols and cholesterol in the blood. Clinical-diagnostic value of their determination in blood.
3. Nitrogen-containing substances of blood.
4. Definition of a total blood protein. Physiological functions of blood proteins, normal concentrations of total blood protein. Causes of hypo- and hyperproteinemia.
5. The main protein fractions of blood serum. Normal values of their concentration in the blood. Give 1-3 examples of proteins for each fraction. Dysproteinemia and paraproteinemia. Age dynamics of protein fractions. Embryospecific proteins and their diagnostic value.
6. Definition of the proteinogram. Change in the ratio of protein fractions in acute and chronic inflammation; pathology of kidneys, tumor, liver diseases.
7. The key enzymes of plasma and serum. What is enzyme diagnostics? True plasma enzymes. Two groups of organ specific enzymes: enzymes of cellular metabolism and excretory (secreted) enzymes.
8. Residual nitrogen of blood. List all components and their quantitative composition. Identify reasons and types of azotemia.

9. Reactions of creatine and creatinine synthesis. Normal concentrations in the blood. Clinical-diagnostic value of determination of creatinine concentration in blood and urine.

10. Reactions of urea synthesis. Normal value of its concentration in the blood. Clinical-diagnostic value of urea concentration in blood and urine.

11. Reaction of uric acid synthesis. Normal concentrations in the blood. Clinical-diagnostic value of uric acid concentration in the blood and urine.

12. Hyperammonemia, causes and consequences. Normal and maximum permissible concentrations of ammonia in the blood. The reasons for toxicity of ammonia.

13. Thymol test for colloid-resistance of serum proteins. Principle of the method. Normal values and clinical-diagnostic value.

14. Protein fractions of blood serum. Principle of the electrophoresis. Normal values and clinical-diagnostic value.

15. With a help of additional material 2 make a table of individual globulins in serum, noting their functions:

α_1-globulins	α_2-globulins	β-globulins	γ-globulins

TOPICS FOR REPORT

1. Age dynamics of protein fractions. Embryospecific proteins and their diagnostic significance.

2. Residual nitrogen: its main components. Dynamics of the level of residual nitrogen fractions in the postnatal period.

Practical 1
THYMOL TEST FOR COLLOID-RESISTANCE OF SERUM PROTEINS

The stability of proteins depends on their charge and the presence of hydration shell. The violation of colloidal stability of proteins under the influence of various agents is manifested firstly by bonding (coagulation) protein molecules, and then by precipitation. Thus, at first, large and less charged proteins are deposited – globulins.

Thymol test

As all coagulation tests, thymol test is a nonspecific reaction. However, it is more acceptable for functional studies of the liver, than other colloidal samples.

The principle

In serum β-, γ-globulins and lipoproteins are precipitated by thymol reagent at pH 7.55 due to the formation of globulin-thymol-lipid complex.

Reagents

Thymol buffer, pH 7.55-7.60.

Material for investigation

Serum.

Procedure

	Test tube, ml
Serum Thymol buffer	0.05 3.0
	Mix and incubate for 15 minutes at room temperature. Mix again and compare with calibration samples. Result is expressed in units of turbidity S-H (authors: Shank-Haagland).

Calibration scale

Solutions with different intensity of turbidity are used as calibration samples. Samples must be thoroughly mixed before use.

N of sample	**Units of turbidity, S-H U**
1	5
2	10
3	15
4	20

Normal values

Serum 0-4 S-H U

Clinical-diagnostic significance

Test is used for the differential diagnosis of liver diseases. In such cases as damage of liver parenchyma (infectious and toxic hepatitis), even at early stage, thymol test is above normal values in 90 100% of cases. Thymol test has normal value in dysfunction of other organs, in other liver diseases.

Design of practical

Write down the principle of the method, the experimental procedure, the normal value and the results of the study, note the clinical and diagnostic value of the index and draw conclusions on the possible pathology.

Practical 2 (in theory)

ELECTROPHORESIS OF PROTEINS ON PAPER AND ACETATE CELLULOSE FILMS

The principle

Protein molecules, negatively charged at pH 6.8, are moved towards the anode in the electric field of direct current. The fastest protein is albumin, then α_1-, α_2-, β- and γ-globulins.

The course of electrophoresis is influenced by the following factors:

- charge (usually depends on pH), size and shape of molecules;
- electrical field – speed of ion movement is directly proportional to the amperage and voltage; inversely proportional to the resistance (depending on type and size of supporting medium and ionic strength of the buffer),

•buffer – composition, concentration, pH and ionic strength (depending on the concentration of ions and their charge),

•supporting medium – its hydrophilicity, adsorption of substances on supporting medium molecules.

Material for investigation

Serum.

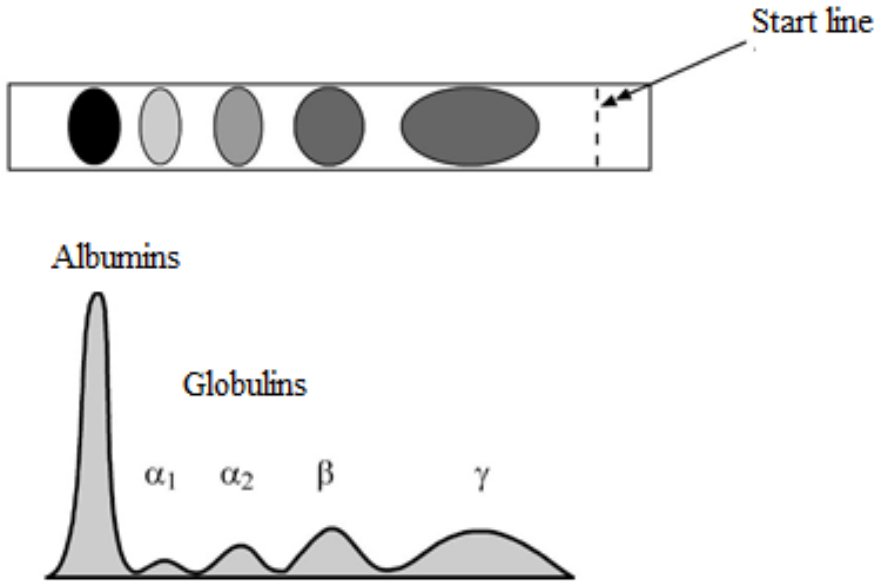
Equipment

Electrophoresis apparatus, densitometer.

Procedure (basic steps)

Serum samples are applied equally at the start line on supporting medium (paper, acetate cellulose film). Medium is placed in the apparatus for electrophoresis, and then the electric current serves. Buffer solution, moving in the electric field, captures protein molecules. Molecules with the most negative charge and smaller size, i.e. albumins, move faster than others. The largest and most neutral molecules (γ-globulins) are the last. After some time (set for each device individually) electrophoresis ends.

Strips of paper or acetate cellulose film are washed from the buffer solution and stained. As a result, areas, containing the protein, stain; area and intensity of the color depend on content of protein fractions. Currently, the quantification of colored zones on the electrophoretogram is performed using densitometer. Operation of the densitometer is based on the transmittance of light through a moving strip of medium. If we have change of the color intensity, there is a "splash" and the presence of a colored zone (protein fraction) is recorded. In parallel, modern devices calculate automatically the percentage of protein fractions.



Clinical-diagnostic value

Albumin:

Decrease in the content of albumin fraction occurs in cases, characterized by:

- reduced synthesis of albumin – congenital analbuminemia, protein starvation, malabsorption, severe liver damage (cirrhosis, degeneration, necrosis, active hepatitis, amyloidosis),
- increased catabolism of albumin – fever, cachexia, severe infections, pancreatitis, collagen diseases, thyrotoxicosis, Itsenko-Kushing disease (hypoadrenalism),
- loss of albumin through the burn surface, kidneys, gastrointestinal tract,
- inflammation due to the release of albumin from the bloodstream into the intercellular space.

The albumin level <20 g/l is accompanied by edema.

α-globulins:

Increase in the content of α_1- and α_2-globulin fractions is associated with acute and subacute inflammatory processes, and some malignant tumors, injuries, as these fractions include the majority of acute phase proteins (C reactive protein, α2-macroglobulin, α1 glycoprotein α1 antitrypsin, ceruloplasmin, haptoglobin).

β-globulins:

The large share of β-globulin fraction is β-lipoproteins (VLDL and LDL), therefore, increase in this fraction is the most often associated with hyperlipoproteinemia. In addition, transferrin, hemopexin and components of the complement system have the influence on dynamics of β-globulin fraction.

γ-globulins:

The content of γ-globulin is increased in pathological conditions associated with chronic inflammatory processes (immunoglobulins G, A and M).

THEME 11.2. IRON EXCHANGE. HEMOPROTEINS. THE SYNTHESIS AND BREAKDOWN OF HEME

INTRODUCTION

A wide variety of biologically important functions of hemoglobin and other hemoproteins (e.g., cytochromes) requires studying the structure and role of these proteins in metabolism. Conditions associated with the heme synthesis and its degradation violations lead to the development of blood and liver diseases.

THE AIM OF THE PRACTICAL CLASS IS:

To study heme synthesis and degradation reactions, bilirubin metabolism.

To determine the bilirubin concentration in the blood serum, bile pigments in the urine.

SELF-STUDY QUESTIONS

1. Iron exchange in the organism: the need, absorption, transport, iron-binding proteins, storage form. Food sources. The iron deficiency symptoms and clinical manifestations. The hemochromatosis concept.

2. The function of these erythrocyte proteins – spectrin, glycophorin, band 3 protein. Glucose oxidation features in the erythrocyte. How are the oxygen active forms neutralized?

3. The structure of heme, reactions and main stages of its synthesis. Regulation of heme and hemoglobin synthesis.

4. Causes of porphyry, clinical manifestations, the basics of porphyry treatment.

5. Thalassemia, their types and causes.

6. The structure of the most abundant hemo containing proteins in the body (hemoglobin, myoglobin, cytochromes, catalase, peroxidase). Their functions and localizations.

7. Pathological and physiological types of hemoglobin (met hemoglobin, sickle cell, glycosylated hemoglobin, carboxyhemoglobin, oxygenated hemoglobin, carbohemoglobin). The importance of determining the concentration of glycosylated hemoglobin, oxyhemoglobin and carbohemoglobin.

8. The scheme of reactions occurring in the erythrocytes in the lungs and tissue capillaries.

9. Mechanisms of carbon dioxide transport. In what form is carbon dioxide transferred? How is it connected with hemoglobin? The role of carbonic anhydrase. The erythrocyte role in the plasma bicarbonate ions concentration changes.

10. The binding of hemoglobin with oxygen. The oxygen normal saturation of hemoglobin. The oxygen transport mechanism. The effect of temperature, pH, CO_2 concentration on the hemoglobin affinity to oxygen, regulation of the process. Cooperation of protomers, the Bohr effect, role of 2.3-diphosphoglycerate.

11. Curves of saturation of hemoglobin with oxygen, or the dissociation of hemoglobin. What does the S-shaped nature of the hemoglobin dissociation curve explain?

12. The decomposition reactions of hemoglobin and heme in the reticuloendothelial system.

13. Indirect (free) bilirubin, its structure, reaction formation. The further fate of the indirect bilirubin.

14. Direct (linked) bilirubin, its structure, reaction formation, its fate. The role of the enzyme UDP-glucuronyltransferase. How is the final breakdown of heme products excreted?

15. Conditions associated with the hemoglobin excessive breakdown. Causes of hemolytic jaundice and its laboratory criteria.

16. Conditions associated with impaired bile flow. Causes of obstructive jaundice and its laboratory criteria.

17. Conditions associated with failure of hepatocytes function. Causes of parenchymatous jaundice and its laboratory criteria.

18. Physiological jaundices of the newborn.

19. Pathological jaundices of newborn:

- hemolytic jaundice, their causes. The physiological basis of the phenobarbital using,

• inherited disorders of bilirubin excretion – Gilbert's, Meulengracht, Dubin-Johnson, Crigler-Nayar syndrome,

• the idea of acquired disorders of bilirubin excretion – the excess of estrogens in milk, infectious and toxic causes,

• the idea of the mechanical jaundice due to cystic fibrosis, Niemann-pick disease, hypoplasia of the biliary tract.

20. Estimation of total bilirubin and its fractions in the blood serum. The method principle, normal values, clinical-diagnostic value.

21. Detection of bilirubin and urobilinogen in the urine. The principle of the method, normal values, clinical-diagnostic value.

TOPICS FOR REPORT

1. Iron deficiency states, their causes. Diagnostics. Effects. Treatment.

2. The congenital and acquired met hemoglobinemia. Causes. Insufficiency of NADH-met hemoglobin reductase. Clinical signs.

3. Porphyria. Classification, causes, pathogenesis, clinical manifestations, the basis of treatment.

Practical 1

DETERMINATION OF THE TOTAL BILIRUBIN AND ITS FRACTIONS CONCENTRATION IN BLOOD SERUM

The principle

The interaction of sulfanilic acid with nitrous sodium acid gives diasoftnisurance acid, and then bilirubin together with it forms a colored azopigment (Ehrlich's diazo reaction). Bound (direct) bilirubin reacts quickly, so its concentration is judged by the initial color intensity. Unbound bilirubin reacts only after addition of an accelerator (caffeine). The last releases bilirubin from complex with proteins and thereby accelerates the reaction of associate (azo coupling).

Material for investigation

Serum.

Reagents

1) Sulfanilic acid in HCl (reagent 1), 2) sodium nitrous acid $NaNO_2$ (reagent 2), 3) caffeine reagent (reagent 3), 4) buffer solution (reagent 4), 5) 0.9% NaCl solution, 6) the albumin solution, 20 g/l.

A standard solution of bilirubin 5 μmol/l.

Procedure

	Common bilirubin, ml	**Direct bilirubin,** ml	**Standard,** ml	**Control,** ml
Sulfanilic acid (reagent 1)	0.2	0.2	0.2	—
$NaNO_2$ (reagent 2)	1 drop	1 drop	1 drop	—
Caffein reagent (reagent 3)	1.0	—	1.0	1.0
NaCl	—	1.0	—	0.2
Blood serum	0.2	0.2	—	0.2
Standard-bilirubin	—	—	0.2	—
	Mix and leave for 10 minutes			
Buffer solution (reagent 4)	1.0	1.0	1.0	1.0
	Measure the optical density of standard sample for direct bilirubin against control at a wavelength of 540 nm (green filter). After 10 minutes measure the optical density of the sample on total bilirubin against control at a wavelength of 540 nm (green filter).			

Calculation

According to the formula the concentration of total and direct bilirubin is calculated, the concentration of indirect bilirubin is found as the difference between the concentration of total and direct bilirubin.

The concentration of bilirubin, μmol/l = $\frac{E_{ex}}{E_{st}} \times C_{st}$, SST, where

E_{ex} and E_{st} – optical density of experiment and standard samples, C_{st} – concentration of standard solution.

Normal values

Serum

Total bilirubin

Children	3.4-17.1 μmol/l
Adults	8.5-20.5 μmol/l

Direct bilirubin

Children	lack
Adults	2.2-5.1 μmol/l

Clinical and diagnostic significance

The table shows the shifts of the content of the main pigments in the blood serum, the urine and feces of healthy people and in different types of jaundices (↑ increase, ↓ the lower, N – normal values):

Types of jaundice	Types of jaundice		
	Hemolytic	**Parenchymal**	**Mechanical**
Blood bilirubin Common Indirect Direct	 ↑ ↑↑ N or ↑	 ↑ ↑ ↑	 ↑↑ N or ↑ ↑↑
Bilirubin in urine	N	N or ↑	↑
The urobilin of urine	↑↑	↑	↓
Feces stercobilin	↑↑	N or ↓	Missing

Blood serum:

The accumulation of bilirubin in the blood (more than 43 mmol/l) leads to its binding by the skin and conjunctiva elastic fibers, which manifests as jaundice. For the differential diagnosis of jaundice, it is necessary to define the fraction responsible for bilirubinemia:

1. **Hemolytic** (suprarenal) jaundice is the accelerated formation of bilirubin as a result of hemolysis *Hyperbilirubinemia* develops due to the indirect fraction of bilirubin. The content of urobilin in *the urine* increases dramatically, bilirubin is absent, sterkobilin is found in *feces*. This type of jaundice can develop with B_{12} deficiency anemia, hemolytic anemias of different origin (porphyria, medications, blood incompatibility, defect in glucose-6-phosphate dehydrogenase).

2. **Parenchymal** (liver cell) jaundice – the extraction of bilirubin in the liver cells, its conjugation and excretion are broken. *Hyperbilirubinemia* develops due to both fractions: the number of indirect bilirubin increases due to the functional insufficiency of hepatocytes or mitigating of them, and direct bilirubin – through cytolysis of hepatocytes. There is bilirubin in the *urine*, moderately increased concentrations of urobilin, the level of *feces* stercobilin is normal or reduced.

This type of jaundice is observed in viral and other forms of hepatitis, cirrhosis and liver tumors, fatty degeneration, and other conditions.

3. **Mechanical** (obstructive) jaundice develops as a result of the bile outflow violations by the blockage of the bile duct. As a result of bile stagnation the bile capillaries are stretched, their permeability is increased. Not having the outflow into the bile, direct bilirubin enters the blood and it results in the development of hyperbilirubinemia. In severe cases, due to the direct bilirubin overflow of hepatocytes the conjugation with glucuronic acid is disrupted and the amount of unbound bilirubin in *blood* increases. Levels of bilirubin in the *urine* are sharply increased; there is virtually no *feces* stercobilin.

In addition to cholelithiasis, obstructive jaundices are identified in tumors of the pancreas and helminthiasis.

Design of practical

Write down the principle of the method, progress and results of research, note clinical-diagnostic value, and make the conclusion about the possible pathology.

Practical 2

DETECTION OF BILIRUBIN AND UROBILINOGEN IN THE URINE USING "ICTOPHAN" DIAGNOSTIC STRIPS

The principle

Strips contain two areas of indication for bilirubin and urobilinogen. The test is based on the reaction of bilirubin combination with stabilized diazoreagent. The reaction zone contains p nitrophenyldiazonium-p-toluensulfonate, sodium bicarbonate and sulfosalicylic acid. The lilac-beige (lilac-pink) colour appears after 30 seconds upon contact with the conjugated (direct) bilirubin. The intensity of coloring depends on the determined bilirubin concentration.

The urobilinogen determination is based on the reaction of urobilinogen azo-combination with the diazonium stabilized salt. In the urobilinogen presence the reaction zone changes color to pink or red.

Material for investigation

Normal urine and urine with bilirubin.

Normal values

Urine

Bilirubin	The test is negative
Urobilinogen	to 17.0 mmol/l

Clinical-diagnostic value

Urine

Bilirubinuria is a characteristic of obstructive and parenchymatous jaundice with the increasing level of direct bilirubin in serum but hemolytic form is not characterized by it. In hepatitis, the bilirubin may be detected in the urine before the appearance of jaundice.

The increasing concentration of urobilinogen in the urine is observed in parenchymal liver disease (hepatitis, cirrhosis, poisoning), hemolytic conditions, intestinal diseases, associated with the excessive absorption of stercobilinogen by the intestine mucous membrane (enterocolitis, constipation).

Design of practical

Write down the principle of the method, and the results in the table, note the clinical-diagnostic value, and made the conclusion about the possible pathology.

The material of study	**Reaction**	**The result**
Normal urine Urine with bilirubin		

THEME 11.3. INORGANIC SUBSTANCES OF THE BLOOD. ACID-BASE STATUS

INTRODUCTION

Blood occupies a special place in metabolism due to a number of specific functions belonging to its chemical components. Irreplaceable role of blood in gas exchange and regulation of the acid-base state of the body, violations of which are often found in clinical practice.

THE AIM OF THE PRACTICAL CLASS IS:

To study the parameters of acid-base status, their normal values, chemical and physiological mechanisms of acid-base status regulation, disorders of acid-base balance.

To obtain practical skills for the quantitative determination of the concentration of inorganic phosphate and chloride ions in serum.

SELF-STUDY QUESTIONS

1. Electrolytes of blood plasma:

- macronutrients: sodium, potassium, calcium, phosphorus, iron, chlorine. What are their distribution and importance in the body? Normal concentrations in blood plasma. What does their concentration in blood plasma depend on?
- micronutrients: iodine, copper, zinc, cobalt and selenium. Examples of their participation in metabolism.

2. Mechanism of carbon dioxide transport. In which form is carbon dioxide transferred? The role of carbonic anhydrase. The role of erythrocytes in the change in concentration of bicarbonate ions in plasma.

3. Mechanism of oxygen transport. How does oxygen bind to hemoglobin? Hemoglobin saturation curve or oxygen-hemoglobin dissociation curve.

4. Scheme of reactions occurring in erythrocytes in capillaries of lungs and capillaries of tissues.

5. Parameters of acid-base status and their normal values.

6. Chemical mechanisms of ABS regulation. How do blood buffer systems work (phosphate, protein, bicarbonate, hemoglobin)? Chemical reactions.

7. Physiological systems of ABS-disorder compensation – role of lungs, kidneys, liver and bone. How do they work?

8. Influence of gastric and pancreas secretion on acid-base status of the body. Role of theliver.

9. The main types of ABS-disorders – respiratory acidosis and alkalosis, metabolic acidosis and alkalosis, and their causes. The change of acid-base status indicators in these disorders. Methods of compensation for violations.

10. Causes of ABS shifts in the following conditions, their chemical and physiological compensation:

- diabetes;
- pneumonia;
- tissue hypoxia;
- alcohol poisoning;
- uncontrollable vomiting;
- diarrhea;
- attack of asthma;
- chronic bronchitis;
- chronic renal insufficiency (decreased kidney function);
- traumatic brain injury with stimulation of the respiratory center;
- high lifting into the mountains;
- right ventricular heart failure.

11. Principle of the quantitative measurement of inorganic phosphate in the serum and urine. Clinical-diagnostic value and normal levels.

12. Determination of the concentration of chloride ions in the serum and urine. Clinical-diagnostic value, normal levels

TOPICS FOR REPORT

1. Combined disorders of the acid-base state. Methods for determining acid-base balance parameters in clinical and laboratory practice.

Practical 1

DETERMINATION OF THE CONCENTRATION OF INORGANIC PHOSPHATE IN THE SERUM

The principle

In acidic environment phosphoric acid reacts with ammonium vanadate and ammonium molybdate with the formation of phospho-vanado-molybdic acid, which has a yellow color. The color intensity is directly proportional to the concentration of inorganic phosphorus in the sample and is determined photometrically.

Material for investigation

Serum. Urine, diluted in 1 to 5.

Reagents

1) 10% trichloroacetic acid, 2) working solution, containing 1 mmol/l ammonium molybdate and 1 mmol/l ammonium vanadate.

A standard solution of KH_2PO_4, 2.5 mmol/l

Procedure

	Test 1, ml	**Test 2,** ml	**Standard**, ml
Serum	0.2	—	—
Urine, diluted in 1 to 5	—	0.2	—
Standard solution	—	—	0.2
distilled water	0.6	0.6	0.6
Trichloroacetic acid	0.8	0.8	0.8
	Filtration in 5-8 minutes or centrifuge 10 minutes in 1500 rpm/minute.		
Supernatant	0.8	0.8	0.8
Working solution	1.0	1.0	1.0
	Mix well and measure the optical density of test tubes and standard in 670 nm (red filter) against the water.		

Calculation

$$\text{Phosphate concentration in serum, mmol/l} = \frac{E_{ex}}{E_{st}} \times C_{st} \times 5 \times D,$$

Where E_{ex} – optical density of experiment samples, E_{st} – optical density of standard samples, C_{st} – concentration of standard solution; 5 – urine dilution, D – value of diuresis (1300-1500 ml per day).

Normal values

Serum	0.81-1.48 mmol/l
Urine	25.8-48.4 mmol/per day

Clinical-diagnostic significance

The concentration of phosphate in serum and urine primarily depends on the function of parathyroid and thyroid glands, kidney function, regulatory influence of calcitriol.

Serum

Hyperphosphatemia is observed in renal failure, hyperthyroidism, hypoparathyroidism, acromegaly, healing bone fractures, bone metastases, overdose of vitamin D, and acute respiratory acidosis in multiple myeloma.

Decrease in the concentration of phosphates is observed during infusion of glucose and hyperinsulinism (as insulin promotes transport of phosphorus in cells); diabetic ketoacidosis (as glucose increases the excretion of phosphates with urine); hypokalemia, hyperparathyroidism in rickets and osteomalacia, acute alcoholism, malabsorption syndrome.

Urine

The release of phosphates *increases* with the acceleration of catabolic processes in the body – hyperthyroidism, meningitis, diabetic ketoacidosis, leukemia, impaired renal function.

The *decrease* in urinary concentration is observed with tuberculosis, hypothyroidism of the parathyroid glands.

Design of practical

Write down the principle of the method, the experimental procedure, the normal value and the results of the study, note the clinical and diagnostic value of the index and draw conclusions on the possible pathology.

Practical 2

COLORIMETRIC METHOD FOR THE DETERMINATION OF CHLORIDE IONS IN THE SERUM

The principle

In the presence of chloride ions in the acidic environment, mercury thiocyanate produces thiocyanate-ions forming the colored complex with Fe^{3+}. The color intensity is proportional to the concentration of chloride ions in the sample and is determined colorimetrically.

The material for investigation

Serum. Urine diluted in 2 times.

Reagents

1) Working reagent containing 2.0 mmol/l of mercury thiocyanate $Hg(SCN)_2$, 30.0 mmol/l of iron nitrate $Fe(NO_3)_3$, 4.0 mmol/l of nitric acid HNO_3.

A standard solution of NaCl, 100 mmol/l.

Procedure

	Test 1, ml	**Test 2**, ml	**Control**, ml
Serum	0.01		—
Urine diluted in 1 to 2.	—	0.01	—
NaCl solution	—	—	0.01
Working reagent	2.0	2.0	2.0
	Mix thoroughly. After 5 minutes measure the optical density of experienced and standard samples against water at a wavelength of 490 nm (blue light filter).		

Calculation

$$\text{Concentration of chloride ions in serum, mmol/l} = \frac{E_{ex}}{E_{st}} \times C_{st} \times 2 \times D,$$

Where E_{ex} — optical density of experiment samples, E_{st} — optical density of standard sample, C_{st} – concentration of standard solution, 2 – urine dilution, D – value of diuresis (1300-1500 ml/per day).

Normal values

Serum	97-108 mmol/l
Urine	120-240 mmol/per day

Clinical-diagnostic significance

Serum

Increase in the concentration of chloride ions is observed during dehydration, caused by insufficient fluid intake, kidney disease, decompensation of heart, hyperventilation (respiratory alkalosis), hypofunction of the adrenal cortex.

Decrease is detected in the dehydration as a result of loss of fluid (vomiting, diarrhea, intense sweating), stenosis of the pylorus, renal diabetes, gastric hypersecretion, insufficiency of the adrenal cortex, increase in the amount of extracellular fluid, infectious diseases and other pathological conditions. Any significant hypochloremia may lead to a compensatory increase in residual nitrogen fractions due to desire of the organism to maintain constancy of osmotic pressure.

Urine

The concentration of chloride ions *increases* with insufficiency of the adrenal cortex, nephritis, the use of diuretics.

Reduction of their concentration is noted with a large loss of chlorine through the gastrointestinal tract, starvation, Itsenko-Cushing syndrome, with severe sweating.

Design of practical

Describe the principle of the method, the experimental procedure and the results of the study, note the practical value of an indicator and draw conclusions about possible pathology.

TESTS

Choose one or more correct answers.

1. THEHYPOALBUMINEMIA DEVELOPS IN _____
   1) dehydration of the body
   2) a case of infectious diseases
   3) poisoning with toxins
   4) violations of the assimilation of protein

2. THEALBUMIN FUNCTION IS_____
   1) transport of endogenous metabolites
   2) participation in immune reactions
   3) participation in blood clotting
   4) regulation of protein metabolism

3. THE GREATEST QUANTITY OF IRON IN THE HUMAN BODY IS IN THE STRUCTURE OF
   1) hemoglobin
   2) ferritin
   3) hemosiderin
   4) transferrin

4. THE GREATEST CONCENTRATION OF FERRITIN IS OBSERVED IN
   1) the liver
   2) erythrocytes
   3) the stomach
   4) the kidneys

5. THE MOST TOXIC EFFECT OF BILIRUBIN PROVIDES ON
   1) hepatocytes
   2) nerve cells
   3) muscle cells
   4) splenocytes

6. _____ IS A SIGN OF HEMOLYTIC JANDISE IN BLOOD
   1) the increased concentration of direct bilirubin
   2) an increase in the number of bile acids
   3) the accumulation of indirect bilirubin
   4) the increased hemoglobin concentration

7. THEARTERIAL BLOOD OF THE HUMAN HAS A PH LEVEL OF _____
   2) 7.37-7.45
   3) 7.00-7.25
   4) 6.85-7.15

8. THE CAUSE OF METABOLIC ACYDOSIS IS_____

1) increase of blood viscosity
2) decrease in the concentration of carbon dioxide
3) ammonia enhancement
4) hyperventilation of the lungs

9. THEMETABOLIC ACYDOSIS DEVELOPMENT MAY BE DUE TO _____

1) severe vomiting
2) use of hypokalemic diuretics
3) the accumulation of bicarbonate ions
4) intensive muscular work

10. METABOLIC ALKALOSIS DEVELOPMENT IS MAY BE DUE TO _____

1) hemolytic anemia
2) intense vomiting
3) diabetes mellitus
4) hyperventilation of the lungs

CASE STUDIES

1. There were two biochemical reports of the protein level in the blood. 30 g/l and 100 g/l, which were made in two patients – a child with extensive burns and men with hypoacid gastritis, pancreatitis (inflammation of the pancreas).

Indicate the patients who own these tests. Justify the conclusion.

2. The patient had a significant increase in residual blood nitrogen.

Propose a complex of biochemical studies to clarify the cause of the increase in residual nitrogen.

3. The patient had difficulties in breath, it became superficial, the blood pH was 7.31, pCO_2 was 52 mmol/l, the $[HCO_3^-]$ was 37 mmol/l, the alkaline reserve was increased.

Note the type of violation of the acid-base state in the patient. Assume mechanisms for compensation violations.

UNIT 12
BIOCHEMISTRY OF KIDNEYS

THEME 12.1. WATER-SALT EXCHANGE. NORMAL AND PATHOLOGICAL COMPONENTS OF URINE

INTRODUCTION

Kidneys are involved in the regulation of water-salt balance, the maintenance of acid-base status, osmotic pressure of body fluids, blood pressure, stimulation of erythropoiesis.

The amount and composition of urine secreted in the kidneys can vary within wide limits, reflecting the state of water-salt metabolism and other aspects of the body metabolism. The examination of every patient, not only in hospital but also in ambulatory conditions should be accompanied by mandatory urine analysis, as this study can help in diagnosis, and often completely change the initial diagnostic assumptions, to assess the effectiveness of the therapy.

THE AIM OF THE PRACTICALCLASS IS:

To study the urine formation mechanisms, common properties, and chemical composition of urine in health and disease, the role of kidneys in the maintenance of acid-base status.

To determine the physic-chemical properties and quantitative composition of urine.

SELF-STUDY QUESTIONS

1. Metabolism of the kidneys. The metabolism differences in the cortex and medulla layers. In what part of the kidney does aerobic and anaerobic oxidation of glucose occur, what is gluconeogenesis? Metabolism features of proteins and lipids in the kidneys. The kidneys role in the synthesis of biologically active substances (creatine, erythropoietin, 1.25-dioxyholecalciferol).

2. The role of enzymes in the kidney function – glycine amidine transaminase, Na^+/K^+-ATPase, glutamate dehydrogenase, glutaminases, alkaline phosphatase, the isoenzymes of lactate dehydrogenase.

3. Nephron structure scheme. Processes of urine formation: filtration, reabsorption and secretion. Action localizations and effect of mineral and water-salt metabolism regulating hormones.

4. Characteristics of filtration, factors influencing its speed and magnitude. Filtration rate evaluation in clinical practice. Clearance. Substances that are used to define clearance.

5. Reabsorption, the biochemical reactions occurring in the tubule lumen and in the cells of the proximal and distal portions of the nephron. The transport maximum for glucose. Counter current multiplying mechanism of the urine concentration.

6. Sources of water in the body and ways of its elimination. The role of skin, lungs, digestive tract and kidneys in the excretion of water. Recirculation of water between the blood and the gastrointestinal tract, blood and kidneys. Children and adults clean water requirements. Water metabolism peculiarities in children.

7. Describe factors affecting the water amount in the body – blood osmolality, blood volume, blood pressure, concentration of sodium and potassium.

8. Regulation of reabsorption of water. The role of antidiuretic hormone. The factors stimulating its synthesis and release. Metabolic consequences of antidiuretic hormone hypofunction, symptoms.

9. The regulation of sodium reabsorption. Activation and functioning of the renin-angiotensin-aldosterone system. The diagram showing the role of renin-angiotensin system in sodium reabsorption. The hypertension mechanism while blood circulation disorders in the kidneys, the causes of such violations.

10. Regulation of calcium reabsorption. The role of 1.25-dioxyholecalciferol, parathyroid hormone and calcitonin in calcium homeostasis.

11. The role of the kidneys in maintaining acid-base balance – reabsorption of bicarbonate, acids, ammonia, the excretion of organic acids.

12. Urine general properties of a healthy man are: quantity, color, transparency, odor, relative density, pH. How are these indicators changed in pathological conditions?

13. Urine organic and inorganic components of a healthy person.

14. The reasons of urine pathological components – protein, glucose, bile pigments, ketone bodies, blood, enzymes.

15. The principle of the methods for the determination of urine physic-chemical properties (density, pH). Clinical-diagnostic value of these indicators. The normal values.

16. The laboratory determination of urine components – protein, glucose, ketone bodies, bilirubin, urobilinogen, hemoglobin, red blood cells. Clinical-diagnostic relevance, the normal values.

17. Assessment of glomerular filtration rate in clinical laboratory diagnosis. What is the definition for creatinine clearance?

TOPICS FOR REPORT

1. Physiological and pathological proteinuria and creatinuria.
2. Mechanism of action of diuretics. Use of diuretics in the clinical practice.

Practical 1

DETERMINATION OF URINE PHYSICO-CHEMICAL PROPERTIES

Material for investigation

Normal urine and urine samples N 1, 2, 3.

Determination of relative density

Equipment

Urometer, a high cylinder for urine.

Procedure

If there is some foam on the urine surface it is removed with filter paper.

If there is a little urine, then prior to testing it can be diluted in 2-3 times with distilled water, and the result is multiplied by the degree of dilution.

The urine is poured along the wall into a high, narrow cylinder and the urometer is carefully immersed in it not touching the walls and bottom of the cylinder. A reading on the scale of urometer is taken using the lower meniscus of the liquid.

In case of a large relative density of urine the second type of urometer (scale 1.030-1.060) is used. If the urine has a temperature that does not meet the conditions noted on the urometer, then for every 3OC above or below the needed temperature, respectively, 0.001 of the indications on the urometer scale are added or taken away.

Normal value

Urine	1.010-1.025

Clinical-diagnostic significance

The relative density of normal urine is directly dependent on the concentration of soluble substances and is in the inverse relationship with the urine amount.

The increase in the urine relative density is observed in diabetes mellitus (glycosuria), injury of the glomerular filter (proteinuria).

The decrease in density is associated with polyuria of any etiology.

Determination of pH

The principle

The principle is based on the indicator color change in accordance with the pH of the solution.

Procedure

A strip of indicator paper is dipped into a test tube with urine and the color change is compared with the reference chart on the package, the pH value of the test urine is set.

Normal value

Urine	5.0-6.5

The influencing factors

With predominantly protein food the reaction of urine is acidic, while plant-based diet – alkaline. The acid reaction of urine is due primarily to the ions $H_2PO_4^-$ and NH_4^+, and alkaline – to ions HCO_3^-.

Clinical-diagnostic value

The prevalence of food animal protein determines the urine pH shift to the acid side, the predominance of plant food – to alkaline.

Strongly acidic reaction of urine is observed during fever, diabetes, starvation, etc. Alkaline reaction of urine is noted with cystitis and pielitis, severe vomiting, diarrhea (diarrhea), administration of sodium bicarbonate and the use of alkaline mineral waters.

The urine acidity determines the possibility of urinary stone formation. Uric acid stones (the urate) tend to occur at pH below 5.5, the oxalate – at a pH of 5.5-6.0, the calcium-phosphate – at pH 7.0 and 7.8.

Design of practical:

Write down the principle of methods and the study results. The results of this work are used for conclusions in practical 2.

	The density			
	The density	**The color**	**Transparency**	**pH**
The norm				
Sample N 1				
Sample N 2				
Sample N3				

Practical 2

QUANTITATIVE DETERMINATION OF THE URINE COMPOSITION

Material for investigation

Normal urine and urine samples N 1, 2, 3.

Equipment

Test strips "Glucophan", "Ketophan", "Albuphan", "Ictophan", and «Hemophan".

Procedure

Without touching the display area by hands, take a strip from the packing box and immerse it for 1-2 seconds in the test liquid. The urine drops from the strip are removed by the edge of the vessel. The strips are left in a horizontal position. After 1 minute the target substance concentration is determined by the color scale on the package.

Determination of glucose by "Glycophan" test strips

The principle

The principle of glucose determination is based on enzymatic reaction glucose oxidase. The display area is impregnated with solutions of the enzymes glucose oxidase, peroxidase and a dye tetramethylbenzidine. Using glucose oxidase glucose is oxidized by air oxygen to gluconic acid with formation of hydrogen peroxide. In the presence of enzyme peroxidase hydrogen peroxide oxidizes a dye, and there is the transition of yellow coloring to green.

Normal value

Urine

Glucose	test strips "Glycophan"	test is negative
	other methods	0.1-0.8 mmol/l

Clinical-diagnostic significance

The glucose level in the urine increases over 10 mmol/l (renal threshold) in all cases of hyperglycemia.

The glycosuria can be physiologic and pathologic.

Physiological glycosuria includes alimentary glycosuria, glycosuria of pregnant and neurogenic glycosuria on the basis of stressful conditions.

Pathological glycosuria is detected:

- by hyperglycemia – diabetes mellitus, thyrotoxicosis, acromegaly, adrenal hyperplasia, myocardial infarction, poisoning with morphine, phosphorus, hemorrhage in internal organs, acute infection and neurological disease,

• when renal tubules are damaged – pielo- and glomerulonephritis, toxic injury of the kidney, renal diabetes (familial renal glycosuria), kidney disease.

Definition of ketone bodies by test strips "Ketophan"

The principle

The test is based on display with test strips that contain an alkaline buffer in the mixture with nitroprusside of sodium, giving acetone and acetoacetic acid red, cherry or violet coloration. The sample is more sensitive to acetoacetic acid than to acetone. With hydroxybyturic acid, the indicator does not respond. Thus, the color intensity reflects only the concentration of acetoacetic acid in the urine.

Normal values

Urine		
Ketone bodies	test strips "Ketophan"	test is negative
	other methods	20-30 mg/per day

Clinical-diagnostic significance

Ketone bodies in urine (ketonuria) appear by ketonemia that occurs in starvation, diabetes, with increasing concentrations of lipid-mobilizing hormones in the blood, in acetonemia conditions in children.

Determination of protein by test strips "Albuphan"

The principle

The test is based on the color change from yellow to blue-green acid-base indicators (tetrabromphenol blue and ether tetrabromophenolphthalein) under the influence of proteins. The test is most sensitive to albumin, is much less sensitive to globulins, mucoproteins, hemoglobin. At strongly alkaline pH of the urine sample it may give false positive results.

Normal values

Urine		
Protein	test strips "Albuphan"	test is negative
	other methods	10-140 mg/l

Clinical-diagnostic significance

A small amount of protein in daily urine is detected in healthy individuals, but in a single urine sample it is impossible to detect such concentration by conventional methods. Part of these proteins has serum origin, the other part is the product of the urinary tract cells.

Proteinuria is usually subdivided, depending on the place of occurrence:

• prerenal, associated with increased tissue protein breakdown or severe hemolysis,

• renal, caused by disorders of the kidneys glomeruli or tubules,

• postrenal associated with inflammation of the urinary tract.

Determination of bilirubin and urobilinogen by test strips "Ictophan"

The principle

Strips contain two areas of indication for bilirubin and urobilinogen.

The test is based on reaction of bilirubin combinations with stabilized diazorea-

gent (see Unit 11.2."Determination of the total bilirubin and its fractions concentration in blood serum"). The reaction zone contains n- nitrophenyldiazonium-St-*n*-toluensulfonate, sodium bicarbonate and sulfosalicylic acid. Upon 30 seconds contact with the conjugated (direct) bilirubin the purplish beige (purplish pink) color appears, the intensity of which depends on the defined bilirubin number.

The determination of urobilinogen is based on the reaction associated with the diazonium stabilized salt. The reaction zone changes color in the urobilinogen presence to pink or red.

Normal values

Urine	
The urobilinogen	Test is negative
The bilirubin	up to 17.0 μmol/l

Clinical-diagnostic significance

The appearance of bilirubin in urine is associated with parenchymal and mechanical jaundice, in which direct bilirubin is filtered into the urine from blood.

Increasing the concentration of urobilinogen in the urine is observed in parenchymal liver disease (hepatitis, cirrhosis, poisoning), hemolytic conditions, intestinal diseases, associated with excessive absorption of stercobilinogen the mucous membrane of the intestine (enterocolitis, constipation).

Determination of hemoglobin and erythrocytes with"Hemofan"test strips

Blood in the urine can be in two types – red blood cells (hematuria, erythrocyturia) or blood pigment (hemoglobinuria).

The principle

The indication zone of the test strips contains an organic hydroperoxide and the indicator is tetramethylbenzidine. Hemoglobin catalyzes the oxidation of the indicator by the hydroperoxide with the formation of dyed blue-green products.

In the presence of free hemoglobin (hemoglobinuria or hemolysis of red blood cells were present initially), the entire touch-sensitive area is colored in more or less homogeneous blue-green color.

Unmodified (whole) red blood cells (microscopic hematuria) appear intensely colored blue-green dots or specks on the undyed reagent zone or a uniform blue-green color of the whole area (gross hematuria).

Negative reactions display area remains yellowish (no green tint).

Normal values

Urine		
Red blood cells and hemoglobin	children	test is negative or slightly positive
	adults	test is negative

Clinical-diagnostic significance

Isolated red blood cells found in the urine of even absolutely healthy people. Up to 1 million red blood cells are allocated every day in healthy people, which corresponds to the content in 1.0 μl of urine 1 red blood cell. Hematuria is detected in lesions of the parenchyma of the kidneys (glomerulonephritis, pyelonephritis, and tumors), intensive physical exertion, and urinary tract infections.

Normal values

Urine

Protein	test strips "Albuphan"	test is negative
	other methods	10-140 mg/l
Ketone bodies	test strips "Ketophan"	test is negative
	other methods	20-30 mg/per day
Glucose	test strips "Glycophan"	test is negative
	other methods	0.1-0.8 mmol/l or 1-15 mg (mg%)
Red blood cells and hemoglobin	children	test is negative or slightly positive
	adults	test is negative
pH		5.0-6.5

Design of practical:

Write down the principle of the methods, the results based on the study of urine samples and on the results of "Laboratory 1", draw conclusions about possible pathologies of the body.

TESTS

Choose one or more correct answers.

1. THENECESSARY QUANTITY OF CLEAN WATER IN THE DAILY RATION OF THE CHILD OF THE FIRST YEAR OF LIFE IS
   1) 15 ml per kg of body weight
   2) 30 ml per kg of body weight
   3) 100 ml per kg of body weight
   4) 500 ml per kg of body weight

2. THEMINIMUM EXCRETION OF WATER IN ADULT TO THE DAY IS COMPOSED BY
   1) 100 ml
   2) 200 ml
   3) 700 ml
   4) 1400 ml

3. THEWATER-SALT BALANCE IS REGULATED BY _____
   1) antidiuretic hormone
   2) the renin-aldosterone system
   3) atriopeptin system
   4) hypothalamic-pituitary-adrenal system

4. A REGULATOR OF THE SMOOTH MUSCLE VESSELS TONUS IS SYNTHESIZED IN THE KIDNEYS
   1) bradykinin
   2) norepinephrine

3) oxytocin
4) vasopressin

5. THE REGULATED REBASORPTION OF CALCIUM IONS IS OCCURRED IN
   1) proximal tubules
   2) distal tubules
   3) the ascending part of the loop of Henle
   4) collecting tubes

6. THE REGULATED REBASORPTION OF WATER IS OCCURRED IN
   1) proximal tubules
   2) throughout the nephron
   3) the ascending part of the loop of Henle
   4) distal tubules and collecting tubes

7. THE INCREASEIN PH BLOOD LEVEL BY KIDNEYS IS CARRIED OUT BY ACTIVATION OF
   1) ammoniogenesis
   2) reabsorption of sodium
   3) reabsorption of water
   4) secretion of uric acid

8. THE SATURATED YELLOW COLOR OF URINE IS A SIGN OF
   1) pregnancy
   2) orotate aciduria
   3) dehydration
   4) intoxication

9. IT IS EXCRETED WITH THE BILE OF THE LIVER
   1) hemoglobin
   2) oxycalciferol
   3) triacylglycerols
   4) cholesterol

CASE STUDIES

1. The patient was bedridden for a long time. He had a cardio-vascular disease. The analysis of urine showed an increase in the content of Ca^{2+} salts.

Find out the reason of salts Ca^{2+} growth in the patient.

2. Several skiers made a big transition in cold weather conditions. The protein in urine was found out in some of them.

Specify the cause of the appearance of protein in the urine of healthy athletes.

3. The woman had sudden pains in the liver, rapidly developed icteric staining of sclera, skin, and feces acquired a light color, urine acquired the color of dark beer.

Indicate disorders of pigment metabolism and type of jaundice.

CHECKLIST FOR FINAL-GOAL ASSESSMENT

(UNITS 10, 11, 12)

1. The hierarchy of regulatory systems. Place of hormones in the regulation of metabolism and function of organs.

2. The difference in membrane and cytosolic mechanisms of transmission of the hormonal signal into the cell.

3. Membrane mechanisms of transmission of the hormonal signal into the cell. Three types of receptors: with enzymatic activity, ion-conductive activity and associated with G-proteins.

4. Describe the receptors associated with G-proteins:

- the system of secondary mediators and their interaction,
- adenylate cyclase the mechanism of action of hormones,
- calcium-phospholipid mechanism of action of hormones.

5. General characteristics guanylate cyclase mechanism of action of hormones.

6. Cytosolic mechanism of action of hormones.

7. Classification of hormones by chemical structure, biological functions and belonging to endocrine glands. The role of releasing hormones and tropic hormones. What is negative feedback in the regulation of synthesis and actions of hormones?

8. Characterization of growth hormone: chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, influence on metabolism and water exchange. How are synthesis and secretion of the hormone regulated? Condition associated with impaired action of the hormone.

9. Characteristics of antidiuretic hormone (vasopressin): the chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, influence on metabolism and water exchange. Regulation of synthesis and secretion of hormone. Condition caused by impaired action of the hormone.

10. Characterization of oxytocin: the chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, effects. How are synthesis and secretion of the hormone regulated?

11. Characterization of parathyroid hormone and calcitonin: chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, influence on exchange of calcium and phosphates. Regulation of synthesis and secretion of hormone. What is their role in the metabolism of calcium and phosphate? What is the role of vitamin D_3?

12. The pancreatic hormones glucagon and insulin: their chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, influence on the metabolism of carbohydrates, proteins, lipids (enzymes regulated by the hormone). Regulation of synthesis and secretion of hormone. Condition caused by absence or surplus of the action of the hormone.

13. Concepts about the mechanisms of development of insulin-independent diabetes. The most important changes in hormonal status and metabolism in diabetes,

the biochemical mechanisms of development of diabetes complications and diabetic coma.

14. Characterization of thyroid-stimulating hormone: chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, effects. How are synthesis and secretion of the hormone regulated?

15. The thyroid hormones- thyroxine and triiodothyronine: chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, influence on the metabolism of carbohydrates, proteins, lipids. Regulation of synthesis and secretion of the hormone. Condition caused by absence or surplus of the action of the hormone.

16. Adrenaline: chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, influence on the metabolism of carbohydrates, proteins, lipids (enzymes regulated by the hormone). Regulation of synthesis and secretion of hormone. Condition caused by impaired action of the hormone. What is the role of adrenaline in the adaptive reactions of the body under the stress conditions?

17. Characteristics of adrenocorticotropic hormone: chemical nature, site of synthesis, target organs, localization of receptors and mechanism of action, influence on metabolism. How are synthesis and secretion of the hormone regulated? Condition caused by absence or surplus of the action of the hormone.

18. Glucocorticoids: chemical nature, the place and the stages of synthesis, target organs, localization of receptors and mechanism of action, influence on the metabolism of carbohydrates, proteins, lipids (enzymes regulated by the hormone). Regulation of synthesis and secretion of hormone. Condition caused by absence or surplus of the action of the hormone. Use of glucocorticoids as anti-inflammatory and anti-allergic drugs. The role of glucocorticoids in the adaptive reactions of the organism under the stress conditions.

19. Mineralocorticoids: their chemical nature, the place and the stages of synthesis, target organs, localization of receptors and mechanism of action, influence on the exchange of electrolytes and water. How are the synthesis and secretion of hormones regulated? The role of the renin-angiotensin system. Condition caused by absence or surplus of the action of the hormone. Biochemical mechanisms of development of renal hypertension.

20. Lactotropic hormone: chemical nature, the place and the stages of synthesis, target organs, localization of receptors and mechanism of action, effects on metabolism. How are synthesis and secretion of the hormone regulated?

21. Characterization of gonadotropic hormones: follicle-stimulating and luteinizing hormones: chemical nature, the place and the stages of synthesis, target organs, localization of receptors and mechanism of action, effect on female monthly cycle. Regulation of synthesis and secretion of hormones.

22. Androgens and estrogens: chemical nature, the place and the stages of synthesis, target organs, localization of receptors and mechanism of action, influence on the metabolism of carbohydrates, proteins, lipids. How are synthesis and secretion of hormones regulated? The use of analogues of androgens and estrogens as drugs.

23. Qualitative reactions to detect insulin. The principle of method for the determination of prolactin and testosterone in serum. Clinical-diagnostic value.

24. What is the total blood protein, the components included in its composition. Physiological functions of proteins of the blood, normal levels of concentration. The reasons for the change in the concentration of total protein in the blood.

25. Protein fractions of blood serum. Characteristics of albumin, causes of Hypo-, hyper- and analbuminemia. Globulins and their main faction. The main representatives of the globulin fractions. The biological role of albumins and globulins. Normal levels of protein fractions. Dysproteinemia and paraproteinemia. The reasons for the changes in the concentration of protein fractions in the blood. The change in the ratio of protein fractions in diseases of the liver, kidney, acute and chronic inflammation, tumors (proteinogram).

26. Non protein nitrogenous components of blood – fractions of residual nitrogen, their characteristics. The role and metabolism of urea, creatinine, uric acid. Clinical diagnostic value of determination of these substances in the blood, their normal performance. Causes and consequences of hyper ammoniemia and nitrogenemia.

27. Characteristics of enzymes the blood – plasma specific, indicator, excretory enzymes. Examples. The use of blood enzymes for diagnosis of diseases.

28. The principle of separation of serum proteins by electrophoresis.

29. Characteristics of metabolism of the erythrocyte. The role of glycolysis and pentose-phosphate pathway.

30. Exchange of iron. Food sources, consumption, transportation, deposition and mobilization, the role of transferrin and ferritin. What are clinical and laboratory signs of iron deficiency?

31. The structure of the hemoglobin molecule. The structure of heme. Normal and pathological forms of hemoglobin. The mechanism of the regulation of hemoglobin affinity for oxygen – cooperative effect, Bohr effect, role of 2.3-diphosphoglyceric acid.

32. The reaction of synthesis of heme and hemoglobin. Regulation of the synthesis processes. Characteristic of disorders of hemoglobin – porphyria, thalassemia, pathological forms of hemoglobin.

33. The reaction of degradation of heme, formation of bilirubin and bilirubin glucuronide, their localization. The main stages of transformation of bile pigments in the body. Way of excretion of bilirubin and other bile pigments.

34. Metabolic disorders of bile pigments. Laboratory criteria for the various types of jaundice (suprahepatic, hepatic, subhepatic).

35. Violations of pigment metabolism in children: 1) hemolytic jaundice; 2) physiologic jaundice of newborn and premature; 3) hereditary disorders of bilirubin metabolism – Gilbert's syndrome, Dubin-Johnson’s syndrome, Crigler-Naya’s syndrome.

36. The principle of the method the detection of bilirubin and urobilinogen in the urine. Clinical-diagnostic value.

37. Methods for the quantitative determination of total bilirubin and its fractions in the blood serum clinical-diagnostic value.

38. Respiratory function of blood. Mechanisms of transport of oxygen and carbon dioxide.

39. Indicators of acid-base status. The chemical and physiological mechanisms of regulation of acid-base status. The relationship between transport of oxygen and carbon dioxide with the mechanisms of maintaining acid-base balance.

40. The role of kidneys in regulation of acid-base balance: bicarbonate reabsorption, acidogenesis, ammoniagenesis.

41. Disorders of acid-base status, its causes, the change of indicators in acid-base status. Methods of compensation for various violations of ABS.

42. The principle of quantitative measurement of inorganic phosphate, and chloride ions in serum and urine. Clinical-diagnostic value normal values.

43. Characterization of the biochemical processes in the nephron. Counter current multiplier mechanism of formation of urine. Features of reabsorption of electrolytes and water in different parts of the nephron. The role of hormones in the processes of reabsorption.

44. Composition and physical-chemical properties of urine. Normal and pathological components of urine, their clinical-diagnostic value normal values.

45. The principle techniques and process of determining the physical-chemical properties of urine:

- determination of relative density of urine,
- determination of urine pH using indicator paper.

46. Principle of the method and the course of determination of pathological components of urine:

- determination of the concentration of protein, glucose, ketone bodies, bile pigments, hemoglobin with test strips.

ANSWERS TO TEST TASKS

Unit 1. "Structure, features and functions of proteins"

Question number	Number of an answer	Question number	Number of an answer
1	2	6	4
2	3	7	1
3	3	8	2, 3, 5
4	5	9	1
5	1, 2, 3, 4, 5	10	4

Unit 2. "Structure, classification and role of vitamins"

Question number	Number of an answer	Question number	Number of an answer
1	3	6	2
2	3	7	3
3	2	8	3
4	1	9	1
5	1	10	3

Unit 3."Enzymology"

Question number	Number of an answer	Question number	Number of an answer
1	4	6	1
2	1	7	5
3	3	8	2
4	1	9	1
5	3	10	2

Unit 4. "Biological oxidation"

Question number	Number of an answer	Question number	Number of an answer
1	4	6	2
2	3	7	1
3	2	8	3
4	4	9	2
5	1	10	1

Unit 5. "Amino acid and protein metabolism"

Question number	Number of an answer	Question number	Number of an answer
1	2	6	3
2	3	7	4
3	2	8	2
4	1	9	1
5	4	10	1

Unit 6. "Structure and metabolism of purine and pyrimidine nucleotides"

Question number	Number of an answer	Question number	Number of an answer
1	3	6	1
2	2	7	4
3	2	8	2
4	1	9	4
5	1	10	2

Unit 7. "Matrix biosynthesis"

Question number	Number of an answer	Question number	Number of an answer
1	2	6	1
2	1	7	1
3	3	8	2
4	2	9	1
5	1, 3, 4	10	1

Unit 8. "Structure and metabolism of carbohydrates"

Question number	Number of an answer	Question number	Number of an answer
1	3	6	3
2	3	7	3
3	2	8	3
4	4	9	1
5	3	10	2

Unit 9. "Structure and metabolism of lipids"

Question number	Number of an answer	Question number	Number of an answer
1	1	6	1
2	3	7	4
3	3	8	2
4	2	9	1
5	1	10	4

Unit 10. "Hormonal regulation of human metabolism and functions"

Question number	Number of an answer	Question number	Number of an answer
1	2	6	3
2	3, 4	7	3
3	1	8	1
4	4	9	1
5	1	10	1

Unit 11. "Biochemistry of blood"

Question number	Number of an answer	Question number	Number of an answer
1	4	6	3
2	1	7	2
3	1	8	1
4	1	9	4
5	2	10	2

Unit 12. "Biochemistry of kidneys"

Question number	Number of an answer	Question number	Number of an answer
1	3	6	4
2	4	7	1
3	1, 2, 3	8	3
4	1	9	4
5	2		

ANSWERS TO CASE STUDIES

Unit 1. "Structure, features and functions of proteins"

1. It will move to cathode at pH 30 and will move to anode at pH10.5; the charge of this peptide is negative at water solution. It will be positive at pH 3.0 and move to the cathode at electric field. The charge of this peptide will increase at pH 10.5 and it will move to the anode.

2. Protein: A – will move to the anode; B – it will move to the cathode at pH 5.0 and will move to the anode at pH 7.0; it will stay at the pH 9.5; it will move to the anode at pH 11.0;

3. The first peptide is similar to the hydrocarbon-containing compound, it has hydrocarbon radicals. This protein is better soluble in nonaqueous medium, because the radicals are hydrophobic. The first peptide reacts with Biuret and ninhydrine reagents. The second one cloud participates in salt bridges formation due the presence of free NH_2-group of lysine and COOH-group of glutamic acid.

Unit 2. " Structure, classification and role of vitamins"

1. Pyridoxine participates in amino acids transamination reactions. Initially the formation of essential amino acids from these four happens. Then it is not needed.

2. Competitive inhibition of microbe’s enzymes occurs, which are required for folic acid synthesis. The disorders of tetrahydrofolate by bacteria can be seen as a result of the process. It is known that the treatment after bacterial infection consists of vitamin B medicines and probiotics for microflora restoration

3. It is prescribed to take vitamin A participating in tissues regeneration, vitamin K for reducing the possibility of bleeding and vitamin D for providing the body with calcium.

Unit 3. "Enzymology"

1. The regulatory center breakdown happens due to a heat dissociation of protein subunits. At the same time an active center preserves its activity.

2. The dry preparation and dissolution with distilled water ensures that no impurities and heavy metals can be contacted with the functional group of the protein. Gentle stirring is necessary to avoid protein's mechanical denaturation. A low temperature provides low activity of enzyme as well as escape of spontaneous unwinding of the protein chain. It also reduces the possibility of bacterial contamination. The drug drying and storage in special evacuated vial protects the enzyme from exposure to oxygen and prevents oxidation of the sulfur-containing groups

3. The effect of adrenaline is the activation energetic metabolism. Its action is intracellular disintegration of lipids metabolism during muscular work. It is also known that the hormone increases the phosphorylation of enzymes. So, the lipase is activated by phosphorylation

Unit 4. "Biological oxidation"

1. The speed of electrons transfer in the respiratory chain is strictly relevant with the ATP requirements. If ATP consumption is small (ATP concentration is high), the rate of electron transfer is small. The level of electrochemical gradient could be reduced. The uncoupling agents (uncouplers) change the rate of electron transfer due a decrease of proton gradient without effect on ATP synthase. The ratio of P/O is reduced. High speed of electron transfer in the respiratory chain causes a decrease of the free energy, part of which is dissipated as heat. Its effects are sweating and high body temperature. Application of uncouplers in vivo leads to reduce of a total amount of ATP especially in cells with great number of mitochondria. Nervous cells belong to them. Consequently, disturbances of the nervous system are main sign of unclouplers action.

2. Low coefficient of P/O means that most of the energy is dissipated as heat, instead of being spent on the synthesis of ATP. This allows the animals to maintain body temperature at the right level. The mechanism responsible for low coefficient of P/O is the uncoupling of oxidation and phosphorylation. It provides thermogenin - protein mitochondrial membrane of brown fat cells

Unit 5. "Amino acid and protein metabolism"

1. The low acidity of gastric juice reduces pepsin activity and leads to the development of intestine microflora. The rotting processes with the formation of hydrogen sulfide are results of this process. To normalize digestion, it can be recommend, the intake of hydrochloric acid with pepsin and gastric juice.

2. Glutamic acid is dicarboxylic mono amino acid. It is able to bind ammonia to form a non-toxic glutamine. When the reamination process occurs it turns into ketoglutaric acid. It is an important substrate of TCA, which is able to oxidize and forms a 4 molecules of ATP. Glutamic acid has an antioxidant effect, reducing the content of lipid peroxides, damaging cell membranes.

3. Patient has a reduced urea formation in liver. So he has a decreased level of urea in blood and its excretion is also low. The urea enzymes activity should be investigated: ornithine carbamoyl transferase and arginase.

Unit 6. "Structure and metabolism of purines and pyrimidines"

1. High content of uric acid in the urine could be found in hyperuricemia of different origin. It is observed in adults with gout or in a case of purine containing foodstuff intake. Another cause is a defect of hypoxanthine-guanine-phosphoribosyl transferase that leads to the alteration of purine nucleotudes salvage pathway (The Lesch-Nyhan syndrome). The excess of uric acids is a result of this defect.

2. Folic acid active form is N^5,N^{10}-methylene-THFA. It participates in thymidylate monophosphate synthesis (TMP), the required component of DNA replication and cell division. The lack of erythrocytes division leads to the reduction of erythrocytes number (megaloblastic anemia is developed). The same trend is found in leucocytes (leucopenia is developed) and in epithelial cells (the division of mucosa membrane and skin cells is decreased).

Unit 7. "Matrix biosynthesis"

1. Sickle cell anemia is a disease of glutamic acid substitution on valine in hemoglobin β-chain. It is a CTT change on CAT in sequence of mRNA.

2. Topoisomerase is an enzyme that participates in the over winding or under winding of DNA in replication. If the enzyme is blocked, the over winding and under winding could not be happened, the replication will be stopped and cell will not be divided. This is the basic of enzyme inhibition application in medicine.

3. It is well-known that the proteins in human body differ from one another. They have different amino acids sequence, nucleic acid number in genes (DNA structure). In this case one gene is responsible for functioning of two proteins. So, there is another regulation level. For example, it may be at processing stage of mRNA formation. The terminator codon will be included in processing stage in enterocytes, that stops protein synthesis despite the translation is not finished. The apoB-48 protein will be developed. The mRNA processing is not modified in hepatocytes, the protein translation will not be changed, and the apoB-100 protein is produced.

Unit 8. "Structure and metabolism of carbohydrates"

1. The level of glucose in the blood can increase due to a stress reaction, which is characterized by an increase in the adrenaline level in the blood and tissues.

2. The tissues are provided with oxygen due to an increase in the rate of blood flow in running at a distance of 5000 m better than a 100-meter, the oxygen debt of the body is less, therefore, the aerobic metabolic processes are activated, the level of lactic acid in the blood and tissues is lower than that of the athlete who ran 100 m.

3. One of the functions of the pentose-phosphate cycle is the supply of pentose phosphates for the synthesis of nucleic acids. After bleeding, the regeneration of blood elements is intensive, for the synthesis of which nucleic acids are needed. Therefore, the processes of the pentose phosphate cycle will be intensified, accordingly, its enzymes, in particular glucose-6-phosphate dehydrogenase, transketolase and others, are activated, which it is have to investigate to check the conclusion.

Unit 9. "Structure and metabolism of lipids"

1. When the bile enters the duodenum, the activation of pancreatic lipase is disturbed (inhibited), the digestion of fats, the absorption of fatty acids and fat-soluble vitamins deteriorates, the motor function of the intestine worsens, and hypercholesterolemia develops.

2. The myocardium is better provided with energy reserves than the skeletal muscle. It contains more amount of lipids, with oxidation of which gives a lot of energy (1 g of carbohydrates – 4.1 kcal (17.2 kJ), fats – 9.3 kcal (38.9 kJ). But the amount of oxygen molecules in a fatty acid is less than in carbohydrates. The oxidation of 1 g of fat requires 2019 ml of oxygen, compared to the oxidation of 1 g of glycogen. It is only required there are only 829 ml of oxygen. In the energy balance of cardiac metabolism, the more effective aerobic processes are leading, but they make it very sensitive to hypoxia (oxygen starvation). In a case heart attack the skeletal muscle is active in the oxygen deficiency.

3. Reducing the carbohydrates in a child's diet leads to a violation of the chain "Glucose $\rightarrow$ pyruvate $\rightarrow$ oxaloacetate" and slowing down the speed of the CTA. The former intake of fat and the oxidation of fatty acids caused a relative excess of acetyl-SCoA, which cannot now burn to the CTA ("fats burn in the flame of carbohydrates"). This leads to ketonemia, the metabolic acidosis is developed. Simultaneously, the increase in gluconeogenesis from amino acids intensified deamination processes and accumulation of ammonia. But urea synthesis is inhibited due to the lack of ATP energy in case of CTA alteration and the hyperammonemia develops. It is expedient to determine the content of glucose and urea in the blood, ketones in the urine, to study the state of acid-base balance before the treatment.

Unit 10. "Hormonal regulation of human metabolism and functions

1. The slowing down of physical and mental development, a decrease in the intensity of metabolic processes is characteristic of hypothyroidism of the thyroid gland. In a pronounced degree the disease is called "cretinism".

2. These complaints are characteristic of diabetes mellitus. To diagnose the pathological states and to evaluate the metabolic changes in diabetes it is expedient to determine the following most informative indicatives. In the blood it should be detected the level of: glucose on an empty stomach, ketone bodies, cholesterol and li-

pids, if necessary, a test for glucose tolerance. In the urine it should be detected the level of: glucose, urine's density, ketone bodies.

3. The high fatigue, frequent hypoglycemic conditions, the increased skin pigmentation and other symptoms are characteristic of the primary chronic insufficiency of the adrenal cortex (Addison's disease).

Unit 11. "Biochemistry of blood"

1. In extensive burns due to fluid loss by tissues, the dehydration develops. There is a thickening of the blood, hyperproteinemia (100 g/l). If the function of the gastrointestinal tract is impaired, the intake of amino acids is decreased, followed by hypoproteinemia (30 g/l) development.

2. To clarify the diagnosis, it is necessary to determine the blood content of urea nitrogen, which is normally half of the residual nitrogen. If the excretory function of the kidneys is disturbed, there is an increase in residual nitrogen, mainly due to urea nitrogen. The urea-forming function of the liver is disturbed and it leads to decrease in urea nitrogen content.

3. The pH is lowered; the acidosis is detected. An increase in the partial pressure of CO_2 indicates the cause of acidosis – the accumulation of carbonic acid due to a violation of its excretion. The excess of HCO_3^- ions is a reflection of the accumulation and dissociation of carbonic acid, and, first of all, compensatory reabsorption of carbonates by the kidneys. Based on the clinical picture, the diagnosis of respiratory acidosis is confirmed. Compensation occurs through "metabolic alkalosis", i.e. excretion by the kidneys of the H^+ ion by activation of ammoniogenesis and acidogenesis. The alkaline reserve corresponds to the sum of all the buffer bases of the blood and is increased by the excess of carbonate ions.

Unit 12. "Biochemistry of kidneys"

1. The reason for increasing excretion of Ca^{2+} ions in the urine may be hypodynamic osteoporosis – the "washing away" of Ca^{2+} from the bones into the blood and excretion of it into the urine.

2. In a healthy person, the protein in the urine is absent (its content is so small that it is not detected by laboratory methods). Proteinuria can appear at very high physical exertion – "marching", "cold" proteinuria.

3. These symptoms are typical of mechanical (obstructive) jaundice, probably caused by a blockage of the common bile duct by a stone. The blood content of bilirubin is increased due to direct (bilirubin glucuronide), since the outflow of bile in the intestine is disturbed. Therefore, feces are colorless (acholia), does not contain stericobilinogen and the urine does not contain stericobilinogen and urobilinogen. The dark color of urine is due to the penetration of direct bilirubin from the blood, it is also possible the foaming of urine due to the presence of bile acids.

RECOMMENDED LITERATURE

List of main literature:

1. Harper's illustrated biochemistry [Text] : textbook / V.W. Rodwell [et al.]. – 30th ed. – New York : McGraw-Hill, 2015. – 817 p.

2. Zurabyan, S. E. Fundamentals of bioorganic chemistry [Электронный ресурс] : учебное пособие / S. E. Zurabyan. – Электрон. текстовые дан. – M. : GEOTAR-MED, 2015. – 304 p. : access mode: http://studentlibrary.ru

List of additional literature:

1. Zurabyan, S. E. Fundamentals of bioorganic chemistry [Текст] : textbook for foreign students of Medical Higher Educational Institutions / S. E. Zurabyan. – 2th. ed. – M. : GEOTAR-MED, 2004. – 320 p.

List of internet resources:

1. Clinical Key: Access: www.clinicalkey.com.
2. Electronic database of Scientific medical library of SSMU Access: http://medlib.tomsk.ru.

ANNEXES

ANNEX 1

CLASSIFICATION AND NOMENCLATURE OF ENZYMES

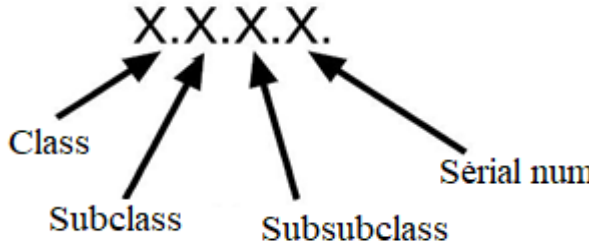
In 1961 in Moscow, the Commission on Enzymes of the International Biochemical Union (IUBM) adopted a modern systematic classification of enzymes.

In accordance with the systematic classification, the reaction and substrate specificity of the enzymes are taken into account. Enzymes are divided:

- on classes – according to the type of catalyzed reaction,
- each class is divided into subclasses – by the nature of the attacked chemical group,
- subclasses are divided into sub-subclasses – by the nature of the attack or by the nature of the acceptor or coenzyme.

There are 6 classes of enzymes:

I. Oxidoreductases,
II. Transferases,
III. Hydrolases,
IV. Lyases,
V. Isomerases,
VI. Ligase.



Each enzyme is assigned a four-digit classification number.

For example, *alcohol dehydrogenase* has a number of EC 1.1.1.1. – this oxidoreductase, acts on the OH group of the donor with NAD^+ as an acceptor with the first ordinal number in its subclass; *lactate dehydrogenase* – EC 1.1.1.27.

Enzymes can have a trivial or systematic name:

1. Systematic name – according to modern classification (*http://www.chem.qmul.ac.uk/iubmb/enzyme/).* It is often difficult to use, then it is simplified and the working name of the enzyme is introduced.

2. The trivial name is a name that has developed historically, which is more commonly used, for example, **pepsin**, **trypsin.** Sometimes the name of the substrate is added with the ending "-ase" – **urease**, **amylas**e, **lipase**. Nevertheless, all such enzymes have a systematic name.

I. CLASS OXIDOREDUCTASE

Enzymes catalyze the oxidation-reduction reactions underlying the biological oxidation. The class has 22 subclasses. Coenzymes of this class are NAD^+, $NADP^+$, FAD, FMN, ubiquinone, glutathione, lipoic acid.

Subclasses are allocated to enzyme groups acting on:

1.1. on the CH-OH group of donors;
1.2. on the aldehyde or oxo group of donors;
1.3. on the CH-CH group of donors;
1.4. on the CH-NH_2 group of donors;

1.5. on CH-NH group of donors
1.6. on NADH or NADPH group of donors
1.8. on a sulfur group of donors;
1.9. on a heme group of donors;
1.10. on diphenols and related substances as donors;
1.11. on peroxide as an acceptor;
1.12. on hydrogen as donors;
1.13. on single donors with incorporation of molecular oxygen;
1.14. on paired donors with incorporation of molecular oxygen;
1.15. on superoxide radicals as acceptors;
1.17. on CH or CH_2 groups;
1.18. on iron-sulfur proteins as donors.

Subclasses are divided into **subclasses** depending on the acceptor – NAD^+ or $NADP^+$ (1.1.1., 1.2.1., 1.3.1., 1.4.1.), disulfides (1.2.4.), oxygen (1.3.3.).

Common names include:

1. **Dehydrogenase** – oxidoreductase, catalyzing the dehydrogenation of a substrate using as a hydrogen acceptor any molecules other than oxygen.

2. If the transfer of hydrogen from the donor molecule is difficult to prove, then such oxidoreductases are called **reductases**.

3. **Oxidase** – oxidoreductase, catalyzing the oxidation of substrates with molecular oxygen as an electron acceptor without the inclusion of oxygen in the substrate molecule.

4. **Monoxygenase** – oxidoreductase, catalyzing the introduction of one oxygen atom in a substrate molecule with molecular oxygen as an oxygen donor.

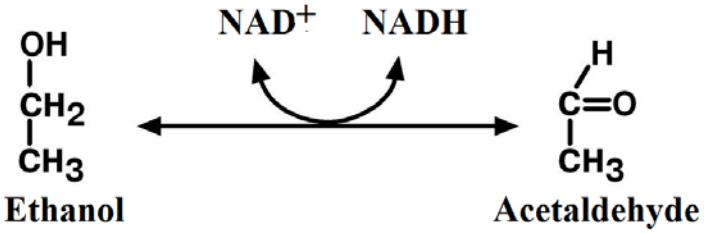
5. **Dioxygenase** – oxidoreductase, catalyzing the introduction of two oxygen atoms in a substrate molecule with molecular oxygen as an oxygen donor.

6. **Peroxidase** – oxidoreductase, catalyzing reactions with hydrogen peroxide as an electron acceptor.

The systematic name of oxidoreductases is formed as follows:

The electron donor : the electron acceptor – oxidoreductase

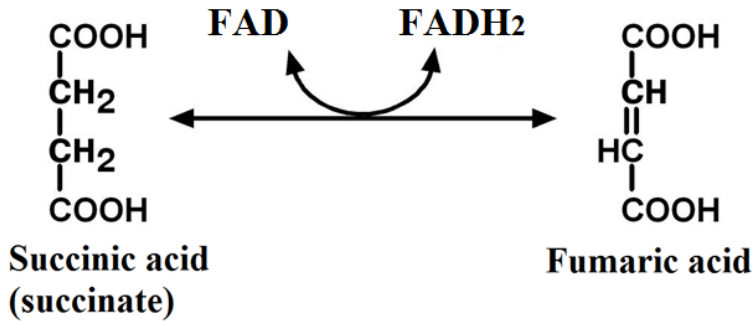
Example 1



	Enzyme characteristic
The systematic name	Alcohol : NAD-oxydoreductase
The working name	Alcoholdehydrogenase
Class	1. Oxydoreductase
Subclass	1.1. acting on the CH-OH group of donors;

Subsubclass	1.1.1. with NAD^+ or $NADP^+$ as an acceptor
Serial number	EC 1.1.1.1.
Cofactors	Nicotinamide adenine dinucleotide. Iron or zinc.

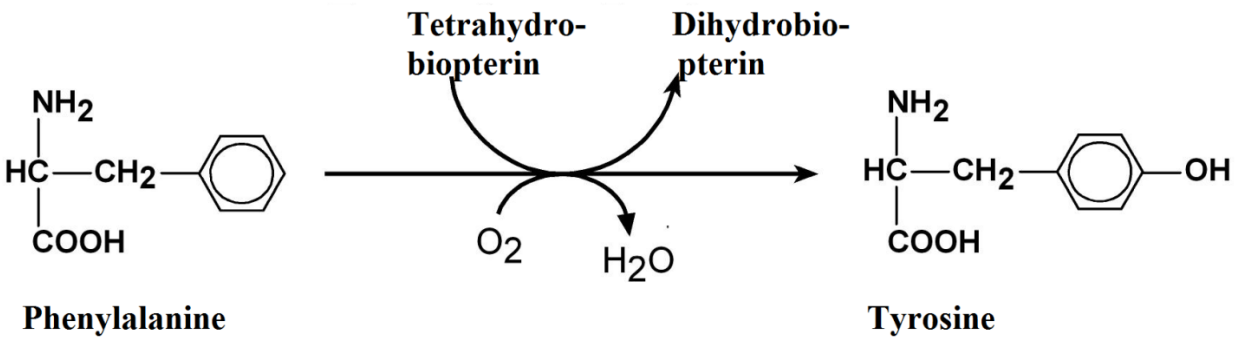
Example 2



Enzyme characteristic

The systematic name	Succinate : FAD-oxydoreductase
The working name	Succinatedehydrogenase
Class	1. Oxydoreductases
Subclass	1.3. acting on the CH-CH group of donors
Subsubclass	1.3.99. with FAD as an acceptor
Serialnumber	EC 1.3.99.1.
Cofactor	Flavin adenine dinucleotide

Example 3



Enzyme characteristic

The systematic name	Phenylalanine. Tetrahydrobiopterin : oxygen-oxydoreductase
The working name	Phenylalanine-4-monooxygenase
Class	1. Oxydoreductase
Subclass	1.14. Acting on paired donors with incorporation of molecular oxygen
Subsubclass	1.14.16. With the reduced pteridine as a donor and the inclusion of one oxygen atom
Serialnumber	EC. 1.14.16.1.
Cofactors	Tetrahydrobiopterin. Iron.

II CLASS. TRANSFERASE

Transferases catalyze the transport reactions of various groups from one substrate (donor) to another (acceptor), participate in the reactions of interconversion of various substances, neutralization of natural and foreign compounds. Coenzymes are pyridoxal phosphate, coenzyme A, THFA, methylcobalamin. The class is divided into 9 subclasses depending on the structure of the groups carried by them:

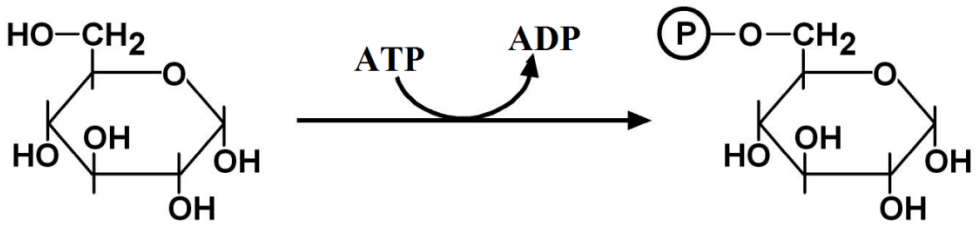
2.1. single-carbon groups;
2.2. aldehyde or ketone groups;
2.3. acyl groups or groups that become alkyl groups during transfer;
2.4. glycosyl groups, as well as hexoses and pentoses;
2.5. alkyl or aryl groups, other than methyl groups;
2.6. nitrogenous groups;
2.7. phosphorus-containing groups;
2.8. sulfur-containing groups;
2.9. selenium-containing groups.

The division into subclasses depending on the nature of the group being transferred, for example: subclass 2.1.1. – methyl, subclass 2.1.2. – carboxymethyl or formyl, subclass 2.6.1. – amino, amidino, hydroxyamino.

The systematic name of transferases is formed as follows:

Group donor : group acceptor – transferable transferase group

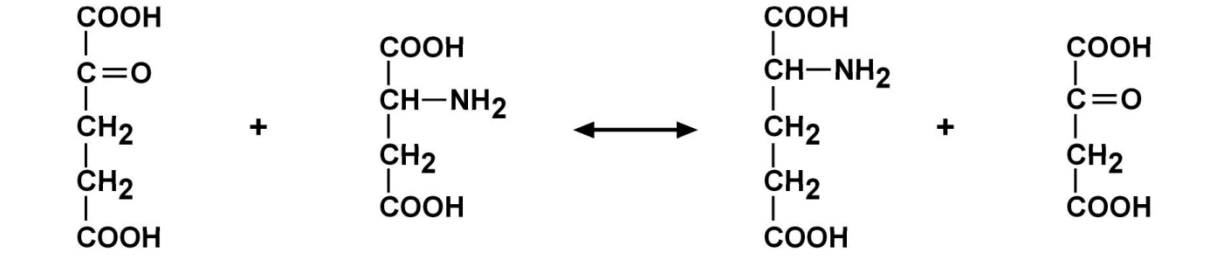
Example 1



Glucose **Glucose-6-phosphate**

Enzyme characteristic	
The systematic name	ATP : D-hexose-6-phsopho-transferase
The working name	Hexokinase
Class	2. Transferase
Subclass	2.7. Transferingphosphorus-containinggroups
Subsubclass	2.7.1. With an alcohol group as an acceptor
Serialnumber	EC 2.7.1.1.
Cofactor	Magnesium

Example 2

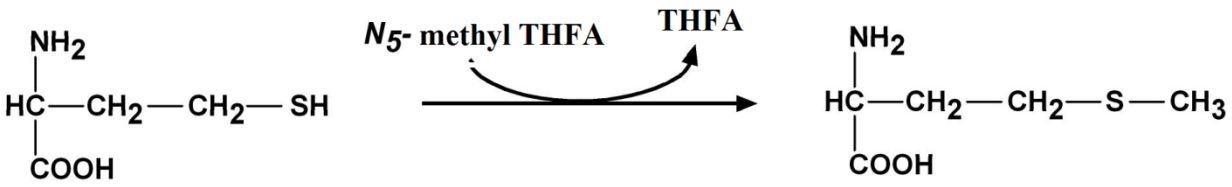


α-Ketoglutaric acid (2-oxoglutarate) **Aspartic acid** **Glutamic acid** **Oxaloacetic acid (oxaloacetate)**

Enzyme characteristic

The systematic name	L-aspartate : 2-oxoglutarate aminotransferase
The working name	Aspartate aminotransferase
Class	2. Transferase
Subclass	2.6. Transferring nitrogenous groups
Subsubclass	2.6.1. Fminotransferase
Serialnumber	EC 2.6.1.1.
Cofactor	Pyridoxal phosphate

Example 3



Homocysteine **Methionine**

Enzyme characteristic

The systematic name	5-methyltetrahydrofolate : L-homocysteine S-methyltransferase
The working name	Methionine synthase
Class	2. Transferase
Subclass	2.1. Transferring single-carbon groups
Subsubclass	2.1.1. Methyltransferases
Serialnumber	EC. 2.1.1.13.
Cofactors	Cobalamin. Zinc.

III CLASS. HYDROLASE

Hydrolases are enzymes that break the intramolecular bonds in the substrate (with the exception of the C-C bonds) by the addition of H_2O molecules. They are divided into 13 subclasses. Enzymes retain trivial names due to the complexity of many substrates: pepsin, trypsin. Coenzymes are absent.

Hydrolases are mainly concentrated in the gastrointestinal tract and lysosomes of tissue cells. They carry out the decomposition of macromolecules, forming easily adsorbed monomers.

Hydrolases can be further classified into several subclasses, based upon the bonds they act upon:

3.1. ester bonds;
3.2. O-glycosides;
3.3. simple ether bonds;
3.4. peptide bonds;
3.5. non-peptide carbon-nitrogen bonds;
3.6. acid anhydrides;
3.7. carbon-carbon bonds.

There are the **subsubclasses**, for example, the carboxylic acid hydrolases (3.1.1.), the phosphono-ester hydrolases (3.1.3.)

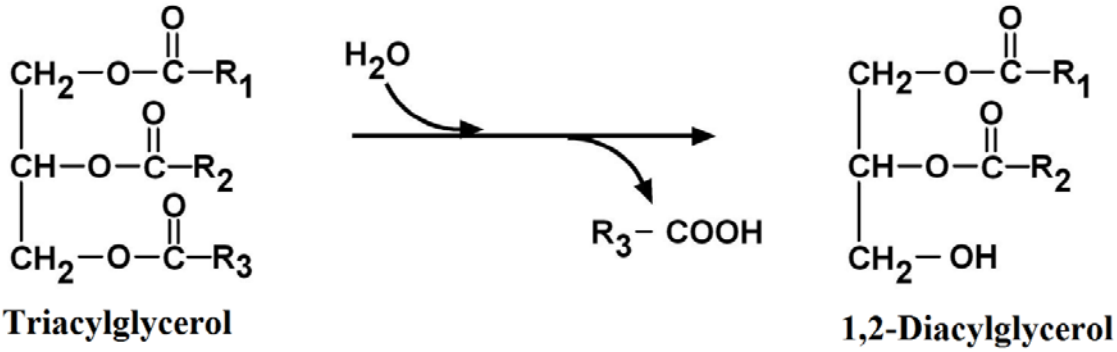
The most common are the following hydrolases:

1. Esterases – hydrolysis of ester bonds.
2. Lipases – hydrolysis of neutral fats.
3. Phosphatase – hydrolysis of monoesters of phosphoric acid.
4. Glycosidases – hydrolyses O- and N-glycosidic bonds.
5. Proteases, peptidases – hydrolysis of proteins and peptides.
6. Nuclease – hydrolysis of nucleic acids.

The systematic name of hydrolases is formed:

Hydrolyzable substrate : hydrolase release group

Example 1

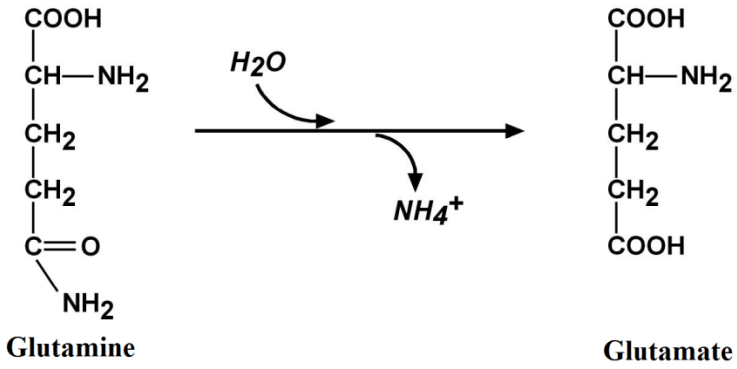


Enzyme characteristic

The systematic name	Triacylglycerol : acyl hydrolase
The working name	TAG-lipase
Class	3. Hydrolase

Subclass	3.1. acts on esters
Subsubclass	3.1.1. Hydrolases of carboxylic acids
Serialnumber	EC 3.1.1.3.

Example 2

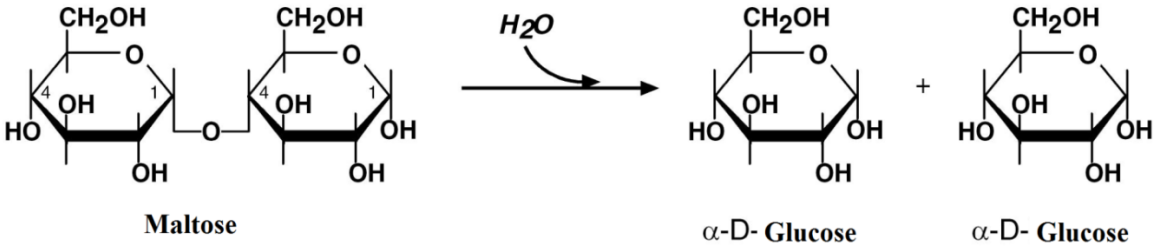


Glutamine **Glutamate**

Enzyme characteristic

The systematic name	L-glutamine : amide hydrolase
The working name	Glutaminase
Class	3. Hydrolase
Subclass	3.5. acts on non-peptide carbon-nitrogen bonds
Subsubclass	3.5.1. Operating in linear amides
Serialnumber	EC 3.5.1.2.

Example 3



Maltose **α-D- Glucose** **α-D- Glucose**

Enzyme characteristic

The systematic name	α-D-glucoside : glucohydrolase
The working name	Maltase
Class	3. Hydrolases
Subclass	3.2. Glycosidase
Subsubclass	3.2.1. acts on O-glycosidic bonds
Serialnumber	EC 3.2.1.20.

IV CLASS. LYASE

Liases are enzymes that catalyze the rupture of C-O, C-C, C-N and other bonds. They carry out the *reversible* reactions of the non-hydrolytical cleavage of substrates with various groups. There are 7 subclasses. These reactions are accompanied by the formation of a double bond or by the attachment of groups to the double bond site.

The class has about 230 enzymes. Lyases are complex enzymes, pyridoxal phosphate, thiamine diphosphate, magnesium, cobalt serve as coenzymes.

Lyases can be further classified into seven **subclasses** according the bond being broken:

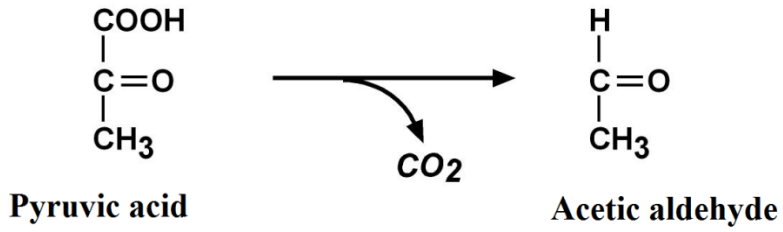
4.1. lyases that cleave carbon-carbon bonds;
4.2. lyases that cleave carbon-oxygen bond;
4.3. lyases that cleave carbon-nitrogen bonds;
4.4. lyases that cleave carbon-sulfur bonds;
4.5. lyases that cleave carbon-halide bonds.

There are the **subsubclasses**, for example, carboxylases (4.1.1.), hydrolases 4.2.1.)

The systematic name of lyases is formed:

Cleavable substrate : detachable group – lyase

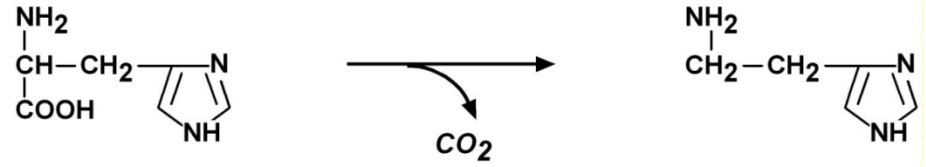
Example 1



Pyruvic acid **Acetic aldehyde**

Enzyme characteristic	
The systematic name	2-oxo acid : carboxylase
The working name	Pyruvate decarboxylase
Class	4. Lyase
Subclass	4.1. Lyases that cleave carbon-carbon bonds
Subsubclass	4.1.1. Carboxyl aliases
Serial number	EC 4.1.1.1.
Cofactor	Thiamine diphosphate

Example 2

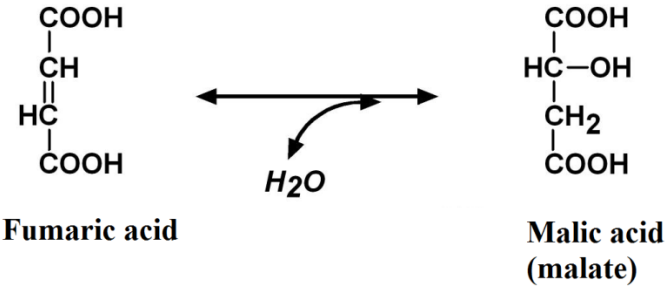


Histidine **Histamine**

Enzyme characteristic	
The systematic name	Histidine : carboxylase
The working name	Histidine-decarboxylase
Class	4. Lyase
Subclass	4.1. Lyases that cleave carbon-carbon bonds

Subsubclass	4.1.1. Carboxylase
Serial number	EC 4.1.1.22.
Cofactor	Pyridoxalphosphate

Example 3

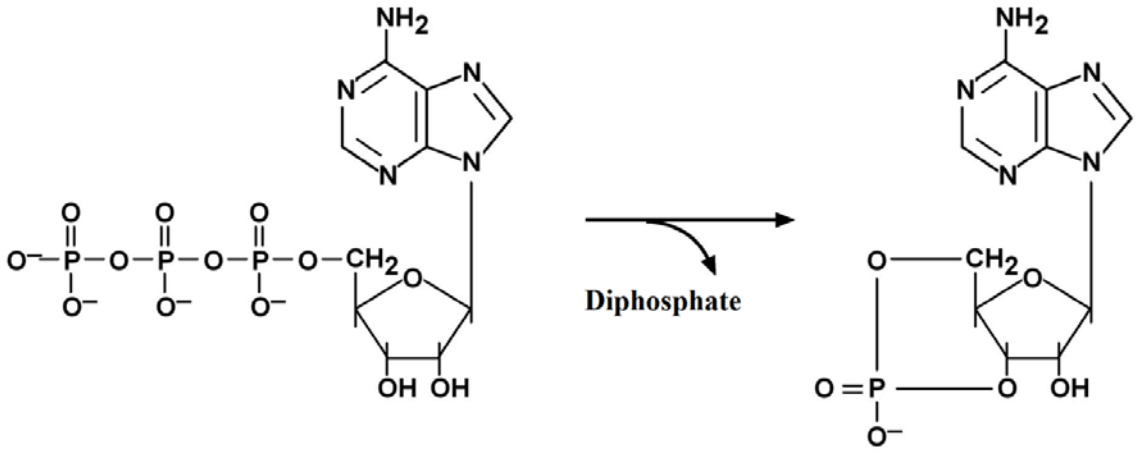


Fumaric acid **Malic acid (malate)**

Enzyme characteristic

The systematic name	(*S*)–Malate : hydrolase
The working name	Fumarase
Class	4. Lyase
Subclass	4.2. Lyases that cleave carbon-oxygen bond
Subsubclass	4.2.1. Hydrolyase
Serialnumber	EC 4.2.1.2.

Example 4



Adenosine triphosphate (ATP) **3'-5'-Cyclic adenosine monophosphate (cyclic AMP)**

Enzyme characteristic

The systematic name	ATP : Lipase diphosphate (cyclization)
The working name	Adenylate cyclase
Class	4. Lyase
Subclass	4.6. Lyases that cleave phosphorus-oxygen bonds
Subsubclass	4.6.1. Phosphorus-oxygen lyase
Serial number	EC 4.6.1.1.

V CLASS. ISOMERASE

Isomerases are enzymes that catalyze isomeric transformations within a single molecule. The class has more than 80 enzymes, in which 6 subclasses are distinguished. Isomerases are complex enzymes. Their coenzymes include pyridoxal, deoxyadenosylcobalamin, peptide (glutathione), monosaccharide phosphates (glucose-1.6-diphosphate), and others.

Isomerases are further classified into six subclasses:

5.1. Racemases and epimerases.

Racemases are responsible for the interconversion of L- and D-isomers, S- and R-isomers. Epimerases change the configuration at one of the chiral carbon atoms, for example: the interconversion of the α- and β-isomers, the conversion of ribulose ↔ xylulose, galactose ↔ glucose, mannose ↔ galactose.

5.2. Cis-trans isomerase;

5.3. Intramolecular oxidoreductases;

5.4. Intramolecular transferase – mutases, makey the transfer of chemical groups within the molecule;

5.5. Intramolecular lyases.

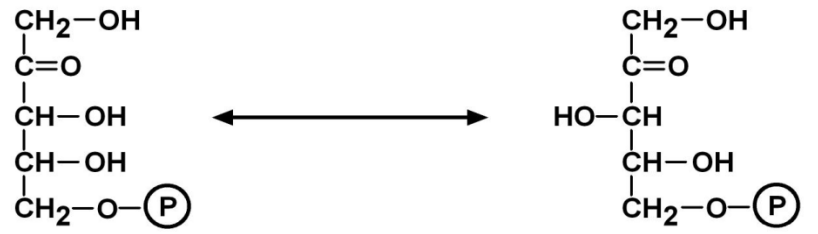
There are the subsubclasses, for example: acting on amino acids and their derivatives (5.1.1.), carbohydrates and their derivatives (5.1.3.), transfering the double (C=C) bonds (5.3.3.).

The systematic name of isomerases is formed:

Substrate – [ ] – reaction,

where [ ] – a designation reflecting the essence of the reaction, for example, "the number of the altered carbon atom", the change in "cis-trans," the change in "keto-enol," the change in "aldose-ketose."

Example 1

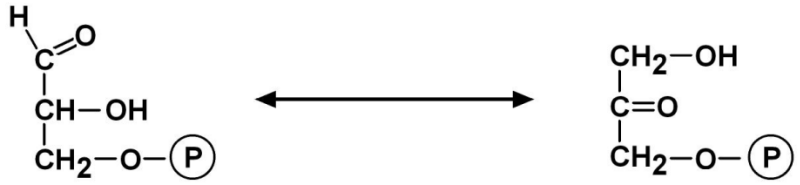


Ribulose 5-phosphate **Xylose 5-phosphate**

Enzyme characteristic

The systematic name	D-ribulose-5-phosphate-3-epimerase
The working name	Ribulose phosphate-3-epimerase
Class	5. Isomerase
Subclass	5.1. Racemases and epimerases
Subsubclass	5.1.3. acting upon on carbohydrates and their derivatives
Serial number	EC 5.1.3.1.

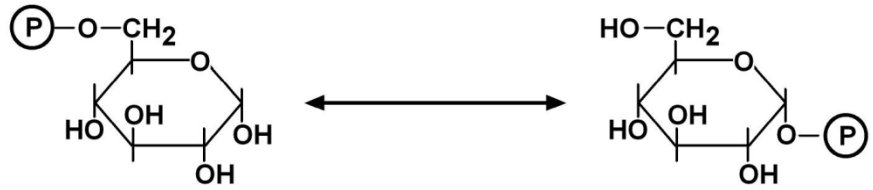
Example 2



Glyceraldehyde phosphate **Dihydroxyacetone phosphate**

Enzyme characteristic	
The systematic name	D-glyceraldehyde-3-phosphate-aldose-ketose isomerase
The working name	Triosephosphate isomerase
Class	5. Isomerase
Subclass	5.3. Intramolecular oxidoreductases
Subsubclass	5.3.1. Catalyzing interconversion of aldose and ketoses
Serial number	EC 5.3.1.1.

Example 3



Glucose-6-phosphate **Glucose-1-phosphate**

Enzyme characteristic	
The systematic name	α-D-glucose-1.6-phosphomutase
The working name	Phosphoglucomutase
Class	5. Isomerase
Subclass	5.4. Intramolecular transferases
Subsubclass	5.4.2. Phosphotransferases
Serial number	EC 5.4.2.2.
Coenzyme	Glucose-1.6-diphosphate

VI CLASS. LIGASE

Ligases (synthetases) are enzymes that catalyze the attachment of two molecules to one another using the energy of high-energy ATP bonds (or other macroergs). Ligases – complex enzymes, contain nucleotide, biotin, folic coenzymes.

Itisisolatedsixenzymesubclasses, dependingontypeoftheformingbond:

6.1. Carbon-oxygen.

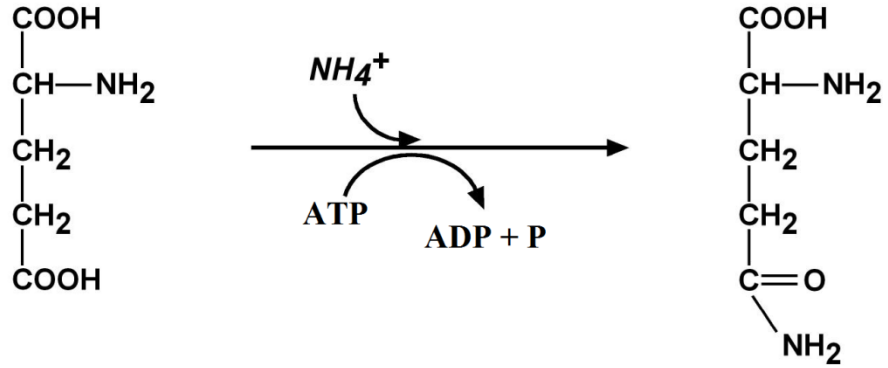
6.2. Carbon-sulfur.
6.3. Carbon-nitrogen.
6.4. Carbon-carbon.
6.5. Phosphorus-oxygen.
6.6. Nitrogen-metal.

There are subsubclasses, for example forming the aminoacyl-tRNA bond (6.1.1.), synthesizing compounds acid-thiol (6.2.1.), amides (6.3.1.).

The systematic name of the enzyme:

Substrate 1 : substrate 2 – ligase

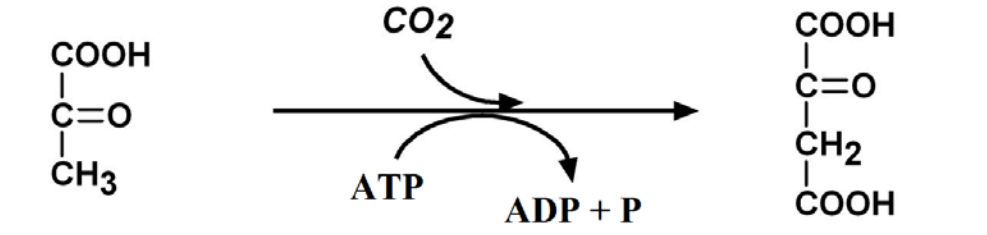
Example 1



Glutamate **Glutamine**

Enzyme characteristic	
The systematic name	L-glutamate : ammonia ligase
The working name	Glutamine Synthesis
Class	6. Ligases
Subclass	6.3. Forming carbon-nitrogen bonds
Subsubclass	6.3.1. Amide synthase
Serial number	EC 6.3.1.2.

Example 2

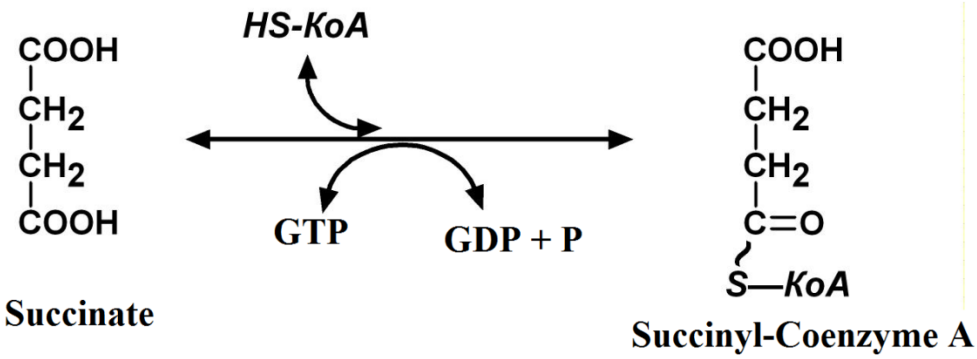


Pyruvic acid **Oxaloacetic acid (oxaloacetate)**

Enzyme characteristic	
The systematic name	Pyruvate : carboxyl ligase (ADP-forming)
The working name	Pyruvate carboxylase

Class	6. Ligases
Subclass	6.4. Forming carbon-carbon bonds
Subsubclass	6.4.1. Forming carbon-carbon bonds
Serialnumber	EC 6.4.1.1.
Cofactors	Biotin. Magnesium. Zinc.

Example 3



Succinate **Succinyl-Coenzyme A**

Enzyme characteristic

The systematic name	Succinate : CoA ligase
The working name	Succinyl-CoA synthetase Succinate-thiokinase
Class	6. Ligases
Subclass	6.2. Forming bonds of carbon
Subsubclass	6.2.1. Ligase acid
Serial number	EC 6.2.1.4.

ANNEX 2

PROTEINS OF BLOOD PLASMA

Protein fractions		Key members of protein fractions	Acute-phase protein
Albumins		Pre- and postalbumins. Albumin	
Глобулины	α_1	α_1-Lipoprotein α_1-Acid seromucoid α_1-Glycoprotein Transcortin Prothrombin Antiplasmin α_1-Antitrypsin Vitamin B_{12} binding protein	α_1-Glycoprotein α_1-Antitrypsin
	α_2	C-reactive protein Haptoglobin (Hp-1, Hp-1-2 Hp-2-2) Ceruloplasmin α_2-Lipoprotein α_2-HS-Glycoprotein α_2-Macroglobulin Cholinesterase Alkaline phosphatase Proaccelerine The Christmas factor	C-reactive protein α_2-Macroglobulin Haptoglobin Cerulo-plasmin
	β	β_1A-globulin β-Lipoprotein β_1B-globulin Transferrin Plasminogen Proconvertin Fibrinogen Complement components C_1 C_4, C_9 Hemopexin	Plasminogen Complement compo-nents C_1 C_4, C_9
	γ	G-immunoglobulin A-immunoglobulin D-immunoglobulin E-immunoglobulin	

CHARACTERISTICS OFSOME BLOOD PROTEINS

Fibrinogen

Fibrinogen is synthesized in the liver. It is a protein of blood clotting.

Normal values

Serum 2.0-4.0 g/l

Clinical and diagnostic significance

Increased concentrations cause acute inflammatory processes and cardiovascular diseases (atherosclerosis). Decrease – hyperfibrinolysis (Disseminated Intravascular Coagulation, DIC) or inherited insufficiency.

α_1-GLOBULINS

Acid α_1-glycoprotein

Acid α_1-glycoprotein (orosomucoid) has acidic properties and contains high amounts of carbohydrates. The protein has a high affinity for polyanions (for example heparin) and probably regulates the amount of free heparin in the plasma. α_1-Glycoprotein binds medicines (propranolol and lidocaine), steroids (progesterone, testosterone). It is synthesized in the liver.

Normal values

Serum 0.55-1.40 g/l

Clinical and diagnostic significance

An increase in the level of protein is observed in acute and chronic inflammatory processes, rheumatoid arthritis, malignant tumors, fevers, injuries, myocardial infarction, exercise training, pregnancy, nephrotic syndrome. Increase in the protein level in blood is observed in acute and chronic inflammatory processes, rheumatoid arthritis, malignant tumors, fever, trauma, myocardial infarction, physical exertion, pregnancy, nephrotic syndrome.

α_1-Antitrypsin

α_1-Antitrypsin is a glycoprotein, is formed in the liver, the acute-phase protein. It is an inhibitor of proteinases (trypsin, chymotrypsin, kallikrein, plasmin) and accounts for 92-94% of the total blood antiproteolytic function. Its autosomal recessive inherited disorder is one of the factors of the emphysema pathogenesis in lungs, bronchiectasias and chronic bronchitis, early cirrhosis of the liver. Obviously, the absence of an inhibitor leads to unrestricted proteolysis of cells in the inflammation zone, which lengthens and deepens the destructive processes in the tissues.

Normal values

Serum 2.0-2.4 g/l

Clinical and diagnostic significance

α_1-Antitrypsin level in the blood increases in acute infections, inflammatory processes, malignant formations, hormones (pregnancy, steroid therapy), systemic lupus erythematosus and cancers.

α_1-Antichymotrypsin

α_1-Antichymotrypsin is one of the first reacting acute-phase proteins (the serum level can be doubled for several hours), it is a weak specific inhibitor of chymotrypsin, but it acts to other proteases is also noted.

Normal values

Serum 0.3-0.6 g/l

Clinicalanddiagnosticsignificance

The increase in protein content is due to acute phase reactions: inflammation, trauma after surgery, myocardial infarction, bacterial infections.

α_2-GLOBULINS

C-reactive protein

C-reactive protein (CRP) is a mesenchymal protein that has undergone partial denaturation due to tissue disintegration in inflammatory and destructive processes. It takes part in the activation of the classical complement pathway, immune reactions, is an inhibitor of platelet aggregation, binds lipids, carbohydrates, and participates in catalase activity.

Normal values

Serum < 50 mg/l (absense)

Clinicalanddiagnosticsignificance

The level of this acute-phase protein rises rapidly in 15-25 times in acute and chronic infections, cell necrosis, myocardial infarction, rheumatoid arthritis, gout.

Haptoglobin

Haptoglobin is an acute-phase protein synthesized in the liver. It has the following functions: binds free hemoglobin of plasma and protects the body from loss of iron, this complex is destroyed in cells of RES and liver; performs a nonspecific protective function, integrating with protein and non-protein substances that appear during the decay of cells; is a natural inhibitor of cathepsin B; participates in the transport of vitamin B_{12}. Haptoglobin in low concentrations is present in many body fluids: cerebrospinal fluid, lymph, synovial fluid, bile.

Normal values

Serum 0.8-2.7 g/l

Clinical and diagnostic significance

The protein level non-specific *increases* in response to tissue damage, inflammation, and the oncogenesis (especially to metastasis development). High indicators are observed in diabetes mellitus, nephrotic syndrome, pyelonephritis, burns, acute and chronic inflammatory conditions, tissue necrosis, myocardial infarction, active autoimmune diseases, systemic rheumatoid diseases.

A decrease in the protein amount is noted in the lesions of liver, hemolytic anemia. The level of haptoglobin is considered to be a sensitive indicator of hemolytic conditions: the release of hemoglobin causes a decrease in the haptoglobin level.

α_2-Macroglobulin

α_2-Macroglobulin is a high-molecular zinc-containing protein, consists of 4 identical subunits and includes a carbohydrate component, is synthesized in the liver. It is an inhibitor of proteinases (both the blood coagulation system and others) - plasmin, pepsin, trypsin, chymotrypsin, endopeptidases, cathepsin D, thrombin, kallikrein. It transports enzymes and hormones, the receptor of lymphocytes, participates in the interaction of the mother and fetus, has an immunomodulating effect, the inhibitor of the complement component.

Normal values

Serum	children (1-3 years)	about 4.5 g/l
	men	1.50-3.50 g/l
	women	1.75-4.20 g/l

Clinical and diagnostic significance

Protein controls the development of infections and inflammatory processes. *An increase* in its level is revealed in cirrhosis of the liver, acute and chronic hepatitis, pregnancy, congenital heart diseases, endocrine diseases (diabetes mellitus, myxedema), pneumonia, nephrotic syndrome. *A decrease* – in rheumatic polyarthritis, loss of protein or its deficiency in nutrition, disseminated intravascular coagulation, fibrinolytic therapy, acute pancreatitis, myocardial infarction, stomach and duodenal ulcers.

Ceruloplasmin

Ceruloplasmin contains 8 copper atoms. This is an acute-phase protein, the copper metabolism regulator in the body (it aggregates 90% of all plasma copper) - transports copper ions from the liver to other organs. Ceruloplasmin is an oxidase of polyphenols and diamines, promotes iron saturation of apotransferrin, participates in the exchange of biogenic amines (adrenaline, norepinephrine, serotonin) and ascorbic acid, regulates the level of sympathetic brain mediators, as a serum antioxidant eliminates superoxide radicals of oxygen, restores O_2 to water and prevents oxidation of unsaturated fatty acids.

Normal values

Serum	0.15-0.50 g/l

Clinicalanddiagnosticsignificance

Elevated level of protein is determined in rheumatoid arthritis, systemic lupus erythematosus, chronic inflammatory processes, cholestasis, hepatitis, liver cirrhosis, myocardial infarction, acute infections, malignant tumors with metastases, melanoma, schizophrenia.

A decrease in the protein content is revealed in reduction in the synthesis of the enzyme (Wilson Konovalov's disease), increased loss (gastrointestinal disease, nephrotic syndrome), a decrease in absorption in the intestine (impaired absorption, malnutrition).

β-GLOBULINS

Transferrin family

The protein called transferrin belongs to the transferrin family, as well as ovotransferrin, lactoferrin, melano- transferrin, and others.

Proteins of this family, binding iron ions (III) and preventing their recovery, are an important component of the body's antioxidant defense. In addition, the binding of iron by transferrin prevents its use by microorganisms, which determines the bacteriostatic activity of these proteins.

Transferrin

Transferrin is synthesized in the liver and reticuloendothelial system. Transferrin transports trivalent iron along with the anion of bicarbonate from the duodenum and spleen to all tissues.

Normally, only 1/3 of the total amount of transferrin is saturated with iron.

Normal values

Serum	Childern	2.0-3.6 g/l
	Men	2.1-3.6 g/l
	Women	2.5-3.8 g/l

Clinical and diagnostic significance

Its level increases in a lack of iron in the body, pregnancy, estrogen, lipoid nephrosis.

Reduction is observed in inherited synthesis failure, testosterone intake, nephroses, malaria, hemochromatosis, malnutrition, tumors.

Lactoferrin

Protein is widely represented in blood plasma, secretory fluids: milk, saliva, tear, bile, secrets of nasal and bronchial glands.

The main biological function of lactoferrin is the binding and transport of iron ions, but also the protein has broad antibacterial, antiviral and antifungal activity.

Normal values

Serum	0.2-0.6 mg/l
Human milk	to 7.0 g/l

Clinical and diagnostic significance

An increase in the protein content in the blood is noted in pregnancy, gestosis, skin diseases, cancers of the gastrointestinal tract.

ANNEX 3

NORMAL VALUES OF STUDIED BIOCAMICAL INDICATORS

Serum

Indicator	Sex, age, etc	Normal values	
Amylase		16-30 g/l·h	
ALT activity		0.10-0.68 mmol/l·h	
AST activity		0.10-0.45 mmol/l·h	
The De Ritis Ratio		1.33±0.40	
Residual nitrogen		14.3-28.6 mmol/l	
Urea	Children	1.8-6.4 mmol/l	
	Adults	2.5-8.3 mmol/l	
Creatinine	Children		
	up to 1 year	18-35 μmol/l	
	from 1 year to 12 years	27-62 μmol/l	
	Adults		
	women	44-97 μmol/l	
	men	52-132 μmol/l	
Uric acid		0.12-0.32 mmol/l	
	Meat Diet	0.16-0.45 mmol/l	
Protein total	Children		
	newborns	51-60 g/l	
	children up to 1 year	51-73 g/l	
	children from 1 to 3 years	54-85 g/l	
	from 4 years	65-85 g/l	
	Adults	65-85 g/l	
Fractions of proteins			
albumins		30-50 g/l	50-70%
α_1-globulins		1-3 g/l	3-6%
α_2-globulins		6-10 g/l	9-15%
β-globulins		7-11 g/l	8-18%
γ-globulins		8-16 g/l	15-25%
The albumin / globulin ratio		1.2-1.8	1.2-1.8
The albumin /α1+α2-glibulins coefficient		3.9-6.1	3.9-6.1
Timole sample		0-4 S-H units	

Glucose			3.5-5.5 mmol/l
Glucose Tolerance Test			
	On an empty stomach	3.5-5.5 mmol/l	100%
	After 60 min	5.3-9.6 mmol/l	150-175%
	After 120 minutes	below 5.3 mmol/l	about 100%

Triacylglycerols	Children		0.15-1.56 mmol/l
		0-5 years	0.2-1.1 mmol/l
		6-11 years	0.3-1.3 mmol/l
		12-15 years	0.4-1.6 mmol/l
		16-19 years	0.5-1.8 mmol/l
	Adults		
		20-29 years	0.5-2.1 mmol/l
		30-39 years	0.5-3.2 mmol/l
		40-49 years	0.6-3.4 mmol/l
		50-59 years	0.6-3.4 mmol/l
Cholesterol total	Children		
		newborns	1.2-2.7 mmol/l
		0-19 years	2.9-5.2 mmol/l
	Adults		
		20-29 years	3.70-6.51 mmol/l
		30-39 years	4.25-7.04 mmol/l
		40-49 years	4.37-7.70 mmol/l
		over 50 years	4.55-8.24 mmol/l

Prolactin	Adults		
		women	follicular phase 4.5-33 ng/ml (98-784 micro Units/l) middle of cycle 6.3-49 ng/ml (134-975 micro Units/l) luteal phase 4.9-40 ng/ml (104-848 micro Units/l)
		men	2.5-17 ng/ml (53-360 micro Units/l)
Testosteron	Adults		
		women over 10 years old	0.45-3.75 nmol/l
		men over 14 years old	5.76-28.14 nmol/l

Hemoglobin	Children	100-140 g/l
	Adults	
	women	120-140 g/l
	men	130-160 g/l
Total bilirubin	Adults	8.5-20.5 μmol/l
	Fullterm babies	
	blood from the umbilical cord	< 34.2 μmol/l
	age up to 5 days	< 205.2 μmol/l
	age up to 5 days	3.4-17.1 μmol/l
	Preterm babies	
	blood from the umbilical cord	< 34.2 μmol/l
	age up to 5 days	< 273.6 μmol/l
Direct Bilirubin	Children	absense
	Adults	2.2-5.1 μmol/l

Potassium	Children	
	newborns	3.7-5.9 mmol/l
	up to 2 years	4.1-5.3 mmol/l
	over 2 years old	3.4-4.7 mmol/l
	Adults	3.5-5.1 mmol/l
Sodium	Children	
	newborns	134-146 mmol/l
	children	138-146 mmol/l
	Взрослые	136-146 mmol/l
Ferrum	Children	
	newborns	17.9-44.8 μmol/l
	up to 2 years	7.1-17.9 μmol/l
	over 2 years old	8.9-21.4 μmol/l
	Adults	
	men	8.9-28.6 μmol/l
	women	7.1-26.8 μmol/l
Phosphates	Children	
	newborns	1.13-2.78 μmol/l
	young age	1.45-2.16 μmol/l
	school-age	1.46-1.76 μmol/l
	Adults	0.81-1.48 μmol/l
Calcium		2.0-2.6 μmol/l
Chlorides		97-108 mmol/l

pH	Newborns	7.21-7.38
	Children and adults	
	arterial blood	7.37-7.45
	venus blood	7.35-7.43

pCO_2	Newborns and children	27-41 mmHg
	Adults	
	men	35-48 mm Hg or 4.66-6.38 кilo Pa
	women	32-45 mm Hg or 4.26-6.00 kilo Pa
Buffer bases		44-48 mmol/l
Bicarbonates	Newborns	17-24 mmol/l
	Childern	19-24 mmol/l
	Adults	
	arterial blood	21-28 mmol/l
	venus blood	22-29 mmol/l
Residual anions		12-16 mmol/l
Excess buffer bases	Newborns	from –10 to –2 mmol/l
	Children up to 2 years old	from –7 to +1 mmol/l
	Children	from –4 to –2 mmol/l
	Adults	from –2 to +3 mmol/l
pO_2	Adults	83-108 mm Hg or 11.04-14.36 kilo Pa
Oxyhemoglobin (HbO_2)	Adults	94-97%
Saturation of hemoglobin with oxygen (HbO_{SAT}, SO_2)	Newborns	40-90%
	Adults	94-98%

Urine

Amylase		28-160 g/l·h
Urea		330-580 mmol/day
Creatinine		4.4-17.7 mmol/day
Uric acid	regular diet	1.46-4.43 mmol/day
	meat diet	2.36-5.90 mmol/day
Protein		50-150 mg/day
Glucose		0.06-0.83 mmol/l
pH		5.0-6.5
Phosphates		25.8-48.4 mmol/day
Calcium		2.5-7.5 mmol/day
Chlorides		120-240 mmol/day

Gastric juice

Hydrochloric acid	Total acidity	40-60 mmol/l
	Free HCl	20-40 mmol/l
	Bound HCl	10-20 mmol/l

Clearance of endogenous creatinine

	Men	Women
Children up to 1 year old	65-100 ml/min	65-100 ml/min
from 1 to 30 years	88-146 ml/min	81-134 ml/min
Adults		
from 30 to 40 years	82-140 ml/min	75-128 ml/min
from 40 to 50 years	75-133 ml/min	69-122 ml/min
from 50 to 60 years	68-126 ml/min	64-116 ml/min
from 60 to 70 years	61-120 ml/min	58-110 ml/min
over 70 years old	55-113 ml/min	52-105 ml/min

CONTENTS

Educational edition

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Tutorial

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