Ministry of Health of Byelorussia
Establishment of education «Gomel state medical university»

Faculty of biochemistry

A.I. Gritsuk, A.N. Koval, V.T. Svergun

PRACTICAL BIOCHEMISTRY

Part 1

Gomel 2005

УДК 577.1 (075.8)
ББК 28.072 я7
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Reviewers: **A.A.Chirkin**, the laureate of the State premium of Byelorussia, Dr. Sci. Biol., the professor managing faculty of chemistry of Vitebsk state university; faculty of biochemistry of the Grodno state medical university/

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The first part of the manual includes brief theoretical information, tasks for self-contained work of students, the description of laboratory works and situational problems on structure, properties and functions of protein, enzymology, bioenergetics, biochemistry of carbohydrates, lipids, proteins and nucleic acids.

It is intended for students of the highest medical educational institutions, and also can be beneficial to students of medicobiological profile of universities.

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FOREWORD

The manual consists of two parts, meets the program on biochemistry for students of the higher medical educational institutions, approved by Ministry of Health of Republic Belarus, and is intended for the students of 2-nd course studying biochemistry.

All course provides 36 occupations, 23 from them are placed in the first part of the manual and 13 — in the second.

The theoretical part includes the list of educational questions treated in the theme which the student should know.

In **a practical part** laboratory works are given. The detailed description of laboratory works includes such important for comprehension and understanding of methods of biochemical researches elements, as principle of the method, course of work, in some cases the accident prevention techniques necessary for performance of work, clinic-diagnostic value of the work which comes to the end with the conclusions based on obtained results.

In **the first part** of the manual themes of the practical occupations concerning the general questions of biochemistry, in particular, of such partitions, as introduction in biochemistry, modern methods of biochemical researches, structure and functions of proteins, enzymology, bioenergetics, biochemistry of carbohydrates, lipids, proteins and nucleic acids are described.

In **the second part** the themes directly concerning problems of clinical biochemistry are surveyed: biochemistry of hormones and vitamins, and also separate organs, tissues and systems of the organism, biochemistry of blood, kidney, liver, nervous and muscular system, myocardium and connective tissue.

Partition “Biochemistry of lipids” is composed together with the senior lecturer of faculty of biochemistry GSMU, Ph. D. V. T. Svergun.

The author thanks assistant of the faculty of biochemistry, Ph. D. A.N. Koval, and also to everyone who helped in preparation of the manuscript for the edition.

1. INTRODUCTION IN BIOCHEMISTRY

Date: ___/___/_____

Lesson 1

Introduction in biochemistry. Modern methods of research. Structure and functions of proteins

The purpose of the lesson is to give an idea about biochemistry as fundamental medico-biological science. Study the structure, physical and chemical properties of proteins. Learn to determine the contents of blood plasma whole protein with the biuretic method.

Initial level of knowledge and skills

The student should know:

1. Basic accident prevention rules of work in chemical laboratory.
2. Structure and classification of alpha-amino acids.
3. Acid-base properties of amino acids. Reactions on their functional groups.
4. Levels of the structural organization of protein.
5. Features of peptide bond structure.
6. Qualitative reaction on proteins and peptides.
7. Complex compounds (copper complex in biuret reaction).

The student should be able:

1. To carry out qualitative reactions on proteins and peptides.

Lesson structure

1. Theoretical part

1.1. Introduction. Brief history of biological chemistry (biochemistry), history of inland biochemistry. A general characteristic of a metabolism. Concept about an anabolism, catabolism, and metabolism. Biochemistry and health. Biochemistry and medicine.

1.2. General strategy used to elucidate biochemical processes:

a) studies at the whole-animal level:

— removal of an organ (eg, hepatectomy);

— alteration of diet (eg, fasting-feeding);

— administration of a drug;

— administration of a toxin;

— use of an animal with a specific disease (eg, diabetes mellitus);

— use of sophisticated techniques such as NMR spectroscopy and positron emission tomography.

b) isolated perfused organ (liver, heart and kidney);

c) tissue slice (eg, liver slices);

d) use of whole cells (eg, blood cells, tissue culture);

e) homogenates (for free-cell studies);
f) isolated cell organelles;
g) subfractionation of organelles;
h) isolation and characterization of metabolites and enzymes;
i) cloning of genes for enzymes and proteins.

1.3. Proteins — the major components of organism. Functions of proteins. Structure, classification and properties of amino acids. Review of levels of the structural organization of protein molecule. Molecular weight of proteins. Shape and size of protein molecules.

2. Practical part

2.1. Specific of working in biochemical laboratory.
2.2. Safety instructions rules.
2.3. International System of Units (SI) in the international laboratory practice.
2.4. Problem solving.
2.5. Laboratory works.
2.6. Final level of knowledge monitoring.

Laboratory works

Laboratory works 1 and 2 are carried out under the conforming instructions.

Laboratory work № 3. **Quantitative determination of whole protein in blood serum with biuretic method**

ATTENTION! Follow safety instruction rules manipulating with sodium hydroxide.

Principle of the method. In alkaline condition peptide bonds of protein form violet colour complex with ions of bivalent copper. Colour intensity of the solution is directly proportional to the concentration of protein determined photometrically.

Course of work. In the tube pour 0.05 ml of blood serum, then add 2.5 ml of biuretic reagent. Contents of the tube cautiously stir (avoid foaming), and measure optic density in 30 minutes in 5 mm flasks at 540 nanometers (green light filter) against control solution (distilled water). Having measured an extinction of researched solution, on standard plot determine concentration of the protein.

Clinico-diagnostic value. The normal contents of protein in blood serum in adult people is 65–85 g/l, in children — 58–85 g/l.

The increased contents of protein in blood serum (hyperproteinemia) occurs rarely. It is observed at rheumatic disease, a multiple myeloma. The short-term relative hyperproteinemia is marked at rheumatism because of significant losses of fluid, for example, at profuse sweating, pernicious vomiting, profuse diarrhea, diabetes, cholera, severe combustions.

Drop of blood protein level (hypoproteinemia) is observed at nephritises, malignant tumors, long starvation, etc.

Conclusion. Copy standard plot and write down obtained result and explain its clinico-diagnostic meaning.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 3–27.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 19–32.

2. ENZYMOLOGY AND BIOLOGICAL OXIDATION

Date: ___/___/_____

Lesson 2

Structure and functions of proteins. Structure and properties of enzymes

The purpose of the lesson is to fix knowledge on protein structure, to give an idea about the structure and properties of enzymes. Learn to carry out qualitative tests on activity of some hydrolases.

Initial level of knowledge and skills

The student should know:

1. Characteristic of levels of structural organization of protein molecule (primary, secondary, tertiary, and quaternary structures).
2. Vitally essential microelements.
3. Vitamins B_1, B_2, B_3, B_6, PP.
4. Structure of coenzymes NAD^+, $NADP^+$.
5. Concepts: coagulation, threshold of coagulation, colloid protection.

The student should be able:

1. To carry out qualitative tests on proteins and peptides.

Lesson structure

1. Theoretical part

1.1. Concept about enzymes. History of enzymology. Features of enzyme catalysis. Structure of enzymes. Proofs of protein nature of enzymes.

1.2. Characteristic of levels of the structural organization of protein molecule (primary, secondary, tertiary, quaternary) and the interactions holding it. Salting-out, denaturation, causes, mechanism and attributes.

1.3. Cofactors of enzymes: metal ions and coenzymes. Participation of vitamins in coenzyme formation.

1.4. The structure functional organization of enzymes: catalytic and allosteric sites.

2. Practical part

2.1. Problem solving.

2.2. Laboratory works.

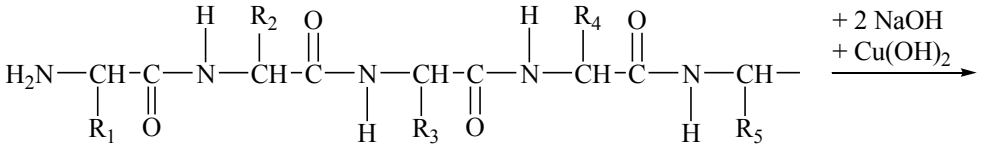
2.3. Monitoring procedure of a final level of knowledge.

Laboratory works

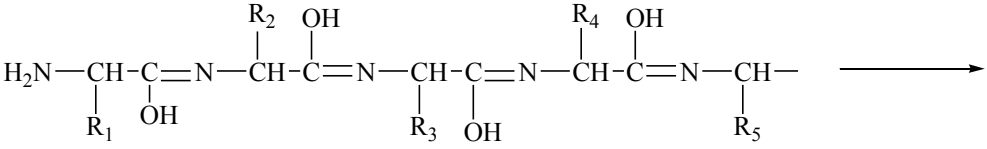
Laboratory work № 1. **Color reactions on proteins and amino acids**

Biuret reaction.

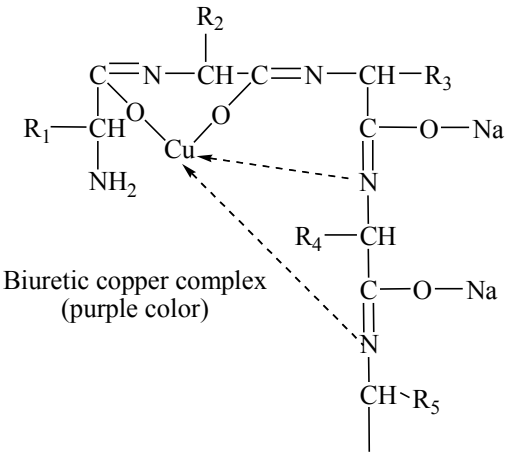
Principle of the method. In alkaline condition peptide bonds of protein form with ions of bivalent copper a complex of violet colour (see equation).



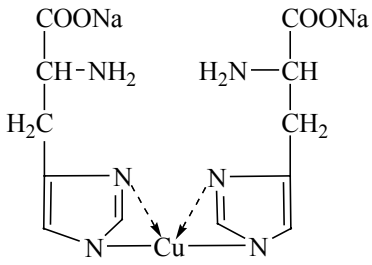
Polypeptide



Enolic form of polypeptide



Biuretic copper complex
(purple color)



Copper complex with histidine
in case of biuretic reaction

ATTENTION! Follow safety instruction rules manipulating with sodium hydroxide.

Course of work. In three tubes pour on 5 drops of solutions: in 1-st — ovalbumin, in 2-nd — gelatin, in 3-rd — myosin. In each tube add on 5 drops of 10% solution of sodium hydroxide and 1 drop of 1% solution of copper vitriol. In all tubes observe a steady blue-violet staining.

Conclusions by results of work.

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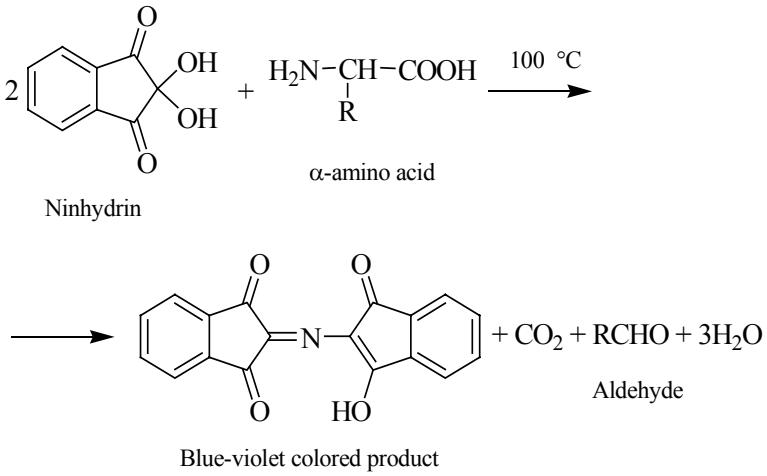
Ninhydrin test

Principle of the method. It is based on formation of dimer of ninhydrin and nitrogen of amino group of blue-violet colour (Ruhemann's complex — see equation).

Course of work. To 5 drops of solution of protein add 5 drops of ninhydrin solution and boil 1–2 minutes. There is a blue-violet staining.



Conclusions by results of the work.

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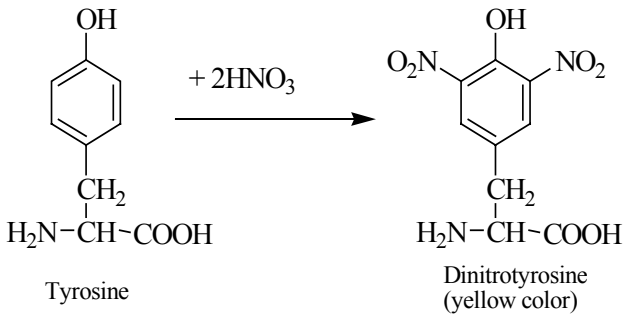
Xanthoproteic reaction (Mulder)

Principle of the method. It is based on formation of nitro compounds of the aromatic and heterocyclic amino acids of bright yellow colour (see the equation).

ATTENTION! Follow safety instruction rules manipulating with concentrated hydrogen nitrate.



Course of work. In three tubes pour on 5 drops of solutions: in 1-st — ovalbumin, in 2-nd — gelatin, in 3-rd — myosin. In each tube add on 3 drops of concentrated nitric acid and cautiously boil. In the first tube the deposit of yellow colour, and in the second — a weak staining, are formed, as gelatin does not contain some cyclic amino acids. In 3-rd tube the deposit of white colour passing in yellow colour is formed. Tubes cool and add in everyone till 10–15 drops of 20% caustic soda before decolourization of solutions owing to formation of sodium salt of dinitrotyrosine.

Conclusions by results of work.

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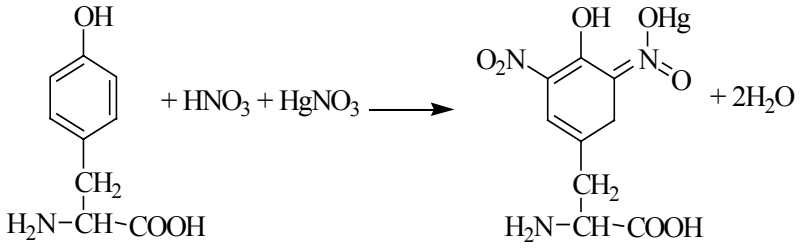
Reaction to tyrosine (Millon's reaction)

Principle of the method. It is based on sludging of dinitrotyrosine mercurial salt of ensanguined (red) colour (see the equation).



Tyrosine

Dinitrotyrosine mercurial salt (red color)

ATTENTION! Follow safety instruction rules manipulating with Millon's reagent (contains Hg and HNO_3).

Course of work. In three tubes pour on 5 drops of solutions: in the 1-st — ovalbumin, in 2-nd — gelatin, in 3-rd — a myosin. In each tube add on 3 drops of reagent of the Millon (solution of mercury in nitric acid) and cautiously heat up. Observe discolouration in the tubes, describe the presence in the specified proteins of tyrosine.

Conclusions by results of work.

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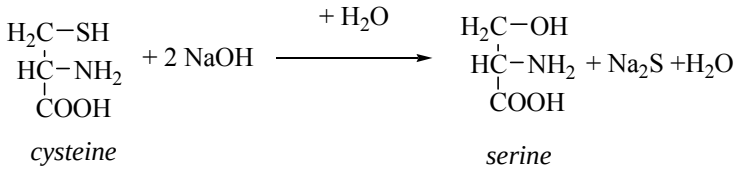
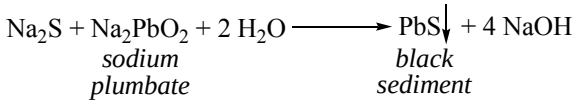
Foll's Reaction (on cysteine)

Principle of a method. Is based on alkaline hydrolysis of sulfhydrile groups SH of protein after subsequent degradation of sulfur as lead sulfide (PbS) of black-brown colour (see equation).

ATTENTION! Follow security rules manipulating with Foll's reagent (contains NaOH and Na_2PbO_2).





Course of work. In three tubes pour on 5 drops of solutions: in 1-st — ovalbumin, in 2-nd — gelatin, in 3-rd — myosin. In each tube add on 5 drops of Foll's reagent. Then intensively boil and yield to stand 1-2 minutes. Thus in the 1-st and in the 3-rd tubes the black or brown deposit of lead sulphide is formed. Gelatin does not form such a deposit, since there is no sulfur-containing amino acids in it.

Conclusions by results of work.

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Laboratory work № 2. **Precipitation reactions of proteins**

Principle of the method. It is based on denaturation and deposition of proteins by various factors.

Deposition of proteins at boiling.

ATTENTION! Follow safety instruction rules manipulating with warming tubes.

Course of work. In 5 tubes pour on 5 drops of protein solution. Heat the first tube to boil. Fluid clouds, since aqueous environments around protein molecule are blasted, and there is an integration of its particles. Micellas of protein carry charge and are kept in suspension.

Heat up solution in the 2-nd tube to boil and add 2 drops of 1% solution of acetic acid up to weak acidification. At settling the deposit of protein drops out. Particles of protein lose charge and come nearer to an isoelectric state.

In the 3-rd tube add 5 drops of acetic acid till strong-acid reaction. At boiling of the fluid deposit is not formed, as protein micellas are recharged and carry positive charge that rises their stability.

In the 4-th tube pour 5 drops of solution of acetic acid, 2 drops of saturated solution of sodium chloride and heat up. The white flocculent deposit drops out, i.e. particles of protein lose charge.

In the 5-th tube add 2 drops of solution of sodium hydroxide. At boiling the deposit is not formed, since in alkaline condition negative charge on particles of protein is increased.

Conclusions by results of work.

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Sedimentation of proteins by concentrated inorganic acids.

ATTENTION! Follow safety instruction rules manipulating with concentrated nitric and sulfuric acids.

Course of work. In 2 tubes pour on 10 drops of concentrated acids: the nitric and the sulfuric.

Having inclined tubes at angle of 45 degrees, cautious on a wall of the tube flow peer volume of protein solution so that both fluids were not admixed. On the border of two fluids the deposit as the small white ring is formed. Addition of excess of nitric acid does not dissolve the deposit, and addition of sulfuric acid solves it.

Conclusions by results of work.

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Deposition of proteins by organic solvents.

Course of work. In 2 tubes bring on 5 drops of protein solution and add on 15–20 drops of alcohol and acetone.

Conclusions by results of work.

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Deposition of proteins by organic acids.

ATTENTION! Follow safety instruction rules manipulating with trichloroacetic acid.

Course of work. In two tubes pour on 5 drops of solution of protein and add on 2 drops of TCAA (trichloroacetic acid) solution in one and 2 drops of sulfosalicylic – in another. Watch the change of solutions.

Conclusions by results of work.

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Laboratory work № 3. **Separation of albumins and globulins with salting-out method.**

Principle of the method. It is based on reversible reaction of protein deposition from solutions with the help of high concentrations of neutral salts (NaCl, NH_4Cl, $MgSO_4$, etc.).

Course of work. To 1 ml of undiluted ovalbumin add 1 ml of saturated solution of ammonium sulfate and stir. Semi saturated solution of ammonium sulfate results in which the deposit of egg globulin drops out. In 5 minutes filter off the deposit, in filtrate there is an ovalbumin. For salting-out of albumins add to filtrate powder of ammonium sulfate up to complete saturation i.e. while the new portion of powder remains undissolved. The dropped out deposit of albumine filter off. With the filtrate make biuret reaction. Negative reaction specifies absence of protein.

Conclusions by results of work.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 27–31.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 30–33.

Date: ___/___/_____

Lesson 3

The mechanism of enzyme action

The aim of the lesson: fix knowledge on enzymes structure, give an idea about the mechanism of enzyme action.

Initial level of knowledge and skills

The student should know:

1. Theoretical bases of chemical kinetics.
2. Structure of monosaccharides. Qualitative tests on aldehydic and alcohol groups.

3. Structure of polysaccharides. Property and qualitative tests.
4. The mechanism of Shiff bases formation.
5. Structure and catalytic mechanism of coenzymes NAD^+ and $NADP^+$.

The student should be able:
1To carry out qualitative tests on products of enzymatic hydrolysis.

Lesson structure

1. Theoretical part

1.1. Properties of enzymes (thermolability, specificity, etc.). The mechanism of enzyme action. Stages of interaction of enzyme and substrate. E. Fisher, D. Coshland's hypotheses and modern views.

1.2. The theory of intermediate compounds. Bases of thermodynamics of catalysis. Activation energy. Energy barrier.

1.3. Kinetics of enzymatic reactions (the factors influencing rate of enzymatic reactions: the nature of enzyme and substrate, their concentration, pH, temperature, medicines, etc.). K_m – definition, physiological value.

1.4. Regulation of enzyme activity. Role of hormones, cAMP, activators, inhibitors. Regulation of activity by chemical modification of enzymes (limited proteolysis, phosphorylation, methylation, etc.). Kinds of inhibition. Allosteric regulation and properties of allosteric enzymes.

2. Practical part

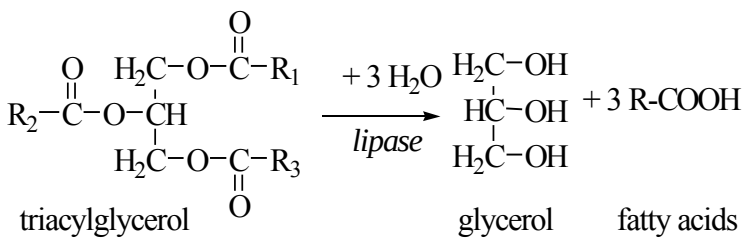
2.1. Problem solving and monitoring procedure of final level of knowledge.
2.2. Laboratory works.

Laboratory works

Laboratory work № 1. **Studying of enzyme action.**

Lipase action.

Lipase is a part of pancreatic juice. In gastric juice it contains in a small amount and reacts only with preliminarily emulsified lipids. In an intestine bile acids and proteins promote emulsification of lipids. Action of lipase can be found, having added its solution to the milk preliminarily weakly alkalized by solution of soda at the presence of phenolphthalein. Light pink colouring occurs.



Principle of the method. Lipase accelerates hydrolysis of neutral lipids on glycerine and fatty acids (see the equation), that results in drop of pH and to disappearance of pink colouring of the indicator — phenolphthalein.

Course of work. In two tubes pour on 10 drops of milk. In the 1-st the tube add 5 drops of pancreatine, which contains lipase, in the 2-nd — 5 drops of water. In both tubes pour on 1 drop of 0.5% solution of phenolphthalein and dropwise 1% solution of soda before appearance of light pink colouring at pH 8,0 (avoid of flowing excess of soda solution). Tubes seat in a thermostat at temperature 38°C for 30 minutes. Observe a decolorization of solution in the tube containing lipase.

Conclusions by results of work.

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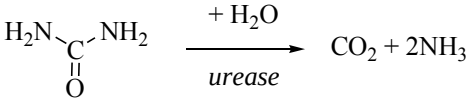
Action of urease.

Principle of the method. Urease catalyzes hydrolysis of urea on carbon dioxide and ammonia (see the equation), that results in rise of pH which is registered by the indicator — phenolphthalein.



Course of work. Take two tubes. In the 1-st measure off 1 ml of 1% urea solution, and in the 2-nd — 1 ml of 1% thiourea solution. In each tube add on 2 drops of phenolphthalein and on 1 ml of urease solution. Contents of both tubes shake up, leave for some minutes at room temperature and observe the appearance of pink colouring in the tube with urea and the absence of colouring in the tube with thiourea. Contents of the 1-st tube get pink colouring owing to shift of pH of ghe solution to the alkaline due to formation of ammonia.

Conclusions by results of work.

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Laboratory work № 2. **Studying of influence of various factors on rate of enzymatic reactions**

The enzyme reaction rate depends on many factors: substrate and enzyme concentration, pH, activators and inhibitors, coenzymes and ions concentration.

Alpha-amylase enzyme contains in saliva and catalyses α-(1–4)-glycoside bonds hydrolysis of starch and glycogen to maltose. Saliva also contents maltase

enzyme, which is splitting maltose to glucose. The starch hydrolysis by α-amylase occurs to dextrines. Intact starch reacts with iodine and dark blue colouring occurs. Dextrines reaction whith iodine make dark blue colouring, amilodextrines — violet, erythrodextrines — red-brown, achrodextrines and maltose — yellow. The final starch hydrolysis products are maltose and glucose. They have free aldehyde groups and can be found out by Trommer reaction (redox reaction). Thus aldehyde group is oxidated into gluconic acid, and copper — into CuO hydrate of yellow colour. When heated the CuO hydrate becomes red Cu_2O.

Thermolability is the one of characteristic properties of enzymes , i.e. the enzyme temperature sensitivity , when the enzyme reaction occurs. For many enzymes the temperature optimum is 38–40°C.

At heating over 70°C enzymes lose its properties because of protein molecule thermal denaturation. The inactivation rate depends on duration of thermal influence. At low temperatures enzymes are well kept, but the enzyme action rate is strongly decreased. The enzyme thermolability is easily tested by saliva amylase action. Enzyme action is proved by changing of the concentrations of substrate or reaction products.

Trommer reaction.

ATTENTION! Follow safety instruction rules manipulating with oxyhydroxide of sodium and warming.

Course of work. To 5 drops of undiluted saliva add 3 drops of starch, and leave at room temperature for 15 minutes. Then add 5 drops of 10% NaOH and 3 drops of 1% $CuSO_4$. Then cautiously heat up to boiling. Red colouring specifies positive Trommer reaction.

Conclusions by results of work.

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Influence of temperature on amylase activity.

The starch degradation by amylase is tested with iodine.

ATTENTION! Follow safety instruction rules manipulating with the boiling water bath.

Course of work.

1. Add 0.5 ml of starch solution in 4 tubes. Then add 0.5 ml of diluted (1: 10) saliva in 4 tubes.

2. Take the first pair of tubes (the one — with the enzyme, another — with starch) and place in the ice-bath. The second pair leave at room temperature. A third pair place in thermostate (40°C), and fourth — in boiling water-bath.

3. After10 minutes merge together the content of each tube pair, carefully mix and leave for 10 minutes in the same conditions.

4. Take 3 drops of fluid from the 3-rd tube and make test with iodine drop on glass. If there is dark blue colouring, the solutions leave for the next 10 minutes, after that repeat the test with iodine on glass. Then add 2 drops of iodine solution in all tubes and observe colouring occurrence.

Conclusions by results of work.

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Influence of activators and inhibitors on amylase activity.

Course of work.

1. Bring in 10 drops of distilled water to one tube, and 8 drops of water and 2 drops of 1% NaCl solution — in the 2-nd; 8 drops of water and 2 drops of $CuSO_4$ solution — in the 3-rd.

2. In every tube add 20 drops of the diluted saliva (1:10); mix the tubes, add 5 drops of starch solution and leave at room temperature for 5 minutes.

3. Prepare 3 tubes with water (1 ml) in the meantime, tinted drop of iodine solution, and add in them 3 drops of contents of skilled tests. Observe colouring depending on a degree of splitting of starch with amylase. In the 1-st tube violet colouring occurs, in the 2-nd tube is red-brown, where the ions of chlorine play role of the activator, there is a yellow colouring, and in the 3-rd, where Cu ions brake the amylase action — the colouring remains dark blue. If all this is not observed, repeat the experiment in 10–15 minutes.

Conclusions by results of work.

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Influence of pH on saliva amylase activity.

Principle of the method. The optimal pH for saliva amylase action is estimated by interaction with starch at various pH values. Degree of starch degradation is tested with iodine. At optimum pH value starch degradation occurs completely (no colouring with iodine). pH shift in the acid or alkaline side results in partial starch degradation into dextrines (red-brown or violet colouring) or starch will not be degradated at all (dark blue colouring).

Course of work.

1. In 4 numbered tubes measure by separate pipettes 2 ml of the phosphate buffer with various pH (6.0; 6.4, 6.8; 7.4).

2. In every tube add 1 ml of starch solution and 0.5 ml of diluted (1:10) saliva, place in thermostat on 10 minutes at 38°C.

3. Mix a drop of a liquid from every tube with a drop of iodine solution on glass and compare colouring in everyone tube. Repeat this test after 1–2 minutes till the test from the 5-th tubes will give red-brown colouring with iodine on glass. After 1–2 minutes add in each tube 2–3 drops of iodine solution (to begin with the 1-st tubes). well shake up the contents of the tubes. Compare colouring in all tubes and estimate degree of starch degradation. Make conclusion about enzyme activity at this pH.

Conclusions by results of work.

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Recommended literature

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 31–43.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 33–37.

Date: ___/___/_____

Lesson 4

The nomenclature and classification of enzymes. Medical enzymology

The aim of the lesson: to give an idea about principles of the nomenclature and classification of enzymes, the basic aspects and problems of medical enzymology. To learn to determine amylase activity in urine.

Initial level of knowledge and skills

The student should know:

1. Structure of cell and the basic organelles.

2. Concept about mutations and mechanisms of a mutagenesis. The basic stages of protein biosynthesis.

3. Principles and methods of measuring of rate of chemical changes.

The student should be able:

1. To carry out qualitative tests on activity of enzymes of biological fluids.

Lesson structure

1. Theoretical part

1.1. Localization of enzymes in a cell (cellular membrane, cytoplasm, mitochondrion, nucleus, lysosome, ribosomes). Marker enzymes. Organo-specifice enzymes. Enzyme preparation and purification. Qualitative detection and quantitative determination. Units of measurement of enzyme quantity and activity. The nomenclature and classification.

1.2. Isoenzymes, their biological role. Polyenzyme complexes. Concept about metabolone.

1.3. Change of activity of enzymes in ontogenesis.

1.4. The basic directions of clinical enzymology.

1.4.1. Enzymopathies. Definition. Classification. Initial (inheritable) enzymopathies. The causes of occurence. The mechanism of development of metabolic disorders at enzymopathies. Degree of clinical manifestations of enzymopathies. The secondary (acquired) — toxic, nutritional enzymopathies. The causes of occurence. The mechanism of development of metabolic disorders. Clinical manifestations.

1.4.2. Enzymodiagnostics, principles and objects of enzymodiagnostics (blood, urine, saliva, liquor, sweat, etc.). The characteristic of the basic enzymes of blood serum (cellular, secretory and excretory). Types of change of enzyme activity in pathology (hypo-, hyper-and disfermentemias). Problems of the enzymodiagnostics.

1.4.3. Enzymotherapy. Use of enzymes for substinutional therapy. The treatment of surgical, cardiovascular and oncological diseases. Immobilized enzymes. Liposomes and their application.

1.4.4. Use of enzymes in laboratory practice for substrate concentration and enzyme activity determination.

1.4.5. Use of enzymes in industry and manufacture.

2. Practical part

2.1. Problem solving.

2.2. Laboratory work.

2.3.Monitoring procedure of final level of knowledge.

Laboratory work **Quantitative determination of amylase activity in urine (by Wolgemuth)**

Principle of the method. Determination of amylase activity in biological fluids (urine, liquor, saliva, blood serum) is based on determination of minimum activity (quantity) of the enzyme catalyzing hydrolysis of added starch in reference conditions. Amylase activity of urine is expressed by quantity of Starch (in milliliters) which is split by enzyme containing in 1 ml of undiluted urine, at temperature 45°C for 15 minutes.

Course of work. Take 10 numbered tubes and flow in everyone on 1 ml of physiological solution. Then in the 1-st tube add 1 ml of urine. Contents of the

tube stir, some times involving and tapping from the pipette. Measure 1 ml of the mixture in the pipette and transfer in the 2-nd tube, and procedure retry down to 10-th tube. From the 10-th tube 1 ml of the mixture reject. Thus the following cultivation of urine is received:

№ of tube	1	2	3	4	5	6	7	8	9	10
Dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Conversion										

In each tube add on 1 ml of physiological solution and on 2 ml of 0.1% of starch solution. After stirring of the contents of the tube thermostat for 15 minutes at temperature of 45°C. After incubation of the tube cool with tap water and put in rack in order. Add in each tube on 1 drop of iodine solution and stir. Mark the tube with the greatest urine dilution at which there was a complete starch degradation with iodine. The received data bring in the table.

Account. Let's assume, that complete hydrolysis of starch has taken place in the first 4 tubes. In fourth tube (where urine cultivation is 1:16) 1/16 ml of urine hydrolyses 2 ml of 0.1% starches. Means, 1 ml not diluted urine will split 32 ml of 0.1% starches. Hence, amylase activity in urine is equal to 32 units. In normal amylase activity in urine is equal to 16–64 units.

Clinico-diagnostic value. Determination of amylase activity of urine and blood serum is widely used in clinical practice for diagnostics of diseases of pancreas. At acute pancreatitis amylase activity of urine and blood serum is increased ten times, especial in the first day of disease, and then is gradually reverted to norm. At renal failure amylase in urine is absent. At children's increase of amylase activity is observed at endemic parotitis that specifies a simultaneous lesion of pancreas with a parotitis virus.

Conclusion: Write down received result and state its clinico-diagnostic evaluation.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 29–30, 43–44.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 37–38.

Date: ___/___/_____

Lesson 5

Biological oxidation. Krebs cycle

The aim of the lesson: to formulate modern views about ways and mechanisms of reception, depoting and utilization of energy in living organisms.

Initial level of knowledge and skills

The student should know:

1. Elements of chemical thermodynamics. The first and second laws of thermodynamics. Concept about Gibbs energy.
2. Essence and the mechanism of redox reactions.
3. Structure of coenzymes NAD^+, $NADP^+$, FAD their role and the mechanism of participation in redox reactions.

The student should be able:

1. To carry out qualitative tests on substrates of energy metabolism.

Lesson structure

1. Theoretical part

1.1. History of development of the doctrine about biological oxidation (BO). Views of A. Lavoisier, M.V. Lomonosov, F. Schoenbein, A.N. Bakh, K. Engler, V.I. Palladin, G. Wiland.

1.2. The theory of peroxide compounds (Bakh-Englera), its essence and critical analysis.

1.3. Palladin-Viland theory, its essence and critical analysis.

1.4. Further development of the doctrine about biological oxidation. Modern understanding about biological oxidation. Principles of transformation and transfer of energy in living systems. Redox reactions, redox potential. Macroergic bonds, structure of ATP, the causes of macroergity.

1.5. Substrates of biological oxidation. The scheme of substrate formation from carbohydrates, lipids, proteins. Stages of biological oxidation — cytoplasmic and mitochondrial. Enzymes, coenzymes of biological oxidation — NAD^+-, $NADP^+$-, FAD-and FMN-dependent dehydrogenases.

1.6. Structure and functions of mitochondrion. The comparative characteristic of mitochondrial membranes. Enzyme composition of various compartments.

1.7. TCA –tricarbonic acids cycle (Krebs cycle) as the common final item point of substrate utilization in biological oxidation. History of discovering. Reaction sequence, enzymes, coenzymes. Substrate-level phosphorylation. Regulation of TCA. Value of TCA (plastic, energy and regulatory role).

1.8. Vitamins PP, B_2. Structure and role in energy metabolism.

2. Practical part

2.1. Problem solving.

2.2. Laboratory works.

2.3. Monitoring procedure of a final level of knowledge.

Laboratory works

Laboratory work № 1. **Discovering of some TCA substrates (citric and succinic acids)**

Principle of the method. Di- and tricarbonic acids with near located carboxylic groups, interact with resorcine and concentrated sulfuric acid, and produce fluorescence in ultra-violet light.

ATTENTION! Follow safety instruction rules manipulating with source of ultraviolet radiation, concentrated sulfuric acid and warming on spirit-lamp.

Course of work. In two tubes add 1 drop of water (surplus of water prevents reaction) and dissolve: in 1-st — some crystals of citrate , and in the 2-nd — succinic acid. Then add in both tubes 10–12 drops of the concentrated sulfuric acid and some crystals of resorcine. Tubes cautiously heat up (but NOT BOIL!) till occurrence of yellow colour. Tubes cool and add 20 drops of distilled water. In ultra-violet light observe fluorescence: light-blue in tube with citrate, and green — with succinate.

Conclusions by results of work.

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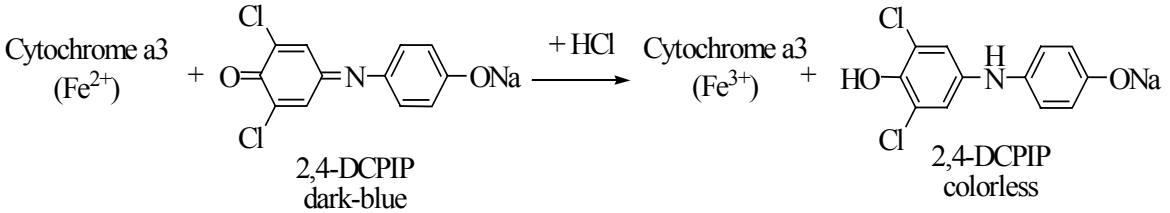
Laboratory work № 2. **Quality detection of cytochrome oxydase.**

Principle of the method.

Cytochromoxidase is contained in skeletal muscles. The enzyme decoloures sodium 2,6-dichlorphenolindophenolate (2,6-DCPIP, Tilmans' paint), restoring it (see equation).



Course of work. 1 g of fresh skeletal muscles exempted from a fatty tissue, carefully pound in ступке during 10 minutes. Smashed muscle filter through a layer of a gauze and repeatedly wash out a solid deposit with distilled water till decolouration of the water.

On the smashed and dried with filter paper muscle, put 2–3 drops of a solution 2,6-DCPIP and observe its decolouration because of activity of cytochromoxidase of muscle tissue (reduction of Tilmans' paint in leukoform).

Conclusions by results of work.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 54–59, 64–70, 83–86.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 1–10, 99–116.

Date: ___/___/_____

Lesson 6

Oxygen consumption in organism. Tissue respiration. Oxidative phosphorylation. Microsomal and peroxide oxidation

The aim of the lesson: to formulate modern representations about mechanisms of reception, deposition and utilization of energy in living organisms, the ways of oxygen consumption in organism in norm and pathology.

Initial level of knowledge and skills

The student should know:

1. Concept about electromotive force of redox reactions.
2. Structure NAD^+, $NADP^+$, FAD, FMN, coenzyme Q, cytochromes and their role in oxidation-reduction processes.
3. Electron structure of oxygen atom and its active forms; catalase, peroxidase.
4. Nature of free-radical processes.

The student should be able:

1. To carry out titrimetric analysis.

Lesson structure

1. Theoretical part

1.1. The ways of oxygen utilization in organism (mitochondrial, microsomal and peroxide oxidation).

1.2. Structure and function of a respiratory chain (RC) of mitochondria. RC complexes. The basic principles and mechanism of mitochondrial RC functioning. The enzymes of tissue respiration: NAD^+, $NADP^+$, FAD-depended dehydrogenases, ubiquinone, cytochromes, their structure and role.

1.3. Oxidative phosphorylation (OP). Phosphorilation sites. P/O — parameter of OP coupling. Mechanisms of oxidation and phosphorylation coupling. P. Mitchell's chemiosmotic theory of oxidative phosphorylation coupling. Un-

coupling of oxidation and phosphorylation. The uncouplers. The mechanism of uncoupler action. Biological meaning of OP uncoupling.

1.4. The importance of tissue respiration in cell and organism bioenergetics. Energy balance of one CTA turnover.

1.5. Microsomal oxidation. Concept about microsomas. The endoplasmic reticulum (EPR) characteristic. Microsomal RC. The carriers: NAD^+, $NADP^+$, FAD- and FMN-depended dehydrogenases, cytochromes b5, P_{450}, their function. Substrates and cosubstrates of microsomal oxidation (xenobiotics metabolism).

1.6. Similarity and difference of microsomal and mitochondrial RC. Connection between TCA, RC of mitochondria with microsomal RC. Biological meaning and organ distribution of microsomal oxidation.

1.7. Concept about peroxide processes. Electron structure of oxygen atom. Mechanisms of reactive oxige species formation. Peroxidation in norm and at pathology. Antioxidan protection: enzye (SOD, catalase, peroxidase, etc.) and non-enzyme (glutathione, vitamins A, C, E, metabolites, etc.).

1.8. Vitamins A, C, E their structure and role in metabolism.

2. Practical part

2.1. Problem solving.

2.2. Laboratory work.

2.3. Monitoring procedure of a final level of knowledge.

Laboratory work. **Quantitative determination of catalase (by Bakh and Zubkova)**

Principle of the method. It is based on titrimetric determination of quantity of hydrogen dioxide decomposed by enzyme for a fixed time term, according to the following equation:

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

Quantity of degraded hydrogen dioxide judge on difference of quantity of $KMnO_4$ spent for titration before and after action of catalase.

Activity of catalase express with the help of catalase number and parameter of catalase. Catalase number is a quantity in milligrams of hydrogen dioxide which desomposed in 1 μl of blood.

Course of work. Diluted blood (1:1000) shake up and add on 1 ml in two flasks, flow on 7 ml of distilled water; in test assay add 2 ml of 1% solution of H_2O_2, and in the reference — 5 ml of 10 % solution of H_2SO_4. Action of catalase in acid medium (in reference) is terminated, since an pH optimum = 7.4.

Leave flasks at room temperature for 30 minutes. Then flow in test flask 5 ml of 10% of H_2SO_4, and in the reference — 2 ml of 1% H_2O_2. Contents of each flask titrate with 0.1 n. solution of $KMnO_4$ till appearance of light pink colouring.

Expect the catalase number (CN) under the formula:

$$CN = (A - B) \times 1.7,$$

where: A — quantity of 0.1 n. solution of $KMnO_4$, spent for titration of reference where catalase is decayed, ml;

V — quantity of 0.1 n. solution of $KMnO_4$, spent for titration of test assay, ml.

Titration of reference assay where catalase is decayed, will require more solution of $KMnO_4$, than for titration of test assay. An obtained difference multiply by 1.7 (conversion factor) and obtain catalase number for tested blood.

In norm catalase number makes 10–15 units.

Clinico-diagnostic value. Determination of catalase activity of blood matters for diagnostics of cancer, anemia, tuberculosis. At these diseases activity of catalase in blood drops.

The note — the catalase parameter serves fraction in which numerator is catalase number, and as a denominator — number of millions erythrocytes in 1 μl of tested blood.

Conclusions. Write down received result and state its clinico-diagnostic evaluation.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 70–83.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 116–122.

Date: ___/___/_____

Lesson 7

CONTROL on PARTITIONS «INTRODUCTION IN BIOCHEMISTRY», «ENZYMOLOGY AND BIOLOGICAL OXIDATION»

The aim of the lesson: the control of learned topic in the partition «Introduction in biochemistry», «Enzymology and biological oxidation»

3. BIOCHEMISTRY OF CARBOHYDRATES

Date: ___/___/_____

Lesson 8

Chemistry of carbohydrates. Digestion and absorption. Glycogen metabolism

The aim of the lesson: to give an idea about a biological role, molecular mechanisms of digestion and an absorption of carbohydrates, metabolic pathways of carbohydrates in living organisms.

Initial level of knowledge and skills

The student should know:

1. Structure, classification and properties of carbohydrates.
2. Mechanisms of digestion of nutrition components in gastro-intestinal tract.
3. Molecular mechanisms of transport of substances through biological membranes.

The student should be able:

1. To carry out qualitative tests on carbohydrates.

Lesson structure

1. Theoretical part

1.1. Structure, classification of carbohydrates. The characteristic of mono-, di- and polysaccharides. Homopolysaccharids: starch, glycogen, cellulose. Heteropolysaccharids: acidic (a hyaluronic acid, hondroitynesulfates, heparin) and neutral (neuraminic and sialic acids). Functions of carbohydrates in an organism.

1.2. Digestion and absorption of carbohydrates in GIT. Kinds of digestion (cavitary, luminal and endocellular), their characteristic.

1.3. Mechanisms of transport of carbohydrates through membrane (the simple, facilitated diffusions, active transport). Role of Na/K-ATP-ase in transport of glucose. Transformation of galactose and fructose into glucose.

1.4. Value of phosphorylation of glucose. Pathways (formation and utilization) of glucose 6-phosphate. The scheme of carbohydrate metabolism in organism.

1.5. Metabolism of glycogen (synthesis and mobilization), reactions, enzymes, regulation.

2. Practical part

2.1. Problem solving.

2.2. Laboratory works.

Laboratory works

Laboratory work № 1. **Digestion of carbohydrates in gastrointestinal tract**

Principle of the method. Is based on specific conversion by ptyalin. Thus conversion by enzymes of gastric juice and Pancreatinum (a ferment drug — the extract of pancreas) does not descend. Other polysaccharide of nutrition — cellulose — is not exposed to hydrolysis by any of the named enzymes.

ATTENTION! Follow safety instruction rules manipulating with warming on a spirit-lamp.

Course of work. Prepare for assays according to the table:

№	Starch solution	Cellulose suspension	Saliva	Gastric juice	Pancreatine
1	1.0	—	1.0	—	—
2	—	1.0	1.0	—	—
3	1.0	—	—	1.0	—
4	–	1.0	–	1.0	—
5	1.0	–	1.0	1.0	—
6	–	1.0	1.0	1.0	—
7	1.0	—	—	—	2.0
8	—	1.0	—	—	2.0

Tubes are incubated in thermostat at 37°S for 30 minutes. Then contents of each tube analyze with the help of Trommer reaction on presence of split products of polysaccharide. For this purpose in each of 8 tubes add 1 ml of 10% caustic soda and 5 drops of 1% copper sulphate. Cautiously heat up top of solution in the tube to boiling and boil for 1 minute. Red deposit of copper oxide means positive Trommer reaction and presence of glucose and maltose.

Conclusions. Write down obtained result and state its clinico-diagnostic evaluation.

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Laboratory work № 2. **Extraction of mucin from spit**

Principle of method. It is based on acid denaturation of mucin in spit.

ATTENTION! Follow safety instruction rules manipulating with concentrated sulfuric acid.

Course of work. In the tube collect 1–2 ml of spit and dropwise add acetic acid. The deposit of glycoprotein — mucin — precipitates.

Fluid from the tube cautiously decant, and the clot slightly dry up with a filter paper.

On the clot render 3 drops of 0.2% solution of α-naphthol and 20 drops of concentrated sulfuric acid. Appearance of a violet-red staining testifies to presence of carbohydrates in protein.

Conclusions. Write down obtained result and state its clinico-diagnostic evaluation.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 50–54, 59–60, 87–90, 108–113, 114–117.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 131–149, 163–166.

Date: ___/___/_____

Lesson 9

TISSUE CARBOHYDRATE METABOLISM. QUANTITATIVE DETERMINATION OF BLOOD GLUCOSE

The aim of the lesson: to give an idea about the paths and mechanisms of glucose metabolism in organism. Learn to determine concentration of glucose in blood.

Initial level of knowledge and skills

The student should know:

1. Tricarbonic acid cycle — reactions, enzymes, the energy balance, regulation.
2. Molecular mechanisms of substance transport through biological membranes.

The student should be able:

1. To carry out a colorimetric analysis.

Lesson structure

1. Theoretical part

1.1. Pathways of glucose-6-phosphate in tissues (the scheme of carbohydrate metabolism in an organism).

1.2. Anaerobic glycolysis, glycogenolysis (enzymes, reactions). Kinase reactions of glycolysis. Substrate level phosphorylation.

1.3. Alcoholic fermentation (enzymes, reactions). Resemblance and difference of anaerobic glycolysis and alcohol fermentation.

1.4. Metabolism of ethanol in organism. The mechanism of toxic action of ethanol and path of detoxification (alcohol DG, MEOS, etc.).

1.5. Aerobic glycolysis. Oxidative decarboxylation of pyruvate (enzymes, reactions). Structure of polyferment complex pyruvate DG.

1.6. Regulation of glycolysis and glycogenolysis. Pasteur effect (nature and the mechanism).

1.7. The energy balance of carbohydrate oxidation.

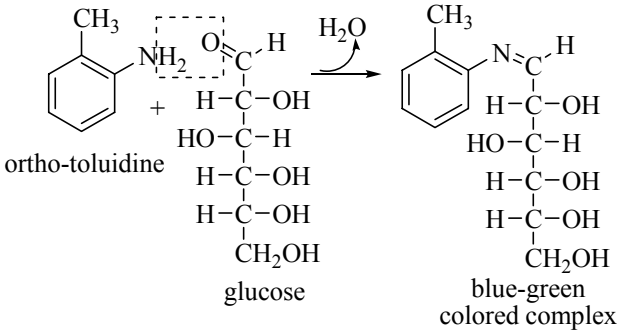
1.8. Vitamin B_1: its structure, role in metabolism, hypo- and avitaminosis.

2. Practical part

2.1. Problem solving.
2.2. Laboratory work.

Laboratory work. **Determination of the of glucose level in blood on staining reaction with ortho-toluidine**

Principle of the method. Glucose yields a blue-green staining when warming with o-toluidine in acid medium. The intensity of the coloring is directly proportional to concentration of glucose and is determined on photoelectrocolorimeter (see the equation).



All aldehydes react with o-toluidine, however their contents in blood is insignificant, therefore the method allows to determine in fact only glucose.

ATTENTION! Follow safety instruction rules manipulating with ortho-toluidine and boiling on the water bath.

Course of work. In two centrifuge tubes pour on 0.9 ml of 3% solution trichloracetic acid (TCAA), then in one of them bring 0.1 ml of blood taken from finger (or serum), and in another — 0.1 ml of standard solution of glucose (5.5 mmol/l). The contents of tubes stir and centrifuge 10 minutes at 3000 rpm.

Supernatant obtained pour in the clear dry tube from where 0.5 ml of supernatant bring in dry tubes, add on 4.5 ml of ortho-toluidine reagent.

Tubes occlude whith foil and seat in the boiling water bath for 8 minutes precisely. It is necessary to watch water in bath continuously boiled. Take out tubes and cool them with tap water up to room temperature. Then measure absorbency of assays on the photometer in 10 mm flasks against water using red light filter (620 nanometers).

Calculation. The glucose contents in experienced assay expect on standard solution of glucose under the formula:

$$C_{as} = C_{st} \cdot (E_{as}/E_{st}),$$

where: C_{as} — concentration of glucose in a blood in assay, mmol/l;
C_{st} — concentration of glucose in a standard test (5.5 mmol/l);
E_{as} — absorbency of assay;
E_{st} — absorbency of the standard of glucose.

Norm. The normal contents of glucose in blood is 3.33–5.55 mmol/l (60–100 mg/%).

Clinico-diagnostic value. The augmentation of glucose concentration in blood (hyperglycemia) is observed at diabetes, an acute pancreatitis, pancreatic cirrhosises, emotional stresses, after an etherization, abundant intake of carbohydrates with food, and rising hormonal activity of some endocrine glands (thyroid, pituitary body, adrenal cortex and medulla).

Drop of glucose level in blood (hypoglycemia) occurs at parenchymatous hepatitis, glycogen storage disease, hypofunction of thyroid, adrenal, pituitary glands, an overdosage of an insulin at treatment of diabetes, carbohydrate malabsorption, phosphorus, benzene, and chloroformium poisoning, lack of carbohydrates in food, extensive losses of blood.

Conclusions. Write down obtained result and state its clinico-diagnostic evaluation.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 91–100, 107–108, 155–156.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 149–157.

Date: ___/___/_____

Lesson 10

TISSUE CARBOHYDRATE METABOLISM. REGULATION OF BLOOD GLUCOSE LEVEL

The aim of the lesson: to give an idea about paths of glucose metabolism, molecular and physiological mechanisms of regulation of glucose level.

Initial level of knowledge and skills

The student should know:

1. Mechanisms of digestion of carbohydrates.
2. Mechanisms of transport of glucose into the cell.
3. Glycolysis.
4. Tricarbonic acid cycle.
5. Mechanisms of action of hormones.

The student should be able:

1. To carry out qualitative tests on presence of ketone bodies.

Lesson structure

1. Theoretical part

1.1. Pathways of glucose-6-phosphate in tissue.

1.2. Pentose cycle: cellular and tissue localization of reactions and enzymes. Biological significance and regulation of pentose cycle.

1.3. Gluconeogenesis (GNG). Cellular and tissue localization of reactions and enzymes. Substrate maintenance of GNG. Glucose-lactate (Cory cycle) and glucose-alanine (Felig cycle) interorgan cycles. Substrate and hormone regulation of GNG. «Futile» cycles, their role in regulation. Biological significance of GNG.

1.4. The scheme of a biosynthesis of the basic classes of glycosaminoglycans, regulation.

1.5. Regulation of glucose level in blood. Normo-, hypo- and hyperglycemias. Characteristics, causes, rise mechanism, clinical manifestations. Insulin role in glucose tissue metabolism. Role of glucose homeostasis in the organism's vital functions.

1.6. Mechanisms of blood glucose level regulation. The urgent mechanism, paths of its realization, role of CNS, hormones, substrates. Biological significance of the urgent mechanism. The constant mechanism, role of hypothalamic-pituitary regulation, hormones and substrates in its realization. Biological significance of GNG in the realization.

2. Practical part

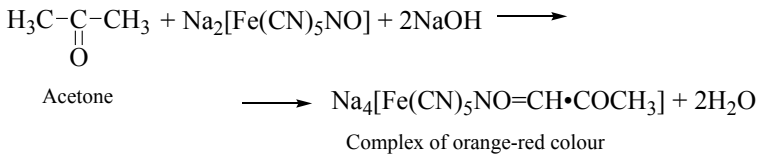
2.1. Problem solving.

2.2. Laboratory work.

Laboratory work. **Qualitative tests on acetone (Legal's assay) and diacetic acid (Gerhardt's reaction)**

a) Legal's Assay for acetone.

Principle of the method. Acetone and diacetic acid in alkaline condition form with sodium nitroprusside an orange-red staining (see the equation):



After acidification with glacial acetic acid the compound of cherry colour is formed.

ATTENTION! Follow safety instruction rules manipulating with sodium hydroxide.

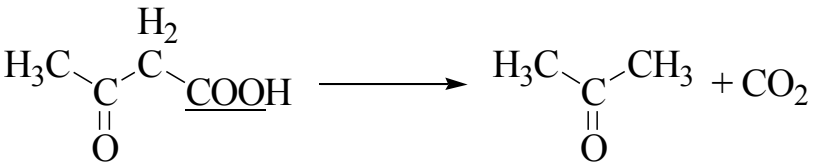
Course of work. In the tube pour 1 drop of urine, 1 drop of 10% solution of NaOH and 1 drop of just prepared Sodium nitroprusside. There is an orange-red staining.

b) Gerhardt's reaction for diacetic acid.

Principle of the method. It is based on formation of iron acetoacetate of cherry-red colour.

Course of work. To 5 drops of urine add dropwise 5% solution of iron chloride, thus the deposit of phosphates in the form of $FePO_4$ drops out.

At presence of diacetic acid from the further adding of iron chloride there is a cherry-red staining. At standing colouring turns pale owing to spontaneous decarboxylation of diacetic acid (see the equation).



Boiling accelerates the process.

Clinico-diagnostic value. Formation of ketone bodies take place in liver, whence they are delivered to other tissues as energy substance. In norm their contents in blood is very insignificant — 13.4–185.2 mcmol/l (0.14–1.9 mg%). They contain in urine in trace amounts and are not revealed by routine reactions.

The increased contents of ketone bodies in blood (ketonemia) and in urine (ketonuria) is observed in lipid or carbohydrate metabolism disorders — diabetes, starvation (deficiency of carbohydrates), hyperproduction of hormones (antagonists of insulin), corticosteroids, Girke disease. Hypoketonemia has no clinical value.

Conclusions. Write down obtained result and to state its clinico-diagnostic evaluation.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 100–107.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 157–163, 166–173.

Date: ___/___/_____

Lesson 11

PATHOLOGY OF CARBOHYDRATE METABOLISM

The aim of the lesson: to give an idea about molecular mechanisms of basic disorders of carbohydrate metabolism, methods of their laboratory diagnostics.

Initial level of knowledge and skills

The student should know:

1. Mechanisms of carbohydrate digestion.
2. Mechanisms of glucose transport into the cell.
3. Paths of glucose tissue metabolism.
4. Mechanisms of blood vital functions glucose level regulation.
5. The general scheme of energy metabolism.

The student should be able:

1. To determine the contents of glucose in biological fluids.

Lesson structure

1. Theoretical part

1.1. Mechanisms of regulation of the blood glucose (urgent and constant). A role of glucose homeostasis in vital functions of organism.

1.2. Type I diabetes (insulin-dependent diabetes mellitus, IDDM; juvenile-onset diabetes). The causes of origin (absolute or relative deficiency of insulin effects). Biochemical alterations in insulin insufficiency, the mechanism of origin and metabolic consequences:

a) Activation of glycogenolysis and GNG, hyperglycemia, glucosuria;

b) Activation of lipolysis — hyperlipemia, ketonemia, ketonuria, ketoacidosis, a hypercholesterolemia, dislipoproteinemia;

c) Activation of proteolysis — hyperaminoacidemia, hyperammonemia;

d) hyperosmolarity — disorder of water-electrolytic and acid-base balance.

1.3. The basic clinical manifestations of diabetes and its connection with disorder of metabolism (polydipsia, polyuria, polyphagia), complications of diabetes — disorder of tissue regeneration, drop of barrier functions of skin and mucosa, caries, atherosclerosis, angiopathies, neuropathies, blindness, etc.

1.4. Diagnostics of diabetes:

a) Clinical diagnostics — change of water-electrolytic balance, appetite, multiple caries, etc.

b) Laboratory diagnostics:

— determination of glucose level, ketone bodies in blood and urine on an empty stomach;

— glucose tolerance test, technics of accomplishment and interpreting;

— determination of contents of blood glycated hemoglobin, insulin, C-peptide.

1.5. Hyperinsulinism — the causes, metabolic consequences, clinical manifestations (hypoglykemia, obesity, type II diabetes).

1.6. Disorder of digestion and absorption of carbohydrates in gastrointestinal tract, disaccharidase insufficiency, the mechanism of development of diarrhea, ketoacidosis and hyperosmolarity, the basic clinical manifestations.

1.7. Galactosemia, fructosuria. The causes of origin. Mechanisms of development of complications. The basic clinical manifestations.

1.8. Glycogenoses — the basic types, the causes and clinical manifestations.

1.9. Mucopolysaccharidoses — the causes and the basic clinical manifestations.

2. Practical part

2.1. Problem solving.

2.2. Laboratory work.

Laboratory work. **Glycemic curve plotting**

Principle of the method. The glucose level in blood routinely characterizes insulin function. The peroral load with glucose entails augmentation of blood glucose level which stimulates insulin activity that results in normal glycemia. Analysis of glycemic curve plot allows to reveal latent forms of diabetes and disorders of glycogenetic functions of liver.

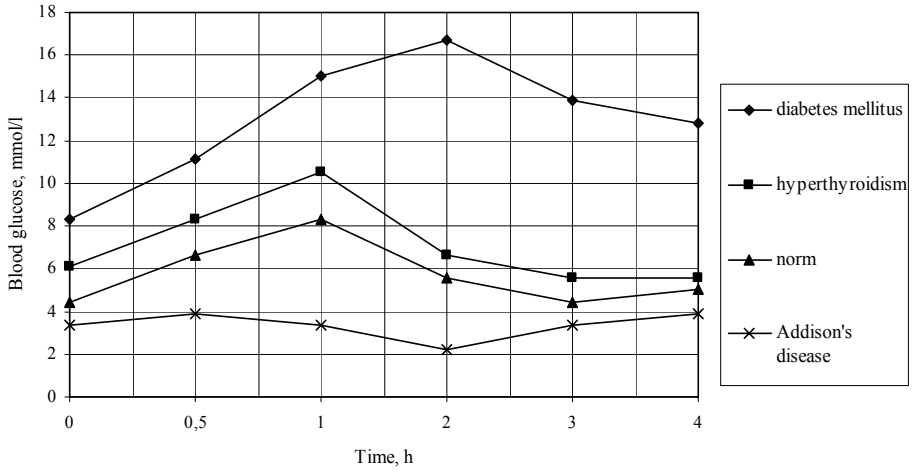
ATTENTION! Follow safety instruction rules manipulating with ortho-toluidine and boiling on the water bath.

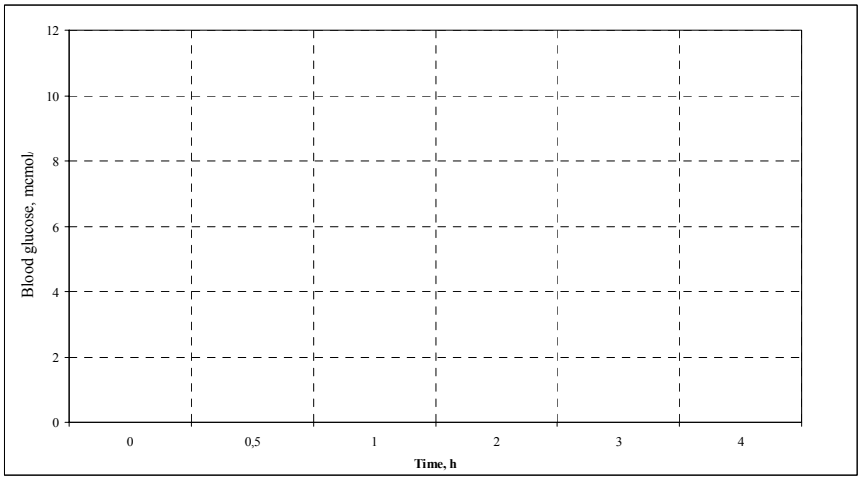
Course of work. Blood is taken from the patient's finger in the morning on an empty stomach and the contents of glucose is determined with ortho-toluidine method (see Lesson 10). Then the patient takes (during no more than 5 minutes) 50–100 g of glucose in 200 ml of warm boiled water (1 g of glucose per 1 kg of body weight). It is possible to replace glucose with saccharose (1,5 g of sugar per 1 kg of body weight). Then repeatedly research the contents of blood glucose, sampling blood from finger every 30 minutes (sometimes every 15 minutes) within 2,5 h (if it was accepted 50 g of glucose) and within 3 h (if it was accepted 100 g of glucose). Children are tested in a similar way as adults, variating only doses of glucose uptaken. On the data received make plot (see a figure), postponing on ordinate axes the contents of blood glucose (mmol/l), and on absciss axis — time of assay capture in minutes or hours.

Glycemic curves plots analysis: healthy person in 15 minutes after glucose administration reveals the augmentation of blood glucose, and between 30-th and 60-th minutes it achieves its maximum. Then downstroke begins and by 120-th minute the contents of glucose returns to the initial level marked on an empty stomach, or with small deflections aside both risings, and downstrokes. In 3 h the contents of blood glucose achieves its initial value.

In diabetes mellitus glycemic curves have extremely high peak and the increased level of glucose remains after 3 h after the load. Diseases accompanying with a hypoactivity of «couter-insuline» hormones (Addison's disease, hypothy roidism), and also lesion of liver parenchyma, serious anemias, diseases of the central nervous system, infectious diseases, toxic states, the flattening of the plot as small peak and low glycemic curve before and after a load are marked.

Conclusions. Write down obtained result, plot the curves and state its clinico-diagnostic evaluation.





Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 90–91, 113–117.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 173–174.

Date: ___/___/_____

Lesson 12

CONTROL ON PARTITION «BIOCHEMISTRY OF CARBOHYDRATES»

The aim of the lesson: the control of learned topic in the partition «Biochemistry of carbohydrates».

4. BIOCHEMISTRY OF LIPIDS

Date: ___/___/_____

Lesson 13

CLASSIFICATION, BIOLOGICAL FUNCTIONS. DIGESTION AND ABSORPTION. LIPOPROTEIN METABOLISM

The aim of the lesson: to give an idea about structure, classifications of the basic lipids, their biological function, about molecular mechanisms of digestion and absorption of lipids in gastrointestinal tract. To study structure, chemical composition, metabolism and functional role of the basic classes of lipoproteins.

Initial level of knowledge and skills

The student should know:

1. Structure and properties of the basic classes of lipids (fatty acids, their derivates, derivates of isoprene).
2. Structure of membranes, models of membranes.

The student should be able:

1. To carry out qualitative tests on hydrolysates of lipids.

Lesson structure

1. Theoretical part

1.1. Lipids – their structure, classifications and biological role.

• Fatty acids and their derivates (PG, LT, TxA), and also:

— simple lipids: wax, diols, triacylglycerols (triglycerides, TG);

— complex lipids: phosphoglycerids — phosphatides (phosphatides: kephalins, lecithines, serinphosphatides, inositolphosphatids, cardiolipins, plasmalogens); sphingolipids (sphingomyelins, cerebrosides and gangliosides); glycolipids, sulfolipids, lipoproteins.

• Derivates of isoprene:

— steroids (sterines and sterids);

— carotenoids (vegetable pigments, vitamins);

— terpenes.

1.2. Role of lipids in membrane formation. Modern models of membranes, their biological role.

1.3. Digestion and absorption of lipids in gastrointestinal tract (structure and functions of bile acids). The mechanism of emulsification of fat. A hepatoenteric cycle of bile acids. Value of lipases. Features of digestion of lipids in children. Resynthesis of TG in enterocytes.

1.4. Lipoproteins (LP) — structure, classification, chemical composition, functional role. Norm metabolism of LP. Exogenous and endogenic paths of transport of lipids in organism.

1.5. Role of receptors of LP in metabolism of lipids.

2. Practical part

2.1. Problem solving.

2.2. Laboratory works.

Laboratory works

Laboratory work № 1. **Qualitative test on bile acids**

Principle of the method. Interaction of bile acid with oxymethylfurfurol, formed from cane sugar under the action of concentrated sulfuric acid, results in red violet staining (Pettenkofer's reaction).

ATTENTION! Follow safety instruction rules manipulating with concentrated sulfuric acid.

Course of work. In the dry tube (under which the sheet of white paper is laid) bring 2 drops of bile, 2 drops of 20% solution of sucrose and carefully stir with glass rod, and then flow 7 drops of concentrated sulfuric acid and stir with same glass rod. After 2–3 minutes there is the red coloring passing at standing in red violet.

Conclusions. Write down received result and to state its clinico-diagnostic assessment.

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Laboratory work № 2. **Influence of bile on activity of lipase**

Principle of the method. Lipase accelerates hydrolysis of neutral fats to glycerin and fatty acids (see the equation), that results in drop pH and to petering of pink coloring of the indicator — phenolphthalein. Activity of the pancreatic lipases determined with titrimetric analysis, sharply grows in action of bile acids.

Course of work. Prepare for three flasks — two test and one control. In them admix preparation of lipase and substrate (milk or sunflower-seed oil) as it is specified in table 1.

Table 1

Composition of an incubativ mixture, ml	Experienced assays		The control
	without bile	with bile	
Milk diluted (1:10)	10	10	10
Glyceric extract of pancreas	1	1	1*
Solution of bile	—	1	1
Water	1	—	1

* — The extract is boiled preliminarily for 10 minutes for an inactivation of lipases.

The prepared incubating mixtures carefully stir. Then from each flask take on 1 ml of the mixture in beforehand prepared glasses for titration. Add in everyone glass on 1–2 drops of solution of phenolphthalein and titrate with 0.01M NaOH solution up to pinkish staining. At the first titration organic acids — lactate and others which were present at milk prior to the beginning of action of lipase are neutralized.

The mixture which has stayed in flasks seat in thermostat (at t = 40°C) And in fixed time intervals (15, 30, 90 minutes) take from each flask (not taking them from thermostat) on 2 ml of mixture and titrate with 0.01M NaOH solution. Time of titration and volume spent NaOH fix in the table 2.

Table 2

Incubation period, minutes	Volume (ml) of 0.01M NaOH expended on titration		
	Test assays		Control
	without bile	with bile	
0			
15			
30			
90			

The results of the first titration received prior to the beginning of action of lipases, subtract from result of the subsequent titrations.

Plot the received data, where on absciss axis postpone time (in minutes), and on ordinate axis — the activity of lipase expressed in volume (ml) of 0.01 M of NaOH solution expended on neutralization of the fatty acids formed for the given interval of time. Compare activity of lipase at the presence of bile and without it.

Conclusions. Write down received result and to state its clinico-diagnostic evaluation.

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Laboratory work № 3. **Emulsification of fats**

Principle of the method. Emulsification of fats with various amphiphile materials descends due to their boundary absorption of two phases — hydrophobic and hydrophilic.

Course of work. In five tubes bring on 1 drop of vegetable oil. Then in each tube accordingly flow on 1–2 drops of NaOH solution, $NaHCO_3$, ovalbumin, detergent and bile. Contents of tubes carefully stir and observe formation of fat emulsion. Explain the mechanism of formation of fat emulsion in these solutions and value of emulsification process.

Conclusions. Write down received result and to state its clinico-diagnostic evaluation.

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Recommended literature

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 117–129.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 185–190, 199–202, 206–209.

Date: ___/___/_____

Lesson 14

TISSUE LIPID METABOLISM

The aim of the lesson: to study the main metabolic paths of basic classes of lipids (TG, PL, fatty acids, ketone bodies, cholesterol). To learn to determine the contents of the common lipids of blood.

Initial level of knowledge and skills

The student should know:

1. The characteristic of basic classes op lipoproteins (LP).
2. Normal metabolism of LP.
3. Paths of hormonal signal transduction in the cell (adenylate cyclase, inositoltriphosphate).
4. TCA, its energy balance.
5. Structure and function of polyenzyme complexes (as example the pyruvate dehydrogenase).

The student should be able:

1. To carry out photocolorimeter investigation.

Lesson structure

1. Theoretical part

1.1. The mechanism of fat mobilization (role of hormones, cAMP and Ca^{2+}).

1.2. Properties and physiological role of free fatty acids (FFA). Transport of FFA in blood.

1.3. Oxidation of triglycerids (TG) in tissue, oxidation of glycerol, its energy balance.

1.4. Stages of β-oxidation of saturated fatty acids. The mechanism of activation and transport of fatty acids through mitochondrial membrane. Role of carnitine. Features of β-oxidations of unsaturated fatty acids and fatty acids with an odd number of atoms. The energy balance of oxidation of C_{16}, C_{15}, $C_{18:2}$.

1.5. The energy balance of tristearate oxidation. Physiological role of FFA in stress.
1.6. Metabolism of Acetyl-CoA (paths of formation and utilization).
1.7. Ketone bodies – biosynthesis, utilization, physiological role.

2. Practical part

2.1. Problem solving.
2.2. Laboratory work.

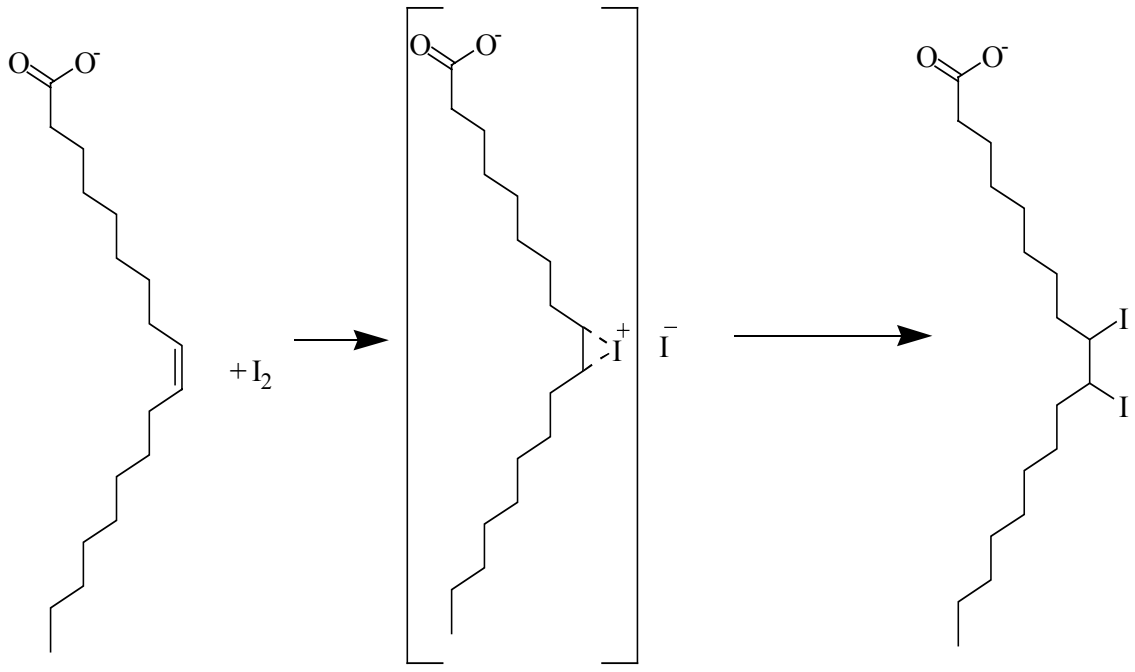
Laboratory work. **Determination of saturation of fats**

Principle of the method: The saturation of fat depends on presence in its structure of unsaturated fatty acids. Unsaturated compounds easily attach in twos atoms of halogen in a place of each double bond. Routinely degree of unsaturation is defined by iodine number. Iodine number is measured by amount of grams of iodine which joins to the 100 gr of fat.

The mechanism of reaction of interaction of unsaturated fatty acids (for example, oleinic) with iodine is shown in figure.

Course of work: In dry 100 ml cone flask with ground-in glass plug is seated with 2 drops of investigated oil. Add 6.3 ml of spirit in the flask for dissolution of the shot. If oil badly solves, it is possible to heat up the flask on the water bath. In the second flask put «blind trial» (control), i.e. take in it 6.3 ml of spirit. In each flask (trial and control) add on 3 ml 0.2 n. Alcoholic solution of iodine (from burette), admix, flow on 25 ml of distilled water and well shake up, having closed plug. In 5 minutes contents of the flasks titrate with 0.1 n. solution of sodium thiosulphate ($Na_2S_2O_3$) till appearance of yellowish staining, then, after adding of 0.25 ml of starch solution, titrate till disappearance of dark blue staining.

Perform parallel investigation with rancid (aged) and irradiated oils.



The scheme of experience:

Components	Control	Trial with oil		
		fresh	aged	irradiated
Oil fresh	—	6.3 ml	—	—
Oil aged	—	—	6.3 ml	—
Oil irradiated	—	—	—	6.3 ml
Spirit	6.3 ml	6.3 ml	6.3 ml	6.3 ml
Solution of iodine	3 ml	3 ml	3 ml	3 ml
Distilled water	25 ml	25 ml	25 ml	25 ml
Stopper and shake up 5 minutes.				
Solution $Na_2S_2O_3$	The first titration			
Solution of starch	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Solution $Na_2S_2O_3$	The second titration			

Difference between amount 0.1 n. solution of sodium thiosulphate spent for titration of trial and control samples, is index of the iodine bonded by shot of oil. Iodine number (in gr.) is calculated under the formula:

$$\text{Iodine number} = \frac{(V_1 - V_2) \cdot 0.0127 \cdot 100}{a},$$

where: V_1 — amount of 0.1 n. solution of $Na_2S_2O_3$, spent for titration of the control (ml);
V_2 — amount of 0.1 n. solution of $Na_2S_2O_3$, spent for titration in trial (ml);
0.0127 — titer of sodium thiosulphate on iodine; a — shot of fat (in gr.).

The divergence in parallel experiments is supposed in the tenth of obtained iodine numbers.

Conclusions. Write down received result and to state its clinico-diagnostic evaluation.

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Recommended literature

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 129–134, 138–141.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 202–206.

Date: ___/___/_____

Lesson 15

BIOSYNTHESIS OF LIPIDS. REGULATION AND PATHOLOGY OF LIPID METABOLISM

The aim of the lesson: to give an idea about the basic types and mechanisms of lipid metabolism disorders. To learn to determine level of the whole blood cholesterol.

Initial level of knowledge and skills

The student should know:

1. Mechanisms of carbohydrate metabolism regulation.
2. Mechanisms of metabolism disorders in diabetes.
3. Structure and biological role of bile acids.
4. The characteristic of basic classes of lipoproteins (LP).
5. Metabolism of LP in norm.
6. Paths of hormonal signal transduction in the cell (adenylate cyclase, inositoltriphosphate).
7. TCA, its energy balance.
8. Structure and function of polyenzyme complexes (as example the pyruvate dehydrogenase).

The student should be able:

1. To carry out photocolorimeter investigation.

Lesson structure

1. Theoretical part

1.1. Biosynthesis of saturated fatty acids. Role of acyl carrier protein protein (ACP), pantothen, biotin, NADPH + H^+ and enzymes. Sources of Acetyl-Coa for biosynthesis of fatty acids (FFA). Regulation of FFA biosynthesis.

1.2. Biosynthesis of triglycerides (TG) and phosphatides.

1.3. Biosynthesis of cholesterol, its regulation, biological role of cholesterol. Poole of cholesterol in the cell, its regulation.

1.4. The mechanism of regulation of lipid metabolism. The hormones which regulate lipolysis and lipogenesis. Integration of lipid and carbohydrate metabolism.

1.5. Lipid-carbohydrate Randle cycle. Triglycerides — fatty acids cycle. Their mechanisms and physiological value. Interactions of ketone bodies, FFA and glucose.

1.6. Disorder of lipid digestion and absorption, manifestations.

1.7. Liver lipid infiltration and degeneration — mechanisms of development and prophylaxis.

1.8. Obesity — types, mechanisms of development and complication.

1.9. Dislipoproteinemias. Fridrikson's classification, biochemical and clinico-diagnostic characteristic of basic groups.

1.10. Lipidoses — inheritable disorders of lipid metabolism.

1.11. Peroxidation of membrane lipids. The mechanism of initiation. Reactions, metabolites. Biological significance in norm and pathology.

1.12. Antioxidant protection (see the topic «Biological oxidation»).

2. Practical part

2.1. Problem solving

2.2. Laboratory work.

Laboratory work. **Quantitative determination of cholesterol in blood serum by the Ilk's method**

Principle of the method. The method is based that HS at the presence of acetic anhydride and mixtures acetic and the chamois of acids (reagent Ilka) forms painted(pigmented) products which intensity of colouring is proportional to concentration and defined(determined) colorimetricly.

ATTENTION! Follow safety instruction rules manipulating with sulfuric acid and acetic anhydride.

Course of work. In the dry tube (!) (presence of traces of water prevents development of colouring) bring 2 ml of reagent Ilka and 0,1 ml of not hemolyzed Serum. Serum add sluggishly so that she(it) flowed off(ran down,drained) on a wall of the tube.

The tube is shaken up aggressively with 10–12 times and seat in a thermostat at temperature 37°C for 20 minutes.

As a check use 2 ml of distilled water.

Colouring of solutions measure on the photometer against the control over a red light filter (wave length of 630–690 nanometers), in the flask in width of 5 mm. Contents HS in assay define(determine) on a standard curve.

Norm. The contents of the general(common) HS in Blood serum of the able-bodied person compounds(makes) 3,7–6,5 mmol/l, or 150–250 mg of %.

Clinico-diagnostic value. The augmentation of contents HS in **a blood plasma** — a hypercholesterolemia — is observed at exuberant consumption of products, rich by a cholesterol, mechanical (obturatsionnoj) an icterus, a nephritis, a myxedema (hypothyroidism), diabetes, an atherosclerosis, a lues, meningitises, some diseases of a liver, and also at ancestral(inheritable) hypercholesterolemias.

Downstroke(Drop) of contents HS in plasma (hypochilesterinemia) is marked at a starvation, an anemia, a tuberculosis, an acute pancreatitis, a parenchymatous icterus, feverish states, acute contagions, a chronic heart failure, a chronic pneumonia, a hyperthyroidism, a cancer cachexia, etc.

Laboratory work. **Determination of low density lipoproteids (LDL) in blood serum.**

Principle of the method: The method is based on ability of LDL to form with heparin complex which under the action of calcium chloride drops out. On degree of opacification of the solution the concentration of LDL in blood serum is estimated.

Course of work: In the tube bring 2 ml of calcium chloride solution and 0.2 ml of blood serum. Stir contents of the tubes. As control sample use 2 ml of distilled water.

Measure absorbency of solution (E_1) on photometer against of 0.27% solutions of calcium chloride at red light filter (wave length of 630 nanometers)

Solution from the cuvette pour again in the tube, add with a micropipette 0.04 ml of 15% heparin solution and exactly after 4 minutes determine again the absorbency of the solution (E_2) in the same conditions.

Calculate the concentration of LDL, gr/l (c) under the formula:

$$c = (E_2 - E_1) \times 10$$

where: 10 is an empirical coefficient.

Clinico-diagnostic value. Content of LDL (β-lipoproteins) in the blood changes in dependence on age, sex and in norm is 3–4.5 gr/l. The increased concentration of LDL is observed in atherosclerosis, mechanical jaundice, acute hepatitises, chronic diseases of liver, diabetes, glycogenoses, xanthomatosis and obesity. Decreased LDL concentration is described in β-plasmacytoma.

Conclusions. Write down received result and to state its clinico-diagnostic evaluation.

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Recommended literature

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 134–138, 141–155.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 190–199, 209–219.

Date: ___/___/_____

Lesson 16
CONTROL ON PARTITION «BIOCHEMISTRY OF LIPIDS»

The aim of the lesson: the control of learned questions in the topic «Biochemistry of lipids».

Date: ___/___/_____

Lesson 17
FINAL (TEST) FOR A SEMESTER

5. BIOCHEMISTRY OF PROTEINS AND NUCLEIC ACIDS

Date: ___/___/_____

Lesson 18

DIGESTION AND ABSORPTION OF PROTEINS. ANALYSIS OF GASTRIC JUICE

The aim of the lesson: to give an idea about alimentary value of proteins, molecular mechanisms of their digestion and absorption in gastrointestinal tract, paths of formation of amino acid pool in tissues and fluids of the organism. To master methods of determination of acidity and pathological components of gastric juice.

Initial level of knowledge and skills

The student should know:

1. Structure, classification and properties of the basic classes of amino acids.
2. Levels of the structural organization of protein molecule.
3. The mechanism of microsomal oxidation.
4. Mechanisms of transmembrane transport of substances.

The student should be able:

1. To carry out titrimetric analysis.
2. To carry out qualitative tests on blood and lactic acid.

Lesson structure

1. Theoretical part

1.1. Role of proteins in nutrition. Valuable and non-valuable proteins. Norms of protein in nutrition. Essential and non-essential amino acids. The nitrogen balance.

1.2. Metabolism of proteins. Digestion of proteins in gastrointestinal tract. Composition and properties of gastric juice. Value of components of the juice in protein digestion (HCl, pepsin, mucus, etc.). The characteristic of pepsin. Mechanisms of formation and secretion of HCl in gastric juice. A regulation of HCl secretion (role of histamine, gastrin, acetylcholine, etc.).

1.3. Intestinal juice. Its composition and properties. The characteristic of pancreatic and intestinal enzymes. The mechanism of activation of trypsin, chymotrypsin, etc.

1.4. Value of pH gradient of the juices of gastrointestinal tract in protein digestion. Mechanisms of protein digestion and absorption of amino acids in gastrointestinal tract.

1.5. Mediators and hormones of gastrointestinal tract — histamin, serotonin, secretin, cholecystokinin, gastric inhibiting peptide, somatostatin, glucagon, enkefalins, etc.

1.6. Putrefaction of proteins in large intestine. Neutralization of putrilages in liver.

1.7. Endogenic pool of amino acids in tissues — paths of formation and utilization.

2. Practical part

2.1. Problem solving.

2.2. Carrying out of accident prevention reinstructing.

2.3. Laboratory works.

Laboratory works

Laboratory work № 1. **Quantitative whole acid estimation; whole, free and combined hydrochloric acid in one assay of gastric juice**

Principle of the method. It is based on titration of stomach contents by solution of 0.1 n. NaOH at the presence of indicators with various zones of transferring. Gastric acidity express amount (in millimol) of NaOH, neutralizing 1 liter of gastric juice.

The basic fractions of acids of gastric juice:

«whole acid» of gastric juice is the sum of all acids of stomachal contents;

«Free hydrochloric acid» — free mineral HCl;

«Combined hydrochloric acid» — acid-reaging salt (chlorides) of proteins and other weekbases;

«Whole hydrochloric acid» — the sum of free and combined HCl.

Quantitative determination of free hydrochloric acid. Free hydrochloric acid is titrated with 0.1 n NaOH solution in presence of the indicator dimethylamine azobenzene, with color transferring region from red up to orange at pH 3.0. Weak acids (lactic, acetic acid, hydrophosphates and combined hydrochloric acid) at pH 2.9–4.0 are in solution in undissociated state and do not react with alkali.

Course of work. To 10 ml of gastric juice add 1–2 drops of spirit dimethylamine azobenzene solution and titrate with solution of 0,1 n. NaOH till orange colouring will appear.

Calculatie on 1000 ml of gastric juice. As the quantity of NaOH spent for titration is equivalent to quantity of hydrochloric acid in assay of gastric juice, the quantity of hydrochloric acid in 1 liter of gastric juice (in a mole/l) will make:

$$X = \frac{a \times 0.1 \times 1000}{b} \quad (1)$$

where: a — quantity of 0.1 n. NaOH solution, spent for titration, ml;

0.1 — quantity of NaOH in 1 ml of 0,1 N. solution, mole;

b — quantity of the gastric juice taken for titration, ml;

1000 — volume of gastric juice, ml.

Quantitative determination of whole acid of gastric juice. Titration of whole acid of gastric juice is carried out by 0.1 n. NaOH solution in presence of phenolphthalein indicator with zone of transferring of colouring between pH 8.2–10.0. At pH 8.2 it is colourless, and at pH higher than 10.0 — it is red.

Course of work. To 10 ml of the filtered gastric juice add 1–2 drops of phenolphthalein solution and titrate with 0.1 n. NaOH solution till appearance of light-pink staining which persists within 1 minutes. Calculate on 1000 ml of gastric juice.

Quantitative determination of whole acid, the whole, free and combined hydrochloric acid in one portion of gastric juice.

Course of work. Measure off in flasks on 10 ml of gastric juice and add on 1–2 drops of dimethylamine azobenzene and phenolphthalein. Titrate with 0.1 n. NaOH solution till appearance of orange staining (the first mark of amount of 0,1н NaOH solution spent). Then continue titrations up to canary (yellow) colour (the second mark) and, finally, up to the pink staining which persists during 1 minute (the third mark).

During titration readout is carried out from initial (zero) point!

The first mark corresponds to amount of free hydrochloric acid, the third — to whole acid. The second mark is used for calculation of the whole hydrochloric acid. Simple average between the second and third marks corresponds to the whole hydrochloric acid. The amount of combined hydrochloric acid is calculated as difference between the whole and free hydrochloric acid. For example, titration with 0.1 n. NaOH it is spent volumes (from the beginning of titration): up to the first mark (orange colour) — 3.3 ml, up to the second (yellow colour) — 4.6, up to the third (pink colour) — 5.6 ml. Simple average between the second and third mark:

$$(4.6 + 5.6)/2 = 5.1 \text{ ml.}$$

Calculate the contents (in moles) of free hydrochloric acid, the whole hydrochloric acid, whole acid on 1000 ml of gastric juice under the formula (1).

This method of calculation is inapplicable at presence of lactic acid in the stomach. Therefore in assays of gastric juice whith lactic acid confine yourself to calculation of free hydrochloric acid and whole acid.

Norm. Parameters of acidity of the filtered stomachal contents of the adult person after standard trial breakfast make:

— whole acid — 40–60 mmol/l (neonatal — 2.8 mmol/l; children about one year — 4–20 mmol/l);

—free HCl — 20–40 mmol/l (neonatal — 0.5 mmol/l);

—combined HCl — 10–20 mmol/l;

—general HCl — 30–60 mmol/l.

The data obtained should be charted:

Problem	Colour	V 0.1 n. NaOH, ml	Maintenance of HCl, mmol/l			Whole acid	Conclusions
			free	combined	whole		
1	Orange						
	Yellow						
	Pink						
2	Orange						
	Yellow						
	Pink						
3	Orange						
	Yellow						
	Pink						

Clinico-diagnostic value. At various diseases of stomach acidity can be increased, dropped and zero. At peptic ulcer of stomach or hyperacid gastritis the hyperacidity — augmentation of the maintenance of free hydrochloric acid and whole acid is observed. At hypoacid gastritis or carcinoma of the stomach the hypochlorhydria — decrease of free hydrochloric acid and whole acid is marked. At carcinoma of the stomach, chronic atrophic gastritis absolute absence of hydrochloric acid and significant drop of whole acid — an achlorhydria — is marked. At malignant anemia, carcinoma of the stomach absolute absence of hydrochloric acid and pepsin — achylia is observed.

Conclusions. Write down received results and state its clinico-diagnostic evaluation.

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Laboratory work № 2. **Investigation of pathological components of gastric juice**

a) Discovery of lactic acid with Uffelmann's reaction.

Principle of the method. At interaction of ferric phenolate having violet colour, with lactate, ferric lactate of yellow-green colour is formed.

Course of work. To 20 drops of phenol solution add 1–2 drops of ferric chloride solution. Solution of ferric phenolate of violet colour is produced. In the tube with ferric phenolate flow dropwise gastric juice (normal and the juice containing lactic acid).

In presence of lactic acid violet colouring passes in yellow-green owing to formation of ferric lactate. Presence of hydrochloric acid decolorizes the fluid. It indicates that strong hydrochloric acid completely blasts ferric phenolate complex, and also replaces more weak lactic acid from its salt; thereof reaction of investigation of lactic acid is negative.

Clinico-diagnostic value. Organic acids (lactic, acetic, butyric, etc.) are routinely of microbial origin and occur in stomachal contents as a result of achlorhydria and the subsequent fermentation of meal components. Presence of organic acids in stomachal contents on an empty stomach frequently meets at atrophic gastritises and carcinoma of the stomach.

Conclusions. Write down obtained results and to state its clinico-diagnostic evaluation.

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b) A benzidine test on blood.

Principle of the method. The haemoglobin reveal catalase activity and decompose hydrogen peroxide forming molecular oxygene which oxidizes benzidine or other stain. Thus there is a colour shifting from colourless to dark blue.

Course of work. In the tube with 1 ml of gastric juice add 4–5 drops of 0.2% spirit of benzidine and 5 drops of 1% solution of hydrogen peroxide. At presence of blood in gastric juice as a result of oxidation of benzidine the dark blue staining developes.

The obtained data should be charted:

Determined component	Used reagents	Assays of gastric juice	
		in norm	in pathology
Whole acid	Phenolphthalein		
Free HCl	Dimethylamine azobenzene		
Lactate (lactic acid)	Ferric phenolate		
Blood	Benzidine		

The note — If results of any work are negative put the crossed out section in the corresponding column.

Clinico-diagnostic value. The blood occurs in stomachal contents at ulceration of walls of stomach at peptic ulcer, erosive, ulcerous gastritis, combustions of stomach mucous and carcinoma of stomach.

Conclusions. Write down obtained result and to state its clinico-diagnostic assessment.

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Recommended literature

1.*Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 156–162.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 231–237.

Date: ___/___/_____

Lesson 19

TISSUE METABOLISM OF AMINO ACIDS. DETOXICATION OF PRODUCTS OF THE METABOLISM

The aim of the lesson: to give an idea about the basic paths of metabolism of free amino acids in tissues. To study mechanisms and significance of detoxification reactions of ammonia in norm and pathology. To master procedure of determination of urea concentration in blood serum and urine.

Initial level of knowledge and skills

The student should know:

1. Structure, classification and properties of the basic classes of amino acids.
2. TCA, reactions, enzymes, mechanisms of regulation.
3. The mechanism of microsomal oxidations.
4. Structure of vitamin B_6 and its coenzyme forms.

The student should be able:

1. To investigate on the photocolorimeter.

Lesson structure

1. Theoretical part

1.1. The basic exchange reactions of amino acids:

1.1.1. Reactions on radical:

a) Hydroxylation (*pro, lys, phe*). The mechanism of microsomal oxidations (the role of ascorbate, NADPH, cytochrome P_{450}, etc.), examples, biological significance;

b) Breakage (the mechanism, biological significance);

c) Methylation, etc.

1.1.2. Reactions on carboxyl group:

a) Decarboxylation (on example of *his*, *tyr*, *trp, glu*) – the mechanism, enzymes, biological role;

b) Reduction — enzymes, biological role.

1.1.3. Reactions on amino group:

a) Types of deamination (oxidative, reduction, hydrolytic, the intramolecular), their medicobiological significance;

b) Direct oxidative deamination — the mechanism, enzymes, coenzymes, biological significance;

c) Reactions of transamination — enzymes, coenzymes, biological significance;

d) Indirect oxidative deamination – the mechanism, enzymes, coenzymes, biological significance.

1.2. Ammonia, paths of its formation and mechanisms of toxicity.

1.2.1. Paths of ammonia detoxification:

a) The reductive amination;

b) Formation of amides (*gln* and *asn*);

c) Ammoniogenesis;

d) Biosynthesis of urea, reaction, enzymes, localization, biological role of urea cycle (UC). Energetics of UC. Integration between UC and TCA, and metabolism of amino acids. Role of UC in regulation of acid-base balance.

1.3. Congenital defects of UC enzymes, general clinical manifestations.

1.4. Paths of amino acids entering in TCA (scheme). Glyco-and ketogenic amino acids.

2. Practical part

2.1. Problem solving.

2.2. Laboratory work.

Laboratory work. **Quantitative urea test in blood serum and urine**

Principle of the method. The urea forms with diacetyl monooxim in strong acid medium in the presence of thiosemicarbazide and ions of trivalent iron the complex of red colour which intensity is proportional to the contents of urea.

Precaution techniques during the work. Pay close attention, as reagent № 2 contains toxicant thiosemicarbazide, and main solution contains sulfuric acid.

Course of work. The work is carried out according to table 1.

Table 1

Reagent	Assay	Standard	Control
Serum or diluted urine	0.01	—	—
Reagent 1	—	0.01	—
Distilled water	—	—	0.01
Reagent 2	2.0	2.0	2.0

In the tube measure off 0.01 ml of blood serum or diluted urine, add 2 ml of main solution (reagent № 2), which contains a mixture of diacetyl monooxim, thiosemicarbazide and iron chloride solution in acidic medium.

Reference assay treat precisely as described above, using 0.01 ml of reference solution of urea (reagent № 1) instead of 0.01 ml of blood serum.

Contents of tubes carefully stir, tubes occlude with aluminium foil and seat precisely (!) for 10 minutes in a boiling bath.

Then tubes quickly cool under tape cool water and not later (!) than 15 minutes after cooling, measure absorbency of assay (A_1) and the standard (A_2) against the control solution (reagent № 2) in the flask 10 mm wide and wave length of 490–540 nanometers (green light filter).

Urine before analysis dilute with distilled water in the ratio 1:100, and the result is multiplied on quotient of dilution.

Calculation:

$$[\text{Urea}] = 16.65 \times (A_1/A_2)\ (\text{mole/l}).$$

Norm. 2.5–8.3 mmol/l.

Warning. If the contents of urea in assay is above 23 mmol/l it is necessary to dilute assay with distilled water, repeat the analysis, and obtained result multiply per dilution factor.

Determination of urea in hemolyzed or hyperlipemic serums requires deproteination before the assay with 5% trichloracetic acid (TCAA) solution. Admix in the tube 0.1 ml of assay, 1 ml of solution TCAA and centrifuge. In the same way dilute the reference solution of urea too. For the analysis itself measure off 0.1 ml of supernatant. Further determination carry out as described above. The same method is possible for the whole blood analyzis.

Clinico-diagnostic value. Urea concentration consists a half of rest (nonprotein) nitrogen of blood, that part which to the greatest degree rests in blood in

disorders of kidney functions. In kidney pathology the level of urea in a blood arise much faster than other components of rest nitrogen. Besides determination of blood urea level is technically easier carried out, than rest nitrogen. That is why the blood urea characterizes, first of all, excretory function of kidney.

Rising of the blood urea is marked at patients with other morbid conditions — reflex anuria, obstruction (renal calculus and malignant neoplasms) in the urinary paths, an extra proteolysis (in acute yellow atrophy of liver, serious infectious diseases, major traumas, etc.).

The upper limit of the blood serum urea level depends on nutrition. At proteins intake in day over 2.5 gr/kg of weight the level of urea can grow up to 10 mmol/l.

Drop of blood urea level is observed rarely and it is marked routinely at deficiency of protein in ration. In pregnancy decreasing of blood urea below 3.33 mmol/l is also possible.

Conclusions. Write down obtained result and to state its clinico-diagnostic evaluation.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 162–173.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 237–245.

Date: ___/___/_____

Lesson 20

FEATURES OF AMINO ACIDS METABOLISM IN NORM AND PATHOLOGY

The aim of the lesson: to give an idea about features of individual amino acids (AA) metabolism in norm and pathology. To give biochemical substantiation of practical application of amino acids in medicine. To master the procedure of determination of blood serum transaminase activity.

Initial level of knowledge and skills

The student should know:

1. Structure, classification and properties of the basic classes of amino acids.
2. TCA, reactions, enzymes, mechanisms of regulation, its interrelation with amino acid, carbohydrates, lipids metabolism, and urea cycle.
3. Mechanisms of mitochondrial and microsomal oxidations.

4. Enzymopathies (general characteristic).
5. Enzymodiagnostics (principles, objects, the purpose and problems).

The student should be able:
1. To investigate on the photocolorimeter.

Lesson structure

1. Theoretical part

1.1. TCA (reactions, enzymes, coenzymes, mechanisms of regulation, biological role). Paths of individual amino acids entering in TCA (glyco-and ketogenic amino acids).

1.2. Features of individual amino acids metabolism — biosynthesis and catabolism, participation GNG or ketogenesis, application in medicine.

1.3. *ala* — the basic paths of metabolism, regulatory role.

1.4. *gly*, *ser* — the mechanism of interconversions, role of tetrahydrofolic acid (THFA) an metabolism, participation in biosynthesis of phosphotides, ethanolamine, choline, purines, porphyrins, glutathione, creatine, hippuric acid, bile acids. Metabolism disorder of *gly* — hyperglycinemia, oxalosis, their basic clinical manifestations.

1.5. *glu* — direct and indirect oxidative deamination, transamination, enzymes and biological significance. Biological significance of glutamatdehydrogenase (GDH).

1.5.1. Adaptive role of *glu*: antihypoxic — formation of GABA, GOBA and succinic acid, power «yield» of *glu* oxidation, antitoxic — neutralization of ammonia, bonding of heavy metals, etc.; antioxidant — synthesis of glutathione; *pro*, purine bases biosynthesis. Role of *glu* in carbohydrate, lipid and nitrogen metabolism integration. Indications to administration of *glu* in medical practice.

1.6. *Pro* — biosynthesis, catabolism, mechanism of *h-pro* formation, reaction, enzymes, role of microsomal oxidation, ascorbate, etc. Clinico-diagnostic value of determination of the contents of *h-pro* in blood and urine. Metabolic disorder of *pro* — hyperprolinemia, the basic clinical manifestations.

1.7. *His* — biosynthesis and the basic pathways, their biological role: formation of histamine, dipeptides – anserine, carnosine. Use of *his* as radioprotector and antioxidant. Metabolic disorder of *his* — hyperhistidinemia, the basic clinical manifestations.

1.8. *Arg* — biosynthesis and the basic pathways, their biological significance: adaptable role of system *arg* — arginase — urea.

1.9. *Asp* — the basic metabolic transformations: transamination, amidation (neutralization of ammonia), β-decarboxylation (biological role of β-alanine), biosynthesis of purine and pyrimidine bases, biosynthesis of urea, participation in purine nucleotide cycle. Indications to administration of *asp* in medical practice.

1.10. *Cis* — the mechanism of biosynthesis from *met*. Antitoxic, antioxidant and radioprotective role: biosynthesis of cystine, taurine, PAPS, glutathione, etc. Metabolic disorder of *cis* — cysinosis, its basic clinical manifestations.

1.11. *Met* — the basic paths of metabolism: formation of S-adenosylmethionine *(SAM)*, vitamin *U* (S-methylmethionine), reactions of transmethylation — synthesis of choline, epinephrine, creatinine, reactions of detoxification, etc. Metabolic disorder of *met* — homocystinuria, cystathioninuria, basic clinical manifestations.

1.12. *Phe* and *tyr* — the basic paths of metabolism: biosynthesis of catecholamins, thyroid hormones, melanin, etc. Metabolic disorder of *phe*, *tyr* — phenylketonuria, albinism, alcaptonuria, a tyrosinosis, their basic clinical manifestations.

1.13. *Trp* — basic pathways: kynurenine, formation of tryptamin and serotonin. Metabolic disorders of *trp* — Hartnup disease, its basic clinical manifestations.

1.14. *Val*, *leu*, *ile* — features of metabolism, regulatory role of these amino acids. Metabolic disorders — maple syrup disease, its basic clinical manifestations.

1.15. Integration of carbohydrate, lipid and protein metabolism, the mechanism of common metabolites formation.

2. Practical part

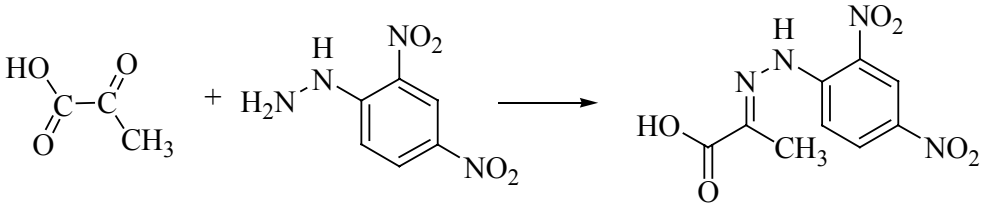
2.1. Problem solving.

2.2. Laboratory works.

Laboratory works

Laboratory work № 1. **Determination of blood serum aspartate aminotransferase activity (AST) by Reitman and and Frenkel**

Principle of the method. As a result of transamination under action of AST, the oxalacetic acid is formed. The oxalacetic acid is spontaneously decarboxylated to pyruvic acid. Addition of 2,4-dinitrophenyl hydrazine in alkaline condition hydrazone of pyruvic acid of red-brown colour is formed. Colouring intensity is determined colorimetrically (see equation)



Pyruvate — 2,4-dinitrophenyl hydrazine (DNP) — 2,4-DNP-hydrazone pyruvic acid

Course of work. The tube containing 0.25 ml of substrate-buffer mixture heat up in thermostat at 37°C during 5 minutes, add 0.05 ml of blood serum and incubate during 60 minutes in thermostat at the same temperature.

Add 0.25 ml of 2,4-dinitrophenyl hydrazine solution and incubate during 20 minutes at room temperature. Then add 2.5 more ml of NaOH, stir and leave for 10 minutes at room temperature.

Measure photometrically extinction of assay sample tube at wave length of 500-560 nanometers (green light filter) in the cuvette of 10 mm layer depth. As a control distilled water is used.

Calculation. Uzie the calibrating graph.

Norm. AST — 0.1–0.45 mmol/h×l (of pyruvate per 1:l of blood serum during 1 hour of incubation at 37°C).

Conclusions. Write down received result and to state its clinico-diagnostic evaluation.

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Laboratory work № 2. **Determination of ALT (alanine aminotransferase) activity in serum and blood plasma with enzymatic method (UV-range)**

Principle of the method. It is based on coupling of two enzymatic reactions (ALT and LDH) — transaminations and the subsequent NADH-dependent regeneration of pyruvate, formed during transamination.

I stage:

$$L\text{-}ala + \alpha\text{-}ketoglutarate \xleftrightarrow{ALT} pyruvate + L\text{-}glu;$$

II stage:

$$pyruvate + NADH{+}H^{+} \xleftrightarrow{LDH} L\text{-}lactate + NAD^{+}.$$

The reaction process is recorded on a loss of the reduced form of the coenzyme — NADH+H^{+}, having the maximum absorption at 340 nanometers.

Course of work. Activity of ALT in blood serum determine in 2 stages.

I stage. Bring in the tube 1 ml of solution № 1 (mixture of LDH, NADH+H^{+} buffer-substrate, pyridoxalphosphate) and 0.1 ml of blood serum, stir and thermostate during 5 minutes at 37°C.

II stage. Contents of the tube pour in the cuvette, preheated up to 37°C, and add 0.1 ml of solution № 2 (α-ketoglutarate).

Measure absorbency at wave length of 340 nanometers, in 10 mm width cuvette, in 3 minutes interval.

Calculation. Calculate change of extinction for 1 minutes ($\Delta A/\Delta t$) In mkkat/l, and also catalytic concentration (activity ALT) under the formula:

$$S = \Delta A/\Delta t \times 31.75.$$

Norm. Activity of blood serum ALT is 0.15–0.96 mkkat/l.

Clinico-diagnostic value. Determination of ALT and AST activities is widely used in early differential diagnostics of various diseases. Both enzymes are highly active in various tissues. However the greatest ALT activity falls at

liver, and AST — on myocardium. In connection with high self-descriptiveness determination of ALT activity is used for early diagnostics of infectious disease (before appearance of jaundice and the first signs of illness — indisposition, weakness, etc.), and also its non- anicteric forms. High activity of the enzyme in blood is sustained during the first 10–15 days, and then gradually drops. The degree of increasing of ALT activity correlates with gravity of illness.

AST is more specific to myocardium, therefore it is used for early differential diagnostics of myocardial infarction. And the increase of its activity is marked in 24–36 hours and drops on 3–7-th day.

Conclusions. Write down obtained result and to state its clinico-diagnostic evaluation.

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Recommended literature:

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 173–182.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 245–256, 262–263.

Date: ___/___/_____

Lesson 21

NUCLEOPROTEINS. STRUCTURE AND FUNCTIONS OF INFORMATION MACROMOLECULES

The aim of the lesson: to give an idea about the structure, metabolism and functions of nitrogen bases, nucleotides and nucleic acids. To master qualitative tests on hydrolysates of nucleoproteins.

Initial level of knowledge and skills

The student should know:

1. Structure, properties and functions of nitrogen bases and nucleotides.
2. E.Chargaff's rules.
3. Structure, classification, properties and functions of nucleic acids.
4. Molecular mechanisms of digestion and absorption of nutrients in gastrointestinal tract.
5. Enzymopathies (general characteristic).

The student should be able:

1. To carry out qualitative tests on proteins and carbohydrates.

Lesson structure

1. Theoretical part

1.1. Digestion and absorption of nucleoproteins in gastrointestinal path. The characteristic and functions of «nuclear» proteins.

1.2. Mononucleotides as structural components of nucleic acids (NA), their basic functions:

1.2.1. energy carriers — ATP, GTP.

1.2.2. coenzymes — NAD^+, $NADP^+$, FAD, FMN.

1.2.3.messengers of hormonal, etc. signals — cAMP, cGMP.

1.3. Metabolism (synthesis and catabolism) of purines and pyrimidines. Reactions, enzymes, regulation.

1.4. Biosynthesis of AMP and GMP. Reactions, enzymes, regulation.

1.5. Structure and functions of NA. Features of structure and role of various types of DNA (nuclear, mitochondrial, ribosomal, sattelite, etc.). Features of viruses and phages DNA structure.

1.6. Features of structure and role of various types of RNA — messenger, ribosomal, transfer, virus.

1.7. Role of minor bases in NA structure. Specific specificity index.

1.8. Polymorphism of the secondary DNA structure — A, B and Z-forms.

1.9. Mechanisms of storage and transfer of the genetic information — reparation, replication (structure of replicative fork), transcription, translation, the characteristic of the basic enzymes and cofactors.

1.10. Stages of DNA biosynthesis — initiation, elongation, termination, role of DNA-polymerases.

1.11. RNA biosynthesis, its regulation, role of RNA-polymerases. RNA processing, its biological significance. Alternative splicing. Regulation of immunoglobulin gene expression.

1.12. Pathology of nitrogen bases and NA metabolism. Disorders of DNA repair processes and their consequence. The causes of origine and the basic clinical manifestations of orotic aciduria, xanthinuria, Lesch-Nyhan syndrome and gout.

2. Practical part

2.1. Problem solving.

2.2. Laboratory work.

Laboratory work **Qualitative tests on nucleoprotein hydrolysates (proteins, carbohydrates, purine bases, phosphate).**

Principle of the method. It is based on carrying out of specific reactions to components of nucleoproteins of the yeast obtained by their hydrolysis: polypeptides, purine bases, carbohydrates and phosphate.

ATTENTION! Follow safety instruction rules manipulating with concentrated sulfuric acid and boiling on the water bath.

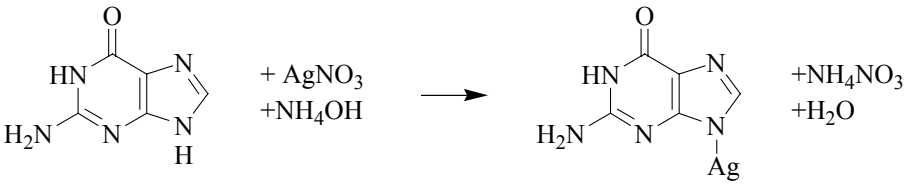
Course of work.

a) Biuret reaction on polypeptides.

To 5 drops of hydrolysate add 10 drops of 10% NaOH solution, then 2 drops of 1% solution of $CuSO_4$. The complex of violet colour is formed.

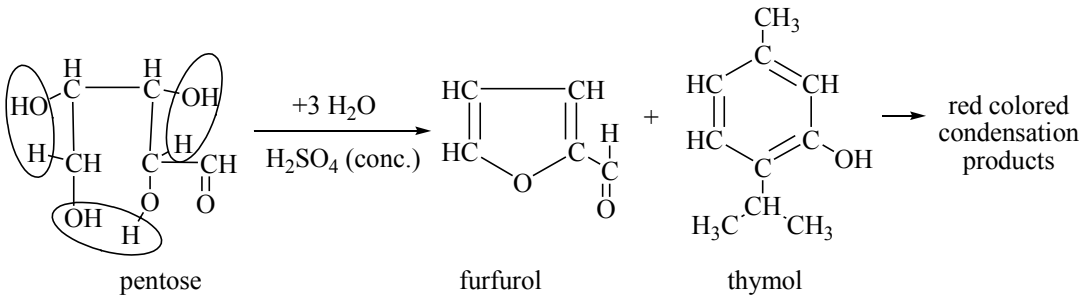
b) Silver assay on purine bases.

To 10 drops of hydrolysate flow 10 drops of concentrated (!) ammonia, then add 10 drops of 2% ammonia solution of silver nitrate. After 3–5 minutes the light brown deposit of silver salts of purine bases (see the equation) is formed.



c) Qqualitative test on pentose (Molicsh).

To 5 drops of hydrolysate add 3 drops of 1% thymol spirit solution, stir and on wall of the tube cautiously couch together 20 drops of concentrated (!) sulfuric acid. At shaking on the bottom of the tube condensation product of furfurol with thymol of red colour (see the equation) is formed.



d) Qualitative test on carbohydrates.

To 5 drops of hydrolysate of yeast flow 3 drops of 0.2% of alpha-naphthol spirit solution and 20 drops of concentrated (!) sulfuric acid. Observe appearance of pink staining.

d) Reaction on deoxyribose and ribose.

To 5 drops of hydrolysate of yeast add 20 drops of 1% solution of diphenylamine and boil on the water bath during 15 minutes, thus the blue-green staining as diphenylamine with desoxyribose yields dark blue staining, and with ribose – green colouring is formed.

e) Molybdenic assay on phosphoric acid.

To 10 drops of hydrolysate of yeast flow 20 drops of molybdenic reagent and boil some minutes on open fire of a spirit-lamp. Thus fluid is staining in yellow colour. The tube cool in stream of cool water. At the bottom of the tube there is a crystalline yellow deposit of ammonium phosphomolybdate.

The results of work. Write down in the table:

Investigated compound	Used reagents	Reaction products	What reaction is caused by?
1			
2			
3			
4			
5			
6			

Conclusions. Write down obtained result and to state its clinico-diagnostic evaluation.

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Recommended literature

1. *Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 182–212.

2. *Marks D. B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 47–67, 256–260.

Date: ___/___/_____

Lesson 22

BIOSYNTHESIS OF PROTEIN. REGULATION OF BIOSYNTHESIS. PROTEIN METABOLISM PATHOLOGY

The aim of the lesson: to give an idea about stages of protein biosynthesis, mechanisms of its regulation and molecular aspects of the basic disorders of nitrogen metabolism. To master refractometer method of blood serum protein determination.

Initial level of knowledge and skills

The student should know:

1. Structure, classification and properties of the basic classes of nucleic acids.
2. Structure of ribosomes.
3. Mechanisms of enzymatic activity regulation.
4. Structure and function of immunoglobulins.
5. Enzimopathies (general characteristic).

The student should be able:

1. To investigate on the refractometer.

Lesson structure

1. Theoretical part

1.1. Principal difference of protein biosynthesis from biosynthesis of other molecules. The general scheme of protein biosynthesis — necessary preconditions:

1.1.1. information stream — the scheme of transfer of the information (the central dogma of molecular biology). Replication and transcription of DNA — enzymes, the mechanism. Reverse transcription, the role of revertases. Processing and splicing of mRNA. The characteristic of genetic code, codon, anticodon;

1.1.2.plastic stream — the mechanism of activation of amino acids, structures of tRNA, characteristic of ARSases — codases;

1.1.3. energy stream. Role of macroergs ATP, GTP, etc. in protein biosynthesis.

1.2. Ribosomes — principles of the organization, structure, composition. The mechanism of translation — stages of ribosomal cycle:

1.2.1. initiation, initiating factors. Formation of initiating complex;

1.2.2. elongation, elongation factors;

1.2.3. termination.

1.3. Types and mechanisms of post-translational modification (processing) of proproteins:

1.3.1. chemical modification (types, examples);

1.3.2. limited proteolysis;

1.3.3. self-assembly of protein.

1.4. Regulation of protein biosynthesis in prokariotes. Features of regulation of protein biosynthesis in eukariotes:

1.4.1. selective transcriptional;

1.4.2. alternative splicing of mRNA;

1.4.3. histone and nonhistone proteins modification.

1.5. Structure of immunoglobulins (Ig). The characteristic of basic classes of Ig — IgA, IgD, IgE, IgG, IgM. Regulation of gene expression of Ig and the causes of their variety.

1.6. Pathology of protein metabolism. Disorder of digestion and absorption, achylia consequence. Protein starvation, kwasiorkor, their consequences and the basic manifestations. The biosynthesis of defect proteins. Primary and secondary defect proteins. Relatively pathological proteins. Damaged proteins.

2. Practical part

2.1. Problem solving.

2.2. Laboratory work.

Laboratory work. **Determination of blood serum whole protein by refractometric method**

Principle of the method. At the heart of refractometry there is a differing refraction of fluids quantitatively expressed by refraction coefficient (the ratio of the dip angle (α) to the angle of refraction (β):

$$n = \frac{\sin \alpha}{\sin \beta},$$

which in blood serum is caused mainly by amount, quality of the dissolved protein and temperature. Influence of other components of blood serum on refraction coefficient is much less. Refraction coefficient is determined by refractometers.

The contents of protein in blood plasma (serum) percentage

Refraction coefficient	The contents of protein	Refraction coefficient	The contents of protein	Refraction coefficient	The contents of protein
1,33705	0,63	1,34313	4,16	1,34910	7,63
1,33743	0,86	1,34350	4,38	1,34947	7,85
1,33781	1,08	1,34388	4,60	1,34984	8,06
1,33820	1,30	1,34420	4,81	1,35021	8,28
1,33858	1,52	1,34463	5,03	1,35058	8,49
1,33896	1,74	1,34500	5,25	1,35095	8,71
1,33934	1,96	1,34537	5,47	1,35132	8,92
1,33972	2,18	1,34575	3,68	1,35169	9,14
1,34000	2,40	1,34612	5,90	1,35205	9,35
1,34048	2,62	1,34650	6,12	1,35242	9,57
1,34086	2,84	1,34687	6,34	1,35279	9,78
1,34124	3,06	1,34724	6,55	1,35316	9,99
1,34162	3,28	1,34761	6,77	1,35352	10,20
1,34199	3,50	1,34798	6,98	1,35388	10,41
1,34237	3,72	1,34836	7,20	—	—
1,34275	3,94	1,34873	7,42	—	—

Calculation. Having determined refraction coefficient according to the table calculate the percentage of protein in blood serum. To convert to SI units (g/l) the result should be multiplied by 10.

Norm. The contents of blood plasma whole protein for the healthy person makes 6,5–8,5%, or 65–85 g/l.

Conclusions. Write down obtained result and to state its clinico-diagnostic evaluation.

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Recommended literature

1.*Danchenko O.A.* Biochemistry: course of lectures. Gomel: VSMU Press, 2004. — P. 212–224.

2. *Marks D.B.* Biochemistry (Board review series), 2-nd edition, Baltimore, USA, 1994. — P. 67–85, 262–264.

Date: ___/___/_____

Lesson 23

CONTROL ON THE TOPIC «BIOCHEMISTRY OF PROTEINS AND NUCLEIC ACIDS»

THE CONTENTS

Учебное издание

Авторы-составители:

Грицук Александр Иванович
Коваль Александр Николаевич
Свергун Валентина Тимофеевна

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