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LABORATORY MANUAL FOR PRACTICAL BIOCHEMISTRY

2-n edition, modified and corrected

Tutorial

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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".

There are laboratory works in each unit, which should be carried out by students during their 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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ЛАБОРАТОРНЫЙ ПРАКТИКУМ ПО БИОХИМИИ

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Л 11 **Лабораторный практикум по биохимии** / О.Л. Носарева, Е.А. Степовая, Е.В. Шахристова, Л.В. Спирина. – 2-е изд., доп. и испр. – Томск: Изд-во СибГМУ, 2024. – 151 с.

Пособие подготовлено по дисциплине «Биохимия» в соответствии с Федеральным государственным образовательным стандартом высшего профессионального образования для студентов, обучающихся по основным профессионально-образовательным программам – программам специалитета: «Лечебное дело».

В каждом разделе приведены лабораторные работы, выполняемые студентами на практических занятиях. Ряд изучаемых методов используется в клинико-диагностических лабораториях для помощи лечащему врачу в постановке диагноза и для контроля эффективности лечения. По каждому разделу студенту предлагаются вопросы для самоподготовки к практическому занятию.

Пособие способствует формированию профессиональных компетенций, а именно выявлению у пациента основных патологических состояний, симптомов, синдромов заболеваний, нозологических форм.

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CONTENTS

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 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 (alpha-amino acids detection)

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

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 detection)

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 nitroprusside and gives a red-purple color 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 received solution 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 be universal (Biuret and ninhydrin reactions) and specific (lead sulfide test, xanthoprotein reaction). They allow to estimate and identify the protein and amino acid content. For instance, ninhydrin is commonly 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 note the change of the 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. Their main 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.

Study the 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). Properties of denatured proteins.
9. Protein renaturation, its mechanism.
10. Protein precipitation methods: salting out, denaturation. The principle of the reaction.

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 solution to 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 rheumatoid arthritis. Decreased levels are detected 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 précised in it.

Practical 2
PROTEIN DENATURATION

Denaturation is a process when proteins lose the quaternary, 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, ultrasound 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 for the investigation

1% Albumin solution.

Principle

Denaturation reduces the protein charge and hydration shells and leads to the change 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. Indicate the intensity of denaturation and its color.

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	Cooper sulfate 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. Cationic groups are neutralized.	
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	

Probe number	Reagents	Number of drops	Mechanism and reaction features	Result
Denaturation by organic acids				
7	Trichloroacetic acid	2	Protein neutralizes the acid charge system, destroy hydrogen bonds and forms complexes with the protein	
8	Sulfosalicylic acid	2		
Denaturation by tannin				
9	Tannin	2	It is formed insoluble salt-like compound with basic amino groups of amino acids	
Denaturation by 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 at home and at work. Their next significant functions are the 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 inhalable. 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 (hem- 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 protein's chemical composition – glycoproteins and phosphoproteins. The principle of methods.

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) 3.75% solution of acidic molybdenum ammonium, 4) concentrated H_2SO_4, 5) 1% $CuSO_4$ solution, 6) 10% CH_3COOH solution.

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 amount of 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 carbohyd -rates)	*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		

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. Eicosanoids, The scheme of the initial reactions of eicosanoids synthesis from the arachidonic acids, their biological role.

8. Make a table for the fat-soluble vitamins, containing the vitamin's title (trivial, chemical abbreviations), chemical formula, biological role and signs of hypo, hyper and avitaminosis.

9. Retinol, tocopherol, menadione, cholecalcipherol quantitative reactions. The principle of the methods.

Practical

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

Vitamin A, 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	Solution's color	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.

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	**Solution's color**	**Result**

UNIT 3
ENZYMOLOGY

THEME 3.1. ENZYME STRUCTURE AND PROPERTIES. CLASSIFICATION AND NOMENCLATURE OF ENZYMES

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 know the enzyme's classification and their specific reactions;
- ✓ 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. Mocern classification and nomenclature of enzymes: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases.

14. Characterize the enzyme class according to the following plan:

- name and class number;
- biochemical role;
- basic coenzymes of the enzyme class;
- rules for the systematic names of the enzymes;
- write down the biochemical reactions of the enzyme class examples (three reactions).

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.

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 37°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 (37°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	37°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

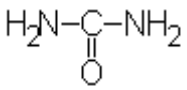
Urease solution made from the seeds of watermelon

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

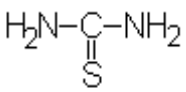
Detection of urease absolute specificity

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.

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

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. ENZYME APPLICATION IN MEDICINE

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 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. Practical usage enzymes in medicine: diagnostics and therapy. Examples.

5. Enzymopathies, inherited and acquired ones. Examples.

5. 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

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.

Procedure

	Test 1, ml	**Test 2, ml**	**Control, ml**
Starch solution	1.0	1.0	1.0
	Incubate 5 minutes at 37°C		
Serum	0.02	—	—
	Incubate 5 minutes at 37°C		
0.01 M Working solution of iodine	1.0	1.0	1.0
Cold distilled water	8.0	8.0	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

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 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.

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. Struc-

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

12. Detection of the phosphoproteins and glycoproteins composite components.

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

14. 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? What are the eicosanoids? Scheme of the initial reactions of the eicosanoids synthesis from the arachidonic acid. Note their role in the body.

15. 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.

16. 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.

17. Enzymes, their role in biochemical reactions. Compare enzymes and inorganic catalysts.

18. 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.

19. Isozymes, structural features. Characteristics and examples of isozymes. Practical application, examples.

20. Classification of enzymes. The enzyme nomenclature. What is the classification number? Systemical enzymes name. Examples of biochemical reactions and enzymes (three examples of the class).

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

22. Units of enzyme activity.

23. 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).

24. 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.

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

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

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

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

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

30. 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).

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. ATP chemical formula, the role of ATP? Significance of cycles ATP-ADP.
4. The main energy-rich substances in cell: ATP, 1.3-diphosphoglycerate, phosphoenolpyruvate, creatinephosphate? Substrate phosphorylation.
5. Sources of key metabolic products – acetyl~S-CoA and pyruvic acid. Further fate of substances.
6. Strucre of the multyenzyme pyruvate dehydrogenase complex, enzymes and co-enzymes. The net reaction of the pyruvate oxidative decarboxylation. The chemistry of the five separate reactions. Regulation of the process.

7. 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.

8. Connection of TCA with catabolism of carbohydrates, lipids, proteins.

9. 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, give the names of the enzymes (trivial and systematic);
- 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.

10. 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).

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

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

13. Causes of hypo energetic states.

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

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 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, gastrin, 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. Amino acids absorbtion in enterocytes (translocase, co-transport with sodium ions).

9. Age characteristics of protein digestion and amino acid absorption in children.

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 cresol and phenol,
- 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.

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:

- in the first tube – solution of the iron phenolate (III),
- drop by drop add the lactic acid solution of the iron phenolate (III) in

the second tube.

- The sample of the normal gastric juice in iron phenolate (III) solution add to the third tube,
- add the pathological gastic juice with lactic acid to the fourth tube with iron phenolate (III).

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.

Samples		Solution color	Presence of lactic acid
N 1	Iron phenolate solution		
N 2	Lactic acid solution		
N 3	Normal gastric juice		
N 4	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.

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

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

7. Reaction of direct oxidative glutamic acid deamination.

8. Indirect oxidative deamination – trans deamination.

9. Features of indirect deamination in muscle tissue (an 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 decaroxylation. 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 creatin urea in children?

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

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 10 min at room temperature		
NaOH	2.5	2.5	2.5
	Mix. 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	AST activity/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 an 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.

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 (reagent 1), 2) nitroprusside salicylate reagent (reagent 2), 3) hypochlorite (reagent 3).

Urea standardized solution (5 mmol/l).

Material of investigation

Serum, Urine (diluted in 1:50).

Procedure and observation

	Test 1, ml	**Test 2, ml**	**Standard, ml**
Reagent 1	0.25	0.25	0.25
Serum Urine (diluted in 1:50) Urea standardized solution	0.01 — —	— 0.01 —	— — 0.01
	Mix well and incubate 5 min at 37°C		
Reagent 2	1.0	1.0	1.0
Reagent 3	1.0	1.0	1.0
	Mix well and incubate 5 min at 37°C. Measure the optical density at 620 nm against the water (red filter).		

Calculation

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

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

E_{test} and $E_{standard}$ – optical density test and standard samples, $C_{standard}$ – urea concentration in standard sample, 50 – 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.

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), as well as inherited disorders with decreased activity of the ornithine cycle enzymes activity.

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) Working solution with picrinic acids, phosphoric acids, NaOH и lauroil sulfate of sodium.

Standardized creatinine solution, 177 μmol/l (0.177 mmol/l).

Material of investigation

Serum, urine (dilution in 1:50).

Procedure and observation

	Test 1, ml	**Test 2, ml**	**Standard, ml**
Working reagent	2.0	2.0	2.0
Serum Urine (diluted in 1:50) Standard-ized creatinine solution	0.4 — —	— 0.4 —	— — 0.4
	Mix well and incubate 10 min at 37°C. Measure the optical density at 540 nm against water (green filter).		

Calculation

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

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

E_{test} and $E_{standard}$ – optical density test and standard samples, $C_{standard}$ – creatinine concentration in standard sample (to calculate the creatinine content in blood serum – 177 μmol/l, in urine – 0.177 mmol/l), 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.

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.

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. Salvage reactions of the adenine, guanmine and hupoxantine.

6. Primary and secondary hyperuricemia:

- Lesch-Nyhan syndrome, its causes, basis of treatment and prognosis;
- urolithiasis, its causes, basis of treatment,
- gout, its causes, clinical manifestation, basis of treatment. The mechanism of allopurinol action in the gout treatment.

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

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.

Standardized solution of uric acid, 0.5 mmol/l.

Material of investigation

Serum. Urine (dilution 1:5).

Procedure and observation

	Test 1, ml	**Test 2, ml**	**Standard, ml**
Serum	0.05	—	—
Urine (dilution 1:5)	—	0.05	—
Standardized solution of uric acid	—	—	0.05
Working reagent	2.0	2.0	2.0
	Wait 10 minutes at 37ºC. Measure the optical density against water at 540 nm (green filter).		

Calculation

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

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

E_{test} and $E_{standard}$ – optical density test and standard samples, $C_{standard}$ – uric acid concentration in standard sample, 5 – dilution of urine, 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 transferase. 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 cytostatic, 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 non-renal 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.

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 led 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,
   - 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,

- 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 (pseudo uridine, dihydrouridine). An adapter role of tRNA.

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

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

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,
- 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. The quantitative determination of protein in the blood serum. Biuret test. The principle of the method, clinical and diagnostic significance, normal values.

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.

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.

THEME 7.3. DISORDERS OF PROTEIN'S METABOLISM AND SOME AMINO ACIDS (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.

13. Origin of kwashiorkor disease. Specific biochemical disorders in the protein's deficiency.

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 amino acids absorption in children.

4. Proteins putrification in the colum. Its origina and consequences. Produced substaces. System of toxic products disposal in the liver: microsomal oxidation and conjugation system. The structure of UDP-glucuronic acid (UDPGA) and phosphoadenosine phosphoric acid (PAPA). Reactions of indican 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. Sources of amino acids in tissues. What is the principle of amino acid division into on glucogenic and ketogenic?

9. Four types of amino acid deamination. Feature of a direct oxidative deamination. Feature of trans deamination – the mechanism of reactions, enzymes, coenzymes, the location. The significance of transdeamination reactions. The role of the cycle IMP-AMP, its reactions.

10. Trans-amination characterictics – mechanism of the reactions, enzymes and co-enzymes, location. Significance of the tran-amination reaction.

11. Characteristics of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), their reactions. The method of quantitative ALT and 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).

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. "Kwashiorkor" disease, origin and cases. Biochemical disorders in protein’s deficiency.

28. 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.

29. 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.

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

31. 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.

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

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

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

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

41. 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.

42. 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: 1) supply of energy (to 67% of daily energy required for the body); 2) building material for cells; 3) precursor molecules for synthesis of lipids, proteins and nucleic acids. Diseases associated with pathology of carbohydrate metabolism include diabetes mellitus, glycogen storage diseases, mucopolysaccharidosis, galactosemia, fructosemia, lactose and sucrose intolerance.

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.
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, cellobiose),
   - polysaccharides (starch, glycogen, cellulose).
4. Monosaccharide derivatives. What are the sialic acids? The chemical structure of N-acetylneuraminic acid, chondroitin-4-acids and chondroitin-6-acids.

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 dicarbohydrate digestion (sucrose-isomaltase, glycoamylase, β-glycosydase complexes and tregalase).

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

8. Transport of monosaccharide through cellular membranes.

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

10. Ways of fructose metabolism. What is the role of fructose in the metabolism? Conversion of fructose into glucose. Essential fructosemia nd fructosuria.

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

12. 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).

13. 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.

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

15. 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.

16. Express detection of glucose level in the blood with glucometer.

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

Practical 1

EXPRESS DETECTION OF GLUCOSE IN THE BLOOD USING GLUCOMETER

Principle

The glucometer working principle is based on the spectrophotometer's coefficient reflection detection in the analytic zone of the strip. The code's plast in the kit has the required data on the diagnostics strips. After the adding the drop of blood to the test zone of the diagnostic strip the reflection coefficient changes. The built optical and electronic system makes the reflection coefficient measurement. Its change is correlated with the glucose content in the blood.

Material

Blood from capillaries.

Equipment

ACCU-CHEK Active glucometer.

Procedure

1. *Preparatory step.*

a) Take the test strip from the kit. Please cover the container with dryer. Use the strip taking the free plastic edge of the layer.

b) Compare the color of the control "window" in the opposite side of the strip with the color scale on the container. The color of the control «window» must be as the color of the scale with 0 mmol/l glucose concentration. *If color of the control "window" differs from the color of scale with 0 mmol/l glucose concentration, do not use the strip.*

c) Take the test strip and be aware that the test zone is in the top, but the linia directions "from your side" – "→". With attention put the strip in to the glucometer till the light click. Do not bend over the strip

d) Turn on the glucometer. After the measurement ending the code number is on the screen. *It is necessary to note that the code number should be matched with the code number of the container of the kit.*

e) The image the strip and waving blood image are appeared on the screen after the code number, then the sound signal performs. The glucometer is ready for the glucose blood content measurement. *For the measument you can have only 90 secs. Then the glucometer could be turned off!*

2. *The blood drop obtaining*

Please wash and clean up the finger. Pierce the skin on the side surface using the scarificator. Stroking the finger with light pressure is helpful

for the blood drop formation.

3. *Glucose content qualitative detection.*

a) Please out the blood drop on the middle of the strip's green zone. Sound signal appears. The measurement starts when you can see the watch on the screen.

b) The result could be on the screen in 5 secs and the sound signal appears.

Normal values

Blood in capillaries (in 12-hours starvation)	3.3–5.5 mmol/l

Clinical and diagnostics significance

Blood in capillaries

The glucose level enhancement could be found in physiological and pathological cases. The glucose measurement in capillaries is prescribed for the patients with diabetes mellitus type 1 and 2.

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,
- thyroid gland hyperfunction, definite liver diseases (infectous hepatitis, liver cirrhosis).

The decreased glucose concentration in the blood is physiological and pathological.

Physiological hypoglycemia

The physiological hypoglycemia could be the result of the increased insulin secretion in the blood after the alimentary hyperglycemia, after the hard and prolonged work, in complete and partial starvation. Hypoglycemia may appear in women in the breast-feeding period due to the high glucose absorbtion by the breast tissues. In infants, the hypogycemia could as the result of the birth (no glucose from the maternal blood), unmatured liver and increased sensitivity of cells to the insulin.

Pathological hyperglycemia

Pathological hyperglycemia is found in a cse of the hyperinsulinemia and hyperplasia of the β-cells in the Langerhans islands (insulinoma, adenoma and cancer of the pancreas). The commen hypoglycemia origin is insulin overdose. Additionally, the lack of the cortisol in the case of the adrenal cortex hypofunction triggers the hypoglycemia (Addison's disease, tumors of the cortex), pituitary gland hypofunction (Simmond's disease), thyroid gland hypofunction. Hypoglycemia may originate in liver toxic damages, glycogenosis, in kidney diseases due to the glucosuria.

Design of the 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.

Practical 2
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

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 body that 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,
- reactions of glycolysis associated with ATP consumption,
- the reaction of substrate phosphorylation, their nature and value,
- glycolytic oxidation reduction of NAD^+ and NADH, its 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. Glucose synthesis from alanine, aspartate and glutamate. What are the reactions features and the process location? What is the value of the process?

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. 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.

11. 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?

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

Practical

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

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 15 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

Clinical and diagnostic significance

Serum

The glucose content increased in the blood is observed both in physiological and in pathological conditions. Types of hyper and hypoglycemia can be found in Practical 1, 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.

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.

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 mus-

cular 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?

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 sample 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 with different glucose concentrations.

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 sample	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:

$$\text{Glucose concentration, mmol/l} = \frac{Eex}{Est} \times C_{ST}, \text{ where}$$

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.

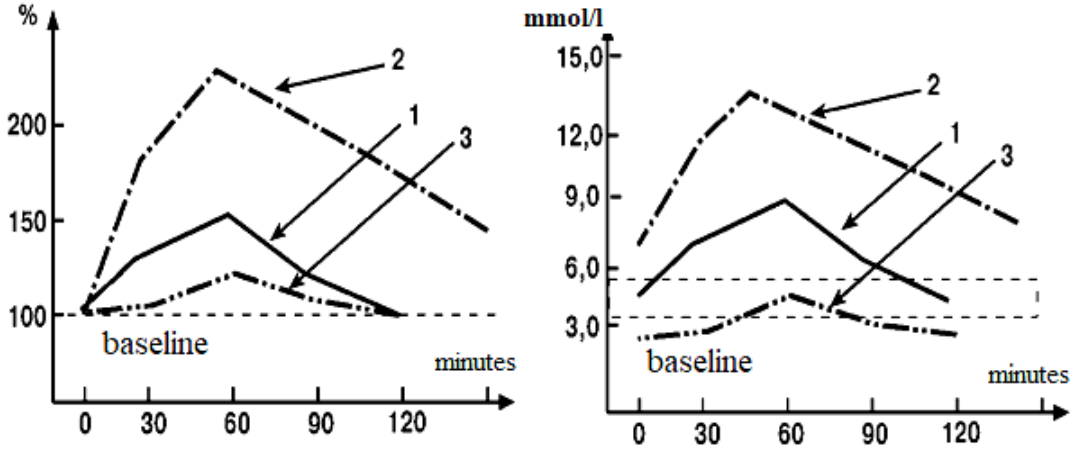
2. By 60-th minute, there is a maximum increase in the glucose concentration in the blood about 50-75% over the baseline. The interval from 30 to 60 minutes is associated with the speed of glucose absorption, and with the general state of the liver and its glycogenesis function.

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.

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: hyaluronic acid, chondroitin-4-sulfate, chondroitin-6-sulfate.

2. 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. Express detection of glucose in the blood using the glucometer.

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

5. 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.

6. 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 glycogenosis and glycogenosis, defects of enzyme and consequences of such defects.

7. 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.

8. The metabolism of ethanol in humans.

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. 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 function-

ing, the source of NADH. The role of the aerobic breakdown of glucose in the brain.

11. 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.

12. 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.

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

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

15. 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.

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

17. 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?

18. 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.

19. 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.

20. 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.

21. 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.

22. 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.

23. 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.

24. 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.

To obtain practical skills for the determination of phospholipids.

SELF-STUDY QUESTIONS

1. Lipids classification. The main goups characteristics according to the plan:
   - chemical structure,
   - biological role.
2. 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.
3. Fatty acids of ω-6 (linoleic, γ-linolenic, arachidonic acids) and ω-3 (α-linolenic, eicosopentaenoic, docosohexaenoic acids). Their length and position of double bonds. The biological role of polyunsaturated fatty acids.
4. Characteristics of derivatives of eicosotrienoic (ω-6), arachidonic (ω-6) and eicosopentaenoic (ω-3) acids – eicosanoids (prostaglandins, leu-

kotrienes, thromboxanes). The biological role of individual types of eicosanoids. Scheme of initial synthesis reactions on the example of arachidonic acid. The role of enzymes – phospholipase A_2, cyclooxygenase, lipoxygenase. What substances affect the synthesis of eicosanoids?

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

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

7. 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, sulpholipids), and their structure. Biological functions of glycolipids.

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

9. 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.

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

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

14.Transport of exogenous lipids. Chylomicron composition, its functions. Role of apo-proteins.

15. Transport of endogenous lipids.

16. Investigation of phosphatidylcholine composition of egg yolk. Principle of determination of constituent elements.

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) 3.75% solution of acidic molybdenum ammonium, 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 3.75% solution of acidic molybdenum ammonium 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. The structure of triacylglycerols, their fatty acid composition. Reactions of synthesis of triacylglycerols (lipogenesis). Lipogenesis activation in the liver and adipose tissue. The relationship of triacylglycerol synthesis with carbohydrate metabolism.

2. Characteristics of very low-density lipoproteins: their composition, proteins, function.

3. Similarity and difference of triacylglycerol biosynthesis in fatty tissue and liver.

4. Characteristics of lipolysis reactions according to the plan:

- localization and conditions of the process,
- sequence of reactions and enzymes,
- end products,
- hormonal regulation of the process,
- transport and use of free fatty acids formed during lipolysis.

5. Hormone-sensitive TAG lipase, the role of the adenylate cyclase system in regulating its activity. Regulation of TAG lipase by hormones adrenaline, glucagon, cortisol and insulin. Allosteric regulation of the activity of lipolysis and lipogenesis enzymes.

6. Glycerol oxidation reactions to pyruvate. Possible pathways of pyruvate metabolism. The energy yield of glycerol oxidation under aerobic and anaerobic conditions.

7. Fatty acid oxidation reactions to carbon dioxide and water:

- the role of carnitine in fatty acid β-oxidation,
- localization and conditions of β-oxidation,
- sequence of oxidation reactions, enzymes,
- participation of vitamins and coenzymes,
- end products,
- connection with CTK and the respiratory chain (CTK reactions, oxidative phosphorylation),
- calculation of the energy value of β-oxidation of palmitic acid.

8. Characteristics of the synthesis of fatty acids from glucose according to the plan:

- localization and conditions of the process,
- reactions of the formation of acetyl~S-CoA from glucose,
- the role of citrate in the transfer of the acetyl group to the cytosol, its further transformations,
- the reaction of the synthesis of malonyl~S-CoA,
- composition of the multi-enzyme complex: fatty acid synthases, chemistry of reactions,
- final product of synthesis,
- participation of vitamins and coenzymes, sources of NADPH,
- regulation of the process with the participation of hormones insulin, adrenalin, glucagon,
- influence of ATP, acyl~S-CoA, malonyl~S-CoA, citrate on the synthesis of fatty acids.

9. Reactions of glycerol-3-phosphate synthesis from glucose. Localization and the role of the process.

10. Reactions of phosphatidic acid synthesis from fatty acids and glycerol-3-phosphate according to the plan:

- localization in the cell,
- sources of glycerol-3-phosphate, fatty acids and energy,
- sequence of reactions,
- connection with carbohydrate metabolism,
- further ways of phosphatidic acid's metabolism.

11. Features of triacylglycerol metabolism in certain conditions (food intake, fasting, muscle activity). What is the peculiarity of brown adipose tissue?

12. Reactions of synthesis of ketone bodies. Conditions, localization and the role of the process. Oxidation reactions of ketone bodies in tissues. Causes of ketoacidosis during fasting and diabetes mellitus. The role of oxaloacetate deficiency for activation of ketogenesis.

13. Determination of the concentration of triacylglycerols in blood serum. Clinical and diagnostic value and normal indicators.

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 quinonimine, painted the product in raspberry pink color, in the presence of chlorophenol.

Material for investigation

Serum.

Reagents

1) 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 = $\frac{Eex}{Est} \times C_{st}$,

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 (SEMINAR)

INTRODUCTION

Phospholipids are part of the cell membrane, and are involved in the formation of lipoproteins, the transmission of hormonal signal in the cell. When phospholipid synthesis is disrupted, cell metabolism and transport lipoprotein formation will change.

THE AIM OF THE PRACTICAL CLASS IS:

To study the processes of biosynthesis and catabolism of phosphorlipids.

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 (see Theme 7.3). 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. Lipidoses or lipid storage diseases. Niemann-Pick's, Gaucher’s and Tay-Sachs diseases.

THEME 9.4. INTRACELLULAR CHOLESTEROL EXCHANGE. LIPID TRANSPORT IN THE BLOOD

INTRODUCTION

Cholesterol is a component of cell membranes, a precursor of steroid hormones, bile acids, vitamin D.

Cholesterol metabolism disorders are manifested by such common diseases as atherosclerosis and bile infection.

THE AIM OF THE PRACTICAL CLASS IS:

To study of cholesterol exchange, function and transport.

To acquire practical skills in determining the concentration of total cholesterol in the blood serum.

SELF-STUDY QUESTIONS

1. Chemical structure and biological role of cholesterol. Food sources of cholesterol. Ways of removing cholesterol from the body.
2. Characteristics of the transport of free cholesterol and its esters in blood plasma.
3. Mevalonic acid synthesis reactions. Scheme of further stages of cholesterol synthesis. The relationship of cholesterol synthesis with the exchange of carbohydrates. Regulation of synthesis.
4. The composition of very low-density lipoproteins, the ratio of lipid fractions, the value. Apoproteins, their function. Where and when are these lipoproteins formed? The metabolism of VLDL. The role of lipoprotein lipase.
5. The composition of low- and high-density lipoproteins. Types of apo-proteins, their functions. Reaction catalyzed by lecithin:cholesterol acyltransferase (LHAT).
6. Localization and role of apo-B100-receptor. The significance of receptor-mediated LDL endocytosis and the pathways of metabolism of their components after endocytosis. The role of acyl~S-CoA:acyltransferase cholesterol (ACAT).
7. Composition of chylomicrons, their functions. The role of apoproteins. The metabolism of chylomicrons. The role of lipoprotein lipase. Activation of the lipoprotein lipase.
8. The relationship of the metabolism of chylomicrons, VLDL, LDL and HDL.

9. Characteristics of cholesterol metabolism disorders – type IIa hyperlipoproteinemia (familial hypercholesterolemia), atherosclerosis (by stages), cholelithiasis. Causes, consequences, fundamentals of treatment.

10. Sources of acetyl~S-CoA formation in the body: catabolism of glucose, amino acids, fatty acids and ketone bodies. Ways of using acetyl~S-CoA: CTC, synthesis of fatty acids, cholesterol, ketone bodies. Under what conditions and in which organs do certain processes occur?

11. The causes of metabolic disorders of triacylglycerols are obesity, hyperlipoproteinemia type I (hyperchylomicronemia) and type V.

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

13. Determination of cholesterol concentration in blood serum. The principle of the method, its clinical and diagnostic value, normal indicators.

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 quinonimine, 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 cholesterol	—	0.02
Working reagent	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.

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 ac-

id) 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, sulpholipids, gangliosides), their biological role and functions.

7. Structure of cholesterol and its esters, and its biological role. Transport of cholesterol in the blood.

8. The role of enzymes and components of bile in the digestion of food lipids in gastrointestinal tract. The scheme of bile acid synthesis, the role of vitamins in this process. Chemical structure of taurocholic and glycocholic acids.

9. Lipid resynthesis in enterocyte. Effects of lipid absorption and digestion disorders.

10. 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?

11. Detection of egg yolk phosphatidyl choline. Principle of the method and course of the measurement.

12. 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.

13. 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.

14. Ways of formation and use of acetyl~S-CoA in the body (schema).

15. Reactions of synthesis and degradation of ketone bodies. Causes of ketonemia and ketonuria in starvation and diabetes.

16. 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).

17. Synthesis reaction of glycerol-3-phosphate from glucose and oxidation of glycerol to pyruvate. Energy yield of the process.

18. 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.

19. Metabolism of triacylglycerols and saturated fatty acids in certain physiological conditions (food intake, hunger, muscular activity, diabetes type 1 and 2).

20. Determination of triacylglycerols concentration in the blood serum. Principle of determination, clinical-diagnostic and normal values.

21. 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 are lipotropic substances? Their functions. Consequences of the lack of lipotropic substances.

22. 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.

23. Determination of concentration in the blood. Principle of the method, definition, clinical-diagnostic value, normal values.

24. 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.

25. 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.

26. 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.

27. 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?

28. 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.

29. 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.

30. FAD-dependent dehydrogenases, carbohydrate metabolism reactions catalyzed by them. Structural formulas of oxidized and reduced forms of FAD. Characteristics of the vitamin, which is a part of the FAD: biological name, signs of insufficiency, daily need, food sources.

31. 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.

32. 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.

33. 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?

34. Uncoulping 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?
- cGMP-mediated mechanism of signal transmission. General characteristics of this mechanism.

5. The structure and sources of secondary mediators of hormonal signal transmission: cAMP, cGMP, inositol triphosphate (IP_3), diacylglycerol (DAG), Ca^{2+}ions.

6. Cytosolic mechanism of the hormonal signal transduction to the target cells. Hormones with this mechanism.

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.

SELF-STUDY QUESTIONS

1. Classes of hormones according to their chemical structure, biological functions and belonging to endocrine glands.

2. Characteristic of the hormones of the hypothalamus (releasing hormones):

- name,
- chemistry,
- synthesis regulation and hormone secretion,
- targets-organ,
- location of the receptors in the cell and mechanism signal tranduction.

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:

- 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,
- impact on the metabolism of carbohydrates, proteins, lipids, minerals, water – reactions and enzymes that are sensitive to the hormone action,
- pathologies associated with the hormone lack and excess.

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: thyroliberin. 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:

- the 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. Make a table or the report for the pituitary gland, thyroid, parathyroid and pancreatic glands hormones according to the following plan: title and chemistry; place of synthesis; hormone's action regulation; target; receptors location; mechanism of action; impact on the carbohydrate, protein's, lipids, water-salt exchange; pathology, associated with the hormone's deficiency (absence) or excess.

Practical

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 acid's 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.

THEME 10.3. HORMONES OF PITUATRY GLAND, 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.

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 or the report for adrenocortical hormones and sex hormones, according to the given scheme: title and chemistry; place of synthesis; hormone's action regulation; target; receptors location; mechanism of action; impact on the carbohydrate, protein's, lipids, water-salt exchange; pathology, associated with the hormone's deficiency (absence) or excess.

Practical

IMMUNOASSAY METHOD FOR TESTOSTERON 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 aspi-

ration 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.

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 examples of the proteins for each fraction (see Appendix 1). Dysproteinemia and paraproteinemia. Age dynamics of protein fractions. Embryo specific 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. C-reactive protein level in the blood. Normal values and clinical-diagnostic value.

15. Proteins fractions of the blood. Principle of the electrophoresis. Normal values and clinical-diagnostic value.

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

1) 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
C-REACTIVE PROTEIN DETECTION IN BLLOD WITH LATEX AGGLUTINATION REACTION

Principle

Latex reagent is the suspension of the latex units with immobilized antibodies to the human C-reactive protein. Mixing the reagent with the blood with C-reactive protein level more than 6.0 mg/l, the agglutination appears as the result of the antigen and antibody reaction. The visible signs of the agglutination are the result of the positive test.

Reagents

1) Latex C-reactive protein reagent (Reagent 1), 2) Buffer-diluter (0.9% NaCl solution) (Reagent 2), 3) positive control (serum) (C-reactive protein level ≤12 mg/l) (Reagent 3), 4) negative control (serum) (C-reactive protein level ≤6 mg/l) (Reagent 4).

Materials

Serum.

Procedure

1. *Preparatory step.*

All reagents, serum must have the room temperature (18–25°C. Mix carefully the latex C-reactive protein reagent (Reagent 1) for the homogenous suspension formation.

2. *Quantitative detection of the C-reactive protein.*

a) Add 20 µl of serum in the tube 1, 20 µl of positive control (Reagent 3) to the "+" tube; and negative control – to the "-" tube (Reagent 4);

b) put 20 µl of reagent 1, latex C-reactive protein, to the place near the the first drop in each of the tube;

c) mix well the content of two drops for the homogenous suspension using the glass stick;

d) wave the test strip, then in 2 to3 minute assess the result of the reaction.

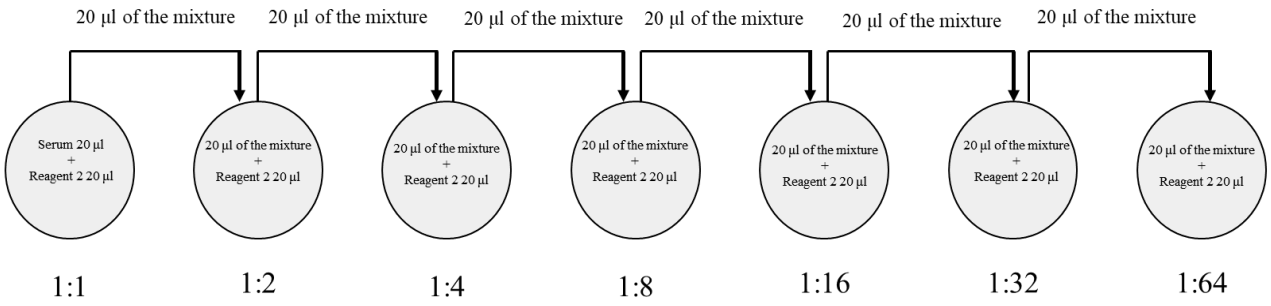
3. *Analysis of the result of the reaction for the quantitative C-reactive protein detection.*

The visible aggregates of the latex units in liquid are the sign of the

C-reactive protein detection with the content ≥ 6 mg/l. This result is evaluated as "+++"; small aggregates – C-reactive protein level is about 6 mg/l ("+"); "milk color" homogenous suspension – C-reactive protein content ≤6 mg/l. If the C-reactive protein level is ≤6 mg/l, the result is negative ("-"). If the C-reactive protein level is ≥6 mg/l, the additional research requires for the semi-quantitative C-reactive protein detection.

4. *Semi-quantitative C-reactive protein detection.*

a) dilute the serum with help of the buffer-diluter (Reagent 2) in: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64;



b) put 20 µl of reagent 1, latex C-reactive protein, to the place near the first drop in each of the tube;

c) mix well the content of two drops for the homogenous suspension using the glass stick;

d) wave the test strip, then in 2 to3 minute assess the result of the reaction.

5. *Analysis of the result of the reaction for the semi-qualitative C-reactive protein detection.*

Choose the dilutions, where the smell aggregates appear and the next dilutions have «milk color» homogenous suspension.

Calculation

$$\text{C-reactive protein level, mg/l} = 6 \times K_{\text{of dilution}},$$

where $K_{\text{of dilution}}$ – the largest dilution with positive signs and visible latex aggregates.

Normal values

Serum	≤ 6 mg/l

Clinical and diagnostics significance

Test is used for the non-specific assessment of the inflammation pres-

ence. Infection induced by bacteria's triggers C-reactive protein release (100 mg/l and upper). Virus induced inflammation is associated with growth in C-reactive protein content till mg/l. It is noted that the growth in the dynamics is a sign of the secondary bacterial infection.

The follow-up in C-reactive protein level is of great importance for the effectiveness in chronic infections treatment.

Design of the work

Indicate the principle of the reaction, procedure, normal values and the result of the test; note the clinical and diagnostic significance of the indicator and make the conclusion.

Practical 3 (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

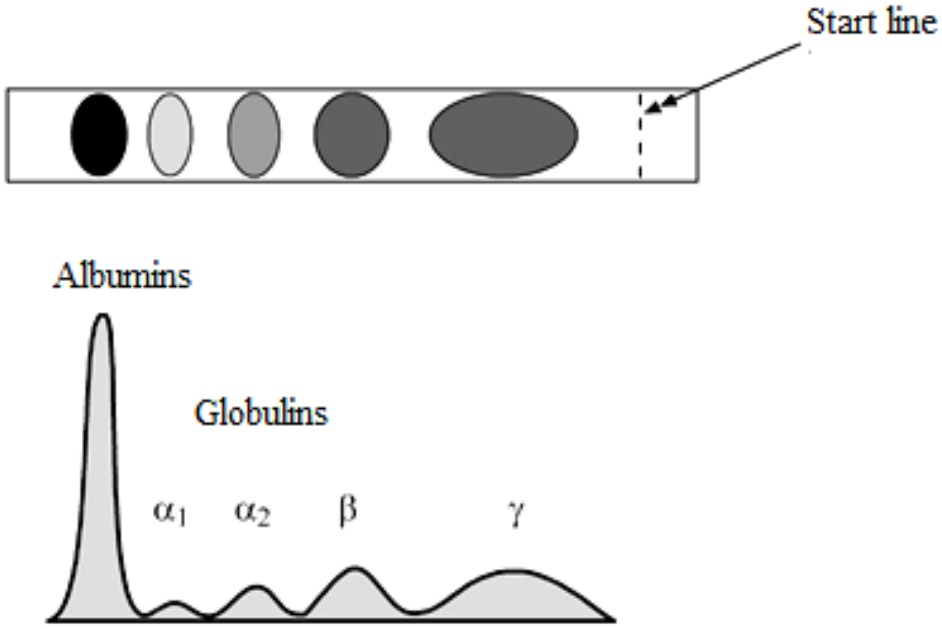
Electrophoresis apparatus, densitometer.

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 an albuminemia, 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 blood-stream 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 concepts.

2. The erythrocyte metabolism features (glycolysis, pentose-phosphate pathway of the glucose oxidation, 2.3-diphosphoglycerate, oxygen active species synthesis, Met-Hb-reductase system, antioxidant system).

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. The structure of the most abundant heme-containing proteins in the body (hemoglobin, myoglobin) and heme-containg enzymes (cytochromes, catalase, peroxidase). Their functions.

6. 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.

7. 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.

8. The binding of hemoglobin with oxygen, cooperation effect. The oxygen normal saturation of hemoglobin. The oxygen transport mechanism. The effect of temperature, pH, CO_2 concentration, 2.3-diphosphoglycerate on the hemoglobin affinity to oxygen.

9. The scheme of reactions occurring in the erythrocytes in the lungs and tissue capillaries.

10. The decomposition reactions of hemoglobin and heme in the reticuloendothelial system.

11. Indirect (free) bilirubin, its structure, reaction formation. The further fate of the indirect bilirubin.

12. 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?

13. Conditions associated with the hemoglobin excessive breakdown. Causes of hemolytic jaundice and its laboratory criteria.

14. Conditions associated with impaired bile flow. Causes of obstructive jaundice and its laboratory criteria.

15. Conditions associated with failure of hepatocytes function. Causes of parenchymatous jaundice and its laboratory criteria.

16. Physiological jaundices of the newborn.

17. Pathological jaundices of newborn:

- hemolytic jaundice, their causes. The physiological basis of the phenobarbital usage,
- inherited disorders of bilirubin excretion – Gilbert's, Meulengracht, Dubin-Johnson, Crigler-Nayar syndrome.

18. Estimation of total bilirubin and its fractions in the blood serum. The method principle, normal values, clinical-diagnostic value.

19. Detection of bilirubin and urobilinogen in the urine. The principle of the method, normal values, clinical-diagnostic value.

Practical 1

DETERMINATION OF THE TOTAL BILIRUBIN AND ITS FRACTIONS CONCENTRATION IN BLOOD SERUM

The principle

The interaction of sulphanilic acid with nitrous sodium acid gives diazo sulphanilic acid, and then bilirubin together with it forms a colored pigment (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) Sulphanilic 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.

A standard solution of bilirubin 5 μmol/l.

Procedure

	Common bilirubin, ml	**Direct bilirubin, ml**	**Standard, ml**	**Control, ml**
Sulphanilic 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 incubate for 5 minutes at the room temperature			
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 15 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 $= \frac{E_{ex}}{E_{st}} \times C_{st}$, 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	↑↑	↑	N or ↑
Direct	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 μmol/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, stercobilin 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 diazo reagent. The reaction zone contains *n*-nitrophenyldiazonic-*n*-toluol sulphate, sodium bicarbonate and sulfosalicylic acid. The lilac-beige (lilac-pink) color appears after 60 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 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	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 (see Appendix 2).

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 the liver.

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

Practical 1
DETERMINATION OF THE BICARBONATES CONTENT IN THE SERUM

The principle

The method is based on the use of conjugated enzymatic reactions carried out by: 1) phosphoenolpyruvate carboxylase, catalyzing the conversion of phosphoenolpyruvate and bicarbonate ion (HCO_3^-) to oxaloacetate

and $H_2PO_4^-$; 2) malate dehydrogenase, catalyzing the reduction of oxaloacetate to malate using NADH. The decrease in NADH concentration is proportional to the concentration of total CO_2 in the test sample.

Material for investigation

Serum.

Reagents

1) A working solution containing phosphoenolpyruvate carboxylase, phosphoenolpyruvate, malate dehydrogenase, NADH.

HCO_3^- standard, 30 mmol/l.

Procedure

	Test, ml	**Standard, ml**
Serum	0.02	—
Standard solution	—	0.02
Working reagent	2.0	2.0
	Mix well and incubate for 10 minutes at room temperature. The optical density of the experimental and standard sample is measured against water at a wavelength of 540 nm (green light filter).	

Calculation

Phosphate concentration in serum, mmol/l = $\frac{E_{ex}}{E_{st}} \times C_{st}$,

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

Normal values

Serum 22-29 mmol/l

Clinical-diagnostic significance

Bicarbonates content measurement is used in acid-base status detection.

Increase in bicarbonates level in blood is found in metabolic alkalosis due the bicarbonates implementation, in a case of vomit, potassium deficiency; in respiratory acidosis due the carbon dioxide release; in emphysema, problems in diffusion in alveolar membrane interruption in cardiac disease with pulmonary edema, in any problems with lungs ventilation, including the drugs overdose or in inadequate artificial lungs ventilation.

Decrease in bicarbonates level is seen in metabolic acidosis (keto and lactoacidosis) in diabetes mellitus, starvation, diarrhea, kidneys insuffi-

ciency, salicylates overdose; in respiratory alkalosis due hyperventilation.

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 LACTATE IN THE SERUM

The principle

The principle of the method is based on the lactate oxidation by-lactate oxidase to pyruvate and hydrogen peroxide, which in the of peroxidase presence reacts with 4-aminoantipirine and forms a colored compound. The color intensity is proportional to the lactate concentration and is determined by spectrophotometer.

The material for investigation

Serum.

Reagents

1) Working solution, containing lactate oxidase, peroxidase, 4-aminoantipyrine.

A standard solution of lactate, 1.10 mmol/l.

Procedure

	Test, ml	**Control, ml**
Serum	0.02	—
Standard solution	—	0.02
Working reagent	2.0	2.0
	Mix and inculbate for 10 minutes at room temperature. Measure the optical density of test and control tubes at 540 nm (green light filter).	

Calculation

$$\text{Concentration of lactate in serum, mmol/l} = \frac{E_{ex}}{E_{st}} \times C_{st},$$

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

Normal values

Serum	0.5-2.2 mmol/l

Clinical-diagnostic significance

Hyperlactatemia is found in a case of hard working, starvation, decompensated diabetes mellitus type 1 and cirrhosis.

Decrease in lactate content has no clinical and diagnostic significance.

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.

UNIT 12
BIOCHEMISTRY OF KIDNEYS AND LIVER

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-dihydroxycholecalciferol).

2. The role of enzymes in the kidney function – glycine amidine transaminase, 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. The assessment of the filtration rate in clinical and laboratory diagnostics. What is the creatinine clearance?

6. Reabsorption. Transport of maximum for glucose.

7. Regulation of reabsorption of water. The role of antidiuretic hormone. The factors stimulating its synthesis and release. Metabolic consequences of antidiuretic hormone hypofunction, symptoms.

8. 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.

9. Regulation of calcium reabsorption. The role of 1.25-dihydroxycholecalciferol, parathyroid hormone and calcitonin in calcium homeostasis.

10. The role of the kidneys in maintaining acid-base balance – reabsorption of bicarbonate, acids, ammonia, the excretion of organic acids.

11. Urine general properties of a healthy man are: quantity, color, transparency, odor, relative density, pH. How are these indicators changed in pathological conditions?

12. Urine organic and inorganic components of a healthy person.

13. The urine pathological components – protein, glucose, bile pigments, ketone bodies, blood, enzymes.

14. The principle of the methods for the determination of urine physicchemical properties (density, pH). Clinical-diagnostic value of these indicators. The normal values.

15. The laboratory determination of urine components – protein, glucose, ketone bodies, bilirubin, urobilinogen, hemoglobin, red blood cells. Clinical-diagnostic relevance, the normal values.

Practical

DETERMINATION OF URINE PHYSICO-CHEMICAL PROPERTIES AND SEMI-QUANTITATIVE DETERMINATION OF THE URINE COMPOSITION

Use the urine analyser Uriscreen and strips «Hospitex Diagnostics» for the urine investigation.

Material for investigation

Normal urine (N 1) and urine samples N 2, 3, 4.

Equipment

Urine analyzer, Uriscreen.

Determination of pH

Principle

Principle is based on the change of the test-strip color.

Impact of factors

The prevalence of proteins in diet results in acidic pH level of urine, plants prevalence – in alkaline pH. Acidic pH level of urine is associated with $H_2PO_4^-$ and NH_4^+ ions; alkaline – HCO_3^- ions.

Clinical and diagnostic significance

The prevalence of proteins in diet results in acidic pH level of urine, plants prevalence – in alkaline pH.

An acidic reaction of urine is observed in feverish conditions, diabetes mellitus, fasting, etc. An alkaline reaction of urine is noted in cystitis and pyelitis, severe vomiting, diarrhea (diarrhea), the introduction of sodium bicarbonate and the use of alkaline mineral waters.

The pH of urine determines the possibility of the formation of certain types of urinary stones. Uric acid stones (urate) are most often formed at a pH below 5.5, oxalate stones – at a pH of 5.5–6.0, calcium phosphate stones – at a pH of 7.0–7.8.

Determination of relative density

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 glucose

The principle

The principle of glucose determination is based on enzymatic reaction glucose oxidase. The display area is impregnated with solutions of the enzyme's 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 oxidizes a dye, and there is the transition of yellow coloring to green.

Normal value

Urine

Glucose	test strips "Hospitex Diagnostics"	test is negative

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.

Determination of protein

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 "Hospitex Diagnostics"	test is negative

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 kidney's glomeruli or tubules,
- postrenal associated with inflammation of the urinary tract.

Blood determination

Blood in the urine can be of two types – blood cells (hematuria, erythrocyte) or hemoglobin (hemoglobinuria).

Principle

The display area of the test strips contains organic hydroperoxide (for example, new hydrogen peroxide) and the tetramethylbenzidine indicator. Hemoglobin catalyzes the oxidation of the indicator with hydroperoxide to form blue-green colored products.

In the presence of free hemoglobin (hemoglobinuria or hemolysis of the primary erythrocytes present), the entire sensory zone is colored in a homogeneous blue-green color.

Unchanged (whole) erythrocytes (microhematuria) are manifested by intensely colored blue-green dots or spots on the unpainted reagent zone or uniform blue-green color of the entire zone (macrohematuria).

In case of a negative reaction, the display area remains yellow (without a green tint).

Normal values

Urine		
Erythrocytes and hemoglobin	children	negative test
	adults	negative test

Clinical-diagnostic significance

Single red blood cells can be found in the urine of healthy people. In practically healthy people, up to 1 million red blood cells are released per day, which corresponds to the content of 1 ml of urine of 1 red blood cell.

Hematuria is found when the kidney parenchyma is affected (glomerulonephritis, pyelonephritis, tumors), with severe physical exertion, with urinary tract damage.

Definition of ketone bodies

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 β-hydroxybutyric 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 "Hospitex Diagnostics"	test is negative

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 bilirubin

The principle

The test is based on reaction of bilirubin combinations with stabilized diazo reagent (see Unit 11.2."Determination of the total bilirubin and its fractions concentration in blood serum"). The reaction zone contains n- nitrophenyldiazonium-St-*n*-toluensulphate, 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.

Normal values

Urine

The bilirubin	negative test

Clinical-diagnostic significance

The appearance of bilirubin in urine is associated with mechanical and parenchymal jaundice, in which direct bilirubin is filtered from the blood into the urine.

Determination of urobilinogen

The principle

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 up to 17.0 μmol/l

Clinical-diagnostic significance

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).

Procedure

1. *Preparatory step*

a) Turn on the analyzer with the switch on the back of the instrument. Wait for the self-test of the instrument to be completed.

b) Test strip to be loaded into a test tube with a urine sample on

1-2 seconds. Remove excess urine sample by soaking test strip edge on filter paper.

2. *Quantification of the indicators studied.*

a) Place the test strip in the holder on the front panel of the instrument.

b) Press the "Start" button, at the end of time to pass the reaction in the reaction zones of the test strip, the instrument will perform the measurement and print the measurement result.

Work design

Write the principle of methods and results of research. Make conclusions about possible pathology of the body.

Indicator	**Sample**			
	N 1	N 2	N 3	N 4
pH				

Relative density				
Glucose				
Protein				
Red blood cells and hemoglobin				
Ketone bodies				
Bilirubin				
Urobilinogen				

THEME 12.2. LIVER INVOLVEMENT IN METABOLISM OF SUBSTANCES. BIOTRANSFORMATION OF XENOBIOTICS (SEMINAR)

INTRODUCTION

The liver is the central element of human metabolism, regulating carbohydrate, lipid, protein and other metabolism. Hepatocyte is a leader in biotransformation of xenobiotics and metabolic products. The involvement of the liver in these processes is important for maintaining homeostasis of the body.

THE AIM OF THE PRACTICALCLASS IS:

To study of liver participation in carbohydrate, lipid and protein exchanges and mechanisms of biotransformation of xenobiotics.

QUESTIONS FOR SELF-STUDY

1. Liver function.
2. Liver's role in carbohydrate metabolism. The processes of maintaining blood glucose homeostasis, clinical and laboratory indicators, disorders.
3. Liver's role in lipid metabolism. Liver's role in the synthesis and transport of endogenous triacylglycerols and cholesterol, clinical and laboratory parameters, disorders. What lipoproteins are formed in the liver?
4. Liver's role in the synthesis of albumins and globulins, clotting factors, plasma-specific enzymes, clinical and laboratory indicators, disorders.
5. Liver's role in the synthesis of biologically active substances (creatine, 25-hydroxycholecalciferol), bile acids. The composition of bile and its role in digestion.
6. Xenobiotics: properties, routes in the body.

7. Biotransformation of xenobiotics. Phases of biotransformation. Factors affecting biotransformation.

8. The first phase of biotransformation is the microsomal oxidation reaction. Microsomal oxidation reactions, note the role of NADPH, cytochrome P_{450}, cytochrome b_5.

9. The second phase of biotransformation is the conjugation reaction. The structure of UDP-glucuronic acid (UDPGA) and phosphoadenosine phosphosulfate (PAPSA). Reactions of direct bilirubin, indican formation.

10. The reason for the increased toxic compounds as a result of biotransformation.

11. Differences between microsomal and biological oxidation.

12. Examples of biotransformation of some medicines.

CHECKLIST FOR FINAL LESSON (UNITS 10, 11, 12)

1. Hierarchy of regulatory systems. The place of hormones in the regulation of metabolism and organ function.

2. Differences between membrane and cytosolic mechanisms of hormonal signal transmission into the cell.

3. Membrane mechanisms of hormonal signal transmission into the cell. There are three types of receptors: with enzymatic activity, with ion-conducting activity and associated with G proteins.

4. Characterize the receptors associated with G proteins:

- systems of secondary intermediaries and their interaction,
- adenylate cyclase mechanism of action of hormones,
- calcium-phospholipid mechanism of action of hormones.

5. General characteristics of the guanylate cyclase mechanism of hormone action.

6. Cytosolic mechanism of action of hormones.

7. Classification of hormones by chemical structure, biological functions and belonging to the endocrine glands. The role of liberins, statins, and tropic hormones. What is the negative feedback in the regulation of hormone synthesis and action?

8. Characteristics of somatotropic hormone: chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action, effect on metabolism and water. How are hormone synthesis and secretion regulated? Conditions associated with a violation of the action of the hormone.

9. Characteristics of antidiuretic hormone (vasopressin): chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action, effect on metabolism and water. Regulation of hormone synthesis and secretion. Conditions caused by a violation of the action of the hormone.

10. Characteristics of oxytocin: chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action, effects. How are hormone synthesis and secretion regulated?

11. Characteristics of parathyroid hormone and calcitonin: chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action, effect on calcium and phosphate metabolism. Regulation of hormone synthesis and secretion. What is their role in the metabolism of calcium, phosphates and calcitriol?

12. Pancreatic hormones glucagon and insulin: their chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action, effect on the metabolism of carbohydrates, proteins, lipids (enzymes regulated by hormones). Regulation of hormone synthesis and secretion. Conditions caused by the lack (absence) or excess of the action of the hormone.

13. Modern ideas about the mechanisms of development of insulin-dependent and insulin-independent diabetes mellitus. The most important changes in hormonal status and metabolism in diabetes mellitus, biochemical mechanisms of the development of complications of diabetes mellitus.

14. Characteristics of thyroid-stimulating hormone: chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action, effects. How are hormone synthesis and secretion regulated?

15. Thyroid hormones (thyroxine and triiodothyronine): chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action, effect on the metabolism of carbohydrates, proteins, lipids. How is the synthesis and secretion of hormones regulated? Conditions caused by the lack (absence) or excess of the action of the hormone.

16. Adrenaline: chemical nature, synthesis reactions, target organs, localization of receptors and mechanism of action, effect on the metabolism of carbohydrates, proteins, lipids (enzymes regulated by hormone). Regulation of hormone synthesis and secretion. Conditions caused by a violation of the action of the hormone.

17. Characteristics of adrenocorticotropic hormone: chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action, effect on metabolism. How are hormone synthesis and secretion

regulated? Conditions caused by the absence or excess of the action of the hormone.

18. Glucocorticoids: chemical nature, place and scheme of synthesis, target organs, localization of receptors and mechanism of action, effect on the metabolism of carbohydrates, proteins, lipids (enzymes regulated by hormone). Regulation of hormone synthesis and secretion. Conditions caused by the lack (absence) or excess of the action of the hormone.

19. Mineralocorticoids: their chemical nature, place and stages of synthesis, target organs, localization of receptors and mechanism of action, effect on the exchange of electrolytes and water. How are hormone synthesis and secretion regulated? The role of the renin-angiotensin-aldosterone system. Conditions caused by the lack (absence) or excess of the action of the hormone.

20. Lactotrophic hormone: chemical nature, place and stages of synthesis, target organs, localization of receptors and mechanism of action, effect on metabolism. How are hormone synthesis and secretion regulated?

21. Characteristics of gonadotropins: follicle-stimulating and luteinizing hormones: chemical nature, place of synthesis, target organs, localization of receptors and mechanism of action. Regulation of hormone synthesis and secretion.

22. Androgens and estrogens: chemical nature, place and stages of synthesis, target organs, localization of receptors and mechanism of action, effect on the metabolism of carbohydrates, proteins, lipids. How hormone synthesis and secretion are regulated.

23. Conducting qualitative reactions to the structural components of insulin. The principle of the method for determining testosterone in blood serum. Clinical and diagnostic value.

24. The concept of total blood protein, fractions included in its composition. Physiological functions of blood proteins, normal indicators of their concentration. Causes of changes in the concentration of total protein in the blood.

25. Protein fractions of blood serum. Characteristics of albumins, causes of hypo-, hyper- and analbuminemia. Globulins and their main fractions. The main representatives of globulin fractions (see Appendix 1). The biological role of albumins and globulins. Dysproteinemia and paraproteinemia. The reasons for the change in the concentration of protein fractions in the blood. Changes in the ratio of protein fractions in liver diseases, kidney, acute and chronic inflammation, tumors (types of proteinograms).

26. Non-protein nitrogen–containing components of blood – fractions of residual nitrogen, their characteristics. The role and metabolism of urine, creatine, creatinine, uric acid. The clinical and diagnostic significance of the determination of these substances in the blood, their normal indicators. Causes and consequences of hyperammonemia.

27. Characteristics of blood enzymes – plasma-specific, indicator, excretory enzymes. Examples. The use of blood enzymes for the diagnosis of diseases.

28. The principle of separation of serum proteins by electrophoresis.

29. Features of erythrocyte metabolism. The role of glycolysis and the pentose phosphate pathway.

30. Iron metabolism. Food sources, consumption norms, transport, deposit and mobilization, the role of transferrin and ferritin.

31. The structure of the hemoglobin molecule. The structure of heme. Normal and pathological forms of hemoglobin. The mechanism of regulation of the affinity of hemoglobin to oxygen. The role of 2,3-diphosphoglycerate.

32. Reactions of heme and hemoglobin synthesis. Regulation of synthesis processes. Characteristics of hemoglobin–porphyria metabolic disorders.

33. Reactions of heme breakdown, formation of bilirubin and bilirubin-diglucuronide, their localization. The main stages of the transformation of bile pigments in the body. Ways of excretion of bilirubin and other bile pigments.

34. Metabolic disorders of bile pigments. Laboratory tests of various types of jaundice (hemolytic, parenchymal, obturation).

35. Disorders of pigment metabolism in children: 1) hemolytic jaundice; 2) physiological jaundice of newborns and infants; 3) hereditary disorders of bilirubin metabolism – Gilbert-Meilengracht, Dubin-Johnson, Kriegle-ra-Nayar syndromes.

36. The principle of the method of detecting bilirubin and urobilinogen in urine. Clinical and diagnostic value.

37. Methods of quantitative determination of total bilirubin and its fractions in blood serum, clinical and diagnostic significance.

38. Respiratory function of the blood. Mechanisms of transport of acid and carbon dioxide.

39. Characteristics of indicators of acid-base state (see Appendix 2). Chemical and physiological mechanisms of regulation of acid-base state.

The relationship of the oxygen and carbon dioxide transport with the mechanisms of maintaining the acid-base state.

40. The role of the kidneys in the regulation of acid-base state: reabsorption of bicarbonates, ammoniogenesis.

41. Violations of the acid-base state, its causes, changes in the indicators of the acid-base state. Methods of compensation for various violations of the acid-base state.

42. The principle of quantitative determination of the content of bicarbonate and lactate in blood serum. Clinical and diagnostic value, normal indicators.

43. Characteristics of biochemical processes in the nephron. The peculiarities of reabsorption and secretion of electrolytes and water in various departments of the nephron. The role of hormones in the processes of reabsorption.

44. Composition, physical and chemical properties of urine. Normal and pathological components of urine, their clinical and diagnostic value, normal indicators.

45. The principle of methods and the course of determining the physical and chemical properties of urine:

- determination of the relative density of urine,
- determination of urine pH.

46. The principle of methods and the course of determination of pathological components of urine:

- determination of the concentration of glucose, protein, blood, keto-new bodies, bilirubin, urobilinogen.

47. Liver biochemistry: functions, role in digestion and metabolism. Participation of the liver in carbohydrate, lipid, protein metabolism, in the synthesis of biologically active substances.

48. The role of the liver in the neutralization of foreign compounds (xenobiotics) and endogenous metabolites. Microsomal oxidation of foreign compounds (xenobiotics). Enzymes. The difference from biological oxidation.

49. The role of cytochrome P-450 in the formation of hydroxyl groups in the synthesis of bile acids, steroid hormones, catabolism of a number of substances.

50. Conjugation reactions in the liver. Examples of neutralization of foreign substances and protein putrefaction products in the intestine (phenol, benzene, cresol, scatol, indole).

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. Zurbaran, S. E. Fundamentals of bioorganic chemistry [Zurbaran Zurbaran]: Zurbaran Zurbaran / 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.

APPENDIXES

APPENDIX 1

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	

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, bronchiectasis 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

Clinical and diagnostic significance

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 <6 mg/l

Clinical and diagnostic significance

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

Clinical and diagnostic significance

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	Children	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, nephrosis, 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.

APPENDIX 2

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	
The albumin /α1+α2-glibulins coefficient		3.9-6.1	
Timole sample		0-4 S-H units	
C-reactive protein		< 6 mg/l	
Glucose			
	Serum	3.5-5.5 mmol/l	
	Capillary blood	3.3-5.5 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

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
	Unmatured 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
	Matured 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
	Adults	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 κilo 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%
Lactate		0.5-2.2 mmol/l

Urine

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
pH		5.0-6.5
Relative density		1.010–1.025
Glucose		test is negative
Protein		test is negative
Erythrocytes and hemoglobin		test is negative
Ketone bodies		test is negative
Bilirubin		test is negative
Urobilinogen		up to 17.0 μmol/l

Educational edition

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