DANYLO HALYTSKYI
LVIV NATIONAL MEDICAL UNIVERSITY

DEPARTMENT OF BIOLOGICAL CHEMISTRY

LABORATORY MANUAL
ON BIOLOGICAL CHEMISTRY

for students of pharmaceutical faculty

PART II

LVIV – 2019

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THEMATIC PLAN OF PRACTICAL LESSONS IN SEMESTER II

N	Theme of the lesson	Hours
1	General pathways of amino acid metabolism (deamination, transamination, decarboxylation of amino acids). Glutathion and creatine, structure and physiological significance.	2
2	Urea biosynthesis and alternative pathways of ammonia detoxification. Specific metabolic pathways of selected amino acids and their disorders.	2
3	Metabolism of cyclic amino acids. Disorders of cyclic amino acids metabolism.	2
4	Biochemical functions of nucleotides and nucleic acids	2
5	Catabolism of purine and pyrimidine nucleotides. Hereditary disorders of nucleotide metabolism.	2
6	DNA replication and transcription of RNA.Mutations and their types, reparations of damaged DNA.	2
7	Biosynthesis of proteins, initiation, elongation and termination steps. Post translational modification of proteins. Principles of gene engineering and production of transgenic proteins of medical significance.	2
8	Functional role of water soluble vitamins.	2
9	Functional role of fat soluble vitamins.	2
10	Molecular mechanisms of action of hormones of protein and peptide nature, as well biogenic amines upon target cells. Humoral regulation of calcium homeostasis in human body.	2
11	Molecular mechanisms of action of steroid and thyroid hormones upon target cells.	2
12	Biochemistry of blood. Proteins of blood plasma, nonprotein nitrogen containing and nitrogen free components of blood plasma. Acid-base equilibrium of blood and its regulation.	2
13	Coagulation, anticoagulation and fibrinolytic systems of blood	2
14	Biological role and metabolism of hemoglobin. Patobiochemistry of porphiria and jandice	2
15	Detoxification function of liver, microsomal oxidation, role of cytochrome P-450 system and flavine containing monooxygenases. Biotransformation of xenobiotics and endogenous toxins.	2
16	Investigation of water and mineral metabolism	2
17	Renal function. Biochemical composition of human urine in norm and pathology.	2
18	Biochemistry of nervous and muscle tissues. Pathochemistry of psychotic disorders**.**	2
19	Principles of pharmaceutical biochemistry.	2
Total		**38**

THEMATIC PLAN OF LECTURES IN SEMESTER II

N	Theme of the lecture	Hours
1	General pathways of amino acid metabolism (deamination, transamination, decarboxylation of amino acids). Urea biosynthesis and alternative pathways of ammonia detoxification	2
2	Specific metabolic pathways of selected amino acids and their disorders. Glutathion and creatine, structure and physiological significance.	2
3	Biosynthesis and catabolism of purine and pyrimidine nucleotides and its regulation. Hereditary disorders of nucleotide metabolism.	2
4	Biosynthesis of nucleic acids, mechanisms of replication and transcription. Biosynthesis of proteins and their post translational modification.	2
5	Modern classification of hormones and molecular mechanisms of their effects. Hormones of central and peripheral glands	2
6	Role of blood and kidneys in mechanisms of homeostasis of human organism	2
7	Hemoglobin, its physiological significance and metabolism. Biosynthesis of porphyrins, ethiology of different forms of porphyrias. End products of heme catabolism, pathobiochemistry of jaundices.	2
8	Biochemical functions of liver. Role of liver in biotransformation and detoxification of xenobiotics and endogenous toxic substances. Metabolism of drugs	2

Topic № 1. General pathways of amino acid metabolism (deamination, transamination, decarboxylation of amino acids). Glutathion and creatine, structure and physiological significance.

Objective: To learn the general pathways of amino acids metabolism, to make an acquaintance with methods of identification of amino acid metabolites, to interpret obtained results.

Actuality of the theme: Protein metabolism plays the most important role in general whole body metabolism. The competence and understanding of general pathways of amino acids transformations, their metabolic intermediates, determination of activity of enzymes, participating in these processes are criteria for evaluation of protein metabolism.

Specific aims:

- ✓ To determine the aminotransferases activity in blood serum;
- ✓ To learn the method of quantitative determination of creatine in blood serum;
- ✓ To evaluate the obtained results and draw the conclusions on possibilities of their application in clinical medicine.

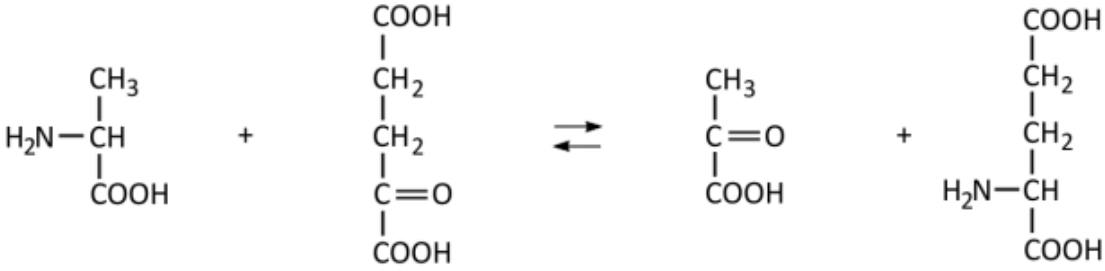
Theoretical questions:

1. Pathways of formation and maintainance of free amino acid pool in human body. General pathways of free amino acid turnover.
2. Transamination of amino acids, substrates for transamination reaction. Mechanism of transamination. Reaction. Aminotransferases, their localization in tissues and organs. Clinical diagnostic significance of determination of aminotransferases activity.
3. Types of reactions of amino acid deamination their final products. Mechanism of oxidative deamination, oxidases of D- and L- amino acids, their enzymatic activity and specificity.
4. Decarboxylation of amino acids, decarboxylases. Production of biogenic amines (GABA, histamine, setrotonin, dopamine). Decarboxylation of amino acids in putrifaction of proteins in intestines. Oxidation of biogenic amines.
5. Glutathion, structure and role in metabolism of organic peroxides.
6. Production of creatine and creatinine, clinical and diagnostic significance of disorders in their metabolism.

Practical part

Experiment 1. Determination of activity of alanine aminotransferase.

Principle. *Alanine aminotransferase* (ALT) catalyses the following reaction:



Pyruvate forms a colored hydrazone after reaction with dinitrophenylhydrazine. In alkaline medium pyruvate hydrazone gives a product of red-brown color, which intensity is proportional to concentration of pyruvate. According to the quantity of formed pyruvate the activity of subsequent amino transferase can be evaluated quantitatively.

Method.

Pipette the solutions into the labelled test tubes according to the table:

	SAMPLE tube 1	**BLANK** tube 2
Substrate mixture	0.5 ml	0.5 ml
Saline	-	0.1 ml
Mix and preincubate at 37 °C for 5 minutes, then introduce:		
Sample (serum)	0.1 ml	-
Mix and incubate at 37 °C for exactly 30 minutes, then introduce:		
0.1% solution of dinitrophenylhydrazine	0.5 ml	0.5 ml
Mix and let stand at the laboratory temperature for 20 minutes, then introduce:		
sodium hydroxide (0.4 M)	5 ml	5 ml
Mix and incubate at the laboratory temperature for 10 minutes. Read the optical density of the sample at 510 nm against the blank.		

Calculate the quantity of pyruvate (in μg) according to the formula:

$$X = D / 0.09 ,$$

where,

X - quantity of pyruvate in μg,

D - Optical density.

The calculation of transaminase activity. 1 unit of alanine aminotransferase is such quantity of enzyme, which produce 1 μg of pyruvate under described conditions. The calculation of enzyme activity to micromoles of pyruvate, formed by 1 ml of serum in one hour is provided according to formula:

$$A = (X \times 2 \times 10) / 88 ,$$

where,

X - quantity of pyruvate in μg,

2 - Coefficient for calculation for an hour of incubation,

10 - Coefficient for calculation for 1 ml of serum,

88 - Molecular weight of pyruvate.

Clinical diagnostic significance. In human body the process of transamination occurs in the liver, hear, skeletal muscles, kidneys and other organs. In blood plasma transaminase activity is very low during nirmal conditions. When a cell membrane is damaged and the integrity of cell is breacked aminotranferase enzymes migrate into the blood. Thus the estimation of aminotranferase activity in blood serum is important in diagnoses, especially in myocardial infarction, viral hepatitis and liver cirrosis.

Considerable increase in ALT activity (10 – 100 times normal values) is observed in cases of viral and toxic hepatitis, blood circulation insufficiency during shock and hypoxia.

Moderate increase of ALT activity occurs during liver cirrhosis, chronic hepatitis during the acute phase, obstructive jaundice, liver swelling during cardiac insufficiency and degenerative processes of the kidneys, lungs, muscles and pancreatic gland.

Considerable increase in AST activity is observed during myocardial infarction, viral hepatitis, toxic liver destruction and blood circulation insufficiency during shock and hypoxia.

Moderate increase of AST activity is detected during liver cirrhosis, obstructive jaundice, liver malignancies, skeletal muscules destruction, pancreatitis, pneumonia and hemolytic anemia.

Normal values in blood serum: ALT - 0.1 - 0.7 μmoles/hr×ml,
AST - 0.1 - 0.45 μmoles/hr×ml.

Experiment 2. Estimation of creatinine according to Jaffe color reaction (Popper et al.).

Principle. After interaction of creatinine and picric acid in alkaline medium a colored compound is formed, the intensity of color is proportional to the concentration of creatinine.

$$\textbf{Creatinine (Yellow)} + \textbf{Picric acid} \xrightarrow{NaOH} \textbf{Creatinine picrate (Orange)}$$

Method.

Pipette the solutions into the labelled test tubes according to the table:

	SAMPLE tube 1	**BLANK** tube 2	**STANDARD** tube 3
Picric acid	6 ml	6 ml	6 ml
Blood serum	2 ml	-	-
standard solution of creatinine	-	-	2 ml
Water	-	2 ml	-
Mix and boil in water bath for 20-30 sec. Eliminate the sediment by filtration, then introduce:			
Supernatant fluid (correspondent to tube)	4 ml	4 ml	4 ml
10% NaOH	0.2 ml	0.2 ml	0.2 ml
Mix and adjast volume to 10 ml and let stand at the laboratory temperature for 10 minutes:			
Read the optical density of the sample at 530 nm against the blank			

Concentration of creatinine is calculated according the formula:

$$X = (D_{exp} / D_{st}) \times 0{,}088 \text{ mMoles/l},$$

where:

D_{exp} - optical descity of tested sample

D_{ST} - optical descity of standard solution

0,088 mMoles/l – concentration of creatinine in standard solution.

Clinical diagnostic significance. Creatinine is an end product of creatine metabolism. Concentration of creatinine in blood plasma is relatively constant and reflects the amount of muscle mass and does not depend from diet or other factors. Creatinine is not

reabsorbed in renal tubules and its clearance value is used for estimation of glomerular filtration in kidneys.

Under normal conditions concentration of creatinine in women – is 0,044 – 0,097 mMoles/l, in men – 0,044 – 0,115 mMoles/l. Increase in creatinine concentration is observed in acute and chronic kidney diseases, a decrease – in the 1-2 trimesters of pregnancy, in lowering of muscle mass due to age or myodystrophic changes.

Examples of Krok-1 tests

1. **Natural peptides can perform various functions. What bioactive peptide is a major antioxidant and fulfills coenzyme functions?**

A. Bradykinin
B. Glutathione
C. Oxytocin
D. Liberin
E. Anserine

2. Indican excretion is a diagnostic criterion of intencified protein putrefaction in the intestine. Name the end product of tryptophan "decay" occurring in the large intestine:

A. Hydrogen sulfide
B. Mercaptan
C. Benzoic acid
D. Putrescine
E. Indole

3. Patients with severe depression demonstrate decreased serotonin levels in brain and cerebrospinal fluid. What amino acid is a serotonin precursor?

A. Tryptophan
B. Threonine
C. Tyrosine
D. Glutamic acid
E. Aspartic acid

4. Structure of proteins includes proteinogenic amino acids. What is the position of the amino group in the structure of these amino acids?

A. ε-position
B. δ-position
C. γ-position
D. β-position
E. α-position

5. A patient with Parkinson's disease exhibits low level of dopamine which is produced from dihydroxyphenylalanine (DOPA). What enzyme catalyzes this conversion?

A. Carboxypeptidase
B. Aminotransferase
C. Decarboxylase
D. Deaminase
E. Hydrolase

Individual students work

1. Clinical diagnostic significance of determination of aminotransferases activity.
2. The synthesis and breakdown of biogenic amines

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
2. Harper′s Illustrated Biochemistry 30th edition / V. W. Rodwell et al.; NY: McGraw-Hill Education, 2015. 817 p.
3. Nelson D.L., Cox M.M. Lehninger Principles of Biochemistry. Fifth edition. NY: W.H. Freeman and Company, 2005. 1010 p.
4. Swanson T. A., Kim S. I., Glucksman M. J. Biochemistry, Molecular Biology, and Genetics 5th edition / Lippincott Williams & Wilkins, 2010. 380 p.
5. Devlin T. M. ed. Textbook of Biochemistry with Clinical Correlations, 7th edition. Hoboken: Wiley-Liss, 2010. 1240 p.
6. Trudy McKee, James R. McKee. Biochemistry. The molecular basis of life. Sixth edition. Oxford University Press, 2015. 928 p.
7. MCQs in biochemistry / A. Ya. Sklyarov et al.: Lviv: Danylo Halytsky Lviv National Medical University Press, 2012. 308 p.

Topic № 2. Urea biosynthesis and alternative pathways of ammonia detoxification. Specific metabolic pathways of selected amino acids and their disorders.

Objective: To interpret the pathways of production, transport and neutralization of ammonia in human body. To learn the methods of determination of urea, phenylpyruvic acid in biological fluids and interpretation of obtained results.

Actuality of the theme: In course of amino acid metabolism are produced metabolites, which can be detected and quantified in blood and urine and may be used in diagnostics and treatment monitoring.

Specific aims:

- ✓ Quantitative determination of urea in biological fluids. Interpretation of obtained results and conclusions;
- ✓ To interpret metabolic pathways of production and neutralization of ammonia, circulatory transport of ammonia, urea biosynthesis;
- ✓ To analyze changes in processes of transport and neutralization of ammonia in hereditary anomalies of enzymes of ammonia turnover;
- ✓ To explain general metabolic pathways of nitrogen free residues of amino acids and peculiarities in transformation of aromatic and heterocyclic amino acids.

Theoretical questions:

1. General metabolic pathways of nitrogen free residues of amino acids in human body. Glucogenic and ketogenic amino acids.
2. Pathways of ammonia production. Toxicity of ammonia and mechanisms of its detoxification. Circulatory transport of ammonia (glutamine, alanine).
3. Biosynthesis of urea: enzymatic reactions, hereditary defects of enzymes involved in urea synthesis (enzymopathias of urea synthesis).
4. Metabolism of sulfur containing amino acids, reactions of methylation.

5. Metabolism of arginine. Biological significance of nitric oxide, NO-synthase.

Practical part

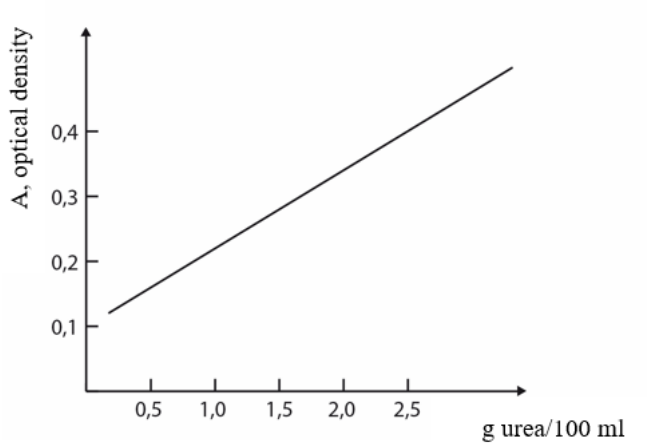
Experiment 1. Determination of urea in urine.

Principle. The method is based on the property of urea to form with paradimethylaminobenzaldehyde in acidic medium complex compound of yellow colour. The intensity of colour is proportional to the concentration of urea in urine and may be determine colorimetrically.

Method.

1. Take a clean dry tube and add there 0.2 ml of urine.
2. Add 1.2 ml of 2% paradimethylaminobenzaldehyde solution.
3. Mix and incubate at the laboratory temperature for 15 minutes.
4. Read the optical density of the sample using a colorimeter with blue lightfilter against water.

Calculate the concentration of urea to calibration curve, prepared for distinct urea concentrations.



Explain the results, draw the conclusion.

Examples of Krok-1 tests

1. Main process of ammonia neutralization occurs in the liver. Arginine decomposition reaction that produces urea as a result is catalyzed with arginase. What group of enzymes does arginase belong to?

A. Synthetases
B. Hydrolases
C. Oxidoreductases
D. Transferases
E. Ligases

2. Disintegration of adenosine nucleotides results in release of ammonia. What enzyme plays the key role in ammonia synthesis from these compounds?

A. Amylase
B. Alanine transaminase
C. Lactate dehydrogenase
D. Alcohol dehydrogenase
E. Adenosine deaminase

3. Catabolism of body's own tissue proteins is intensified during such diseases as thyrotoxicosis and tuberculosis. This process is attended by intensive synthesis in liver and subsequent excretion with urine of the following:

A. Urea
B. Glucose
C. Acetone bodies
D. Fatty acids
E. Nucleotides

4. Fatty degeneration of liver is prevented by lipotropic substances. Which of the following substances relates to them?

A. Cholesterol
B. Methionine
C. Bilirubin
D. Glycine
E. Glucose

5. Examination of a patient revealed an increase in ammonia and citrulline concentration in blood, a decrease in urea concentration in urine as well as citrullinuria. This condition is caused by the deficiency of the following enzyme:

A. Arginine-succinate lyase
B. Glutaminase
C. Ornithine carbamoyl transferase
D. Arginine-succinate synthetase
E. Glutamine synthetase

Individual students work

1. Peculiarities of metabolism of branched chain amino acids, role of vitamin B_{12} in metabolism of amino acids.
2. Special pathways of noncyclic amino acids metabolism.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
2. Harper′s Illustrated Biochemistry 30th edition / V. W. Rodwell et al.; NY: McGraw-Hill Education, 2015. 817 p.
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Topic № 3. Metabolism of cyclic amino acids. Disorders of cyclic amino acids metabolism.

Objective: To study pathways of turnover of of cyclic amino acids. To learn the methods of determination of distinct metabolic intermediates in phenylketonuria, alkaptonuria, albinism.

Actuality of the theme: In course of amino acid metabolism are produced metabolites, which can be detected and quantified in blood and urine and may be used in diagnostics and treatment monitoring.

Specific aims:

- ✓ To explain specific pathways of metabolism of aromatic amino acids phenylalanine and tyrosine.
- ✓ To explain biochemical basis in development and manifestation of genetic anomalies in metabolism of aromatic and heterocyclic amino acids, accumulation of distinct metabolic intermediates in phenylketonuria, alkaptonuria, albinism.
- ✓ To explain specific pathways of turnver of tryptophan.

Theoretical questions:

1. Specific pathways of metabolism of aromatic amino acids phenylalanine and tyrosine, sequence of enzymatic reactions.
2. Hereditary enzymopathias of phenylalanine and tyrosine metabolism – phenylketonuria, alkaptonuria, albinism.
3. Turnver of tryptophan: kinine urenine and serotonin pathways. Hereditary enzymopathias.

Practical part

Experiment 1. Qualitative reaction on phenylpyruvic acid. (Folling test).

Principle. Phenylpyruvic acid forms a complex compound with Fe^{3+} ions , which has a blue-green color.

Method.

1. Take a clean dry tube and place there 2 ml of urine.
2. Add 8-10 droplets of 10% solution of $FeCl_3$.
3. In presence of phenylpyruvic acid a blue-green color appears after 30-60 seconds, which gradually disappear during 5-30 min of standing in dependence from the content of phenylpyruvic acid in urine.

Clinical diagnostic significance. Phenylketonuria (PKU) is an autosomal recessive metabolic genetic disorder characterized by homozygous or compound heterozygous mutations in the gene for the hepatic enzyme phenylalanine hydroxylase (PAH), rendering it nonfunctional. This enzyme is necessary to metabolize the amino acid phenylalanine (Phe) to the amino acid tyrosine (Tyr). When PAH activity is reduced, phenylalanine accumulates and is converted into phenylpyruvate (also known as phenylketone), which can be detected in the urine.

Examples of Krok-1 tests

1. **A man presents with signs of albinism: blonde hair, extreme photosensitivity, impaired vision. What amino acid metabolism is disrupted in the patient?**

A. Valine
B. Histidine
C. Methionine
D. Tyrosine
E. Proline

2. L-DOPA and its derivatives are used in treatment of Parkinson's disease. What amino acid is this substance made of?

A. Glutamate
B. Asparagine
C. Tyrosine
D. Tryptophan
E. Arginine

3. The patient has hypovitaminosis PP. What amino acid taken with meals partially compensates patient's need for vitamin PP?

A. Phenylalanine
B. Tryptophan
C. Valine
D. Arginine
E. Methionine

4. A child with PKU has an unpleasant mouse-like odor, growth retardation, mental retardation. These symptoms are associated with the high concentration of the following substance in blood:

A. Uric acid
B. Adrenaline
C. Cholesterol
D. Glucose
E. Phenylpyruvic acid

5. **A patient with Parkinson's disease exhibits low level of dopamine which is produced from dihydroxyphenylalanine (DOPA). What enzyme catalyzes this conversion?**

A. Deaminase
B. Hydrolase
C. Decarboxylase
D. Aminotransferase
E. Carboxypeptidase

Individual independent students work

1. Hereditary enzymopathias of phenylalanine and tyrosine metabolism.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
2. Harper′s Illustrated Biochemistry 30th edition / V. W. Rodwell et al.; NY: McGraw-Hill Education, 2015. 817 p.
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Topic № 4. Biochemical functions of nucleotides and nucleic acids

Objective: To study chemical structure of nucleotides, structure and functions of nucleic acids. To perform qualitative reactions for detection of nucleoproteins.

Actuality of the theme: DNA is the chemical basis of heredity and may be regardedas the reservebank of genetic information. DNA is exclusivelyresponsiblefor maintaining the identity of different species of organismsover millions of years. Nucleic acids are the polymers of nucleotides (polynucleotides) held by 3' and 5' phosphatebridges.In other words, nucleic acids are builtup by the monomeric units-nucleotides (it maybe recalledthat protein is a polymer of aminoacids).Nucleoproteins are proteins that are associated with nucleic acids. They can serve functional roles as enzymes, for example, telomerase in modifying the nucleic acid, or structural ones, as with histones in packaging chromatin.

Specific objectives:

- ✓ To interpret chemical structure of nucleoprotein compounds, structure and function of nucleic acids, their role in protein biosynthesis.
- ✓ To know methods of isolation of nucleoproteins from tissues and qualitative reactions for detection of their components: a) biuret test for polypeptides, b) Trommer test for sugars (pentoses), c) silver probe for purine bases, d) molybdenum probe for phosphates.

Theoretical questions:

1. Biochemical functions of nucleic acids and nucleotides. Formation of nucleic acid chain from nucleotides.
2. Constituents of nucleotides and nucleosides. Minor nitrogenous bases and nucleotides.
3. Nucleic acids: structure, properties, stages of investigation. Primary structure of nucleic acids, polarity of polynucleotides, specific features of DNA and RNA structure.
4. Structure, properties and biological significance of DNA. Experimental proves of DNA significance in heredity (phenomenon of transformation).
5. Secondary structure of DNA, role of hydrogen bonds in stabilization of secondary structure (Chargaff rules, Watson-Crick model), antiparallelism of chains.
6. Tertiary structure of DNA. Physical-chemical properties of DNA: denaturation and renaturation of DNA.
7. Structure, properties and biological functions of RNA. Types of RNA: mRNA, tRNA, rRNA, snRNA; specific features of structure (secondary and tertiary) of different RNA types.

Practical part

Qualitative reactions on structural components of nucleoproteins.

Principle of the method. For investigation of chemical composition of nucleoproteins yeast cells are a convenient object. After hydrolysis the components of nucleoproteins can be detected in hydrolysate with reactions, which are specific to each constituent of nucleoproteins.

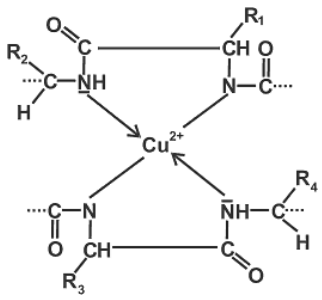
Reagents and materials. 500 mg of fresh yeasts or 100 mg of dried yeasts, 10% solution of sulfuric acid, pipettes, filter paper, water bath.

Preparation of the filtrate.

1. Place 100 mg of dried yeast into a clean, dry tube.
2. Add 4 ml of H_2SO_4 (10%).
3. Place the tube into water bath (100° C) and incubate it for 60 min.
4. Cooland filtrate the liquid.

In the filtrate products of nucleoproteins are detected, i.e: polypeptides, purine and pyrimidine nitrogenous bases, ribose, deoxyribose, phosphoric acid.

Experiment 1. Biuret Test for Protein.



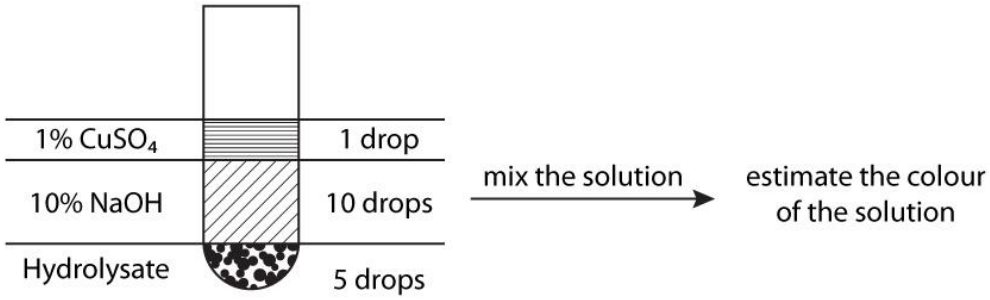
Principle of the method. The biuret test is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a copper (II) ion forms violet-colored coordination complexes in an alkaline solution. The Biuret reagent is made of sodium hydroxide (NaOH) and hydrated copper (II) sulfate, together with potassium sodium tartrate. Potassium sodium tartrate is added to complex to stabilize the cupric ions. Proteins in the alkaline environment reduce Cu^{2+} to Cu^{+}, which forms a coordination complex with proteins, leading to a blue to pink color change.

Method.

Take a clean and dry tube.

1. Introduce 5 drops of hydrolysate (obtained as described previously).
2. Add10 drops of NaOH (10%).Mix the solution carefully.
3. Add 1 drop of $CuSO_4$(1%). Mix the solution carefully.

Estimate the color of the solution. Explain the results. Write conclusions and draw the peptide bond.



The biuret reaction can be used to assess the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide. The intensity of the color, and hence the absorption at 540 nm, is directly proportional to the protein concentration, according to the Beer-Lambert law.

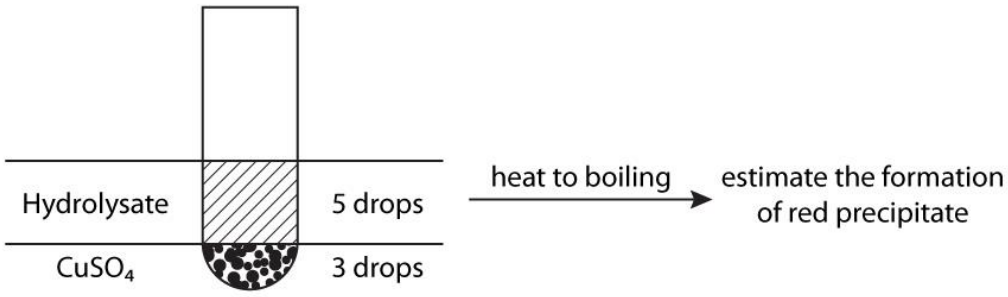
Experiment 2. Trommer's test for ribose and deoxyribose.

Principle of the method. Sugars with free hemiacetal group possess reductive properties and reduce some metal ions with changes in color or other effects. In Trommer test copper ion (blue) is reduced to cuprous ion (orange red) in presence of reductive sugars (ribose, deoxyribose).

Method.

1. Add 3 drops of $CuSO_4$ solution to about 5 drops of hydrolyzate (detected solution of sugar). Mix and then introduce drop by drop $CuSO_4$ solution. Stir incessantly until obtained $Cu(OH)_2$ dissolve.
2. Heat the blue solution to boiling. Red precipitate appears in the presence of reducing sugar. (Trommer's reagent contains copper sulphate with sodium hydroxide).

Draw a conclusion.



Clinical diagnostic significance. Analysis of DNA is a routine investigation in diagnostics of hereditary diseases. It may be used for genotyping of fetal tissue in prenatal diagnostics, as well as in paternity determination.

Derivatives of nitrogenous bases are widely used in modern medicine. Mercaptopurine posses an antileukemic activity, as it serves as purines structural analog and antimetabolite. Fluorouracil and fluorofur also has antitumor activity, as they are transformed into 5-fluoro-2-deoxyuridine 5'-monophosphate, which is a strong inhibitor of thymidylate synthase.

Examples of Krok-1 tests

1. Purine ring biosynthesis occurs in ribose-5-phosphate through gradual accumulation of nitrogen and carbon atoms and closing of the rings. The source of ribose phosphate is the process of:

A. Glycogenolysis
B. Gluconeogenesis
C. Glyconeogenesis
D. Glycolysis
E. Pentose phosphate cycle

2. Primary structure of nucleic acids is a polynucleotide chain that has a certain composition and order of the nucleotides.What bonds stabilize this structure?

A. Peptide
B. 3`,5`-phosphodiester
C. Glycosidic
D. Disulfide
E. Amide

3. Chromatin contains positively charged histone proteins. What amino acid is contained in histone proteins in large amounts?

A. Lysine
B. Alanine
C. Valine
D. Threonine
E. Serine

4. From nitrates, nitrites and nitrosamines in organism is formed nitrous acid which causes oxidative deamination of nitrogene bases of nucleotides. This induce a point mutation by replacement of cytosine to...

A. Uracil
B. Inosine
C. Guanine
D. Thymine
E. Adenine

5. Nowadays about 50 minor bases have been found in the t-RNA structure besides the main four nitrogenous bases. Choose the minor nitrogenous base:

A. Adenine
B. Uracil
C. Dihydrouracil
D. Cysteine
E. Cytosine

Individual independent students work

1. Structure, properties and biological significance of nucleoproteins, phosphoproteins, lipoproteins, glycoproteins
2. Peculiarities in synthesis and in degradation of nucleoproteins, glycoproteins and proteoglycans.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
2. Harper′s Illustrated Biochemistry 30th edition / V. W. Rodwell et al.; NY: McGraw-Hill Education, 2015. 817 p.
3. Nelson D.L., Cox M.M. Lehninger Principles of Biochemistry. Fifth edition. NY: W.H. Freeman and Company, 2005. 1010 p.
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Topic № 5. Catabolism of purine and pyrimidine nucleotides. Hereditary disorders of nucleotide metabolism.

Objective: To learn reactions of synthesis and degradation of purine and pyrimidine nucleotides in normal conditions and in hereditary enzymopathias of this metabolic pathways. To learn the method of determination of uric acid in biological fluids and to interpret the obtained results.

Actuality of the theme: Uric acid is the end product of purinemetabolism in humans. The normalconcentrationof uric acid in the serumof adultsis in the range of 3-7 mg/dl. In women, it is slightly lower (by about 1 mg) than in men. The daily excretion of uric acid is about 500-700 mg. *Hyperuricemia* refers to an elevation in the serum uric acid concentration. This is sometimes associated with increased uric acid excretion (*uricosuria*). Gout is a metabolic disease associated with overproduction of uric acid. At the physiological pH, uric acid is found in a more soluble form assodium urate. In severe hyperuricemia, crystals of sodium urate get deposited in the soft tissues, particularly in the joints. Such deposits are commonly known as tophi. This causes inflammation in the joints result in a painful gouty arthritis. Sodium urate and/or uric acid may also precipitate in kidneys and ureters that results in renal damage and stone formation.

Specific objectives:

- ✓ To analyze the sequence of reactions of metabolism of purine nucleotides, disorders in uric acid metabolism and biochemical principles of gout development.
- ✓ To analyze the sequence of reactions of biosynthesis and catabolism of pyrimidine nucleotides.
- ✓ To conduct quantitative determination of uric acid in biological fluids and to interpret the obtained results.

Theoretical questions:

1. Biosynthesis of purine nucleotides; scheme of reactions of IMP synthesis; synthesis of AMP, GMP, ATP, GTP. Regulation of purine nucleotides synthesis on a principle of feedback inhibition.
2. Biosynthesis of pyrimidine nucleotides: reactions, regulation.
3. Biosynthesis of deoxyribonucleotides. Formation of thymidyl nucleotides; inhibitors of dTMP biosynthesis as antitumor drugs.
4. Catabolism of purine nucleotides; hereditary disorders of uric acid metabolism. Biochemical background of hyperuricemia, gout, Lesch-Nyhan syndrome.
5. Catabolism of pyrimidine nucleotides.

Practical part

Experiment 1. Quantitative determination of uric acid in blood serum.

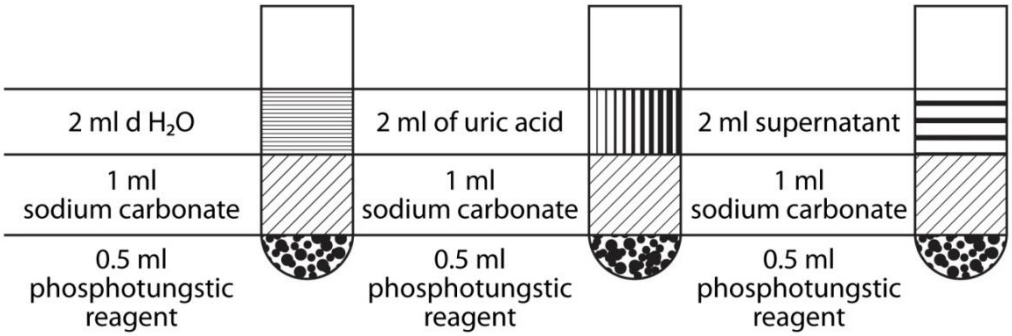
Principle of the method. Uric acid reduces phosphotungsten reagent with formation of blue color compound. The absorbance of light in 640 nm wavelength (optical density) is proportional to the concentration of uric acid in the tested specimen.

Reagents. Blood serum, 10% solution of sodium carbonate, 0.35 M sulfuric acid, phosphotungstic reagent (Folin reagent), 30 μM solution of uric acid.

Method.

1. Transfer0.5 ml of blood serum into centrifuge tube
2. Add 4 ml of water. Mix the solution
3. Add 0.25 ml of 0.35 Mof sulfuric acid and 0.25 ml of 10% solution of sodium dihydrogentungstate.
4. Incubate tubes for 5 min at room temperature
5. Centrifuge samples 10 min at 3000 rpm.
6. Collect a supernatant and determine the concentration of uric acid according to the scheme, as shown in a figure below:

Determination of uric acid in blood serum



After 30 min the optical density of probes is measured in colorimeter on wavelength 640 nm (red filter, 590-700 nm).

Calculation of uric acid concentration is conducted according to the following formula:

$$C = \frac{A_{exp}}{A_{stand}} \text{ x } 30 \text{ x } 10$$

where:

C – concentration of uric acid in a blood serum sample, in mmoles/L

A_{exp} – optical density of the test sample

A_{stand} – optical density of uric acid standard

30 – concentration of uric acid in standard, μmoles/L

10 – dilution of serum sample

Explain the results.

Normal values of uric acid content in blood serum are 240–500 μmoles/L in males and 160–400 μmoles/L in females.

Experiment 2. Quantitative determination of uric acid in urine.

Principle of the method is based on the property of uric acid to reduce phospho tungstic complex anion to a blue color compound. The quantity of phosphotungstic compound is determined by titration with potassium ferricyanide, which oxidizes phosphotungstic complex and decolorize it.

Reagents. Urine, phosphotungstig reagent (Folin reagent), 20% solution of sodium carbonate, 0.01 n solution of potassium ferricyanide ($K_3[Fe(CN)_6]$), stadard solution of uric acid (0.5 mg/ml).

Method.

1. Take two clean, dry flasks. To the first add 1.5 ml of urine (control) and to the second – 1.5 ml of standard solution of uric acid (control).
2. To both flasks add 1 ml of sodium carbonate solution (Na_2CO_3, 20%) and 1 ml of Folin's reagent.
3. Titrate the content of flasks with $K_3[Fe(CN)_6]$ solution (0.01 N) up to disappearance of blue color.

The content of uric acid (in mg) in daily urine is calculated according to formula:

$$x = \frac{0{,}75 \text{ x } B \text{ x } D}{1{,}5 \text{ x } C}$$

where:

0,75 – amount of uric acid in standard, mg;
B – volume of $K_3[Fe(CN)_6]$ used for titration of urine sample, ml;
C – volume of $K_3[Fe(CN)_6]$ used for titration of standard sample, ml;
D – daily diuresis, ml.

Draw a conclusion.

Clinical and diagnostic significance. Normal value of uric acid excretion with urine is 1.60 – 3.54 mmoles/day (270 – 700 mg/day). The normal values of uric acid concentration in blood serum is 0.05–0.06 mg/ml in males and 0.04-0.05 mg/ml in females. The increase of excretion of uric acid is observed in cases of diseases with an enhanced breakdown of nucleoproteins (leukoses, treatment with cytostatic drugs, action of ionizing radiation, combustion, rheumatism, hemolytic anemia, lead intoxication, toxicosis etc). A decrease of uric acid excretion is observed in kidney diseases (nephritis, renal insufficiency), progressive myodystrophie.

An increase in uric acid concentration in blood is called hyperuricemia. It is observed in gout, a disease, caused by hyperproduction of urates and their deposition in tissues, first of all in synovial sheets of joints. Deposition of uric acid is due to its poor solubility in water media. The drug of choice for the treatment of gout is ***allopurinol***. This is a structural analog of hypoxanthine that competitively inhibits the enzyme xanthine oxidase. Further, allopurinol is oxidized to alloxanthine by xanthine oxidase. Alloxanthine, in turn, is a more effective inhibitor of xanthine oxidase. This type of inhibition is referred to as suicide inhibition. Inhibition of xanthine oxidase by allopurinol leads to the accumulation of hypoxanthine and xanthine. These two compounds are more soluble than uric acid, hence easily excreted. Besides the drug therapy, restriction in dietary intake of purines and alcohol is advised. Consumption of plenty of water will also be useful. The anti-inflammatory drug *colchicine* is used for the treatment of gouty arthritis. Other anti-inflammatory drugs-such as *phenylbutazone*, *indomethacin*, *oxyphenbutazone*, *corticosteroids* are also useful.

Examples of Krok-1 tests

1. Purine ring biosynthesis occurs in ribose-5-phosphate through gradual accumulation of nitrogen and carbon atoms and closing of the rings. The source of ribose phosphate is the process of:

A. Pentose phosphate cycle
B. Glycolysis
C. Glyconeogenesis
D. Gluconeogenesis

E. Glycogenolysis

2. An oncological patient was prescribed fluorouracil that is a competitive inhibitor of thymidine synthase. It inhibits the process of:

A. Carbohydrate disintegration
B. Purine nucleotides synthesis
C. Pyrimidine nucleotides synthesis
D. Purine nucleotides disintegration
E. Lipids synthesis

3. Gout develops when purine nucleotide metabolism is disturbed. A doctor prescribed the patient allopurinol that is a competitive inhibitor of:

A. Succinate dehydrogenase
B. Alcohol dehydrogenase
C. Lactate dehydrogenase
D. Xanthine oxidase
E. Hexokinase

4. A 55-year-old man came to a doctor with complaints of acute pain in his big toes. Meat and wine remain permanently in his diet. The doctor suspects gout. What substance must be measured in the patient's blood to confirm this diagnosis?

A. Urea
B. Uric acid
C. Lactate
D. Bilirubin
E. Ketone bodies

5. **A patient undergoes chemotherapy with 5-fluorouracil that is a competitive inhibitor of thymidilate synthase. What process is inhibited by this drug?**

A. Glucose synthesis
B. Purine nucleotides salvage
C. Adenosine triphosphate synthesis
D. Purine nucleotides disintegration
E. Thymidine monophosphate synthesis

Individual independent students work

1. Peculiarities of biosynthesis and degradatyion of purine and pyrimidine nucleotides in health and disease.
2. Role of adenyl nucleotides in regulation of enzymatic activity.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
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Topic № 6. DNA replication and transcription of RNA. Mutations and their types, reparations of damaged DNA

Objective: To learn general principles of nucleic acids synthesis; stages of these processes; mechanisms of mutations and reparations of damaged DNA; development of hereditary diseases. To interpret mechanisms of action of antibiotics and other inhibitors of nucleic acids biosynthesis. To conduct quantitative determination of DNA in biological material.

Actuality of the theme: The biological information flows from DNAto RNA, and from there to proteins. This is thecentral dogma of life. It is ultimatelythe DNA that controls every function of the cellthrough protein synthesis.In the process of nucleic acids biosynthesis may occur various disorders in nucleotide sequence under the action of physical (ionizing and corpuscular irradiations), chemical (mutagens) and biological (viruses) agents. pharmaceutical preparations are widely used in medical practice that can inhibit biosynthesis of nucleic acids in eukaryotes and retard tumor cells proliferation in oncologic patients.

Specific objectives:

- ✓ To conduct quantitative determination of DNA in biological material.
- ✓ To interpret molecular mechanisms of storage and transfer of genetic information, role of enzymatic systems, which provide semiconservative mechanism of DNA replication in prokaryotes and eukaryotes.
- ✓ To explain mechanism of action of enzymatic system of RNA transcription.
- ✓ To interpret biochemical mechanisms of gene recombination, gene amplification, specific features of regulation of gene expression in eukaryotes.
- ✓ To analyze consequences of genomic, chromosomal and gene mutations, mechanisms of action of the most known mutagens, biological significance and mechanisms of DNA reparations (reparation of UV-induced gene mutations).

Theoretical questions:

1. Biological significance of DNA replication. The sense of J.Watson and F.Crick discovery (1953). Semiconservative mechanism of replication, the scheme of Meselsohn's and Stahl's experiment.
2. General scheme of DNA synthesis. Mechanisms of DNA replication in prokaryotes and eukaryotes.
3. General scheme of transcription. Stages and enzymes of RNA synthesisin prokaryotes and eukaryotes.
4. Posttranscriptional modificationsof RNA.
5. Inhibitors of replication and transcription as medical drugs; their mechanisms of action.
6. Regulation of gene expression in prokaryotes. Structure of Lac-operon of *Escherichia coli*: structural and regulatory genes, promotor, operator, regulator; repression and induction of Lac-operon function.

7. Mutations: genomic, chromosomal, gene (point mutations), their significance in appearance of enzymopatias and human hereditary diseases.
8. Biological significance and mechanisms of DNA reparation. Reparation of UV-induced mutations. Xeroderma pigmentosum.

Practical part.

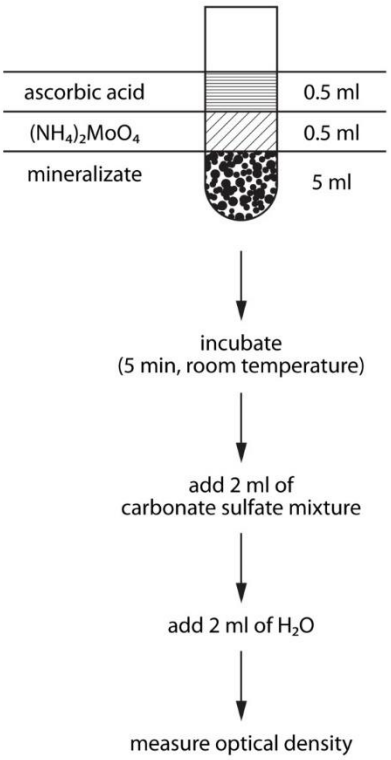
Experiment 1*. *Determination of DNA on basis of phosphorus content in a sample.

Principle of the method. The method consists on determination of phosphorus content as phosphate anion produced after mineralization of DNA specimen with concentrated sulfuric acid. The quantity of phosphorus is determined by ammonium molybdate in the presence of reducer (ascorbic acid). The product of reaction has a blue color; its intensity is proportional to quantity of phosphorus in a sample.

Method.

Isolation of DNA.

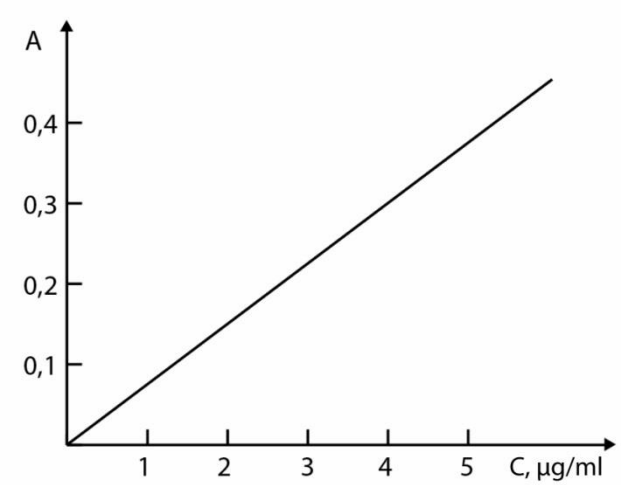
1. The equivalent of no more than 100 μL of liver tissue is placed in a microcentrifuge tube.
2. Add 200 μL of grinding buffer and dounce the tissue using a commercial or homemade dounce.
3. Add 200 μL of mini lysis solution.
4. Place tubes at 65°C for 30 min.
5. Add 60 μL of 5M potassium acetate and mix the solution by inverting.
6. Place tubes on ice for 30 min.
7. Spin tubes at max speed in a microcentrifuge and transfer supernatant to a new tube.
8. Add 1 mL of ice-cold 95-100% ethanol and mix solution by inverting. Let tubes sit for 30 min at room temperature
9. Pellet the DNA by spinning at maximum speed in a microcentrifuge and remove ethanol supernatant
10. Resuspend pellet in 100 μL of 0.5M ammonium acetate (NH_4OAc).
11. Add 400 μ1 of 70% ethanol and mix the tubes by inverting. Let tubes sit at room temperature for 10 min.
12. Pellet the DNA by spinning at maximum speed in a microcentrifuge for 10 min.
13. Pour off ethanol and allow pellet at bottom of tube to air dry for 30 min to one hour.
14. Resuspend the pellet in 50 to 100 μL of dH_2O or TE buffer solution.



Determination of phosphorus.

1. Transfer 5 ml of obtained sample (mineralizate) into clean and dry tube.
2. Add 0.5 ml of $(NH_4)_2MoO_4$(5%)and mix the tubes.
3. Add 0.5 ml of 1% sol of ascorbic acid. Resuspend solution carefully.
4. Incubate tube for five min in room temperature.
5. Add 2 ml of carbonate-sulfite mixture and adjustthe volume to 10 ml with dH_2O.
6. Incubate tube (10 min at room temperature).
7. Measured the optical density (extinction) on a photocolorimeter at a red filter.

8. Determine the content of phosphorus according to calibration curve.



Concentration of DNA in mg% is calculated according to formula:

$$C_{DNA} = a \times 10 \text{ mg\%}$$

where,

a – is a concentration of P in mg/ml.

The normal content of DNA in rat liver is 25–35 mg%.

Explain the results. Write conclusions

Diagnostic and practical application. Determination of DNA content in tumor tissues has some prognostic significance in treatment of patients. More important is investigation of DNA in samples of biological origin (tissue bioptates, blood cells, spermatozoa, sediment obtained from urine, human hair, etc.) by the method of polymerase chain reaction (PCR) in diagnostics of viral, hereditary diseases and in identification of a person (DNA diagnostics). The content of DNA is used also for control the process of purification of subcellular fractions (microsomes, lysosomes, Golgi vesicles etc.).

Examples of Krok-1 tests

1. A number of hereditary diseases is caused by mutations in gene areas that determine beginning or end of an intron. What process results in removal of introns and joining of exons?

A. Splicing
B. Transcription
C. Recombination
D. Replication
E. Translation

2. What enzyme allows for synthesis of various genes from template-RNA to DNA in genetic engineering (this enzyme catalyzes the process discovered in RNA-viruses)?

A. Reverse transcriptase
B. Exonuclease
C. DNA-ligase
D. Helicase
E. Endonuclease

3. Accidental ingestion of death cap mushrooms containing α-amanitin causes intoxication. What enzyme is inhibited with this toxine?

A. RNA polymerase II
B. DNA polymerase
C. DNA synthetase
D. Peptidyl transferase
E. Translocase

4. Primary structure of nucleic acids is a polynucleotide chain that has a certain composition and order of the nucleotides. What bonds stabilize this structure?

A. 3′,5′-phosphodiester
B. Peptide
C. Glycosidic
D. Disulfide
E. Amide

5. Nucleoproteins contain significant amount of alkaline proteins. What proteins carry out structural function in chromatin?

A. Protamines and histones
B. Albumines and globulines
C. Prolamines and glutenins
D. Hemoglobin and myoglobin
E. Interferones and mucin

Individual work of students

1. Modern methods of DNA and RNA investigation, their clinical significance.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
2. Harper′s Illustrated Biochemistry 30th edition / V. W. Rodwell et al.; NY: McGraw-Hill Education, 2015. 817 p.
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Topic № 7. Biosynthesis of proteins, initiation, elongation and termination steps. Post translational modification of proteins. Principles of gene engineering and production of transgenic proteins of medical significance

Objective: To learn general principles of protein synthesis, stages of this process, possible mechanisms of appearance and development of hereditary diseases. To interpret the mechanisms of antibiotics action as well as other inhibitors of protein synthesis. To know principles of gene engineering and gene cloning, its application in modern medicine. To learn principle of polymerase chain reaction (PCR),its applications in diagnostics.

Actuality of the theme: There are wide variations in the cells with respect to the quality and quantity of proteins synthesized. This largely depends on the need and ability of the cells. Erythrocytes lack the machinery for translation, and therefore cannot synthesize proteins. In general, the growing and dividing cells produce larger quantities of proteins. Some of the cells continuously synthesize proteins for export. For instance, liver cells produce albumin and blood clotting factors for export into the blood for circulation. The normal liver cells are very rich in the protein biosynthetic machinery, and thus the liver may be regarded as the protein factory in the human body. Due to gene engineering technology

the production of interferon, human insulin, somatotropin, somatostatin, protein preparations for diagnostics of AIDS is achieved. In particular, in recent years in diagnostics of many diseases and detection of bacilli-cariers is used an special method – polymerase chain reaction.

Specific objectives:

- ✓ To interpret the conception of protein synthesis on the ribosomes.
- ✓ To explain biochemical processes of posttranslational modification of proteins.
- ✓ To explain the influence of physiologically active substances and antibiotics on translation.
- ✓ To explain biochemical and molecular biology principles of methods used in recombinant DNA technologies, recombinant gene technology and formation of hybrid DNA molecules.
- ✓ To explain principles of gene cloning for production of drugs with biotechnologies methods.

Theoretical questions:

1. Features of genetic code; triplet structure, its properties.
2. Protein synthesis on the ribosomes. Components of protein synthesis system.
3. Transfer RNA and amino acid activation, aminoacyl-tRNA synthetase.
4. Stages and mechanisms of translation: initiation, elongation, termination. Initiating and terminating codons of mRNA.
5. Post-translational modification of polypeptide chains. Regulation of translation. Molecular mechanisms of translation control on example of globin synthesis.
6. The influence of biologically active compounds on translation. Antibiotics as inhibitors of transcription and translation in prokaryotes and eukaryotes, their biomedical application.
7. Biochemical mechanisms of antiviral activity of interferon. Block of protein synthesis by diphtheria toxin (ADP-ribosylation of translation factors).
8. Gene engineering or recombinant DNA technology: general principles, biomedical significance. The employment of enzymes. Gene cloning for obtaining of medicinals and diagnostic tools using methods of biotechnology (hormones, enzymes, antibiotics, antigens, interferons etc.).
9. Polymerase chain reaction, its biomedical application in diagnostics of contagious and hereditary diseases, identification of a person (DNA-diagnostics).

Practical part

Experiment № 1. The polymerase chain reaction (PCR).

The polymerase chain reaction (PCR) is an in vitro oligonucleotide primer-mediated enzymatic (thermostable DNA polymerase) amplification of genomic DNA sequences. Obviously, PCR is a cell-free amplification technique for synthesizing multiple identical copies (billions) of any DNA of interest. Developed in 1984 by Karry Mullis (Nobel Prize, 1993), PCR is now considered as a basic tool for the molecular biologist.

Applications:

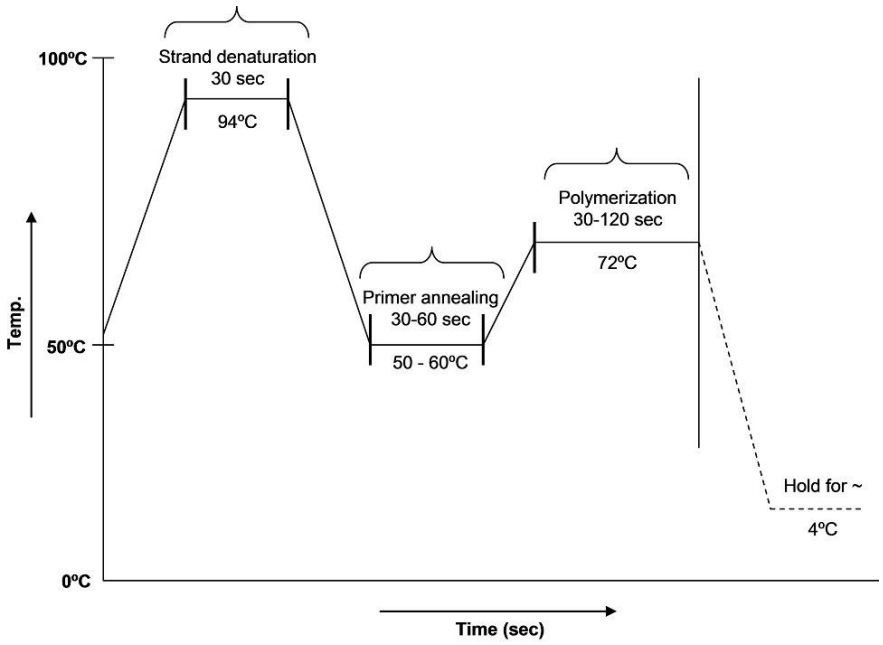
- PCR is widely used in molecular biology andgenetic diseasestudies to identify new genes.

- With its exquisite sensitivity and high selectivity, PCR has been used in wartime human body identification.
- Validation of individual identities in forensic and crime labs from mixed-samples can be performed.
- Environmental and foodpathogenscan be quickly identified and quantitated at high sensitivity.

The PCR process requires a repetitive series of the three fundamental steps that defines one PCR cycle: double-stranded DNA template denaturation, annealing of two oligonucleotide primers to the single-stranded template, and enzymatic extension of the primers to produce copies that can serve as templates in subsequent cycles.

PCR Enzymes:

Use of conventional DNA polymerases in PCR is not practical, because at the high temperature necessary for strand separation, the polymerase is itself irreversibly denatured and becomes inactive. However, DNA polymerase isolated from certain organisms is heat stable because the organisms normally found in hot springs at temperatures well above 90°C, such as is found in Yellowstone National Park. Such organisms are said to be thermophiles. The most widely used heat-stable DNA polymerase is called *Taq*polymerase, because it was originally isolated from the thermophilic bacterium *Thermus aquaticus*. The choice of the DNA polymerase is determined by the aims of the experiment. There are a variety of commercially available enzymes to choose from that differ in their thermal stability, processivity, and fidelity. The most commonly used and most extensively studied enzyme is *Taq* DNA polymerase.



Examples:

PCR Primers:

PCR Primers are short oligo deoxyribonucleotides, or oligomers, that are designed to complement the end sequences of the PCR target amplicon. The procedure need 2 primers named as Forward (F) and Reverse (R).

Unique features:

- These synthetic DNAs are usually 15–25 nucleotides long
- Should have approximately 50–60% GC content
- Special care must be taken to assure that the primer sequences do not form primer-dimer or hairpin loops within them
- The 3′ end of the primer must match the target in order for polymerization to be efficient
- The 5′ end of the primer may have sequences that are not complementary to the target and that may contain restriction sites

Primer design softwares: Primer3, BLAST, Oligo®

Primer Types:

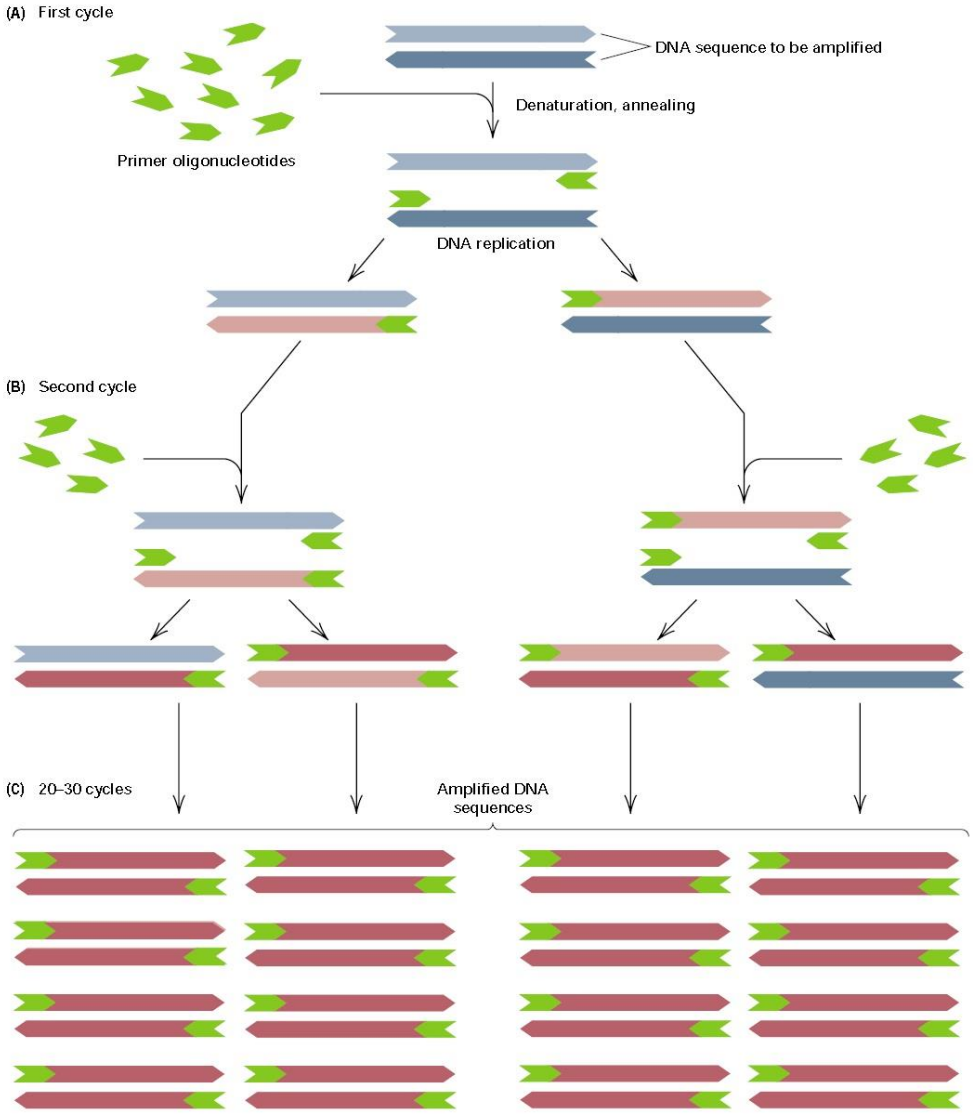
Homopolymer (oligo dT) – used to prime the RNA templates.

Random decamers (10 bases) – used in a technique called RAPDS (randomly amplified polymorphic DNAs), used to produce a diverse array of PCR products that form a fingerprint of a genome.

Other components:

1. **Template DNA:** polymerisation is carried out based on a DNA template which is obtained as genomic DNA or RNA isolated from various tissues.
2. **dNTPs or deoxynucleotide-triphosphate:**dNTP cocktail prepared of equal ratio of A,T,G and C (or supplied as ready-made) are the building blocks of new strands added to the PCR mix in sufficient amount to allow polymerisation to take place.
3. **$MgCl_2$ or bivalent metal ion:**Magnesium chloride ($MgCl_2$) is an essential cofactor for the DNA polymerase used in PCR.
4. **DH_2O or deionised water:** water is used as solvent for the components in a PCR mix. It also dissolves the PCR products.
5. **Polymerase buffer:**a buffer is used to support with best conditions for polymerisation by the enzyme.

Strand denaturation:



DNA strand separation can be performed in in vitro by heating the sample at 94°C. The temperature tolerant polymerase is used for the purpose. At this high temperature evaporation of material is prevented by using oil. 30 sec is optimum for strand denaturatio.

Primer annealing:

The annealing temperature is the value at which the primer pair binds to the template successfully. The annealing temperature is chosen a few degrees below the melting temperatures (within 2–4°C) of the two primers. Lower annealing temperature may allow primer binding but the PCR process loss specificity. Primer annealing may take 30sec to 1 min.

Polymerization: Extension of primer by sequential addition of dNTPs is done by *Taq* polymerase at 72°C. Time spent in extension step can range from 30 sec to 2 min based on the length of the template.

Tm or melting temperature:

Melting temperature, or Tm, is defined for a given DNA duplex as the temperature at which half the molecules are single-stranded and half are double-stranded is called the Tm of the complex. Because of the greater number of H bonds present in higher GC content. Often, GC content alone is used to predict the Tm of the DNA duplex. The generic formula for calculating the Tm is: Tm = 4(G+C) + 2(A+T)°C.

1. 1st cycle of thermal cycling, 3’ end of the newly produced strands becomes longer than actually required amplicon, however soon after 2nd cycle desired length is restored in most of the DNA copies. Longer hybrid copies get outnumbered in the crowd of correct copies.
2. The number of PCR amplification cycles should be optimized with respect to the starting concentration of the target DNA. In general, there are 20 to 35 cycles of PCR is needed to produce optimum quantity of copies for further manipulation.
3. The power of PCR amplification is that the number of copies of the template strand increases in exponential progression: 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, and so forth, doubling with each cycle of replication. For example, starting with a single molecule, 25 rounds of DNA (Fig 3).
4. replication will result in 225 = 3.4 x 107 molecules.

Clinical and diagnostic significance. The laboratory tests based on DNA analysis can specifically diagnose the inherited diseases at the genetic level. DNA-based tests are useful to discover, well in advance, whether the individuals or their off springs are at risk for any genetic disease. Further, such tests can also be employed for the prenatal diagnosis of hereditary disorders, besides identifying the carriers of genetic diseases. Although not in routine use in the laboratory service, methods have been developed or being developed for the analysis of DNA in the diagnosis of several genetic diseases. These include sickle-cell anemia, cystic fibrosis, Duchenne's muscular dystrophy, Huntington's disease, certain cancers (e.g. breast cancer, colon cancer), type ll diabetes, obesity, Parkinson's disease and baldness.

Prenatal diagnosis of inherited diseases: PCR is employed in the prenatal diagnosis of inherited diseases by using chorionic villus samples or cells from amniocentesis. Thus, diseases like sickle-cell anemia, p-thalassemia and phenylketonuria can be detected by PCR in these samples.

Diagnosis of retroviral infections: PCR from cDNA is a valuable tool for diagnosis and monitoring of retroviral infections, e.g., HIV infection.

Diagnosis of bacterial infections: PCR is used for the detection of bacterial infections e.g., tuberculosis by *Mycobacterium tuberculosis*.

Diagnosis of cancers: Several virally-induced cancers (e.g., cervical cancer caused by human papilloma virus) can be detected by PCR. Further, some cancers which occur due to chromosomal translocation (chromosome 14 and 18 in follicular lymphoma) involving known genes are identified by PCR.

Examples of Krok-1 tests

1. A local general practitioner recommends taking interferon for influenza prevention. What is the mechanism of action of this drug?

A. Blocks virus stripping
B. Inhibits virion exit from cells
C. Prevents adsorption of virus in cell receptors
D. Blocks virus protein synthesis
E. Disrupts the process of virus assembly

2. Interferons are natural antiviral and antitumor agents. What is their mechanism of action?

A. Protein synthesis increase
B. Protein synthesis depression
C. Replication activation
D. Transcription activation
E. Repair activation

3. Streptomycin and other aminoglycosides by binding with 30S subunit of ribosome prevents formylmethionyl-tRNA joining. What process is disrupted due to this effect?

A. Translation termination
B. Transcription initiation
C. Translation initiation
D. Transcription termination
E. Replication initiation

4. During long-term carbon tetrachloride poisoning of animals significant activity drop of aminoacyl tRNA synthetase in hepatocytes was detected. What metabolic process is disrupted in this case?

A. DNA replication
B. RNA transcription
C. Post-translational modification of peptides
D. Protein biosynthesis
E. Post-transcriptional modification of RNA

5. Patient who lives on specific geochemical territory has a diagnosis – endemic goiter. What type of posttranslational modification of thyroglobulin is violated in the organism of a patient?

A. Iodation
B. Methylation
C. Acethylation
D. Phosphorylation
E. Glycosylation

Individual work of students

1. Transcription processes in normal and pathological conditions. Programmed cell death-apoptosis. Biochemical mechanisms of apoptosis.
2. Gene engineering. An application of gene engineering methods in modern medicine.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
2. Harper′s Illustrated Biochemistry 30th edition / V. W. Rodwell et al.; NY: McGraw-Hill Education, 2015. 817 p.
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Topic № 8. Functional role of water soluble vitamins

Objective: To learn the structure, principles of classification, functional significance of vitamins, vitaminoids, antivitamins, biologically active supplements. To master methods of qualitative and quantitative determination of water soluble soluble vitamins.

Actuality of the theme: water-soluble vitamins are involved in metabolism as coenzymes and activators of many enzymatic and non-enzymatic processes. Disorders in vitamins turnover reduce intensity of energetic and plastic metabolism, what results in violations of the functions of several organs, decline of immunity to infectious diseases, loss of ability to adopt to unfavorable environmental factors.

Specific objectives:

- ✓ To interpret the role of water soluble soluble vitamins and their precursors as nutritional components in metabolic and physiological processes.
- ✓ To explain application of antivitamins as enzyme inhibitors in contagious diseases and in disorders of homeostasis.
- ✓ To evaluate the role of water soluble soluble vitamins in metabolism, development of hypo- and hyper- vitaminoses, their prevention and treatment.
- ✓ To explain the role of biologically active supplements as nutritional components and their effect in the organism.

Theoretical questions:

1. Vitamins as essential nutritional components. History of vitamins discovery and development of vitaminology.

2. Vitamin B_1 and B_2: structure, biological function, sources, daily requirement. Symptoms of hypovitaminosis.
3. Structure and properties of vitamin H and pantothenic acid. Their envolvement in metabolism, sources, daily requirement. Metabolic significance of CoA.
4. Antianemic vitamins (B_{12}, folic acid): structure, biological function, sources, daily requirement. Symptoms of hypovitaminosis.
5. Vitamins B_6 and PP, structure, biological function, sources, daily requirement. Symptoms of hypovitaminosis.
6. Vitamin C and P: structure, biological function, sources, daily requirement. Functional interrelations between vitamin C and P, manifestations of insufficiency in human organism.
7. Provitamins, antivitamins, mechanism of action and application in practical medicine.
8. Modern vitamin drugs, their application in treatment and prevention of diseases. Biologically active supplements.

Practical part

Experiment 1. Reduction of methylene blue by ascorbic acid (vitamin C).

Principle of the method. Methylene blue is reduced by ascorbic acid to a colorless leuko-form.

Method.

1. Take 2 tubes and add one droplet of 0.01 % sol. of methylene blue and one droplet of 10% sol. of Na2CO3 to each of them.
2. Add to the first tube several drops of the extract of canine rose fruits.
3. Add to the second tube several drops of water.
4. Heat the tubes above fire flame.
5. Observed decoloration of the first tube during heating due to reduction of dye.

Clinical and diagnostic significance. The daily need for ascorbic acid is 80-100 mg. People, living in the conditions of hot or cold climate or working in factories with hot temperature inside have higher demands for vitamin C. Ascorbic acid is essential for the hydroxylation of proline and lysine, which compose collagen. Vitamin C also has outstanding antioxidant properties. The deficiency of vitamin C results in the disease, called scurvy, accompanied by fragility of small vessels, bleeding of gums etc.

Examples of Krok-1 tests

1. Nicotinic acid amide fulfills important metabolic function. What disorder develops, when it is deficient in the organism?

A. Rickets
B. Anemia
C. Xerophthalmia
D. Pellagra
E. Beri-beri

2. A patient suffers from hyperchromic B_{12}-deficiency anemia. What vitamin preparation should be prescribed in this case?

A. Cyanocobalamin
B. Riboflavin
C. Vicasol (Menadione)
D. Thiamine chloride
E. Retinol acetate

3. Avidin - an egg white protein inhibits reception of biotin (carboxylase coenzyme) by the body. What reaction will be blocked by avidin administration?

A. NH_3 attachment to glutamate
B. NH_3 detachment from glutamine
C. CO_2 attachment to pyruvate
D. Detachment of phosphate residuals
E. Beta-oxidation of fatty acids

4. Diet of an individual must contain vitamins. What vitamin is usually prescribed for treatment and prevention of pellagra?

A. Vitamin C
B. Vitamin PP
C. Vitamin A
D. Vitamin B_1
E. Vitamin D

5. A patient demonstrates symmetrical dermatitis on the palms. A doctor made a diagnosis of pellagra. What vitamin deficiency can result in such symptoms?

A. Cobalamin
B. Ascorbic acid
C. Folic acid
D. Cholecalciferol
E. Nicotinic acid

Individual work of students

1. Endogenous hypovitaminoses. Causes and mechanisms of development in diseases of digestive or cardiovascular systems.
2. Biologically active dietary supplements. Their significance in pathology prevention and in well balanced nutrition.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
2. Harper′s Illustrated Biochemistry 30th edition / V. W. Rodwell et al.; NY: McGraw-Hill Education, 2015. 817 p.
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Topic № 9. Functional role of fat soluble vitamins

Objective. To learn the structure and functional significance of fat soluble vitamins, to recognize methods of qualitative and quantitative determination of fat soluble vitamins.

Actuality of the theme: Fat soluble vitamins are involved in many enzymatic and non-enzymatic processes. Disorders in vitamins metabolism, reduces intensity of energetic and plastic metabolism, which is accompanied with violations in function of several organs, decline of immunity to the viral and infectious diseases, loss of organism ability to adapt oneself to the different unfavorable factors or development of specific pathologic conditions, such as rickets, xerophthalmia, infertility and miscarriages, bleeding.

Specific objectives:

- ✓ To interpret the role of fat soluble vitamins as nutritional components in metabolic and physiological processes.
- ✓ To evaluate the role of fat soluble vitamins in metabolism, development of hypo- and hyper- vitaminoses, their prevention and treatment.
- ✓ To explain the role of vitaminoids in metabolic processes.
- ✓ To explain the role of biologically active supplements as nutritional components and their effect in the organism.

Theoretical questions:

1. Vitamins of D group: structure, biological function, nutritional sources, daily requirement. Symptoms of hypo- and hyper-vitaminosis, avitaminosis.
2. Vitamin A: structure, biological function, sources, daily requirement. Symptoms of hypo- and hypervitaminosis.
3. Vitamins E, F: structure, biological role, nutritional sources, mechanism of action, daily requirement. Symptoms of insufficiency, application in medicine.
4. Antihemorrhagic vitamins (K2, K3) and their water soluble forms: structure, biological function, nutritional sources, mechanism of action, daily requirement, symptoms of insufficiency, application in medicine.
5. Provitamins, antivitamins, mechanism of action and application in practical medicine.
6. Vitaminoids: structure and biological activity.
7. Modern vitamin drugs, their application in treatment and prevention of diseases. Biologically active supplements.

Practical part

Experiment 1. Detection of vitamin E with the use of ferric chloride.

Principle of the method. Alcohol solution of α-tocoferol is oxidized by ferric chloride to tocoferylquinone, which is of red color.

Method.

1. Place 4-5 droplets of 0.1% alcohol solution of tocoferol to a dry tube.
2. Add 0.5 ml of 1% solution of ferric chloride.
3. Mix intensively the content of the tube.
4. Heat the tube on a flame till the change of the colour.

Clinical and diagnostic significance. Due to the modern outlook, the main function of tocopherols is to serve as antioxidants. Hydrogen atom in the molecule of α-tocopherol

cooperates with the peroxide radicals of lipids, reducing them into hydroperoxides and thus breaks the chain reaction of peroxidation.

Vitamin E is also involved in the regulation of reproductive functions in males and females.

Daily need of vitamin E is 20 – 30 mg, its concentration in blood is 3,500 – 8,000 nmole/l.

Examples of Krok-1 tests

1. An ophthalmologist has detected increased time of dark adaptation in a patient. What vitamin deficiency can result in such symptom?

A. C
B. K
C. A
D. B_1
E. B_6

2. A patient consulted an ophthalmologist about deterioration of twilight vision and xerophthalmus. What drug should the doctor prescribe?

A. Pyridoxine
B. Retinol
C. Tocopherol
D. Ascorbic acid
E. Cocarboxylase

3. Increased concentration of active oxygen forms is a mechanism of pathogenesis in a number of diseases. To prevent this process, antioxidants are prescribed. Select an antioxidant from the list below:

A. Glucose
B. Calciferol
C. Cobalamine
D. Glicerol
E. Alpha-tocopherol

4. A woman noticed that a cut on her skin was still bleeding even after 20 minutes had passed. What vitamin deficiency causes such condition?

A. Vitamin A
B. Vitamin D
C. Vitamin E
D. Vitamin K
E. Vitamin B_{12}

5. A patient suffers from mucosal dryness and mesopic vision disorder. What vitamin deficiency causes these symptoms?

A. A
B. P
C. E
D. C
E. D

Individual work of students

1. Vitamin-like substances. Their significance in pathology prevention and in well balanced nutrition.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.

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Topic № 10. Molecular mechanisms of action of hormones of protein and peptide nature, as well biogenic amines upon target cells. Humoral regulation of calcium homeostasis in human body.

Objective: To learn biochemical and physiological function of hormones in a system of intercellular integration in the organism. To learn structure of hormones of protein and amino acid nature, derivatives of amino acids and steroid hormones, mechanisms of their action upon the target cells, role of secondary messengers in cell response to the hormones of protein or amino acid nature.

Actuality of the theme: Hormones are conventionally defined as organic substances, produced in small amounts by specific tissues (endocrine glands), secreted into the blood stream to control the metabolic and biological activities in the target cells. Hormones may be regarded as the chemical messengers involved in the transmission of information from one tissue to another and from cell to cell.

Understanding of biochemical mechanisms of realization of hormones effect on functioning of cell systems allows explaining mechanisms of development of pathological states, caused by disorders in functioning of endocrine glands and target cells as well as it also forms in students considerations about the correction of hypo- or hyperfunction of endocrine glands.

Specific objectives:

- ✓ To interpret the biochemical and physiological functions of hormones and bioregulators in a system of intercellular integration of vital functions of human organism.
- ✓ To analyse and to explain correspondence between the structure of protein and peptidehormones to their function and mechanism of action on target cells.
- ✓ To interpret the molecular mechanisms of action of hormones of protein and peptide nature, derivatives of amino acids (catecholamines) up on target cells with involvement of signaling mediator molecules.
- ✓ To interpret the molecular mechanisms of direct regulatory effect of steroid hormones on the genome of targets cells.

Theoretical questions:

1. Hormones in a system of intercellular integration of physiological functions in human organism. Classification of hormones.
2. Mechanisms of hormonal action – amino acid derivatives, peptide and protein hormones, steroid hormones. Regulatory sites in DNA, which interacts with hormone-receptor complexes.Messenger function of cyclic nucleotides, Ca/calmodulin system phosphoinositides. Serine, threonine and tyrosine proteinkinases in effectory response of the cell.
3. Hormones of hypothalamus. Mechanism of their action.
4. Tropic hormones of the anterior pituitary:
   - Group "growth hormone (somatropin) - prolactin - Chorionic somatomamotropin"; pathological processes associated with impaired growth hormone, somatomedin, prolactin;
   - a group of glycoproteins – pituitary trophic hormones (TSH, gonadotropins, FSH, LH), chorionic gonadotropin;
   - POMK hormones– processing products of POMK (adrenokortykotropin, lipotropin, endorphins).
5. Hormones of the posterior pituitary: vasopressin (antidiuretic hormone) and oxytocin. Mechanism of action. The use of oxytocin in medical practice.
6. Characteristics of pancreatic hormones:
   - endocrine function of the pancreas (insulin, glucagon, somatostatin, pancreatic polypeptide);
   - insulin – structure, biosynthesis and secretion; characterization of insulin receptors, molecular mechanisms of action (effect on metabolism of carbohydrates, lipids, amino acids and proteins);
   - glucagon – the chemical nature and the biological effect of the hormone;
7. Catecholamines: epinephrine, norepinephrine, dopamine. Chemical nature, biological effect, receptors. Their role in the stress response.
8. The mechanism of action of parathyroid hormones and calcitonin. Parathyroid hormone – structure, mechanism of action. Calcitriol: biosynthesis; effects on the absorption of Ca2 + and phosphate in the intestine. Calcitonin - structure, effect on calcium and phosphate.
9. Clinical and biochemical characteristics of disorders of calcium homeostasis (rickets, osteoporosis). The distribution of Ca^{2+} in the body; molecular forms of calcium in the blood plasma. The role of bone, small intestine and kidney in calcium homeostasis.

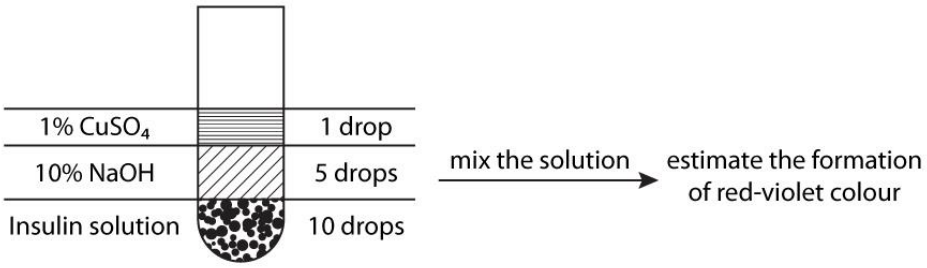
Practical part

Experiment 1.Qualitative reactions of insulin.

Insulin is a polypeptide hormone produced by the B-cells of islets of Langerhans of pancreas. It has profound influence on the metabolism of carbohydrate, fat and protein. Insulin is considered as anabolic hormone, as it promotes the synthesis of glycogen, triacylglycerols and proteins. This hormone has been implicated in the development of diabetes mellitus. Insulin gives all characteristic reactions of proteins.

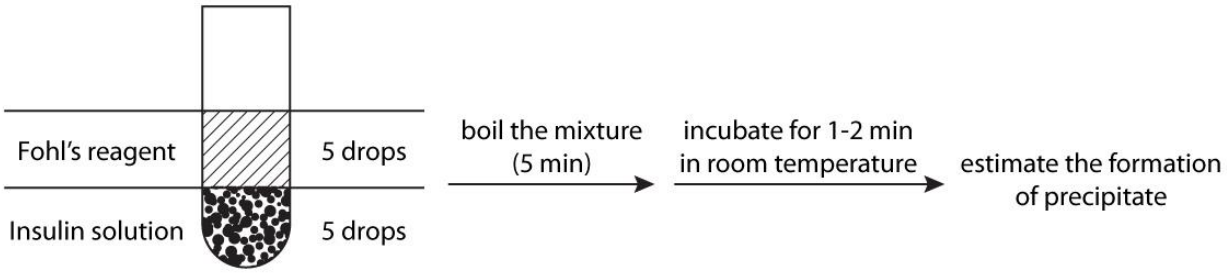
Biuret probe.

1. Into a clean, dry tube add 10 droplets of insulin solution.
2. Add 5 drops of 10 % solution of NaOH and one drop of $CuSO_4$ solution. Mix the tube carefully.
   A red-violet color appears which is characteristic for proteins and peptides.



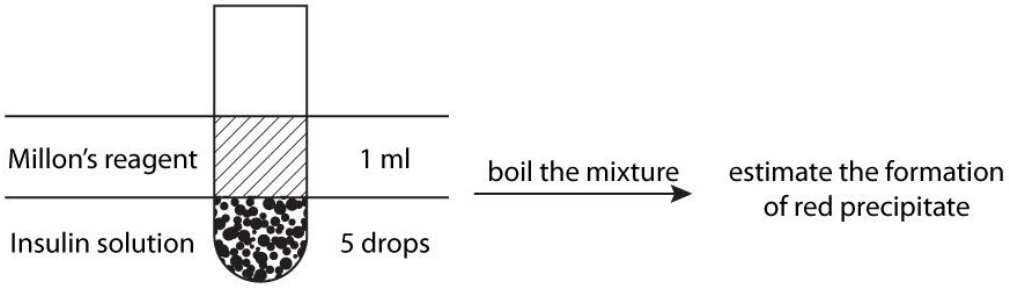
Fohl's reaction.

1. Into a clean, dry tube add 5 droplets of insulin solution.
2. Add 5 droplets of Fohl's reagent (alkaline solution of lead acetate).
3. Boil the mixture 5 min. Incubate the solution for 1-2 min at room temperature. Observe the formation of precipitate.



Millon's reaction.

1. Into a clean, dry tube add 5 droplets of insulin solution.
2. Add 1-2 ml of Millon's reagent.
3. Carefully heat the mixture on a flame. Observe the formation of red precipitate. Explain the results.



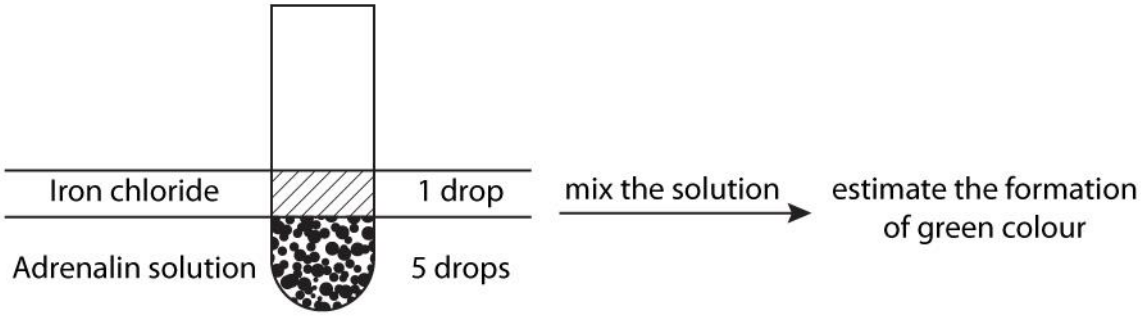
Experiment 2. Qualitative reactions of adrenalin.

2.1. Principle of the method. Adrenalin is readily oxidized by air oxygen to adrenochrome which gives a characteristic green color with ferric chloride or red color with diazotisized sulfanilic acid (diazoreagent).

Reaction with iron chloride.

Method.

1. Into a clean, dry tube add 5 droplets of adrenalin solution.
2. Add 1 droplet of iron chloride solution. Green color appears, which after addition of ammonia changes to red, and thereafter - to brown.

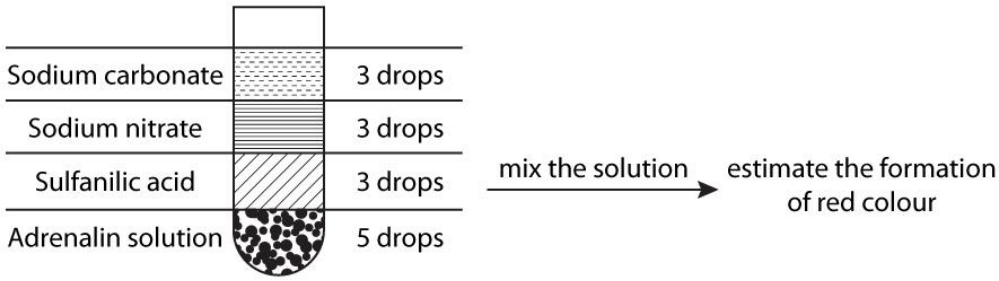


Diazoreaction on adrenalin.

Method.

1. Into a clean, dry tube add 5 droplets of adrenalin solution.
2. Add 3 drops of 1% solution of sulfanilic acid and 3 droplets of sodium nitrite.
3. Add 3 droplets of 10 % solution of sodium carbonate. Mix the solution. Observe the formation of red color.

   Explain the results



Clinical and diagnostic significance. The investigation of hormones and mediators metabolism has an important significance in diagnostics of endocrine disorders, as well as for evaluation of body status in different pathological conditions, connected with alteration of function of central and vegetative nerve systems, heart, liver, kidneys and other parenchimatous organs.

Whatever disorders in a system hypothalamus-hypophysis-cortical adrenals lead to a change in corticosteroid secretion.

In interpretation of results it is useful to remember, that adrenalin secretion in females and males is almost equal, exception is in children in age of 12-15 years (in boys excretion is higher than in girls) and in 41-50 years old persons (in men higher then in women).

Secretion of noradrenalin is equal in boys and girls up to 8-11 years of life, but thereafter its secretion in women is higher, then in men.The secretion of adrenalin and

noradrenalin is not constant during the day. In day time it is respectively 7.5- and 30.9 mg/min, in night it corresponds to 1.9 and 11.3 mg/min.

Smoking, physical load, emotional stress cause increase in excretion of catecholamines in urine. Enhanced catecholamines excretion is observed in liver cirrhosis, acute phase of ulcer disease of stomach and duodenum, Disorders in excretion have significance in pathogenesis of uremia.

Examples of Krok-1 tests

1. Parents of a 10-year-old child have made an appointment with an endocrinologist due to complaints of the child's low height. The child's appearance is corresponding with that of a 5-year old. What hormone causes such changes in physical development, if its secretion is disturbed?

A. Adrenocorticotropic hormone
B. Somatotropic hormone
C. Thyroxin
D. Testosterone
E. Insulin

2. A patient has been receiving Theophylline (inhibitor of cyclic adenosine monophosphate phosphodiesterase) for a week. What hormone can increase its action due to such treatment and cause hyperglycemia?

A. Testosterone
B. Aldosterone
C. Insulin
D. Glucagon
E. Estradiol

3. During routine preventive examination the local pediatrician noticed a boy of short stature. Mental development of the child corresponds with his age. What endocrine disorder is it?

A. Cretinism
B. Acromegalia
C. Pituitary nanism
D. Gigantism
E. Rickets

4. Information transfer from peptide hormones to intracellular second messengers occurs involving adenylate cyclase. What reaction is catalyzed by adenylate cyclase?

A. ATP breakdown into ADP and inorganic phosphate
B. ATP synthesis from adenosine monophosphate and pyrophosphate
C. ADP breakdown with adenosine monophosphate and inorganic phosphate production
D. ATP breakdown into adenosine monophosphate and pyrophosphate
E. Cyclic adenosine monophosphate production

5. A parturient woman diagnosed with uterine inertia has been delivered to the maternity ward. The doctor gave her an injection of the drug that activates the contraction of smooth muscles of the uterus. What hormone is a component of this drug?

A. Gastrin
B. Secretin

C. Angiotensin
D. Oxytocin
E. Bradykinin

Individual work of students

1. The role of hormone-receptor interaction in the development of hormonal effect.

References:

1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
2. Harper′s Illustrated Biochemistry 30th edition / V. W. Rodwell et al.; NY: McGraw-Hill Education, 2015. 817 p.
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Topic № 11. Molecular mechanisms of action of steroid and thyroid hormones upon target cells.

Objective: To analyze changes in carbohydrate, lipid and protein metabolism which occur in endocrine glands dysfunction and to interpret prognostic significance of distinct disorders.. To know biochemical mechanisms of pathological processes development and typical manifestations in endocrine diseases, with special attention to thyroid gland dysfunction.

Actuality of the theme: Hormones play an important role in mechanisms of homeostasis. These substances regulate the activity of enzymes in cells, influence the expression of cell genome and change the intensity of metabolism in target cell and in the body as well. The knowledge of mechanisms of the neurohumoral regulation of metabolism gives basis for diagnostics and rational therapy at endocrinological disorders.

Specific objectives:

- ✓ To analyse metabolic changes and biochemical indexes, which characterise the carbohydrate metabolism, metabolism of proteins and lipids in disorders of endocrine glands and to summarise the prognostic estimation of these disorders.
- ✓ To interpret the mechanisms of hormonal regulation of calcium homeostasis: distribution of calcium in the body, fractions of calcium in human blood plasma, the role of bone tissue, small intestine and kidneys in support of calcium homeostasis.

- ✓ To explain the biochemical mechanisms of development and manifestation of pathological processes and typical disorders of endocrine system.

Theoretical questions:

1. The mechanism of action of thyroid hormones thyroid and steroid hormones (cytosolic and nuclear receptors).
2. Thyroid hormones:
   - Structure and biosynthesis of thyroid hormones;
   - biological effects of T4 and T3;
   - thyroid pathologies; disorders of metabolism under conditions of hyper- and hypothyroidism;
   - endemic goiter and its prevention.
3. Steroid hormones: nomenclature, classification. Biosynthesis of steroid hormones from cholesterol.
4. Steroid hormones of the adrenal cortex (C21-steroids):
   - structure, physiological and biochemical effects of glucocorticoids (cortisol, corticosterone), the role of cortisol in the regulation of metabolism (carbohydrates, proteins, lipids);
   - biochemical basis of glucocorticoid anti-inflammatory properties;
   - structure, physiological and biochemical effects of mineralokortykoids (aldosterone); role of aldosterone in regulation of water-salt metabolism;
   - Cushing's disease, Adison’s disease (bronze), aldosteronism, Kron's disease.
5. Steroid hormones of gonads:
   - female hormones: estrogen - estradiol, estrone (C18-steroids), progesterone (C21-steroids); physiological and biochemical effects; connection with the phases of the menstrual cycle; regulation of synthesis and secretion;
   - male sex hormones (androgens) - testosterone, dihydrotestosterone (S19-steroids); physiological and biochemical effects, regulation of synthesis and secretion;
6. General characteristics of hormone-like substances. Biochemical basis of hormonal regulation of digestion. Gastrin. Cholecystokinin. Secretin.
7. Biogenic amines with hormonal and neurotransmitter properties: structure, biosynthesis, physiological effects, biochemical mechanisms of action (serotonin, melatonin, histamine). Receptors of biogenic amines; receptor action of drugs, receptors of histamine antagonists.
8. Eicosanoids: general characteristics; nomenclature (prostaglandins, prostacyclines, thromboxanes, leukotrienes):
   - biosynthesis of prostaglandins and thromboxane; cyclooxygenase, peroxidase, their biological and pharmacological properties;
   - biosynthesis of leukotrienes andtheir biological properties; 5-lipoxygenase;
   - clinical application of eicosanoids, aspirin and other nonsteroidal anti-inflammatory drugs as inhibitors of prostaglandin synthesis.

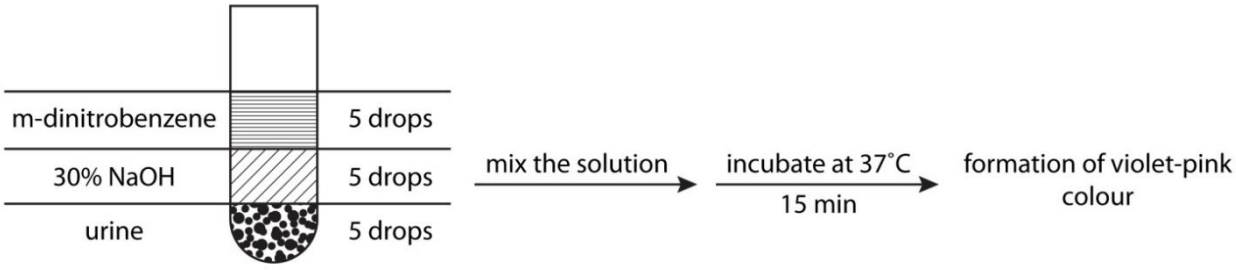
Practical part

Experiment 1. Determination of 17-ketosteroids in urine.

Principle of the method. 17-ketosteroids after reaction with m-dinitrobenzene in strong alkaline medium give a condensation product with a violet-pink color (maximum of absorption at 530 nm). The intensity of color is proportional to a quantity of 17-ketosteroids in urine.

Method.

1. Into a clean, dry tube add 5 drops of urine and 5 drops of 30 % of NaOH solution (caution, hazard!).
2. Add 5 drops of m-dinitrobensene. Mix the solution.
3. Put the test tubes into the incubator (37 ˚C) for 15 minutes.
4. Observe the formation of violet-pink colored solution.
5. Write a conclusion. Explain the results.



Clinical significance. Maximal excretion of 17-ketosteroids is observed at 25 years age, thereafter the quantity of excreted steroids diminishes. During stress situation quantity of 17-ketosteroids in blood and urine is increased. In pathology the content of ketosteroids is changed depending from the function of suprarenal glands. At hypofunction of suprarenal glands (Addison's disease) excretion of 17-ketosteroids consists of 20-30 % of normal value. The excretion of ketosteroids is also decreased at hyperthyreosis, hepatic cyrrhosis, suprarenal gland tumors. 17-ketosteroids are excreted mainly in conjugated form (with sulfuric or glucuronic acid). Simultaneous determination of free and conjugated 17-ketosteroids in urine permits more precisely evaluate functional state of suprarenal glands.

Normal values (17-ketosteroids excretion per 24 hours):

- men – 12–18 mg/24 h
- women – 7–14 mg/24 h

The urine contains mainly two steroids-17-hydroxysteroids and 17-ketosterio – derived from the metabolism of glucocorticoids and mineralocorticoids. Androgens synthesized by gonads also contribute to the formation of17-ketosteroids.

Addison's disease: impairment in adrenocortical function results in Addison's disease. This disorder is characterized by decreased blood glucose level (hypoglycemia, loss of weight, loss of appetite (anorexia), muscle weakness, impaired cardiac function, low blood pressure, decreased Na^+ and increased K^+ level in serum, increased susceptibility to stress etc.

Cushing's syndrome: hyperfunction of adrenal cortex may be due to long term pharmacological use of steroids or tumor of adrenal cortex or tumor of pituitary. Cushing's syndrome is characterized by hyperglycemia (due to increased gluconeogenesis), fatigue,

muscle wasting, edema, osteoporosis, negative nitrogen balance, hypertension, moon-face etc.

Experiment 2. Qualitative reaction on iodine in the solution of thyroxin.

Principle of the method. Degradation of thyroxin molecules leads to potassium iodide formation. The released Iodine gives a blue color with starch.

$$5\ KI + KIO_3 + 3\ H_2SO_4 \rightarrow 3\ I_2 + 3\ K_2SO_4 + 3\ H_2O$$

$$I_2 + \text{starch} \rightarrow \text{blue colour}$$

Materials and reagents: thyroxin, 10% solution of H_2SO_4, starch.

Method:

1. Take a clean, dry tube and add 25 drops of thyroxin hydrolysate.
2. Add H_2SO_4 (10 %) mix the solution and add KIO_3 (10 %).
3. Add 5 drops of starch. Observe the formation of blue colore.
4. Write a conclusion. Explain the results.

Clinical significance. Among the endocrine glands,thyroid is the most susceptible forhypo- or hyperfunction. Three abnormalities associated with thyroid functions are known.

Goiter: Any abnormal increasein the size of the thyroid gland is known as goiter. Enlargement ofthyroid gland is mostly to compensatet he decreased synthesis of thyroid hormones and is associated with elevated TSH. Goiter is primarily due to a failure in the autoregulation of T3 and T4 synthesis. This may be caused by deficiency of excessof iodide.

Goitrogenic substances (goitrogens): These are the substances that interfere with the production of thyroid hormones. These include thiocyanates, nitrates and perchlorates and the drugs such as thiourea, thiouracilt, thiocarbamid.

Simple endemic goiter : This is due to iodine deficiency in the diet. lt is mostly found in the geographical regions a way from sea coast where the water and soil are low in iodine content. Consumption of iodized salt is advocated to overcome the problem of endemic goiter. In certain cases, administration of thyroid hormone is also employed.

Examples of Krok-1 tests

1. Eicosanoids synthesis begins with freeing polyene acids from membrane phospholipids by means of a specific phospholipase. Name this enzyme:

A. Cyclooxygenase
B. Phospholipase C
C. Phospholipase A_2
D. Protein kinase
E. Arginase

2. A patient complains of tachycardia, insomnia, weight loss, irritability, sweating. Objectively: the patient has goiter and slight exophthalmos. What gland is affected, and what functional disorder is it?

A. Hypothyroidism
B. Hyperthyroidism
C. Hyperparathyroidism
D. Hypoparathyroidism
E. Adrenomedullary hyperfunction

3. A patient with hyperproduction of thyroid hormones has been prescribed Merkazolilum. This drug inhibits the following enzyme participating in iodothyronine synthesis:

A. Aromatase
B. Reductase
C. Iodide peroxidase
D. Decarboxylase
E. Aminotransferase

4. A 48-year-old patient has been intravenously administered prednisolone solution to arrest severe attack of bronchial asthma. What group of hormonal agents does prednisolone belong to?

A. Gestagenic drugs
B. Estrogenic drugs
C. Mineralocorticoid
D. Glucocorticoids
E. Anabolic steroids

5. In response to the administration of protein drugs, a patient developed an allergic reaction. The development of the allergic reaction is caused by the increased synthesis of the following compound:

A. Histamine
B. Choline
C. Adrenaline
D. Histidine
E. Serotonin

Individual work of students

1. Transformations of arachidonic acid in human organism and the influence of products on metabolic processes.

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1. Satyanarayana U., Chakrapani U. Biochemistry. Fifth edition, N.Delhy: Elsevier, co-published with Book and Allied, 2017. 788 p.
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Topic № 12. Biochemistry of blood. Proteins of blood plasma, nonprotein nitrogen containing and nitrogen free components of blood plasma. Acid-base equilibrium of blood and its regulation.

Objective. To analyze biochemical composition of blood and to explain the role of blood plasma proteins, to analyze changes in the composition of the end products of blood metabolism. To know about nonprotein nitrogen containing blood compounds, which represents rest nitrogen; to learn methods of determination of residual nitrogen in blood.

Actuality of the theme: In blood plasma over 100 proteins was found out and described which differ on their physical, chemical and functional properties. Plasma proteins determine properties of plasma: colloid-osmotic pressure and permanent blood volume, viscidity of blood, support of permanent blood pH value and functions, namely: protective, regulatory, termoregulatory, respiratory, trophic etc.

Rest nitrogen is an important indicator of the state of protein's metabolism. The detection of nonprotein nitrogen is conducted with the purpose of diagnostics of disorders of kidneys and liver, endogenous intoxication at the row of diseases and estimation of kidney insufficiency degree.

Specific objectives:

- ✓ To analyze the state of health on the basis of biochemical indexes of the intermediates and end products of metabolism in the blood.
- ✓ To explain biochemical basis of the functioning of systems which regulate blood pressure (kallikrein-kinin system and renin-angiotensin system).
- ✓ To analyze biochemical composition of blood plasma proteins, non-protein nitrogen compounds (rest nitrogen), nitrogen-free organic components in normal conditions and during pathologies.
- ✓ To interpret obtained results and their application in diagnostic purposes.
- ✓ To explain mechanism of oxygen and CO_2 transport by hemoglobin in lung and tissue capillaries.

Theoretical questions:

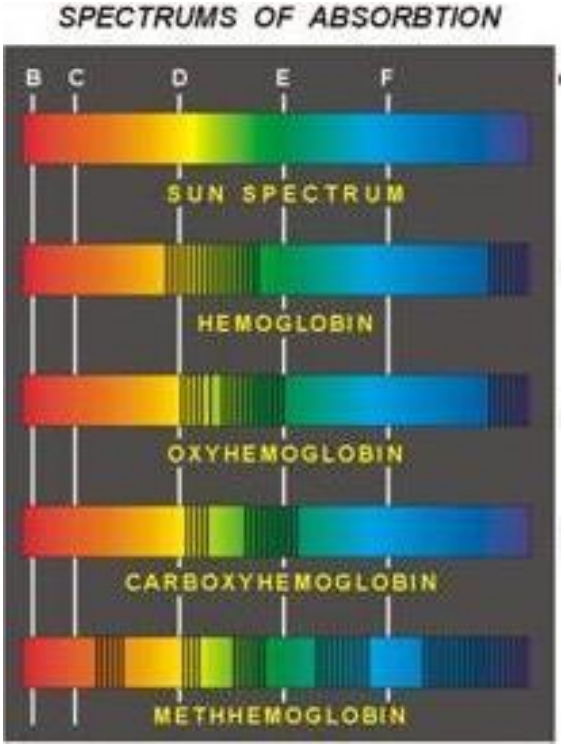
1. Principal groups of blood proteins, their composition and content in normal conditions and in pathology.
2. Albumins and globulins. Resolution of blood plasma proteins by method of protein electrophoresis.
3. Glycoproteins: their structure, biological role, changes in diseases.
4. Proteins of acute phase of inflammation: C-reactive protein (CRP), ceruloplasmin, haptoglobin, cryoglobulin, alpha-1 antitrypsin, alpha-2 macroglobulin, interferon, fibronectin, their diagnostic validity.
5. Enzymes of blood plasma: genuine (secretory), excretory, indicatory (tissue) enzymes. Kallicrein-kinine and renin-angiotensin systems, their biological significance.
6. Diagnostic value of investigation of enzyme and isoenzyme activity in blood plasma: creatine kinase (CK), LDH, AST, ALT, amylase, lipase, cholinesterase.
7. Definition of total and rest nitrogen in blood. Nonprotein nitrogen containing compounds of blood, their diagnostic significance.
8. Nitrogenemia, its kinds and causes of development, differentiation in clinical conditions.

9. Hemoglobin: derivatives, mechanisms of the transport of oxygen and carbon dioxide. Pathological types of hemoglobin in humans.
10. Acid-base equilibrium of blood. Regulation of pH in biological fluids, disorders of acid-base equilibrium: metabolic and respiratory acidosis, metabolic and respiratory alkalosis, mechanisms of their development.
11. Buffer systems of blood, their types, role of different buffer systems in providement of constant pH of blood.

Practical part

Experiment 1. Detection of oxyhemoglobin (Fe^{2+}).

Principle of the method (for experiments 1,2). For obtaining of hemoglobin derivatives (oxyhemoglobin, carbhemoglobin, carboxyhemoglobin, methemoglobin) the following properties are used: HbO_2 is not stable substance, MetHb is produced under the influence of oxidants, HbCO is very stable substance. These derivatives are identified by spectral analysis. The blood pigments selectively absorb the light at distinct wavelength giving a characteristic spectrum, what may be supervised using a special device for spectral analysis - spectroscope.



Method.

1. Add 1.0 ml of defibrinated blood into graduated cylinder and dilute to 100 ml with distilled water.
2. Note the color of the solution.
3. Place the tube with 10 ml of HbO_2 solution against the slit of the spectroscope.
4. Observe two distinct dark bands of light absorption in yellow-green part of spectrum.

Divide HbO_2 solution between 5 tubes in a quotes of about 10 ml for obtaining other derivatives.

Experiment 2. Detection of hemoglobin (Hb)Fe^{2+}.

Method.

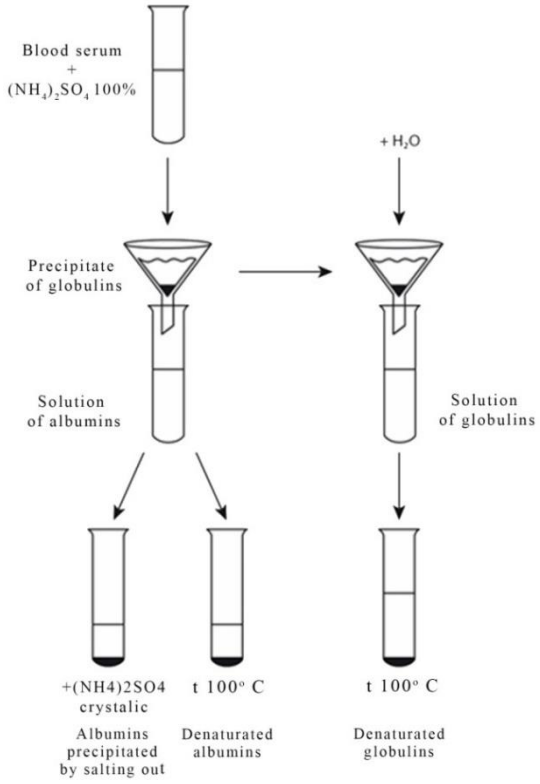
1. Add 2-3 pieces of sodium dithionite $Na_2S_2O_4$ to HbO_2 solution in the second tube and carefully mix.
2. Register the change of the color and using the spectroscope examine the absorption spectrum.
3. Observe a broad absorbtion band between Fraungoffer's lines D and E characteristic for deoxy-Hb.
4. Carefully shake the tube with Hb solution in order to saturate Hb with oxygen.
5. Observe the change of the colour to scarlet red.
6. Using spectroscope observe again the appearance of two dark bands, characteristic for HbO_2.

Experiment 3. Fractionation of blood plasma proteins using a method of salting out: determination of albumins and globulins in blood plasma.

Principle of the method. Proteins can be separated from the solution in the form of precipitates. There are many different ways to precipitate proteins. Precipitation of proteins using concentrated solutions of neutral salts (ammonium sulfate, sodium chloride, etc.) is called salting out. Most reactions of the salting aout and reactions with alcohol, acetone are reversible, because protein precipitate can be dissolved again by diluting it with water or the concentration of salts may be reduced using dialysis.

Method:

1. Place 2.0 – 3.0 ml of blood serum to a test tube.
2. Add equal volume of saturated ammonia sulphate solution and mix well.
3. Observe the precipitation of globulins (50 % of the solution saturation).
4. Filtrate the precipitation.
5. Add some water to the precipitate, which remained on filtrating paper.
6. Boil obtained solution and observe the appearance of albumins precipitate.
7. Filtrate albumins precipitate, add some water and obtained filtrate devide into 2 tubes.
8. To the first tube add crystal ammonium sulphate till the complete saturation. Observe albumins precipitate.
9. Boil the content of the second tube, observe the appearance of albumins precipitate.



Fraction separation of blood serum proteins.

Name of the protein	Used salt	Level of saturation	Appearance of precipitate
Globulins			
Albumins			

Explain obtained results. Draw a conclusion. Pay attention at the use of fractionation of proteins in medical practice.

Clinical and diagnostic significance. Identification of certain fractions of serum proteins is important for clinical diagnosis. Normally blood albumin content is 40-45 g/l, globulin - 20-30 g/l. In various diseases of the liver (cirrhosis, hepatitis), in nephrosis, chronic diseases of the stomach, digestive tract tumors of blood, albumin concentration decreases. In acute infectious diseases, rheumatism concentration of α_2-globulins is increased. The concentration of β-globulins increases in hepatitis, multiple myeloma, and γ –globulins in chronic diseases, chronic polyarthritis.

Fractionation of proteins using a method of salting out is used in clinical practice for obtaining native proteins from biological fluids.

Examples of Krok-1 tests

1. A 46-year-old patient was found to have hyperactivity of creatine kinase in his blood serum. What kind of pathology can be suspected?

A. Acute pancreatitis
B. Chronic hepatitis
C. Myocardial infarction
D. Haemolytic anemia
E. Renal failure

2. Examination of a patient revealed toxic hepatitis developed on the background of the use of medicines. This diagnosis can be confirmed by the activity of the following enzyme of blood serum:

A. Alanine amino transferase
B. Creatine phosphokinase
C. Pyruvate dehydrogenase
D. Maltase
E. Malate dehydrogenase

3. During ultrasound investigation a patient has been diagnosed with bilateral stenosis of renal artery with atherosclerotic genesis. Specify the bioactive substance that due to its excessive secretion is the key component of arterial hypertension pathogenesis in the given case:

A. Cortisol
B. Renin
C. Vasopressin
D. Noradrenaline
E. Thyroxin

4. A patient was found to have an increased blood serum LDH-1 activity. In which organ is the pathological process localized?

A. Liver
B. Kidneys
C. Stomach
D. Heart
E. Muscles

5. A hospital admitted a patient with arterial hypertension induced by renal artery stenosis. The patient complains of persistent nausea and headache. The main element in the pathogenesis of hypertension is the activation of the following system:

A. Hypothalamic-pituitary
B. Kallikrein-kinin
C. Renin-angiotensin
D. Sympathoadrenal
E. Parasympathetic

Individual work of students

1. Role of proteins and indicatory enzymes of blood in normal conditions and in pathology.
2. Evaluation of data of nitrogen metabolism and changes in content of nonprotein nitrogen containing components of blood.

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Topic № 13. Coagulation, anticoagulation and fibrinolytic systems of blood

Objective: To learn the role of components of coagulation, anticoagulation and fibrinolytic systems of blood in providement of physiological condition of blood. To characterize biochemical components of immune system. To learn mechanisms of immunodeficiencies.

Actuality of the theme: Blood coagulation is a difficult physiological and biochemical process which is the protective reaction of our organism on bleeding. Knowledge of biochemical description of coagulatory, anticoagulatory and fibrinolytical systems of blood is necessary for understanding of mechanisms of support of the aggregate

state of blood in normal state and at numerous diseases, and also for their timely correction by medical drugs.

Specific objectives

- ✓ To interpret biochemical principles of functioning of coagulation, anticoagulation and fibrinolytic systems of blood.
- ✓ To learn methods of investigation of coagulation and fibrinolytic systems of blood, to interpret obtained results.
- ✓ To characterize cellular and humoral factors of immune system.
- ✓ To explain mechanisms of development of immunodeficient states.

Theoretical questions:

1. Functional and biochemical characteristics of intrinsic and extrinsic blood coagulation pathways.
2. Blood coagulation system; characteristics of coagulation factors. Cascade mechanism of activation and function of blood coagulation; intrinsic and extrinsic blood coagulation pathways. Role of vitamin K in reactions of hemocoagulation (carboxylation of glutamic acid residues, its role in Ca binding). Medical preparations as vitamin K agonists and antagonists.
3. Hereditary disorders of hemocoagulation.
4. Anticoagulation system of blood, functional characteristics of its components – heparin, antithrombin III, citric acid, prostacycline. Role of vascular endothelium. Changes in biochemical characteristics of blood in prolong treatment with heparin.
5. Fibrinolytic system of blood: stages and factors of fibrinolysis. Pharmacological modulation of fibrinolytic process. Activators and inhibitors of plasmin.

Practical part

Experiment 1. Detection of vicasol with cysteine.

Principle of the method. Solution of vicasol in alkaline medium in the presence of cystein turns into lemon yellow colour.

Method.

1. Add 5-10 droplets of 0.05 % alcohol solution of vicasol into a tube.
2. Add 2.5 ml of NaOH.
3. Observe the appearance of lemon yellow colour.

Clinical and diagnostic significance. The most clarified function of vitamin K is its interrelationship with blood coagulation. Vitamin K is essential for the synthesis of blood coagulation factors, such as prothrombin (factor II), proconvertin (factor VII), Christmas factor (factor IX), Stuart's factor (factor X). Vitamin K contributes to the synthesis of the full molecule of prothronbin, providing its posttranslational modification.

Current scientific data also gives evidence that vitamin K as other fat soluble vitamins affects the state of cell membranes and subcellular structures, being the constituent of lipoproteins of these membranes.

Daily need in vitamin K makes 1-2 mg, its concentration in blood serum makes 400 – 600 nmol/l.

Deficiency of vitamin K in most cases is endogenous and results from the disturbance of its production in the intestine (use of antibiotics or sulphamides), impaired absorption

due to the lack of bile production, obstruction of bile ducts, liver disorders. The use of antivitamins of vitamin K may also cause its deficiency. The signs of vitamin K deficiency include bleedings after small injuries, coagulopathias in newborns (before the appearance of appropriate microflora in the small intestine).

In medical practice the drugs of vitamin K are widely used and its water soluble analogue – vicasol. These drugs are prescribed in pathologies, accompanied by hypoprothrombinemia and bleedings.

Examples of Krok-1 tests

1. A 45-year-old woman, who for two weeks has been taking neodicoumarin (ethyl biscoumacetate) due to trombophlebitis, during a regular examination was detected to have decreased blood content of prothrombin, in urine there is microhematuria. What drug should be administered as a neodicoumarin antagonist?

A. Protamine sulfate
B. Sodium citrate
C. Vicasol (Menadione)
D. Heparin
E. Aminocapronic acid

2. A patient with myocardial infarction has been administered intravenously a direct anticoagulant, namely:

A. Vikasol
B. Heparin
C. Thrombin
D. Calcium gluconate
E. Neodicumarinum

3. A woman noticed that a cut on her skin was still bleeding even after 20 minutes had passed. What vitamin deficiency causes such condition?

A. Vitamin A
B. Vitamin D
C. Vitamin E
D. Vitamin B_{12}
E. Vitamin K

4. Which of the following drugs would be best to use on a patient who has just had a heart attack?

A. Tissue plasminogen activator
B. Dicoumarol
C. Heparin
D. Warfarin
E. Thrombin

5. In a patient with symptoms of enhanced blood coagulability (thromboses, thrombophlebitis) heparin was injected, never the less coagulation was not inhibited. What protein factor deficiency of anticoagulant system may exists in a patient?

A. Antithrombin III
B. α_2-Macroglobulin
C. αI – inhibitor of proteinases
D. Antithromboplastine
E. Anticonvertin

Individual work of students

1. Anticoagulants, classification, mechanism of action.

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Topic № 14. Biological role and metabolism of hemoglobin. Patobiochemistry of porphyria and jaundice.

Objective: To learn pathways of heme biosynthesis and its catabolism with production of bile pigments, role of liver in turnover of bile pigments. To interpret the results of determination of bilirubin content in blood in differential diagnostics of jaundices.

Actuality of theme: The metabolism of bilirubin – the product of heme degradation – take place in a liver. Disorders in bilirubin metabolism cause a jaundice, which are recognized and investigated with the use of biochemical tests on blood and urine. Liver plays a central role in general metabolism, supporting the stability of internal milieu of organism. The most important reactions of detoxification of endogenous and exogenous harmful substances are performed in this organ.

Specific objectives:

- ✓ To describe principal stages of heme biosynthesis, use of succinate and glycine as precursors of porphyrin.
- ✓ To explain mechanisms of hereditary disorders of heme biosynthesis and development of different forms of porphyrias.
- ✓ To describe transformation of heme into different forms of bile pigments. Role of liver in this process, mechanisms of development of different forms of jaundices.
- ✓ To learn biochemical method of determination of direct and indirect bilirubin in blood plasma and its application for diagnostics of different forms of jaundices.

Theoretical questions:

1. Biosynthesis of heme, precursors and scheme of enzymatic reactions. Regulation of porphyrin/heme synthesis.
2. Hereditary disorders of porphyrin metabolism (porphyrias).
3. Hemoglobin catabolism: production of bile pigments, biliverdin, its transformation to bilirubin, synthesis of bilirubin diglucuronide and excretion with bile.

4. Pathobiochemistry of jaundices; hemolytic (prehepatic), parenchimatous (hepatic), occlusive (posthepatic).
5. Enzymatic congenital jaundices: Crigler-Najjar syndrome, Gilbert disease, Dubin-Johnson.
6. Diagnostic significance of determination of total bilirubin and its fractions in blood plasma.

Practical part

Experiment 1. Determination of bilirubin in blood serum.

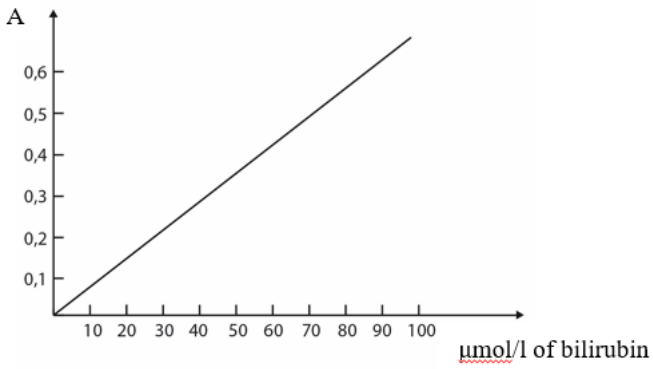
Principle of the method. Bilirubin reacts with diazoreagent with the formation of pink colored azobilirubin. The intensity of color is proportional to bilirubin concentration and can be measured with the use of photocolorimeter at 530-560 nm wavelength. Conjugated bilirubin reacts rapidly (direct reaction), non-conjugated bilirubin reacts slowly. In presence of accelerators (e.g. caffeine) non-conjugated bilirubin reacts as rapidly as conjugated one. For determination of total bilirubin (conjugated and free) buffer solutions with accelerators (caffeine) are used.

Method.

Fill 4 tubes accordingly to the table below:

Reagents	Conjugated bilirubin Test	Conjugated bilirubin Control	Total bilirubin Test	Total bilirubin control
reagent 1 without accelerator	3 ml	3 ml	-----	-----
reagent 1 with accelerator	-----	-----	3 ml	3 ml
reagent 2 (diazoreagent)	0.075 ml	0.075 ml	0.075 ml	0.075 ml
blood serum	0.4 ml	-----	0.4 ml	-----
distilled water	-----	0.4 ml	-----	0.4 ml

Quickly mix the components in each tube and after 5 min (exactly) measure the optical density at green light filter (560 nm) against control probes. The quantity of bilirubin is estimated according to calibration curve.



The curve of dependence of optical density (A) of the solution of bilirubin from its concentration.

Explain the result, write the conclusion.

Clinical and diagnostic significance. Normal values of total bilirubin concentration in blood serum are 0.1-1.2 mg % (1.7-20.5 μmol/l), non-conjugated – 0.1-1.0 mg% (1.7-17.1 μmol/l) , conjugated – 0.05-0.25 mg% (0.86-4.3 μmol/ml.). High concentrations of bilirubin exhibit toxic effects, which are caused due to it photosensibilization properties. As a metabolite of protoporphyrine, a very active photosensibilizer, bilirubin transform molecular oxygen to singlet form, which is extremely active oxidant. Singlet oxygen induce damage of biological membranes, nucleic acids, proteins; it partially is expressed as hemolysis.

The increase in blood bilirubin concentration over 30 μmol/l causes the jaundice. The last one can be: hemolytic (prehepatic), parenchymatous (hepatic), occlusive (posthepatic, obturation of bile ducts).

In hemolytic jaundice liver is not able to transform all the quantity of free bilirubin in blood, which is produced due to increased hemolysis. In this case in blood is noted an increase in concentration of free (non conjugated) bilirubin up to 100 μmol/l. This form of jaundice is characteristic to hemolytic and pernicious anemias.

In parenchimatous jaundice the conjugating capacity of liver cells is suppressed due to damage of liver cells. Production of bile is decreased, conjugated bilirubin partially returns back to blood. Bilirubinemia in this case is caused by increase in concentration of direct and indirect fractions of bilirubin. Parenchimatous jaundice is observed in fat degeneration of liver (steatosis), hepatitis (viral, toxic), cirrhosis of liver.

In occlusive jaundice bile is accumulated in bile ducts due to occlusion of bile duct with bile stones or tumor and bile is returned back to blood. Bilirubinemia in this type of jaundice is characterized by a marked increase of bilirubin level in blood (170-700 μmol/l) mainly due to conjugated fraction of bilirubin.

In neonates at first days of life jaundice is developed due to several causes. Bilirubin is not transformed to derivatives due to sterility of intestines and is intensively absorbed to blood from digestive tube. In neonates frequently is observed transient insufficiency of glucuronyl transferase, which causes the increase of nonconjugated bilirubin in blood.

Congenital disorders of bilirubin turnover and transport are observed in congenital jaundices, as such: Crigler-Najjar syndrome (absence or insufficiency of glucuronyl transferase), Gilbert disease (insufficiency of glucuronyl transferase and block of absorption of bilirubin from blood by liver cells – “absorption jaundice”); Dubin-Johnson syndrome (disorder of transport of bilirubin diglucuronide from liver cells to blood - “excretory jaundice”).

Examples of Krok-1 tests

1. A patient presents with icteric sclera and mucous tunics; urine is dark; feces are light-colored. Blood content of direct and indirect bilirubin is increased, urine content of direct bilirubin is increased. What pathology can be characterized by these signs?

A. Hemolytic jaundice
B. Hepatocellular jaundice
C. Obstructive jaundice
D. Jaundice of the newborn
E. Atherosclerosis

2. A 71-year-old woman developed mechanical jaundice due to obstruction of the bile duct with a chololith. Decrease of blood pressure and bradycardia are detected. These changes in functioning of the patient's cardiovascular system are caused by increased blood content of the following substance:

A. Direct bilirubin
B. Indirect bilirubin
C. Urobilin
D. Bile acids
E. Stercobilin

3. Hemoglobin break-up begins in the cells of reticuloendothelial system. What enzyme catalyzes the reduction reaction of biliverdine into bilirubin?

A. Beta-glucuronidase
B. Biliverdine reductase
C. Xanthine oxidase
D. Heme oxygenase
E. Hexokinase

4. A patient has icteric skin; unconjugated bilirubin content in blood is high; conjugated bilirubin in urine is not detected. There is significant amount of urobilin in urine and stercobilin in feces. Name the pathology characterized by given symptoms:

A. Jaundice of the newborn
B. Hemolytic jaundice
C. Obstructive jaundice
D. Hepatocellular jaundice
E. Atherosclerosis

5. A newborn child born from Rh-negative mother in the result of her third pregnancy presents with gradually worsening jaundice, irritated central nervous system, anemia. What type of jaundice does the infant suffer from?

A. Hemolytic
B. Hepatocellular
C. Obstructive
D. Parasitic
E. Toxic

Individual work of students

1. Pathobiochemistry of hereditary jaundices.

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Topic № 15. Detoxification function of liver, microsomal oxidation, role of cytochrome P-450 system and flavine containing monooxygenases. Biotransformation of xenobiotics and endogenous toxins.

Objective: To learn main pathways of xenobiotics detoxification. To learn reactions of microsomal oxidation. To understand the mechanisms of development of drug tolerance in interrelationship with the processes of microsomal oxidation.

Actuality of the theme: Liver detoxifies endotoxins and xenobiotics, including drugs, which are foreign for the body and can change or damage the course of metabolic processes. At the same time drugs and medicinals are used in pathology in order to normalize metabolism and favor reconvalescence. Knowledge of kinetics of absorbtion, transport, distribution and metabolism of drugs in human body is necessary for the development of new phamaceuticals with desired properties.

Specific objectives:

- ✓ To learn the role of biotransformation and metabolism of xenobiotics.
- ✓ To explain mechanism of action of cytochrome P450, and the role of monooxygenase systems in the biotransformation of endogenous and exogenous substrates.
- ✓ To analyze types of conjugation reactions of xenobiotics and endogenous toxins in hepatocytes.

Theoretical questions:

1. Homeostatic role of liver in human body. Involvement of liver in glucose turnover (glycogenesis and glycogen breakdown, gluconeogenesis), proteins and lipid metabolism.
2. Detoxification function of liver; biotransformation of xenobiotics and endogenous toxins.
3. Types of reactions of biotransformation of foreign substances in liver.
4. Reactions of microsomal oxidation; inducers and inhibitors of microsomal monoxygenases.
5. Conjugation reactions in hepatocytes: biochemical mechanisms, functional significance.
6. Electron transport chains of endoplasmic reticulum. Genetic polymorphismus and induction of biosynthesis of cytochrome P-450.
7. Development of tolerance to medicinal and drugs and its causes.

Practical part

Methods of qualitative detection of phenacetine and its metabolites.

Phenacetine, 1-ethoxy-4-acetamidobenzene, possess an anti-inflammatory, fever and pain relief effects. It is metabolized by dealkylation and hydroxylation and formation of paracetamol, p-phenetidine, p-aminophenol. Part of phenacetine is excreted with urine in unchanged form, another part is transformed to glucuronides and sulphates and also excreted with urine.

Most of reactions for detection of phenacetine in biological material are connected with detection of p-aminophenol. The last is obtained in a way of extraction of phenacetine with chloroform in acidic conditions and subsequent hydrolysis of dried extract with concentrated HCl. In hydrolyzate p-aminophenol is determined with several characteristic reactions.

Experiment 1. Formation of indophenol dye.

Method. Add a droplet of 2% solution of chromic acid anhydride or 5 % solution of potassium bichromate to the aliquote of tested sample.

Observe the appearance of violet color of the solution, which is gradually transformed to cherry red, indicating on the presence of p-aminophenol.

Side effects of xenobiotics oxidation, significance for medicine and pharmacotherapy. Consumption of some drugs may lead to a state of tolerance, in which there is a decrease in responsiveness to the medication. This phenomenon is caused by proliferation of smooth endoplasmic reticulum membrane as well as increase in total protein and microsomal protein, especially cytochrome P-450 per gram of liver. Cross-tolerance may also occur. The use of the anticoagulant warfarin is a classical example of such an interaction. When phenobarbital and warfarin are administered together, warfarin soon becomes ineffective. If phenobarbitol is withdrawn after warfarin dosage has been increased, there is an increased risk of internal hemorrhage in a patient. That is why medical personnel should be aware of such cases.

Examples of Krok-1 tests

1. The second stage of detoxification involves joining certain chemical compounds with functional groups of toxines. Select one such compound:

A. Higher fatty acids
B. Cholesterol
C. Glucuronic acid
D. Glucose
E. Pyruvate

2. Universal system of biological oxidation of nonpolar compounds (numerous drugs, toxic agents, steroid hormones, cholesterol) is microsomal oxidation. Name the cytochrome that is included in oxygenase chain of microsomes:

A. Cytochrome C
B. Cytochrome A_3
C. Cytochrome A
D. Cytochrome C_1
E. Cytochrome P-450

3. A patient with chronic constipation had been prescribed bisacodyl. After 3 weeks of treatment, the patient noticed a reduction of laxative effect. This is caused by the development of the following side-effect:

A. Tolerance
B. Dysbacteriosis
C. Cumulation
D. Sensibilization
E. Dependence

4. Due to prolonged taking of phenobarbital the epileptic patient has developed tolerance for this drug. What is this phenomenon based on?

A. Biotransformation acceleration
B. Absorption process weakening
C. Increase of receptor sensitivity
D. Biotransformation suppression
E. Substance accumulation in body

5. Certain drugs can stimulate liver to synthesize enzyme systems taking part in drugs and toxines metabolism. What compound stimulates drug metabolism in liver microsomes?

A. Phenobarbital
B. Heparin
C. Aspirin
D. Sulfanilamide
E. Menadione sodium bisulfite

Individual work of students

1. Reactions of microsomal oxidation and conjugation in biotransformation of xenobiotics and endogenous toxins.

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Topic № 16. Investigation of water and mineral metabolism

Objective: To learn processes of water and minerals turnover in human body. To learn methods of qualitative and quantitative determination of inorganic substances in blood serum and to interpret obtained results with diagnostic purpose and in treatment of some diseases.

Actuality of the theme. Water and minerals are of vital importance for normal functioning of human body. Changes in water and minerals turnover occur in different diseases, thus knowledge and understanding of these processes as well as their qualitative and quantitative determination is necessary for evaluation of water-mineral metabolism.

Specific objectives:

✓ To learn hormonal regulation of water and mineral metabolism in the body;

- ✓ To analyse the major characteristics of principle elements;
- ✓ Discuss the biochemical functions, dietary requirements, sources and absorption of macroelements and microelements.

Theoretical questions:

1. Biological role of water and its distribution in human body. Water balance, its types.
2. Regulation of water and mineral metabolism, its disorders. Dehydratation and rehydratation, biochemical mechanisms of their development.
3. Mechanism of Na,K-ATPase action and its regulation.
4. Biogenic elements, their classification, pathways of their providement.
5. Biological role of macroelements, trace elements and ultramicroelements.
6. Human microelementoses: endogenous and exogenous causes (technogenic, yatrogenic, etc.). Oligotherapy.

Practical part

Experiment 1. Determination of calcium in urine (Sulkovitz probe).

Principle of the method. The method is based on formation of insoluble Ca compounds in its increased quantity in urine. Sulkovitz reagent contains oxalic acid 2,5 g, ammonium oxalate – 2,5 g, conc. acetic acid – 5,0 ml, the volume is adjusted to 150 ml with water.

Method.

1. Place 5 ml of urine into the tube and add 2.5 ml of Sulkovitz reagent to it.
2. Observe the development of milky turbidity in a tube after 30 sec.
3. Make a conclusion, knowing that the quantity of calcium in urine is relevant to the intensiveness of the turbidity. If the content of calcium in urine is decrased – urine after adding Sulkovitz reagent remains transparent.

Clinical and diagnostic significance. Calcium is an essential macroelement for the maintenance of homeostasis in human body, being a structural constituent of bones and envolved in the processes of muscular contraction. Calcium turnover in human body is regulated by parathormon, derevatives of vitamin D3 and calcitonin. The normal level of calcium in blood is 2.2-2.6 Mmol/l. The normal level of calcium in daily urine is 2.5-8.0 Mmol/day. An increase or diminishing of the level of calcium ions in blood plasma can result in the development of different pathologic conditions. Pharmacologic remedies that contain salts of calcium have got wide application in different fields of medicine.

Experiment 2. Colorimetric method of potassium determination in blood serum. (Lasarev method).

Principle of the method. Potassium ions in presence of $Pb^{=2}$, $Cu^{+@}$ and NO_2^- ions form water insoluble compound $K_2Pb[Cu(NO_2)_6]$, which can be dissolved in a mixture of rivanol and concentrated acetic acid. Optical density of solution is determined, which is proportional to the quantity of NO_2 ions.

Method.

1. Add 1 ml of rivanol solution and 2 ml of concentrated acetic acid to the sediment of blood serum sample.

2. Stir the mixture and when sediment is dissolved adjust the volume of the solution to 25 ml with water.
3. Measure the optical density of the obtained solution at 540 nm (green filter). Estimate the level of potassium according the formula:

$$K = A \times 36 \text{ (mg\%)},$$

where:

A is the value of extinction.

Clinical and diagnostical significance. Potassium is a main intracell cation.The normal level of potassium in blood serum makes 3.5-5.0 Mmol/l. Decline of potassium level in blood (less than 3 mM/l) results in general fatigue, weakness of legs, myalgias, disturbances of heart action, in severe cases violation of breathing, paralysis and intussusception may occur. Hypopotassemia may be caused by the use of diuretics, evacuants, after intensive vomiting, diarrhea. Increase of the potassium level (hyperpotassemia) mainly occurs in cases of kidney insufficiency and causes bradycardia, that in severe cases may lead to heart stoppage.

Examples of Krok-1 tests

1. Tetanic spasms of skeletal muscles occur under low calcium concentration in blood. What endocrine disorder can this condition be associated with?

A. Hyperfunction of adrenal cortex
B. Hypofunction of adrenal cortex
C. Hypofunction of parathyroid gland
D. Hyperthyroidism
E. Hypothyroidism

2. As a result of an emergency situation (shipwreck) a man had to drink sea (salty) water. What form of water-salt imbalance may occur in this case?

A. Hypoosmolar hyperhydration
B. Hypotonic hyperhydration
C. Hyperosmolar hyperhydration
D. Isoosmolar hyperhydration
E. Isotonic hyperhydration

3. A 40-year-old patient has developed polyuria (10-12 liters per day) and polydipsia induced by damage to the hypothalamo-hypophyseal tract. What hormone deficiency causes such disorders?

A. Vasopressin
B. Oxytocin
C. Corticotropin
D. Somatotropin
E. Thyrotropin

4. Drugs that block certain channels can prevent the transmission of excitation from presynaptic membrane to the postsynaptic memebrane of synapse. What channels are blocked?

A. Calcium
B. Sodium
C. Potassium potential-dependent
D. Potassium ATP-dependent
E. Chlorine

5. Hemoglobin catabolism results in release of iron which is transported to the bone marrow by a certain transfer protein and is used again for the synthesis of hemoglobin. Specify this transfer protein:

A. Albumin
B. Transferrin (siderophilin)
C. Transcobalamin
D. Haptoglobin
E. Ceruloplasmin

Individual work of students

1. Human microelementosis.
2. Diagnostics of water-electrolyte composition.

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Topic № 17. Renal function. Biochemical composition of human urine in norm and pathology.

Objective: To know physical and chemical properties of urine; principal normal and pathological constituents of urine and pathways of their appearance. To be able to conduct biochemical analysis of urine and to interpret obtained results.

Actuality of the theme: A biochemical analysis of urine is one of the most important and commonly performed laboratory investigations in medical practice, that is very informative in complex diagnosis of numerous diseases and monitoring of effectiveness of treatment.

Specific objectives:

- ✓ To interpret the biochemical mechanisms of water and minerals turnover regulation and role of kidneys in the formation of urine.
- ✓ To analyze the biochemical composition of urine in health and during pathological processes; to estimate the functional value of the final products of nitrogen turnover (urea, uric acid, creatinine) and products of detoxication (animal indican, hippuric acid), changes in their daily secretion.

- ✓ To analyze the state of health of a patient on a basis of biochemical parameters of blood and urine.

Theoretical questions:

1. Role of kidneys in regulation of volume, composition of electrolytes and acid-base equilibrium of biological fluids. Biochemical mechanisms of urine production (filtration, reabsorption, secretion and excretion). Characterization of renal clearance and renal threshold, their diagnotic significance.
2. Humoral mechanisms of regulation of water and mineral metabolism and kidney function; antidiuretic hormone; aldosteron.
3. Renin-angiotensine system. Natriuretic factors of heart atrium and other tissues. Biochemical mechanisms of development of renal hypertonia.
4. Physical and chemical properties of urine: volume, color, odor, transparency, acidity (pH), its dependence from diet. Role of kidneys and lungs in regulation of acid-base equilibrium. Ammoniogenesis.
5. Chemical composition of urine in health (organic and mineral components), their diagnostic significance and causes of changes of stable indices of urine analysis.
6. Pathological constituents of urine – blood, protein, sugar, bile pigments, ketone bodies. Causes and pathways of their appearance in urine.
7. Glucosuria, galactosuria and pentosuria, causes of their development. Clinical significance of their detection.
8. Clinical significance of detection and determination of indicane, phenylpyruvic and homogentisinic acids in urine.
9. Clinical significance of detection and determination of ketone bodies in urine.
10. Differentiation of jaundices on the appearance of bile acids and bile pigments in urine.

Practical part

Biochemical analysis of urine.

For the biochemical analysis a middle portion of urine from morning urination is used. Urine collection requires sterile conditions in order to avoid contamination with bacterias and flagellas. The urine analysis is conducted, beginning from the estimation of physical and chemical properties: amount, color, smell, transparency, reaction (pH) and density of urine.

Experiment 1. Determination of physical and chemical properties of urine.

A. Urine volume.

Measure the volume of tested urine using graduated cylinder. The quantity of urine excreted in 24 hours is defined as diurhesis.

Normally healthy person excretes daily in average 1,100-1,600 ml of urine. Increase in volume of urine excreted is called polyuria, decrease - oliguria, complete absence of urine - anuria.

B. Color.

Estimate the colour of tested urine.

Normally urine has straw-yellow color due to the presence of specific pigments - urochrome, urobilin, uroerythrin. In pathological conditions color may be changed due to

occurence of bile pigments (bilirubinuria) or hemoglobin (hemoglobinuria, hematuria). Color of urine also depends on diet.

C. Odor.

Estimate the odor of urine.

Usually urine has specific odor due to the presence of traces of volatile compounds. Ammonia odor appears in bladder inflammations, purulent - in gangrenous processes, fruit odor - in diabetes mellitus. Odor of urine also depends on diet.

D. Transparency.

Estimate the transparency of urine.

Normally fresh urine is transparent. During standing some sediment may appear due to formation of mucin clots. Turbidity may be caused by sediments of phosphates (in alkaline medium), urates (in acidic medium), mucin, cellular elements, microorganisms.

E.The acidity of urine (pH)

Using pH-meter or sensitive indicator papers determine pH of urine.

Normally urine has pH in ranges 4.5 – 8.0 and depends on a character of diet or metabolic disorders. Alkaline urine may occur in case of hyperaciditas of gastric juice, in loss of acid equivalents in massive vomiting, in cystitis or pyelitis. Acidic reaction of urine occurs in diabetes mellitus, starvation (due to the presence of ketone bodies), in renal insufficiency. Highly acidic urine is estimated in gout and fever.

Urine density is measured with urometer with subsequent scale.

In health urine density makes 1008 – 1025 g/l.

Experiment 2. Detection of protein in urine.

Principle of the method. The detection of protein in urine is based on the precipitation of protein with sulfosalicylic acid.

Method.

To 2 ml of urine add 5 droplets of 20 % solution of sulfosalicylic acid.

If white precipitate or turbidity appears in a tube, it proves the presence of protein.

Experiment 3. Detection of sugar in urine.

Principle of the method. All mono- and disaccharides with a free hemiacetal hydroxyl possess reducing properties and reduce metal ions (silver, copper, bismuth) with degradation of carbon chain and polymerization. Reactions are performed after elimination of protein, as it is an interfering substance.

1. To 10-15 ml of urine add 10 droplets of 10% acetic acid and boil.
2. Cool and separate the appeared sediment by filtration.

Test obtained filtrate for the presence of sugar using Trommer' reaction:

3. To 1-2 ml of test sample add 1 ml of 5% NaOH and 2-3 drops of $CuS0_4$ solution.
4. Observe the formation of precipitate of $Cu(OH)_2$.
5. Boil the tube.

In case of presence of sugar in the tested sample the precipitate will become yellow during heating due to formation of colored precipitate of cuprous hydroxide. In course of boiling the color of the solution changes to red as a result of cuprous oxide formation.

Experiment 4. Detection of blood (benzidine probe).

To 3 ml of urine add 2-3 droplets of 3% H_2O_2 and 2-3 droplets of benzidine in acetic acid. In presence of blood the color of the solution will change to green-blue.

Examples of Krok-1 tests

1. A victim of a traffic accident is hospitalized at a resuscitation unit. Objectively: the patient is unconscious, BP is 90/60 mm Hg, high blood content of creatinine and urea is observed, diurnal diuresis is 80 ml. Characterize the patient's diurnal diuresis:

A. Oliguria
B. Polyuria
C. Anuria
D. Pollakiuria
E. Nocturia

2. A patient has obstruction of the common bile duct. Which of these substances is usually found in urine in such cases?

A. Bilirubin
B. Ketone bodies
C. Uric acid
D. Creatinine
E. Glucose

3. A 40-year-old patient has developed polyuria (10-12 liters per day) and polydipsia induced by damage to the hypothalamo-hypophyseal tract. What hormone deficiency causes such disorders?

A. Oxytocin
B. Corticotropin
C. Somatotropin
D. Thyrotropin
E. Vasopressin

4. A patient's blood shows an increased concentration of pyruvate which is excreted with urine for the most part.This is typical for the following vitamin deficiency:

A. B_1
B. B_2
C. B_3
D. B_6
E. E

5. A patient has oliguria caused by acute renal failure. What daily amount of urine corresponds with this symptom?

A. 100-500 ml
B. 1500-2000 ml
C. 1000-1500 ml
D. 500-1000 ml
E. 50-100 ml

Individual work of students

1. Pathobiochemistry of kidneys. Clinical and biochemical changes at glomerulonephritis, amyloidosis, pyelonephritis, sharp and chronic kidney insufficiency.
2. Inhibitors of angiotensin converting enzyme, side effect.

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Topic № 18. Biochemistry of nervous and muscle tissues. Pathochemistry of psychotic disorders.

Objective. To learn the composition and peculiarities of metabolism in muscle tissue, its function in normal and pathological conditions. To perform a quantitative determination of creatinine and creatine in urea and its diagnostic significance in some pathology.

Actuality. Muscle tissue has specific features of metabolism depending on age of a person, pathological states, caused with both endogenous and exogenous factors, and that is why in a clinic the special place beloungs to biochemical methods of diagnostics. For example, at some diseases of muscles it is important to know the excretion of creatine and creatinine in 24-hours urine.

Specific objectives:

- ✓ To analyze biochemical composition of muscles and the role of proteins in formation of muscle cell structure.
- ✓ To explain biochemical mechanisms of contraction and relax of muscle fiber.
- ✓ To analyze pathways of energetic support of muscle contraction, role of ATP and creatine phosphate in these processes.

Theoretical questions:

1. Fine structure and biochemical composition of myocytes; structural organization of sarcomers. Myofibril proteins: myosine, actin, tropomyosine, troponine complex. Molecular organization of thick and thin filaments.
2. Nitrogen containing and nitrogen free water soluble organic compounds, their structure and functional significance. Molecular mechanisms of muscle contraction: modern data on interaction of muscle filaments. Role of Ca^{2+} ions in regulation of contraction and relax of striated and smooth muscles.
3. Modern ideas on energetics of muscle contraction and relaxation. Macroergic compounds of muscles. Structure, production and role of ATP, creatine phosphate, creatine phosphokinases, sources of ATP in muscle cell; role of creatine phosphate in energetic supply of contraction. Pathobiochemistry of muscles - myopathias.
4. Metabolic changes in muscles in certain disorders.

5. Biochemical composition and metabolism in brain: neurospecific proteins and lipids (gangliosides, cerebrosides, cholesterol), peculiarities of amino acid composition of brain tissue, role of glutamate system.
6. Energetic metabolism in human brain.
7. Biochemistry of neuromediators (acethylcholine, norepinephrine, dopamine, serotonine, excitory and calming amino acids), their role in nerve impulse transduction and memory regulation.
8. Receptors for neuromediators and physiologically active compounds.
9. Peptidergic system of brain.
10. Opioid receptors (enkephalins, endorphins, dinorphins) and ther receptors.
11. Biochemical mechanisms of the development of human neuro-psychic disorders (alcoholism, narcomany, Alzheymer's disease, multiple sclerosis, Parkinson's disease, epilepsy).

Practical part

Experiment 1. Quantitative determination of creatinine in urine due to unified Jaffe method.

Principle of the method. Creatinine combine with picric acid in alkaline medium and forms colored complex (Jaffe reaction). The intensity of color is proportional to creatinine quantity and is measured in photocolorimeter.

Method. Into one calibrated cylinder 0.5 ml of urine is measured (test),to another – 0.5ml of water is added (control). To both cylinders 0.2 ml of 10 % NaOH and 3 ml of saturated solution of picric acid are added, after five min distilled water is added to the final volume 100 ml. Solutions are mixed and the intensity of color is measured in photocolorimeter in 1 cm cuvette at green light.

The content of creatinine is calculated according to calibration curve. A daily excretion of creatinine is calculated, using known values of daily volume of urine and volume of tested specimen of urine.

Explain the results.

Clinical and diagnostic significance. Normal daily excretion of creatinine is 8.8-17.7 mmole (1.0-2.0 g) in males and 7.1-15.9 mmoles (0.8-1.8 g) in females. The enhancement of creatinine excretion is observed in excessive ingestion of meat (exogenous source), in breakdown of proteins in cytoplasm of cells, in intensive physical efforts, diabetes mellitus and diabetes insipidus, nfectious diseases, etc. Creatinine is not reabsorbed in kidney tubules, which permits to evaluate the glomerular filtration index.

Examples of Krok-1 tests

1. Patients with severe depression demonstrate decreased serotonin levels in brain and cerebrospinal fluid. What amino acid is a serotonin precursor?

A. Threonine
B. Tyrosine
C. Tryptophan
D. Glutamic acid
E. Aspartic acid

2. Inhibitors of one of the amides metabolism enzymes are used to treat depression. What enzyme inhibition has such an effect?

A. Acetylcholinesterase
B. Formylkynureninase (Arylformamidase)
C. Kynurenine 3-hydroxylase
D. Lactate dehydrogenase
E. Flavin adenine dinucleotide (FAD) containing monoamine oxidase (MAO)

3. The patient with parkinsonism has been prescribed a drug - dopamine precursor - to relieve muscular rigidity. Name this drug.

A. Aminazine
B. Paracetamol
C. Scopolamine hydrobromide
D. Levodopa
E. Atropine sulphate

4. Antidepressants can increase the concentartion of catecholamines in the synaptic cleft. What is the mechanism of action of these drugs?

A. Inhibition of monoamine oxidase
B. Activation of monoamine oxidase
C. Inhibition of xanthine oxidase
D. Activation of acetylcholinesterase
E. Inhibition of acetylcholinesterase

5. Stable contraction of myofibrilla of muscle fibers takes place due to accumulation of the following ions in the cytoplasm:

A. Calcium
B. Potassium
C. Sodium
D. Magnesium
E. Hydrogen

Individual work of students

1. Antidepressants, mechanism of action.

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Topic № 19. Principles of pharmaceutical biochemistry.

Objective: To evaluate the biochemical transformation of drugs. To master the methods of determination of some medicinal drugs and their metabolites and to use the obtained knowledge in biopharmacy.

Actuality of the theme: Due to the capability of medicinal drugs, which are forein for the normal metabolic pathways, to change and alter the course of turnover processes as well as to normalize metabolism under pathologic conditions and contribute to the recovery of the patients, the knowledge on the kinetics of the absorbtion, transpoprt, distribution and turnover of the compounds in human body is the background for the elaboration of medicinal drugs with indicated properties.

Specific objectives:

- ✓ To explain the role of the processes of metabolism and biotransformation of xenobiotics.
- ✓ To know the mechanisms of action of cytochrome P450 and the role of monooxygenase system in biotransformation of endogenous substances.
- ✓ To explain the phases of xenobiotics metabolism.
- ✓ To explain the types of reactions of conjugation of xenobiotics.

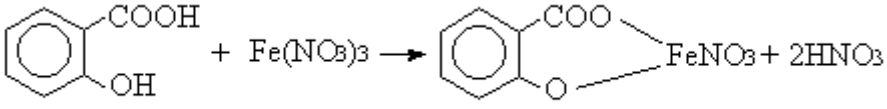
Theoretical questions:

1. Homeostatic role of the liver in the metabolism of the whole organism. Biochemical functions of hepatocytes. Glucogenic and lipid-regulating functions of liver, protein and urea synthesis.
2. Detoxification function of the liver. The concept of the biochemistry of foreign compounds - "xenobiochemistry"; mechanisms of biotransformation of xenobiotics and endogenous toxins.
3. Types of reactions of biotransformation of foreign compounds in the liver.
4. Reactions of microsomal oxidation, inducers and inhibitors of microsomal monooxygenases.
5. The biological role of cytochrome P-450. Electron transport chain in the membranes of endoplasmic reticulum of hepatocytes.
6. Reactions of conjugation in hepatocytes: biochemical mechanisms, functional significance.
7. The origin and nature of the development of drugs tolerance.

Practical part

Experiment 2. Detection of salicylic acid in biological fluids.

Salicylic acid is used in medicine in a form of sodium salts, ointments or chemical derivatives (aspirin) for the treatment of different inflammatory conditions, pain and fever relief, in skin diseases as well as with the purpose of prevention of blood thrombosis. Salicylic acid is excreted from the body partially in unchanged form, as well as in the form of conjugates with glycine or glucuronic acid. Metabolites of salicylic acid include 0-



hydroxybenzoylglucuronide and 0-carboxyphenylglucuronide. In the course of hydroxylation 2,3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid and 2,3,5-trihydroxybenzoic acid are also formed, which are eliminated with urine.

Method.

Add 4.5 ml of 0.55% solution of iron nitrate in 0,04 n solution of nitric acid to 0.5 ml of urine. Development of purple red color indicates the presence of salicylic acid in tested sample.

Examples of Krok-1 tests

1. A patient with chronic constipation had been prescribed bisacodyl. After 3 weeks of treatment, the patient noticed a reduction of laxative effect. This is caused by the development of the following side-effect:

A. Cumulation
B. Sensibilization
C. Tolerance
D. Dysbacteriosis
E. Dependence

2. Due to prolonged taking of phenobarbital the epileptic patient has developed tolerance for this drug. What is this phenomenon based on?

A. Absorption process weakening
B. Increase of receptor sensitivity
C. Biotransformation suppression
D. Substance accumulation in body
E. Biotransformation acceleration

3. Certain drugs can stimulate liver to synthesize enzyme systems taking part in drugs and toxines metabolism. What compound stimulates drug metabolism in liver microsomes?

A. Sulfanilamide
B. Phenobarbital
C. Heparin
D. Aspirin
E. Menadione sodium bisulfite

4. Examination of a patient revealed an increase in 17-ketosteroid concentrationin urine. Hydroxylation of 17-ketosteroids is possible with the enzymes of the following system:

A. Krebs cycle
B. Protein synthesis system
C. Microsomal oxidation
D. Pentose phosphate cycle
E. Ornithine cycle

5. Detoxication rate is 4 times lower in children than in adults. What enzyme necessary for toxic compounds conjugation has low activity in children?

A. Glucuronyl transferase
B. ALAT
C. AspAT
D. Creatine phosphokinase
E. LDH_1

Individual work of students

1. Biotransformation of drugs.
2. The influence of harmful habits (smoking, alcoholism, narcomania) on human organism.

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