

BIOCHEMISTRY

Manual for international students
of pharmaceutical faculty

Minsk BSMU 2018

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА БИОЛОГИЧЕСКОЙ ХИМИИ

БИОХИМИЯ

BIOCHEMISTRY

Практикум
для иностранных студентов фармацевтического факультета



Минск БГМУ 2018

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Содержит рекомендации по подготовке к лабораторно-практическим занятиям по биологической химии. По каждой теме даны: цель занятия, вопросы для обсуждения, литература для подготовки, описание лабораторных работ и их клинко-диагностическое значение. Включены вопросы для подготовки к коллоквиумам, перечень экзаменационных вопросов.

Предназначен для иностранных студентов 3-го курса, обучающихся на английском языке по специальности 1-79 01 08 «Фармация».

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SAFETY RULES FOR WORK IN BIOCHEMICAL LABORATORY

Work in a laboratory involves certain degree of risk: accidents can and do happen. Strict adherence to the following rules is essential for preventing — or minimizing — the effects of accidents.

1. Students should pay full attention to all instructions, written or spoken, before and during experimental work in the student laboratory. No unauthorized experiments are allowed. In case of any unclear instruction student has to ask the teacher for further information.

2. Eating, drinking and smoking in the laboratory is strictly forbidden. Bringing food and beverages to the laboratory is not allowed either.

3. Students must use adequate footwear for the laboratory and always wear a laboratory coat, which must be kept button-down.

4. Every student works at his/her working place, where he/she is obliged to keep everything clean and in order. Every student is responsible for the condition of used equipment and reagents. Any loss or damage must be immediately reported to the teacher or technician.

5. Any heated or chemically contaminated tools can only be put aside on a specified place. Any reagent bottle should be returned to its place immediately after use.

6. In case of spillage or leakage of any reagent or biological material or any other unexpected event, every student is obliged to perform all possible measures to minimize harm to human health, equipment or environment, and notify the instructor immediately.

7. Spilled acid is immediately diluted with water, and washed out with water or removed by absorption into an inert material. Likewise, spilled alkali is washed out with water or removed by absorption into an inert material, and disposed as a dangerous waste.

8. For work with biological material and some hazardous substances the single-use latex gloves are used. Contaminated gloves should be changed immediately.

9. When heating and observing a sample in a test tube, the tube orifice must always be directed out from the student's face, as well as faces of any other persons around.

10. In case of any injury every student is obliged to provide an adequate first aid and notify the teacher or technician immediately.

11. After finishing the experiments every student is supposed to clean his/her working place, rinse used glassware and put all tools to their appropriate places. Only if the teacher or technician checks the working place and finds it in an acceptable condition, the student is allowed to leave.

First Aid in Laboratory Accidents

Chemicals in the eye

If any reagent or biological material enters the eye, immediately (!) wash the affected eye with plenty of tap water. The teacher will decide on further treatment. No neutralizing solutions or eye drops are used in the first aid.

Corrosion of the skin

Remove the stained clothes, and wash with plenty of tap water. In case of severe burns the teacher will provide further treatment.

Burns

As soon as possible cool the affected area of the skin with a lot of cold tap water. The teacher will provide further treatment.

Open wound

Stop bleeding and prevent wound infection. Small wounds, e.g. cuts by pieces of glass are washed with stream of water; further treatment, including disinfection and sterile cover, is provided by the teacher. Do not try to remove foreign bodies in the wound, such as glass shivers, during the first aid.

1. INTRODUCTION TO PRACTICAL WORK. INTRODUCTION TO BIOCHEMISTRY. THE STRUCTURE OF AMINO ACIDS AND PEPTIDES. DETERMINATION OF THE PROTEIN CONTENT IN BIOLOGICAL FLUIDS

Objective. To study the structure, physical and chemical properties of amino acids as structural elements of proteins and peptides and to learn general principles of evaluating the content of protein in biological fluids.

Problems for discussion:

1. The subject, objectives and tasks of Biochemistry. Objects and research methods in Biochemistry.
2. Amino acids, their classification. General properties. Formulae for proteinogenic amino acids, terminology. Use of amino acids as medicaments (examples).
3. Peptides, their structure. The peptide bond. Properties of the peptide bond postulated by L. Pauling and R. Corey. Classification and biological role of peptides, their use in medicine.
4. Proteins as a class of organic substances, their biological functions. Classification of proteins.
5. Determination of total protein by a biuret method.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 3–5.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 4–5.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 14–21.
4. Lecture material.

PRACTICAL PART

Determination of total protein by a biuret method

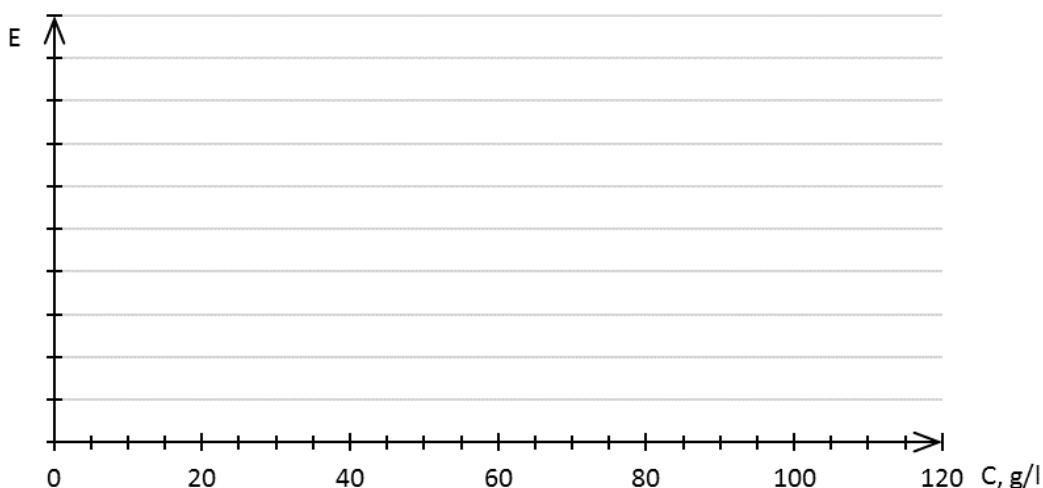
There are various methods to measure total protein in the blood serum including colorimetric methods that have become widely spread and among them is the method based on a biuret reaction. It is characterized by specificity, precision and availability. The other colorimetric method, Lowry's method, uses both a biuret reaction and a reaction of Folin's reagent reduction by cyclic amino acids (tyrosine, phenylalanine, tryptophan). Although this method is more sensitive, it has a complex procedure of preparing stock solution and is less specific, as a great number of other substances come into reaction with Folin's reagent and form complexes with specific staining.

Principle of the method. The method is based on the formation of a complex of protein peptide bonds and ions of bivalent copper (copper sulfate) with violet staining in alkaline medium. The intensity of stained solution is proportional to serum protein concentration and is determined photometrically.

Procedure. The first test-tube (tested) is filled with 0.1 ml of analyzed serum, the second (control) — with 0.1 ml of 0.9 % NaCl. 5 ml of biuret reagent are added into both test-tubes. The content of the test-tubes is carefully stirred avoiding foam formation, and in 30 minutes the tested sample undergoes a photometric procedure in 10-mm cuvettes under a green light filter (wave length is 540 nm) versus the control solution. Having determined the tested solution extinction, the protein concentration is determined according to a calibration graph (in g/l).

Construction of a calibration graph. To construct a graph one should prepare working albumin solutions with 20, 40, 60, 80 g/l concentration on the basis of a standard protein solution with a concentration of 100 g/l. So, take 0.2; 0.4; 0.6; 0.8; 1 ml of standard protein solution and add 0.8; 0.6; 0.4; 0.2; 0 ml of NaCl solution. Take 0.1 ml of the solution from every dilution and apply it into test-tubes with 5 ml of biuret reagent. 30 minutes later the extinction of standard samples is checked versus control photometrically. The calibration curve is constructed by marking concentration values of standard protein solutions (in g/l) on the abscissa and corresponding values of optical density on the ordinate.

Results:



<i>C, g/l</i>	20	40	60	80	100
<i>E</i>					

E test=

C test=

Clinical and diagnostic value. The normal content of total protein in the blood serum (normoproteinemia) in adults is 65–85 g/l, in children under 6 years — 56–85 g/l. Changes in total protein concentrations can be absolute and relative. Relative changes are associated with blood volume changes (plasma). Thus, relative hypoproteinemia (reduced protein concentration in the blood) develops in hydremia, i.e. loading with water, while relative hyperproteinemia (elevated protein concentration in the blood) occurs in blood thickening due to considerable loss of fluid in diarrhea, incoercible vomiting, profuse perspiration, cholera, burns. Absolute hypoproteinemia is seen in nephritis, malignant tumors, alimentary dystrophy. Absolute hyperproteinemia occurs relatively rare, e.g. in myeloma, chronic polyarthritis.

Conclusion:

2. STRUCTURE AND FUNCTIONS OF PROTEINS. PHYSICAL AND CHEMICAL PROPERTIES OF PROTEINS. MECHANISMS OF PROTEIN SEDIMENTATION

Objective. To consolidate knowledge of the proteins' primary structure and its role in formation of three-dimensional structure of the molecule. To form the notion of conformational states of a protein molecule and their significance in protein functioning. To acquaint with the usage of protein denaturation in medical practice.

Problems for discussion:

1. Spelling rules for peptides (proteins), their names and their charge definitions.
2. Levels of organization of a protein molecule (concept, varieties and bonds stabilizing the structure).
3. Conformational changes in functioning of proteins. Interaction of proteins with ligands. Cooperativity effect.
4. General physical and chemical properties of proteins (viscosity of solutions, light diffusion, optical activity, mobility in the electric field, absorption of UV rays, solubility in water).

5. Stability of protein solutions (the role of a protein charge, hydrate shell, molecular weight, molecule shape). Isoelectric state.
6. Denaturation. Reversibility of denaturation. Mechanisms of denaturing factors action. Uses of protein denaturation in medicine.
7. Sedimentation of proteins (reversible — “salting-out”, irreversible).

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 5–9.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 6–9.
3. *Harper’s biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 30–49.
4. Lecture material.

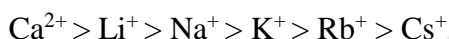
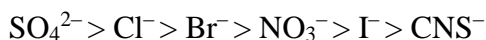
PRACTICAL PART

Laboratory work 1. Ammonium sulfate precipitation (“salting-out” of proteins)

“Salting-out” is a reversible reaction of protein sedimentation from the solution by high concentrations of neutral salts: NaCl, (NH₄)₂SO₄, MgSO₄.

Dehydration of protein molecules occurs in presence of high salt concentrations. A number of factors affect the process of salting-out: hydrophilicity of the protein, its relative molecular mass, its charge. That is why various concentrations of the same salt are needed for precipitation of various proteins. Albumins precipitate in a saturated solution of (NH₄)₂SO₄ and globulins — in a semi-saturated solution of (NH₄)₂SO₄, because globulins have higher molecular weight and a smaller charge compared to albumins.

Salting-out of proteins is a reversible reaction as the protein deposit can be dissolved again when the salt concentration is reduced by dialysis or water dilution. The process of proteins deposition by NaCl is not as active as by ammonia sulfate due to a weaker hydration ability that is characterized by the position of ions in Hoffmeister’s series:



Separation of albumins and globulins of egg-white

Procedure. Add 20 drops of a saturated solution of (NH₄)₂SO₄ to 20 drops of egg-white and carefully stir. Watch the egg-globulin precipitation. Leave for 5 minutes, then filter out the deposit using a paper filter. The filtrate still contains another protein — egg-albumin. Add the fine powder of ammonia sulfate to the filtrate till complete saturation, i. e. till a new portion of the powder stays unsolved. Then filter out the albumin deposit. Expose the filtrate to biuret reaction: add 2 drops of 1% solution of CuSO₄ + 5 drops of 10% solution of NaOH to the filtrate. A negative biuret reaction (blue staining) indicates the absence of protein in the tested solution.

Conclusion:

Laboratory work 2. Irreversible sedimentation of proteins

Denaturation gives irreversible sedimentation of the protein. Denaturation results in breaking the protein native structure and its loss of biological properties, including solubility. In such reactions proteins suffer deep changes and cannot be solved in the primary diluter. Irreversible reactions include: protein precipitation by salts of heavy metals, by mineral and organic acids, alkaloid reagents and sedimentation while boiling.

Protein sedimentation by salts of heavy metals, unlike salting-out, occurs in low salt concentrations. Proteins interacting with salts of heavy metals (lead, copper, silver, mercury etc.) adsorb them forming salt-like and complex compounds soluble in the excess of these salts

(excluding the salts of silver nitrate and mercury chloride), but insoluble in water. Dissolution of the precipitate in the excess of salts is called *adsorption peptisation*. It occurs as a result of acquiring the same positive charge by protein particles

Procedure.

Reagents	1 st test-tube	2 nd test-tube
Egg-white solution	5 drops	5 drops
1 % copper sulfate solution	1–2 drops	–
5 % silver nitrate solution	–	1–2 drops
<i>Mark the formed precipitate</i>		
1 % copper sulfate solution (excess)	5–10 drops	–
5 % silver nitrate solution (excess)	–	5–10 drops
<i>Mark the dissolution of the precipitate</i>		

Conclusion:

The ability of the protein to tightly bind ions of a heavy metal and form insoluble complexes is used as an antidote in poisonings by salts of mercury, copper, lead etc. Immediately after poisoning, when the salts are not absorbed yet but are already in the stomach, the victim is given some milk or egg-white to drink, then vomiting is forced to remove the poison from the organism.

Protein sedimentation by concentrated mineral acids

Concentrated mineral acids cause denaturation of the protein and form complex salts of the protein with acids. The protein precipitate is dissolved in the excess of all mineral acids excluding the nitric acid.

Procedure.

Reagents	1 st test-tube	2 nd test-tube
HNO ₃ (concentrated)	10 drops	–
H ₂ SO ₄ (concentrated)	–	10 drops
Add protein carefully, on the wall of the test-tube	10 drops	10 drops
Mark the appearance of the precipitate on the border between phases		
Excess of HNO ₃ (concentrated)	10 drops	–
Excess of H ₂ SO ₄ (concentrated)	–	10 drops
<i>Mark the dissolution of the precipitate</i>		

Conclusion:

3. METHODS OF SEPARATION, ISOLATION AND PURIFICATION OF PROTEINS. COMPOUND PROTEINS. GEL FILTRATION

Objective. To consolidate knowledge of physical and chemical properties of proteins to understand methods of separation and purification of proteins, as well as principles of proteins functioning in the organism. To master the method of gel filtration.

Problems for discussion:

1. Separation methods for proteins: “salting-out”; chromatography (affinity, gel chromatography); electrophoresis (on paper, in polyacrylamide gel using sodium dodecyl sulfate);
2. Methods of proteins purification from impurities (dialysis, gel chromatography).

3. Western-blot (purpose, steps, molecular probes).
4. The structure and functions of compound (conjugated) proteins.
5. Methods for the synthesis peptide and protein molecules (solid-phase synthesis of peptides, peptide synthesis in solution), their advantages and disadvantages.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 10–11.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 10–17.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 21–30.
4. Lecture material.

PRACTICAL PART

Column gel filtration

For gel filtration the so-called molecular sieves — inert hydrated polysaccharide molecules — are used. They are produced from bacterial polysaccharides (sephadexes), agar or polymerized acrylamide gels (acrylex). Swollen in the solution gel granules acquire pores through which molecules of various sizes can pass (depending on the size of pores). Molecules that penetrate well inside the granules pass through a chromatographic column slower than large molecules. The separation efficiency of the substance mixture is determined by the composition of the solution leaked out of the column (eluate).

Procedure:

1. Close the column for gel filtration with a rubber plug, put it into a test-tube. Mix up the content of the beaker with sephadex and apply it into the column. Leave it to sediment, take off the plug. The fluid will freely leak out of the column.

2. Measure 1 ml of egg-white into a test-tube (high-molecular compound) and add 3–5 drops of riboflavin (low-molecular compound).

3. Measure biuret reagent per 1 ml into 12 clean test-tubes. Place the column into the 1st test-tube with biuret reagent, remove the fluid layer over sephadex and insert the specimen to be separated (mixture: egg-white + riboflavin).

When the applied specimen moves down into sephadex replace the column into the other test-tube with biuret reagent, carefully fill in the dilated part of the column with water and, having counted 5–7 drops of the fluid leaked out of the column, put the column into the next test-tube. Repeat the procedure until proteins come out of the column (a positive biuret reaction), constantly add water into the column.

4. When the work is completed (yellow-green staining appears in the test-tube with biuret reagent that is due to riboflavin flowing out), blow out the content of the column into the beaker and rinse the column with water.

5. Fill in the table with the results of the test:

Test-tube №	1	2	3	4	5	6	7	8	9	10	11	12
Color of the solution												
What is present in the solution												

Conclusion:

4. ENZYMES. CLASSIFICATION, STRUCTURE, PROPERTIES

Objective. To learn how to use the knowledge of enzyme properties and enzyme composition of organs in further study of metabolism of organs and systems as well as to solve problems of diagnosis, prophylaxis and treatment of diseases associated with functional impairment of enzymes.

Problems for discussion:

1. Peculiarities of enzymes as protein catalysts.
2. Modern classification of enzymes and terminology of enzymes (systematic and working names). Enzyme code. General characteristic of classes.
3. The structure of enzymes. An active site of the enzyme and its organization. The theories explaining the work of the active site.
4. Coenzymes, their classification and role in catalysis. Block-structures of the NAD⁺, NADP⁺, FAD and FMN.
5. The mechanism of enzyme action. Enzyme kinetics. The effect of substrate concentration, pH, temperature on enzyme reaction velocity (molecular mechanism, graphical relationship). Michaelis's constant (K_m), usage of K_m for predicting the course of biochemical reactions.
6. Specificity of enzyme action. Types of specificity.
7. Units of enzyme activity.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 13–15.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 18–26, 69–76.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 49–72.
4. Lecture material.

PRACTICAL PART

Work 1. Studying the effect of various factors on the rate of enzyme-catalyzed reactions

1. Evaluation of saliva amylase activity and its thermolability

One of characteristic properties of enzymes is their thermolability, i. e. sensitivity of the enzyme to temperature at which a reaction takes place. For the majority of enzymes the temperature optimum is observed at 38–40 °C. Enzymes heated over 70 °C, as a rule, lose their properties of biological catalysts.

Hydrolysis of starch under the action of saliva α -amylase occurs until the stage of dextrines formation. Starch together with iodine gives blue staining. Dextrines, depending on their size, together with iodine give various staining: amylo-dextrines — violet, erythro-dextrines — red-brown, maltose — yellow. End products of starch hydrolysis — maltose and glucose — have got free aldehyde groups and can be revealed by Trommer's reaction.

The enzyme effect is judged by the decrease of the substrate amount or by the appearance of reaction products.

Procedure. Pre-dilute the saliva with water 1 : 10 (to 1 ml of saliva add 9 ml of water). Apply a small quantity of diluted saliva (2–3 ml) into a clean test-tube and boil it for 5 minutes, then cool it. Take 1 % starch solution and apply into 3 test tubes per 10 drops into each. Add 10 drops of native saliva diluted 1:10 into the 1st test-tube; add 10 drops of boiled saliva into the 2nd tube and 10 drops of water as a control to the 3rd tube. All test-tubes are placed into the thermostat at 38 °C for 10 minutes. Then the content of the test-tubes is exposed to qualitative reactions for starch and products of its disintegration.

Reaction for starch. Add 1 drop of iodine solution in potassium iodide (compound iodine) to 5 drops of tested solution. Blue staining appears in the presence of starch.

Reaction for glucose (Trommer's reaction). Add 5 drops of 10 % NaOH and 3 drops of 1 % copper sulfate to 5 drops of tested solution. Carefully boil it for 1 minute till red staining appears, indicating the presence of glucose.

Fill in the table with the results of the experiment.

Test-tube №	Reaction with compound iodine	Trommer's reaction
1. Native saliva		
2. Boiled saliva		
3. H ₂ O		

2. Effect of pH on the enzyme activity

Various enzymes have their optimum pH when the enzyme is particularly active. For example, pepsin has its optimum pH — 1.5–2.5, arginase — 9.5. Evaluate the pH optimum for saliva amylase according to the following method:

Procedure. Use the diluted saliva (1 : 10). Take 3 test-tubes and apply 2 ml of buffer solution with various pH (6.0; 6.8; 8.0) into each. Then add per 1ml of 0.5 % starch solution and 1 ml of diluted saliva to each of them. Stir the content of the test-tubes and place them into the thermostat at 38 °C for 10 minutes. Then add per 1 drop of iodine into each test-tube, stir, observe staining and mark pH when the amylase behaves most actively.

Fill in the table with the results of the experiment:

pH of the medium	6.0	6.8	8.0
Reaction with compound iodine (color)			

3. Activators and inhibitors of the saliva amylase activity

Procedure. Add 1 ml of saliva diluted 1 : 40 into 3 test-tubes. Add 2 drops of water into the 1st tube, 2 drops of 1 % NaCl into the 2nd tube and 2 drops of 1 % CuSO₄ into the 3rd one. Then add 5 drops of 1 % starch solution into every tube and leave them for 2 minutes at room temperature. Then add 1 drop of compound iodine to every tube, stir, observe staining and say where an activator and where an inhibitor is active.

Fill in the table with the results of the experiment:

Test-tube №	1 (H ₂ O)	2(NaCl)	3(CuSO ₄)
Reaction with compound iodine (color)			

Work 2. Specificity of enzymes

Unlike inorganic catalysts, enzymes possess specificity (absolute, relative, stereospecificity). This property is due to a unique structure of an active center of each enzyme. Determine the type of saliva amylase specificity according to the following procedure:

Procedure. To study the amylase specificity take saliva diluted 1 : 10 and apply per 1 ml of it into 2 test-tubes.

Add 1 ml of 1 % starch solution into the 1st test-tube, 1 ml of 1 % sucrose into the 2nd tube. Place both test-tubes to the thermostat at 38 °C for 10 minutes, then conduct Feling's reaction to reveal glucose.

Feling's reaction: add 15 drops of Feling's reagent to 15 drops of tested solution and bring it to boiling. When the reaction to glucose is positive, red staining is observed, it being caused by cupric oxide.

Fill in the table with the results of the experiment:

Test tube №	Enzyme	Substrate	Feling's reaction
1			
2			

Conclusions:

5. REGULATION OF ENZYME ACTIVITY. DETERMINATION OF ENZYME ACTIVITY

Objective. To learn how enzyme activity can be regulated by specific and nonspecific factors to understand action of medicines regulating enzyme activity, to get acquainted with the role of enzymes in diagnosis and treatment monitoring.

Problems for discussion:

1. Regulation mechanisms of the enzyme-catalyzed processes rate: regulation of the enzymes amount (synthesis, break-down), enzyme activity, change of the substrate amount, the existence of isoenzymes, association of enzymes into multienzyme complexes, compartmentation of processes.
2. Structure peculiarities of allosteric enzymes, allosteric center. The concept of a “key enzyme”.
3. Regulation of enzyme activity: activators and inhibitors (examples). Types of inhibition (irreversible and reversible, isosteric and allosteric), characteristic, examples.
4. Regulation of enzyme activity by covalent modification: partial (selective) proteolysis, phosphorylation/dephosphorylation.
5. Isoenzymes. Examples, their biological role.
6. Medical aspects of enzymology. The use of enzymes as analytical reagents, examples.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 15–18.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 21–22, 27–37.
3. *Harper’s biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 72–80.
4. Lecture material.

PRACTICAL PART

Work 1. Determination of saliva α -amylase activity

The method is based on evaluation of the least amount of amylase (at maximum saliva dilution) that completely digests the whole added starch. Amylase activity of the saliva is expressed by the amount of 0.1 % of starch solution (in ml) that is digested by 1 ml of undiluted saliva at 38 °C for 30 minutes. Normal saliva amylase activity is 160–320. This method is widely used for evaluation of amylase activity of the blood and urine.

Procedure. Apply per 1 ml of water into 10 test-tubes and add 1ml of diluted saliva into the first one. Stir the content of this tube by drawing it in and out from the pipette several times. Take into the pipette 1 ml of the mixture and put it into the 2nd test-tube. Stir the content of this tube and put 1 ml of it into the 3rd tube and so on to the 10th test tube. Take 1 ml of mixture from the 10th test-tube and dispose it. Add per 1 ml of water and 2 ml of 0.1 % of starch solution, stir it shaking the test-tubes and place them into the thermostat at 38 °C for 30 minutes. Cool the test-tubes after incubation under running water, add 1 drop of 0.1 % iodine solution into each tube and stir. The fluid in the tubes is stained in yellow, rose and violet color. Mark the last tube with yellow staining where the hydrolysis has been completed and make calculations. Put down the results into the table:

Starch hydrolysis in the presence of saliva enzymes in various dilutions

	Saliva dilutions									
	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
	Test tubes									
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th	10 th
Staining with iodine										
Conclusions										

Calculation. Having marked the test-tube with a complete starch hydrolysis and the least amount of enzyme (solution of a yellow color) calculate the saliva amylase activity by the amount of undiluted saliva in this test-tube by the following proportion: A ml of saliva digest 2 ml of 0.1 % starch solution, where A — the amount of undiluted saliva. For example, yellow staining appeared in the 4th tube where the saliva was diluted 1 : 160; 1/160 of saliva digest 2 ml of 0.1 % starch solution; 1 ml of undiluted saliva digest x ml of 0.1 % starch solution:

$$x = 2 \cdot 1 \cdot 160 / 1 = 320 \text{ ml of 0.1 \% starch solution.}$$

Hence, amylase activity is 320.

Conclusion:

Work 2. Determination of the urine amylase activity (diastase)

The method is based on calculating the time necessary for complete digestion of starch in the presence of 1 ml of urine. Presumably 1 unit of urine amylase activity is equal to the amount of enzyme splitting 2 ml of starch for 15 minutes. Amylase activity is expressed by the number of units in 1 ml of urine. Normal values are 1–2 units.

The urine of healthy people has a low amylase activity as compared to saliva amylase. Evaluation of α -amylase activity in the urine and blood serum is widely used in clinic while diagnosing diseases of the pancreas. During the first 24 hours of the disease amylase activity increases in the urine and blood serum in tens times, and then it gradually returns to normal. In renal insufficiency amylase in the urine is absent.

In childhood the increase of amylase activity is observed in parotitis (mumps) indicating the affection of the pancreas by the parotitis virus. The influenza virus also affects the pancreas but not so frequently.

Procedure. Apply per 1 drop of 0.1 % of compound iodine solution on a dry slide in several places (8–10 drops all in all). Introduce 2 ml of 0.1% starch solution containing 2 mg of starch, 1 ml of 0.85 % of NaCl solution into a test-tube and place the test-tube into thermostat at 37 °C. In 2 minutes without withdrawing the tube from the thermostat, add 0.5 ml of urine, stir and mark the time when the reaction starts. Then, every 2–3 minutes, transfer a drop of the mixture with a glass stick from the test-tube to the slide into the iodine drop. Continue doing it until the staining of the iodine drop stops changing, i.e. stays yellow. Mark the reaction time in minutes. The urine amylase activity is calculated by the formula:

$$X_{\text{unit}} = 15 / (T \cdot 0.5),$$

where X_{unit} — amylase activity in 1 ml of urine; 15 — the time necessary for complete splitting of 2 ml of starch; 0.5 — the amount of urine taken into reaction mixture, ml; T — the reaction time, min.

Conclusion:

6. COLLOQUIUM “THE CHEMISTRY OF PROTEINS, ENZYMES”

Questions for preparation:

1. Amino acids, their classification. General properties. Formulas of proteinogenic amino acids.
2. Peptides, their structure. Classification and biological role of peptides. Spelling rules for peptides (proteins), their names and their charge definitions.
3. Proteins as a class of organic substances, their biological functions. Classification of proteins.
4. Qualitative determination of total protein in biological fluids.
5. Peptide bond, its properties (postulates of Pauling–Corey).
6. Protein hydrolysis. Role for the organism and practical implementation of protein hydrolyzates in medical practice.
7. Secondary, supersecondary, tertiary, quaternary structures of a protein molecule (concept, varieties and stabilizing bonds).
8. Conformational changes in functioning of proteins. Interaction of proteins with ligands.
9. Denaturation. Reversibility of denaturation. Mechanisms of denaturing factors action.
10. General physical and chemical properties of proteins (viscosity of solutions, light diffusion, optical activity, mobility in the electric field, absorption of UV rays, solubility in water).
11. Stability factors of protein solutions (charge of the protein, hydrate shell, molecular weight, molecule shape). Isoelectric state.
12. Separation and purification methods for proteins: “salting-out”, chromatography (affinity, gel chromatography), electrophoresis (paper, PAGE), dialysis, Western-blot. Principles.
13. Methods for the synthesis peptide and protein molecules. The chemical synthesis and semisynthesis of peptides and proteins for the preparation of medicaments, examples.
14. Compound (conjugated) proteins, their classification, structure and functions.
15. Enzymes as protein catalysts.
16. Modern classification of enzymes and terminology of enzymes (systemic and working names). Enzyme code. General characteristic of classes.
17. The structure of enzymes. Coenzymes, their classification and role in catalysis. Block-structures of the NAD^+ , NADP^+ , FAD and FMN .
18. The mechanism of enzyme action. Enzyme kinetics. The effect of substrate concentration, pH, temperature on enzyme reaction velocity (molecular mechanism, graphical relationship). Michaelis constant (K_m), usage of K_m for predicting the course of biochemical reactions.
19. Specificity of enzyme action. Types of specificity.
20. Units of enzyme activity.
21. An active site of the enzyme, its organization. The theory explaining the work of the active site.
22. Structure peculiarities of allosteric enzymes, allosteric center. The concept of “key enzymes”.
23. Regulation mechanisms of the enzyme-catalyzed processes rate: regulation of the enzymes amount (synthesis, break-down), enzyme activity, change of the substrate amount, the existence of isoenzymes, association of enzymes into multienzyme complexes, compartmentation of processes.
24. Regulation of enzyme activity: covalent modification, activators and inhibitors (examples). Kinds of inhibition (irreversible and reversible, isosteric and allosteric), characteristic, examples.
25. Isoenzymes. Examples, their biological role.
26. Medical aspects of enzymology. Examples of enzymes and inhibitors usage in diagnosis and treatment. The use of enzymes as analytical reagents.

7. INTRODUCTION TO METABOLISM. CENTRAL METABOLIC PATHWAYS (OXIDATIVE DECARBOXYLATION OF PYRUVATE. CITRIC ACID CYCLE)

Objective. To get the notion of metabolism, anabolic and catabolic pathways, their interrelations at various levels. To form the notion of oxidative decarboxylation of pyruvate and citric acid cycle as central metabolic pathways, the significance of a hydrogen-donor function of tricarboxylic acids cycle (TCA cycle) for further oxidation-reduction (redox) reactions in the chain of tissue respiration, to understand a catabolic and anabolic functions of the citric acid cycle.

Problems for discussion:

1. Metabolism, metabolic pathways, regulatory (key) enzymes.
2. Catabolism and anabolism, their distinctions and interrelations.
3. Reactions of dehydrogenation as a basic way of oxidizing substances in the organism. Pyridine-dependent and flavin-dependent dehydrogenases. The role of vitamins PP and B₂ in redox reactions. Block-structures of coenzymes NAD⁺, NADP⁺, FAD, FMN.
4. Oxidative decarboxylation of pyruvate. Pyruvate dehydrogenase complex (enzymes, co-enzymes, scheme of reactions).
5. Tricarboxylic acid cycle as a central metabolic pathway. Cellular localization, reactions, enzymes, co-enzymes.
6. Dehydrogenase reactions of TCA cycle as a source of hydrogen for the system of tissue respiration. Decarboxylation in the citric acid cycle as a cellular CO₂ formation mechanism that is an end product of carbonic compounds catabolism.
7. The functions of TCA cycle: integrative, catabolic, anabolic, energetic, hydrogen-donor. Regulation. Anaplerotic reactions.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 19–24.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 49–68, 86–92.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 80–92, 122–136, 140–144.
4. Lecture material.

PRACTICAL PART

Work 1. Evaluation of TCA cycle functioning by acetyl-CoA decrease

Principle of the method. The first step of TCA cycle — is a condensation reaction of acetyl-CoA that is catalyzed by citrate synthase. The formed citric acid is exposed to conversions in the tricarboxylic acids cycle, and the released CoA-SH can be determined by Folin's reagent (blue staining appears). If TCA cycle is blocked by the malonic acid, acetyl-CoA is not used and CoA-SH is not formed. For work we use a readymade homogenate of the liver.

Experiment scheme:

Content of test-tubes	Control (ml)	Experiment (ml)
Phosphate buffer	2.0	2.0
Acetyl-CoA solution	0.5	0.5
Oxaloacetate solution	0.5	0.5
Malonic acid solution	1.0	–
Saline solution	–	1.0
Homogenate of the liver	0.5	0.5
10 min incubation at room temperature		
Folin's reagent A	0.5	0.5
Folin's reagent B	0.5	0.5
Observed changes (color)		

Conclusion:

Work 2. TCA cycle functioning manifested by the formation of CO₂

Principle of the method. When acetyl-CoA is oxidized in TCA cycle, CO₂ is released. It binds calcium hydroxide and is revealed, when sulfuric acid is added in form of gas bubbles.

Experiment scheme:

Content of test-tubes	Control (ml)	Experiment (ml)
Phosphate buffer ph = 7.4	2.0	2.0
Acetyl-CoA solution	0.5	0.5
Oxaloacetate solution	0.5	0.5
Malonic acid solution	1.0	–
Incubation solution	–	1.0
Ca(OH) ₂ solution	1.0	1.0
Homogenate of the liver	0.5	0.5
10 min incubation at room temperature		
0.1N solution of sulfuric acid	1.0	1.0
Observed changes (CO ₂ bubbles formation)		

Conclusion:

Work 3. TCA cycle functioning revealed by the formation of hydrogen atoms

Principle of the method. When acetyl-CoA is oxidized, 8 atoms of hydrogen are removed from the substrates by corresponding dehydrogenases. In this method 2,6-dichlorophenolindophenol (2,6-DCPI) is used as a hydrogen acceptor. If the cycle is functioning, then 2,6-DCPI is reduced and decolorized.

Experiment scheme:

Content of test-tubes	Control (ml)	Experiment (ml)
Phosphate buffer ph=7.4	2.0	2.0
Acetyl-CoA solution	–	0.5
Oxaloacetate solution	–	0.5
Distilled water	1.0	–
Homogenate of the liver	1.0	1.0
0.001N DCPI solution	1.0	1.0
Incubation at room temperature		
Observed changes (color)		

Conclusion:

8. INTRODUCTION TO BIOENERGETICS. PHOTOSYNTHETIC PHOSPHORYLATION (LIGHT-DEPENDENT REACTIONS)

Object. To get the notion of the ways of oxygen utilization by cells; localization, structure and functioning of components of the respiratory chain and the electron transport chain in chloroplasts, oxidative and photosynthetic phosphorylation. To learn that coupling of respiration and phosphorylation is the basis of normal cell energetic. To learn how to apply this knowledge in further studying of cellular metabolism. To consolidate knowledge of mechanisms of active oxygen species formation in cells and ways of antioxidant protection.

Problems for discussion:

1. Adenylate system of the cell, its participation in energy exchange. The central role of ATP (adenosine triphosphate) in processes coupled with energy consumption. Ways of ATP synthesis: substrate-level, oxidative and photosynthetic phosphorylation. The concept of high-energy compounds.
2. Oxygenase pathway of oxygen utilization by cells (microsomal oxidation). Enzymes, its difference from the oxidase pathway. Biological role of microsomal oxidation.
3. Tissue respiration as the process of substrates' hydrogen oxidation in the respiratory chain with formation of endogenous water in cells. Distinctions between water formation in the process of tissue respiration and similar process in vitro.
4. The structure of the respiratory chain components, enzyme complexes, co-enzymes, functioning mechanism.
5. The diagram of the respiratory chain, phosphorylation points, the mechanism of an electro-chemical gradient formation.
6. Mechanisms of mitochondrial synthesis of ATP. H⁺-ATP-synthase. Coupling of respiration and phosphorylation. The chemiosmotic theory of Mitchell. Phosphorylation ratio (P/O) for various substrates supplying hydrogen to the respiratory chain.
7. Regulation of the respiratory chain and H⁺-ATP-synthase.
8. Causes for the hypoenergetic states development. Uncoupling of oxidative phosphorylation (mechanism, uncoupling agents) and tissue respiration. Inhibitors of electron transport and oxidative phosphorylation.
9. Photosynthesis: the essence of the process, the stage. The total reaction of photosynthesis. Photosynthetic structures in a plant cell. The role of photosynthesis for life on Earth.
10. Pigments of photosynthesis. Photosystems I and II, the general scheme of electron transfer. Photosynthetic phosphorylation is the main pathway for the formation of ATP in plant cells.

Recommended literature

1. *Biological chemistry*. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 24–29.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 69–85.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 86–102.
4. Lecture material.

PRACTICAL PART

Work 1. Reactions of oxidative phosphorylation

Principle of the method. In oxidation of various substrates in the respiratory chain energy is released, a part of which is used for the reaction of oxidative phosphorylation. The degree of the latter (energetic value of substrates) is evaluated by the decrease of inorganic phosphate (ratio P/O = 0.5–2.5). Using various substrates (malate, succinate, ascorbate) we estimate the degree of oxidative phosphorylation. The content of phosphoric acid is determined in reaction with ammonia molybdate and reducing solution of ascorbic acid by the intensity of the resulted “molybden blue”.

Procedure. Introduce reagents into four test-tubes according to the scheme:

Content of test-tubes	Control (ml)	Test (ml)		
		1	2	3
Incubation mixture	1.0	1.0	1.0	1.0
Saline solution	0.5	–	–	–
Malate solution	–	0.5	–	–
Succinate solution	–	–	0.5	–
Ascorbate solution + Cytochrom c	–	–	–	0.5
Mitochondria suspension	0.5	0.5	0.5	0.5

Content of test-tubes	Control (ml)	Test (ml)		
		1	2	3
10 min incubation at room temperature, then add				
Trichloroacetic acid (TCA)	1.0	1.0	1.0	1.0
Ammonia molybdate solution	0.5	0.5	0.5	0.5
Reducing solution of Fiske and Subarow				
Dilute the content of all test-tubes 1:8, 10 min incubation				
Observed changes (staining intensity by four-point scale)				
P/O ratio				

Conclusion:

Work 2. Effect of 2,4-dinitrophenol (2,4-DNP) on oxidative phosphorylation

Principle of the method. 2,4-DNP is an uncoupler of phosphorylation and oxidation. Oxidative phosphorylation is judged by the decrease of inorganic phosphate in the incubation medium, it is determined as described in work 1.

Content of test-tubes	Control (ml)	Experiment (ml)
Malate solution	0.5	0.5
2,4-DNP solution	–	0.5
Saline solution	0.5	–
Mitochondrium suspension	0.5	0.5
10 min incubation at room temperature		
TCA solution	1.0	1.0
Ammonia molybdate solution	0.5	0.5
Reducing solution	1.0	1.0
Observed changes (color)		

Conclusion:

9. DIGESTION OF CARBOHYDRATES. METABOLISM OF GLYCOGEN.

Objective. To consolidate knowledge of the carbohydrates structure of animal tissues and dietary vegetable carbohydrates. To form the notion of carbohydrate digestion, glucose transport to cells, molecular mechanisms of glycogen storage and mobilization, physiological significance and regulation of these pathways. To learn anaerobic pathways of glucose oxidation and their significance.

Problems for discussion:

1. Classification and functions of carbohydrates. Carbohydrates as medicines.
2. Carbohydrates digestion, end products. The role of cellulose and pectin in the human diet.
3. Absorption of carbohydrates digestion products, molecular mechanisms. The fate of absorbed monosaccharides. Glucose transport to cells.
4. General scheme of sources and ways of glucose consumption in the body, their significance and interrelation.
5. Glycogen synthesis, purpose, sequence of reactions, expenditure of energy and regulation. Aglycogenesis.
6. Degradation of glycogen in the liver and muscles, sequence of reactions, regulation. Glycogenoses.
7. Alcoholic fermentation.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 30–35.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 93–98, 112–119.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 136–153.
4. Lecture material.

PRACTICAL PART

Work 1. Alcoholic fermentation

Alcoholic fermentation is a breakdown of glucose with ethanol and carbon dioxide formation:



Glycolysis and alcoholic fermentation include similar reactions till the formation of pyruvate which proceed with heat release and the formation of two molecules of ATP. By the action of yeast decarboxylase (coenzyme — TPP) pyruvate is decarboxylated and converted to acetaldehyde, which is reduced to ethanol by the action of alcohol dehydrogenase.

Procedure

1. Fill 1/3 of a test tube with the yeast solution. Add 5 % glucose solution up to the top and seal with a cork with a glass tube. Place the closed test tube in a thermostat at 37 °C for 30–50 minutes (depending on the enzyme activity of the yeast).
2. *Detection of CO₂*. Remove the test tube from the thermostat and fill up to the top with 10 % NaOH. Stir its content by closing the test tube with your finger. Carbon dioxide is absorbed by an alkali, creating a vacuum, and the finger is sucked into the test tube.
3. *Detection of ethanol*. Alcohol can be found using the reaction of iodoform formation:



Filter 2–3 ml of test tube content into a new test tube. Add several drops of 10 % solution of iodine till appearing yellow color and then heat without boiling. In a short period of time there will be a specific smell of iodoform.

Conclusion:

10. AEROBIC AND ANAEROBIC PATHWAYS OF GLUCOSE OXIDATION. METABOLISM OF PYRUVATE. GLUCONEOGENESIS.

Objective. To consolidate knowledge of pyruvate fate in cells depending on the energetic status and peculiarities of cellular metabolism, gluconeogenesis as an important process of the blood glucose level maintaining. To form the notion of interconnection between central metabolic pathways and aerobic glycolysis. To master the enzymatic method of glucose measurement in blood.

Problems for discussion:

1. Glycolysis, its biological role, subcellular localization, phases (preparatory or unoxidative, oxidative), reactions energy yield and mechanism of ATP formation. Glycolysis regulation, key enzymes. The difference between glycolysis and alcohol fermentation.
2. Pyruvate as a central metabolite. Pathways of pyruvate conversion depending on the energetic status and peculiarities of oxidative cellular metabolism.
3. Restoration of pyruvate in lactate (reaction, isoenzymes LDH, purpose of reactions). Utilization of lactate by cells. Cory's cycle.
4. Aerobic oxidation of glucose to CO₂ and H₂O (steps associated with oxidative phosphorylation, energy yield).
5. Gluconeogenesis (biological role, substrates, key reactions and enzymes, regulation, expenditure of energy).

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 35–41, 21–22.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 98–108.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 136–145, 153–163.
4. Lecture material.

PRACTICAL PART

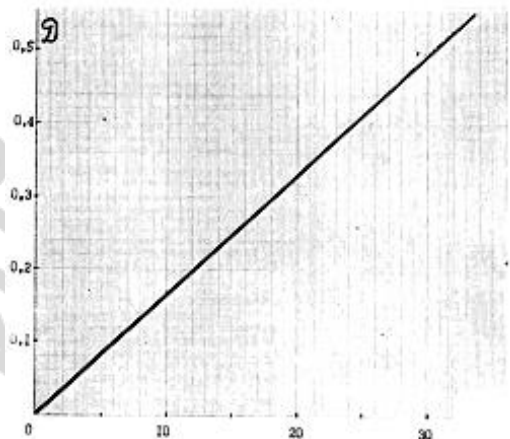
Work 1. Determination of pyruvate in the urine

Pyruvate is one of intermediate products of carbohydrate metabolism. Under anaerobic conditions (hypoxia) pyruvate is reduced into lactate. Under aerobic conditions pyruvate under the influence of a pyruvate dehydrogenase complex (coenzymes: TPP, lipoamide, CoA-SH, NAD⁺, FAD) as a result of oxidative decarboxylation is converted into acetyl-CoA that in the citric acid cycle is oxidized to CO₂ and H₂O.

During 24 hours 113.7–283.9 μM/24 h (10–25 mg) of pyruvate are excreted with urine.

Principle of the method. Pyruvate interacting with 2,4-dinitrophenylhydrazine in alkaline medium forms 2,4-dinitrophenylhydrazones derivatives of yellow-orange color, the staining intensity of which is proportional to concentration of pyruvate.

Procedure. Take 2 test-tubes: apply 1 ml of H₂O into a control one and 1 ml of urine into a test tube. Then add into both test-tubes 0.5 ml of 2,4-dinitrophenylhydrosine solution and leave them for 20 minutes at room temperature. Then add 5 ml of 0.4N NaOH into each tube and in 10 minutes determine the optical density of the test sample versus the control sample using 10 mm cuvettes with a green light filter.



Calibration graph: solution optical density (D) versus the pyruvate concentration in the sample (μg per 1 ml)

Calculation is performed according to a ready calibration graph. Recount the found value by daily diuresis (1500 ml for men and 1200 ml for women) and get the content of pyruvate in daily urine. The factor of recalculation in SI units (μmol/day) — 11.4.

Clinical and diagnostic value. In avitaminosis and hypovitaminosis of B₁ in the blood and other tissues, especially in the brain, a great amount of pyruvate is accumulated and its excretion with urine increases. The content of this acid in the blood increases in diabetes mellitus, cardiac insufficiency, hyperfunction of the pituitary-adrenal system. The amount of pyruvate increases after injection of some medicines — camphor, strychnine, epinephrine. In anesthesia the content of this acid in the blood decreases.

Results and calculation: D =

C =

Conclusion:

11. PENTOSE PHOSPHATE AND GLUCURONIC PATHWAYS OF GLUCOSE METABOLISM. PHOTOSYNTHESIS (THE DARK PHASE). REGULATION OF BLOOD GLUCOSE LEVEL

Objective. To form understanding of the significance of pentose phosphate and glucuronic pathways of glucose metabolism; to learn the role of hormonal regulation in maintaining glucose concentration in the blood to know how to interpret the character of biochemical impairments in patients with pathology of carbohydrate metabolism.

Problems for discussion:

1. Pentose phosphate pathway (subcellular localization, steps, key enzymes, metabolites, biological role). Role of NADP⁺.
2. Uronic acid pathway (tissue and subcellular localization, biological role).
3. Regulation of blood glucose content. Mechanisms of hormonal regulation (insulin, epinephrine, glucagon, glucocorticoids etc.).
4. The dark phase of photosynthesis (the total equation). Carboxylation of ribulose-1,5-bisphosphate, the sequence of reactions to the formation of 3-PGA.
5. Synthesis of carbohydrates in the Calvin cycle.
6. Metabolism of exogenous ethanol (pathways, reaction scheme).

Recommended literature

1. *Biological chemistry*. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 41–43.
2. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 108–111, 119–126.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 153–173.
4. Lecture material.

PRACTICAL PART

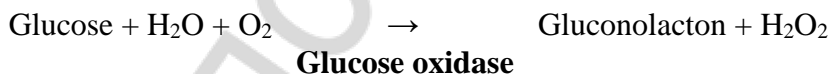
Effect of hormones on blood glucose content

To study the effect of hormones on the blood glucose level take 3 blood samples (tested). One of them was taken before applying hormones, the second — after injecting insulin, the third one — after injecting epinephrine.

1. Evaluate glucose content in every sample.
2. On the basis of received data make a conclusion which of the samples corresponds to the above states.

To evaluate glucose concentrations in the samples, use the **enzymatic (glucose oxidase) method**. The tested and standard samples are performed in parallel.

Principle of the method. The method is based on the following enzyme-catalyzed reactions:



The formed product is of a rose color. Staining intensity is proportional to glucose concentration and is measured photometrically.

Procedure: Serum proteins are precipitated by a deproteinizing agent. Glucose is determined in supernatant after centrifugation. Reagents are added as follows:

	Tested sample, ml	Standard sample, ml
Apply into centrifuge test-tubes:		
Blood serum	0.1	–
Standard glucose solution	–	0.1
Deproteinizing solution (3 % TCA)	1.0	1.0
Stir and centrifuge at 3000 rotations per minute for 15 minutes		
Apply into dry test-tubes:		
Supernatant (overprecipitate fluid)	0.2	0.2
Working solution of enzymes	2.0	2.0
Stir and incubate the reaction mixture for 10 minutes at 37 °C or 30 min at room temperature		

On completion of incubation extinctions of the tested and standard samples are measured photometrically (wave length of 490–540 nm) in 5 mm thick cuvettes versus the control.

The control sample contains 0.2 ml of deproteinizing solution and 2.0 ml of working solution of enzymes. The control sample can be prepared only one for the whole group.

Calculate glucose concentration according to the formula:

$$C_t = E_t \cdot C_s / E_s,$$

where C_t — glucose concentration in serum (mmol/l); C_s — glucose concentration in standard solution (5.5 mmol/l); E_t — extinction of the tested sample; E_s — extinction of the standard sample.

$E_t =$

$E_s =$

$C_t =$

Results:

Sample	Optical density (E)	Glucose concentration (mmol/l)
1		
2		
3		
Standard		

Normal values of glucose concentration in plasma and serum — 3.9–6.1 mmol/l, in cerebrospinal fluid — 2.78–3.89 mmol/l.

Clinical and diagnostic value. Increase of glucose content in the blood (hyperglycemia) is observed in diabetes mellitus, acute pancreatitis, pancreatic cirrhosis, emotional stresses, after ether narcosis, after a meal rich in carbohydrates as well as a result of hyperfunction of thyroid gland, hypophysis, adrenal cortex and medulla.

Decrease of blood glucose level (hypoglycemia) occurs in affection of the liver parenchyma, impairment of enzyme activity in glycogen break-down; hypofunction of the thyroid gland, adrenal glands, pituitary gland; overdosage of insulin during treatment of diabetes mellitus, impairment of carbohydrate absorption, poisonings by phosphorus, benzole, chlorophorm, in insufficient taking of carbohydrates with food, after considerable losses of blood.

Conclusion:

12. COLLOQUIUM “INTRODUCTION TO METABOLISM AND BIOENERGETICS, CARBOHYDRATE METABOLISM. PHOTOSYNTHESIS.”

Questions for preparation:

1. Metabolism, catabolism and anabolism, their distinctions and interrelations. Types of metabolic pathways (examples), regulatory (key) enzymes.
2. Pyridine-dependent and flavin-dependent dehydrogenases. Block-structures of co-enzymes NAD⁺, NADP⁺, FAD, FMN.
3. Adenylate system of the cell, its participation in energy exchange. Ways of ATP synthesis: substrate-level, oxidative and photosynthetic phosphorylation.
4. Oxidative decarboxylation of pyruvate as a central metabolic pathway. Pyruvate dehydrogenase complex (enzymes, co-enzymes, scheme of reactions).
5. Tricarboxylic acid cycle as a central metabolic pathway. Cellular localization of TCA cycle, reactions, enzymes, co-enzymes. Functions of TCA cycle. Anaplerotic reactions.
6. Oxygenase pathway for oxygen utilization in cells. Enzymes. Oxidation in microsomes. Scheme of the microsomal chain. Differences in the oxidase and oxygenase pathways of oxygen utilization. The role of these processes in the cell.
7. Tissue respiration. The structure of the respiratory chain components, enzyme complexes, co-enzymes, functioning mechanism. The diagram of the respiratory chain, phosphorylation points, the mechanism of an electro-chemical gradient formation. H⁺-ATP-synthase. The chemiosmotic Mitchell theory. Phosphorylation ratio (P/O) for various substrates supplying hydrogen to the respiratory chain. Regulation of the respiratory chain and H⁺-ATP-synthase.
8. Causes for the hypoenergetic states development. Uncoupling of oxidative phosphorylation (mechanism, uncoupling agents). Inhibitors of electron transport and oxidative phosphorylation.
9. Carbohydrates, classification, main representatives.
10. Carbohydrates digestion, end products. The role of cellulose and pectines in the human diet. Carbohydrates as medicines (examples).
11. Absorption of carbohydrates digestion products, molecular mechanisms. The fate of absorbed monosaccharides. Glucose transport to cells.
12. Glycogen synthesis, purpose, sequence of reactions, expenditure of energy and regulation. Aglycogenesis.
13. Degradation of glycogen in the liver and muscles, sequence of reactions, regulation. Glycogenolysis.
14. Glycolysis, its biological role, subcellular localization, phases (unoxidative, oxidative), reactions, enzymes, energy yield and mechanism of ATP formation. Glycolysis regulation, key enzymes.
15. Pyruvate as a central metabolite. Pathways of pyruvate conversion depending on the energetic status and peculiarities of oxidative cellular metabolism.
16. Recovery of pyruvate to lactate (reaction, isoenzymes LDH, the purpose of the reactions). Utilization of lactate by cells. Cori's cycle.
17. Gluconeogenesis, biological role, substrates, key reactions and enzymes, regulation, expenditure of energy.
18. Aerobic oxidation of glucose to CO₂ and H₂O (stages, energy yield, mechanisms of ATP formation).
19. Alcoholic fermentation (stages, enzymes, regulation). Differences in alcoholic fermentation and glycolysis.
20. Exogenous ethanol exchange (scheme), tissue localization of the process. Mechanism of development of alcohol hypoglycemia and lactic acidemia.
21. Pentose phosphate pathway, subcellular localization, steps, key enzymes, metabolites, biological role, regulation. The role of NADP⁺ in the cell.
22. Uronic acid pathway (tissue and subcellular localization, biological role).

23. Regulation of blood glucose level. Mechanisms of hormonal regulation (insulin, epinephrine, glucagon, glucocorticoids etc.). Determination of glucose concentration in blood (the principle of glucose oxidase method).

24. Photosynthesis. The total equation. Pigments of photosynthesis. Characteristics of the light stage. Noncyclic and cyclic electron transfer in photosystems. Formation of a proton gradient in thylakoids. Photophosphorylation. The final products of the light stage.

25. Characteristics of the dark phase of photosynthesis. Carbohydrate synthesis in the Calvin cycle (scheme, key reaction). The total equation.

13. LIPID METABOLISM. DIGESTION AND RE-SYNTHESIS. TRANSPORT OF EXOGENOUS LIPIDS. EVALUATION OF LIPASE ACTIVITY

Objective. To consolidate knowledge of lipids chemistry. To learn molecular mechanisms of digestion and absorption of lipids from food, re-synthesis of lipids, transport of exogenous lipids through the blood stream for further analysis of biochemical impairments of these processes.

Problems for discussion:

1. General characteristics and classification of lipids (saponifiable and unsaponifiable, simple and complex). Characteristic of lipid groups (chemical formulas and terminology of acylglycerols and glycerophospholipids; block-structures of waxes, sphingophospholipids, glycolipids, sulfolipids). Biological role of lipids.

2. Use of lipids and their components as medicaments. The structure of liposomes, their use and methods of production.

3. Food lipids. Lipids digestion, phases. Emulsification (purpose, factors, stabilization of fat emulsion). Bile, bile acids (primary, conjugated, secondary). Place of formation, participation in assimilation of food lipids. Enterohepatic re-circulation of bile acids.

4. Hydrolysis of lipids (conversion patterns). Enzymes (place of formation, substrate specificity). Activation of pancreatic lipase. Absorption (mechanisms, micellar dissolution, fate of micelles).

5. Re-synthesis of triacylglycerols and glycerophospholipids in enterocytes. Transport forms of lipids in the blood. Structure and metabolism of chylomicrons.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 47–52.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 127–129, 134–135.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 197–219.
4. Lecture material.

PRACTICAL PART

Work 1. Kinetics of pancreatic lipase

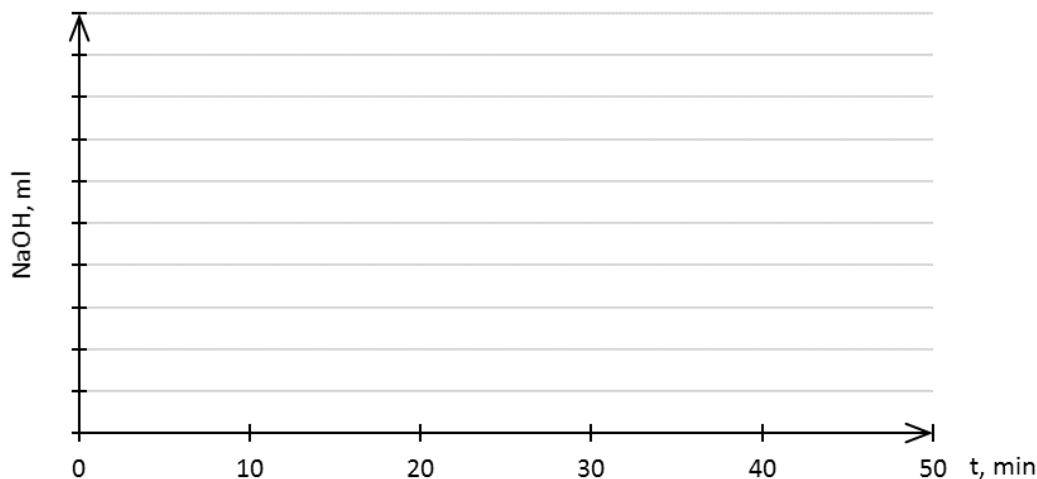
Principle of the method. The lipase action rate in separate portions of milk is evaluated by the amount of fatty acids formed in hydrolysis of milk fat for a definite interval. The amount of fatty acids is determined by alkaline titration.

Procedure. Prepare two test-tubes containing 10 ml of milk. Add 1 ml of water into one test-tube (control) and 1 ml of bile — into the other (tested). Then add 1 ml of 10 % solution of pancreatin (pancreatic juice which contains lipase) in both test-tubes. Quickly stir the mixture in the test-tubes. Take 1 ml of the mixture from every tube and apply into two flasks, add 1-2 drops of a 0.5 % solution of phenolphthalein in both flasks and titrate with 0.05 N solution of NaOH to light-rose color which doesn't disappear within 30 seconds. The obtained value of titration is "reaction time "0". Place both tubes in a thermostat at 38 °C. Every 10 minutes take out 1 ml of the mixture from two tubes into flasks and titrate using 0.05 N solution of NaOH in the presence of phenolphthalein to light-rose color. Perform 5 such determinations. Subtract the value obtained in

first titration (“reaction time “0”) from each obtained value. In this case, the resulting graphs will pass through the point zero, because the result of the titration of the initial amount of fatty acids presented in milk is subtracted. Based on the obtained data, plot two kinetic curves that reflect the process of enzymatic fat hydrolysis by pancreatic lipase vs time, depending on the presence or absence of bile.

Results:

	Incubation time					
	0 min	10 min	20 min	30 min	40 min	50 min
NaOH, ml						



Conclusion:

Work 2. Action of the pancreatic phospholipases

Principle of the method. The pancreatic phospholipases action on glycerophospholipids of egg yolk is manifested by the appearance of free phosphoric acid capable of forming a yellow precipitate in heating with ammonia molybdate.

Procedure. Apply per 5 drops of egg yolk suspension into 2 test-tubes. Add 2 drops of pancreatine into the first tube, and 2 drops of water into the second (control) tube. Place both test-tubes into the thermostat at 38 °C for 30 minutes. After incubation add 5 drops of molybdenum reagent into both tubes, heat them over the burner and cool under running water.

Conclusion:

14. LIPID TRANSPORT IN BLOOD. CHOLESTEROL METABOLISM. STORAGE AND MOBILIZATION OF LIPIDS. DETERMINATION OF PLASMA β -LIPOPROTEINS

Objective. To form the notion of the blood lipid transport system. To study the pathways of lipids synthesis and break-down in the adipose tissue and liver, cholesterol synthesis in cells. To acquire skills of β -lipoproteins determination in serum.

Problems for discussion:

1. Synthesis of TAG and glycerophospholipids in the liver and adipose tissue (reactions, general steps of synthesis, distinctions, the role of lipotropic factors). Transport forms of lipids in

blood. The structure and metabolism of VLDL (very low density lipoproteins), IDL (intermediate density lipoproteins), LDL (low density lipoproteins), HDL (high density lipoproteins).

2. Cholesterol, the biological role, food sources. Elimination of cholesterol from the organism, bile acids as a major end product of cholesterol metabolism, cholelithiasis. Cholesterol biosynthesis (tissue and subcellular localization, substrates, phases, reactions of the 1st phase, regulation).

3. Mechanisms of maintaining cholesterol balance in cells.

4. Biochemistry of atherosclerosis, hypercholesterolemia as a risk factor, other risk factors. Basic principles of prevention and diagnosis of atherosclerosis (atherogenic index).

5. Mobilization of lipids from the adipose tissue. Hormone-sensitive lipase.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 52–53, 60–63.

2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 135–136, 142–153.

3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 205–231.

4. Lecture material.

PRACTICAL PART

Determination of plasma β -lipoproteins (low density lipoproteins)

The majority of lipids are not free in the blood, but compose protein-lipid complexes (lipoproteins). Fractions of lipoproteins differ in their molecular mass, amount of protein, percentage of individual lipid components. Lipoproteins can be separated by various methods: electrophoresis, thin-layer chromatography, ultracentrifugation in density gradient. Electrophoretic separation (on chromatographic paper, acetate cellulose, agar, in polyacrylamide gel) gives fractions of chylomicrons (immobile) and lipoproteins of various density: α -lipoproteins (HDL) have mobility of α -globulins, β -lipoproteins (LDL) have mobility of β -globulins. Pre- β -lipoproteins (VLDL) are located on the electrophoregram before β -lipoproteins from the start line, that's why they are called this way.

Evaluation of β -lipoproteins in the blood plasma is important for diagnosing atherosclerosis, acute and chronic liver diseases, xanthomatosis and other pathologies.

Principle of the method. The method is based on the ability of β -lipoproteins (VLDL) to sediment in the presence of calcium chloride and heparin; the solution turbidity being changed. Concentration of β -lipoproteins in plasma is determined by the degree of solution turbidity.

Procedure. Apply 2 ml of 0.025 M solution of CaCl_2 and 0.2 ml of blood plasma into a test-tube and stir. Evaluate optical density of the solution (E_1) versus CaCl_2 solution in cuvettes 5 mm thick under a red light filter (630 nm). Add 0.1 ml of heparin solution into the cuvette, stir and exactly in 4 minutes evaluate the solution optical density (E_2) under the same conditions.

Calculation. Calculate the difference of optical densities and multiply by 10 — an empiric factor suggested by Ledvina, because the construction of a calibrating curve is associated with a number of difficulties (x (g/l) = $(E_2 - E_1) \cdot 10$). Normal values for β -lipoproteins content are 3–4.5 g/l. The content of β -lipoproteins varies depends on the age and sex.

$$E_1 = \quad E_2 = \quad x \text{ (g/l)} = (E_2 - E_1) \cdot 10 =$$

Conclusion:

15. INTRACELLULAR METABOLISM OF FATTY ACIDS. KETONE BODIES. DETERMINATION OF CHOLESTEROL IN SERUM

Objective. To study the processes of oxidation and synthesis of fatty acids, ketone bodies. To learn the role of hormonal regulation of lipid metabolism for understanding mechanisms of biochemical impairments in fasting and diabetes mellitus. To form the notion of eicosanoids and their functions. To acquire skills of cholesterol and ketone bodies determination.

Problems for discussion:

1. β -oxidation as a central pathway of fatty acids catabolism. Subcellular localization of the process, activation of fatty acids, transport to mitochondria. Oxidation reactions, participation of vitamins. Association with oxidative phosphorylation, energetic yield. β -oxidation of fatty acids with an odd number of carbons, unsaturated fatty acids. Peculiarities of β -oxidation in peroxisomes.
2. Biosynthesis of fatty acids. Subcellular localization, substrates, reactions, regulation. Peculiarities of fatty acid synthase structure. The role of malic-enzyme.
3. Polyunsaturated fatty acids as essential nutritive factors: representatives, biological role.
4. Metabolism of arachidonic acid. Biosynthesis of eicosanoids (prostaglandins, prostacyclins, leukotriens, thromboxans) and their biological role. Inhibitors of phospholipase A₂. The mechanism of action of non-steroidal anti-inflammatory drugs.
5. Ketogenesis: tissue and subcellular localization, substrates, reactions. Molecular mechanisms of ketonemias in diabetes mellitus and fasting. Utilization of ketone bodies (interconversions, activation, involvement into metabolism, energy yield of oxidation).
6. Acetyl-CoA as a central metabolite.
7. Hormonal regulation of lipid metabolism.

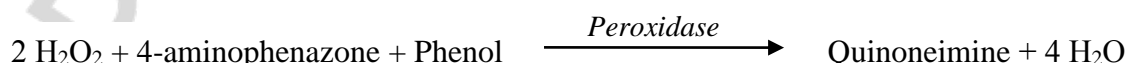
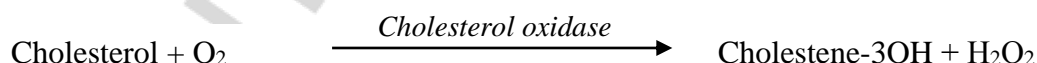
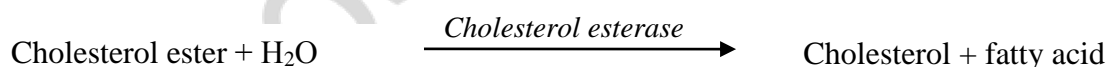
Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 53–59, 61–63.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 129–133, 137–142.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 173–197.
4. Lecture material.

PRACTICAL PART

Work 1. Determination of total cholesterol concentration in serum

Principle of the method. Determination of cholesterol after its catalytic hydrolysis and oxidation. The indicator is quinoneimine which is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase.



The resulting product has pink color. The color intensity is directly proportional to the cholesterol concentration and is measured photometrically.

Procedure. Cholesterol is determined in serum. The reagents should be added according to the scheme:

	Test sample, ml	Standard sample, ml
Test-tubes:		
Serum	0.02	–
Cholesterol standard solution	–	0.02
Enzyme process solution	2.0	2.0
Stir and incubate the reaction mixture for 5 minutes at 37 °C or 10 minutes at room temperature		

Evaluate optical density of the test sample (E_t) and standard sample (E_s) versus control sample in cuvettes 5 mm thick under a green light filter (540 nm).

A **control sample** contains 2.0 ml of enzyme process solution.

Calculate cholesterol concentration according to the formula:

$$C_{\text{cholesterol}} (\text{mmol/l}) = 5.17 \times (E_t/E_s)$$

$E_t =$

$E_s =$

$C_{\text{cholesterol}} =$

Normal values of cholesterol concentration in plasma and serum — 3.9–6.2 mmol/l.

Clinical and diagnostic value. When fat metabolism is impaired, cholesterol may accumulate in the blood. The increased plasma cholesterol level (hypercholesterolemia) is observed in atherosclerosis, diabetes mellitus, mechanic jaundice, nephritis (especially in lipoid nephrosis), hypothyrosis. Decrease of cholesterol in the blood (hypocholesterolemia) is observed in anemias, fasting, tuberculosis, hyperthyroidism, cancerous cachexia, impairment of the central nervous system, feverish states.

Conclusion:

Work 2. Qualitative reactions for acetone

Procedure.

1. Legal's test for acetone. Acetone after addition of sodium nitroprusside in alkaline medium forms orange-red color. After acidation with acetic acid a compound with cherry color is formed.

Apply 1 drop of urine, 1 drop of 10 % NaOH solution and 1 drop of freshly made sodium nitroprusside into a test-tube. Orange-red staining appears. Add 3 drops of glacial acetic acid and observe cherry color appearance.

2. Gerhard's reaction for acetoacetic acid. Add 5 % solution of chloric iron (FeCl_3) drop by drop to 5 drops of urine; phosphate residue sediment in the form of FePO_4 . In the presence of acetoacetic acid, when addition of chloric iron is continued, cherry-red staining appears. Left alone the staining becomes pale due to spontaneous decarboxylation of acetoacetic acid. The process undergoes a very quick course in boiling.

Clinical and diagnostic value. Ketonemia and ketonuria are observed in diabetes mellitus, fasting, overproduction of hormones which are antagonists of insulin.

Conclusion:

16. COLLOQUIUM “LIPID METABOLISM”

Questions for preparation:

1. Lipids, general characteristics, classification. Characteristic and biological role of lipid groups (chemical formulas and terminology of acylglyceroles and glycerophospholipids; block-structures of waxes, sphingophospholipids, glycolipids, sulfolipid structures).
2. Digestion of lipids, phases. Emulsification (purpose, factors, stabilization of fatty emulsion). Bile, bile acids (primary, conjugated, secondary). Enterohepatic re-circulation of bile acids. Hydrolysis of diet lipids (enzymes, conversion patterns). Absorption (mechanisms, micellar dissolution, fate of micelles).
3. Re-synthesis of triacylglyceroles and glycerophospholipids in enterocytes.
4. Transport forms of lipids in the blood. Structure and metabolism of chylomicrons.
5. Synthesis of TAG and glycerophospholipids in the liver and fatty tissue (role of lipotropic factors). Lipotropic factors as medications.
6. The structure and metabolism of VLDL (very low density lipoproteins), IDL (intermediate density lipoproteins), LDL (low density lipoproteins), HDL (high density lipoproteins). Biochemistry of atherosclerosis, atherogenic index.
7. Cholesterol, biological role, biosynthesis (tissue and subcellular localization, substrates, phases, reactions of the 1st phase, regulation). Mechanisms of maintaining cholesterol balance in cells.
8. Mobilization of lipids from the adipose tissue. Hormone-sensitive lipase.
9. β -oxidation of fatty acids. Subcellular localization of the process, activation of fatty acids, transport to mitochondria. Oxidation reactions, association with the process of oxidative phosphorylation, energetic yield. β -oxidation of fatty acids with an odd number of carbons, unsaturated fatty acids. Peculiarities of β -oxidation in peroxisomes.
10. Biosynthesis of fatty acids. Subcellular localization, substrates, reactions, regulation. Peculiarities of the fatty acid synthase structure. The malic-enzyme role.
11. Polyunsaturated fatty acids as essential nutritive factors: representatives, biological role.
12. Metabolism of arachidonic acid. Biosynthesis of eicosanoids (prostaglandins, prostacyclins, leukotriens, thromboxans) and their biological role. Inhibitors of phospholipase A₂. The mechanism of action of non-steroidal anti-inflammatory drugs.
13. Ketogenesis: tissue and subcellular localization, substrates, reactions. Molecular mechanisms of ketonemias in diabetes mellitus and fasting. Utilization of ketone bodies (interconversions, activation, involvement into metabolism, energy yield of oxidation).
14. Acetyl-CoA as a central metabolite.
15. Hormonal regulation of lipid metabolism.
16. Liposomes formation and their use for the delivery of drugs to organs and tissues.

17. PRACTICAL SKILLS OF BIOCHEMICAL ANALYSIS CONTROL. COLOR REACTIONS FOR PROTEIN AND AMINO ACIDS. DETERMINATION OF TOTAL PROTEIN IN SERUM BY BIURET METHOD

Objective. To consolidate acquired practical skills of qualitative and quantitative biochemical analysis. To check the ability of students to use methods of quantitative and qualitative analysis to solve applied medical aspects while evaluating the protein content in plasma and color reactions for proteins and amino acids.

Work 1. Color reactions for protein and amino acids

Color reactions give the possibility to reveal the presence of protein in solutions and biological fluids. These reactions are used both for qualitative and quantitative analysis of proteins and amino acids. Some reactions are peculiar not only for proteins but other substances as well. For example, phenol, like tyrosine, together with Millon's reagent, gives rose-red staining, that is why it is not sufficient to conduct only one reaction to reveal the presence of protein in the studied solution.

There are two types of color reactions:

- 1) universal — biuret (for all proteins) and ninhydrine (for all α -amino acids and proteins);
- 2) specific — only for specific amino acids both in a protein molecule and in amino acids solutions, e. g. Foll's reaction (for amino acids containing loosely bound sulfur), Sakaguchi's reaction (for arginine) etc.

After completing the study protocol on the basis of obtained data student should make a conclusion concerning composition of studied solution. Make a choice among the following options of answers:

- 1) Egg-white solution (contains aromatic, aliphatic and sulfur-containing amino acids);
- 2) Gelatin solution (gelatin is denatured collagen that does not contain aromatic amino acids);
- 3) Solution of aromatic α -amino acids;
- 4) Solution of aliphatic α -amino acids.

Conclusion:

Reaction	Principle of the reaction	Summary of accomplishment	Notes	Result (color)	Conclusion
Biuret reaction	This reaction reveals peptide bonds in protein. It is due to the formation of a biuret complex in alkaline medium as a result of binding copper with a protein peptide group. The solution becomes of a violet-blue color	5 drops of studied solution + 5 drops of 10 % NaOH + 2 drops of 1 % copper sulfate (CuSO ₄). Stir the content of the test-tube	Biuret reaction is produced by substances containing not less than two peptide bonds		
Ninhydrine reaction	Amino acids, polypeptides and proteins while boiling with water solution of ninhydrine give blue or blue-violet staining. As a result of interaction of α -amino acid with ninhydrine a Schiff base is formed that is re-grouped, decarboxylated and split into aldehyde and aminodi-ketohydrinden	5 drops of studied solution + 5 drops of 0.5 % water solution of ninhydrine. Boil the mixture for 1–2 minutes. The appearance of staining is observed	It is characteristic for amino groups in α -position included into protein, polypeptides and free amino acids		
Xantho-protein reaction	While processing the protein solution with concentrated nitric acid a yellow staining appears. Aromatic amino acids in interacting with concentrated HNO ₃ form nitrocompounds stained in yellow color	5 drops of studied solution + 3 drops of concentrated nitric acid (HNO ₃). Carefully boil the mixture	Positive reaction proves the presence of aromatic amino acids in the solution (trp, phe, tyr)		

Reaction	Principle of the reaction	Summary of accomplishment	Notes	Result (color)	Conclusion
Reaction for tyrosine (Millon's)	Tyrosine interacting with Millon's reagent and while boiled forms bloody-red deposit of mercury salt of dinitrotyrosine due to the presence of a phenol ring in tyrosine	5 drops of studied solution + 3 drops of Millon's solution. Carefully heat the mixture	Compounds comprising a phenol ring also give positive Millon's reaction		
Foll's reaction (for cysteine)	Thiol groups (-SH) in protein or in peptide are exposed to alkaline hydrolysis resulting in splitting-off sulfur in the form of lead sulfide that together with plumbite gives black or brown insoluble deposit of lead sulfide (PbS)	5 drops of studied solution + 5 drops of Foll's solution. Boil and leave it to stay for 1–2 minutes	The reaction is positive only with amino acids that contain loosely bound sulfur (cystein)		

18. DIGESTION AND ABSORPTION OF PROTEINS. ANALYSIS OF GASTRIC JUICE

Objective. To form the conception of general nitrogen metabolism in the organism, the protein as a main dietary source of nitrogen and amino acids. To understand the molecular basis of protein digestion in gastrointestinal tract, characteristics of various proteases and usage of their inhibitors in clinical practice, absorption of amino acids and their transport to cells. To master methods of laboratory analysis of gastric juice.

Problems for discussion:

1. Nitrogen balance. Kinds of nitrogen balance.
2. Protein requirements. The biological value of food proteins.
3. Proteolysis. Kinds, biological role.
4. Digestion of proteins. General characteristic of proteases, their substrate specificity.
5. Role of hydrochloric acid in digesting proteins. Mechanisms of formation of hydrochloric acid in the stomach.
6. Processes of decay of proteins in the intestine. Products of decay of proteins and their neutralization.
7. Absorption of amino acids, transport of amino acids into cells.
8. Amino acid pool of the cell — its sources and utilization.

Recommended literature

1. *Biological chemistry*. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 64–66.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 154–156.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 242–249.
4. Lecture material.

PRACTICAL PART

Determination of gastric juice acidity

Principle of the method. Total acidity of gastric juice is measured in milliliters of 0.1 N solution of NaOH spent for neutralization of 1000 ml of gastric juice in the presence of a phenolphthalein indicator (pH transition zone 8.3–10.0; below 8.2 — colorless, above 10.0 — red). Normal total acidity for an adult person is 40–60 mmol/l, for a newborn — 2.8 mmol/l, for children from 1 month to 1 year — 4–20 mmol/l.

The content of free hydrochloric acid in gastric juice is measured in milliliters of 0.1 N solution of NaOH spent for neutralization of 1000 ml of gastric juice in the presence of dimethylaminoazobenzole (pH transition zone is 2.9–4.0; below 2.9 — rose-red; above 4.0 — yellow). Free hydrochloric acid is almost completely neutralized at pH = 3.0; the color of dimethylaminoazobenzole changes from rose-red to orange. Normal content of free hydrochloric acid is 20–40 mmol/l (in newborns — 0.5 mmol/l).

Evaluation of total acidity, content of free hydrochloric acid and bound hydrochloric acid is done on one portion of gastric juice. Titration is performed with two indicators: dimethylaminoazobenzole and phenolphthalein.

Procedure. Add 10 ml of gastric juice by a pipette into a flask; add 1 drop of dimethylaminoazobenzole and 2 drops of phenolphthalein. When free hydrochloric acid is present in gastric juice, it is stained in red color with a rosy shade, when it is absent, orange staining appears.

Titrate by 0.1 N NaOH from a microburette till orange color appears and mark the result (the 1st mark, it is used to calculate the content of free HCl). Without adding alkaline into the burette continue titration till lemon-yellow color appears and mark the result (the 2nd mark; the difference between the 1st mark and the 2nd mark is used to calculate the content of bound HCl). Continue titration till rosy staining appears (the 3rd mark; the total amount of NaOH spent for titration from 0 is used to calculate the total acidity).

Calculation. Calculate the content of free HCl (the 1st mark), bound HCl (the 2nd mark – 1st mark) and total acidity (3rd mark) by the formula:

$$X \text{ (mmol/l)} = A \cdot 1000 \cdot 0.1/10,$$

where A — the amount of 0.1 N solution of NaOH, ml; 10 — the amount of gastric juice taken for evaluation; 0.1 — the amount of alkaline mg/eqv in 1 ml of 0.1 N solution, mmol; 1000 — re-count to 1 l.

Results:

	Gastric juice N 1	Gastric juice N 2	Gastric juice N 3
Free HCl	A= X(mmol/l)=	A= X(mmol/l)=	A= X(mmol/l)=
Bound HCl	A= X(mmol/l)=	A= X(mmol/l)=	A= X(mmol/l)=
Total acidity	A= X(mmol/l)=	A= X(mmol/l)=	A= X(mmol/l)=

Clinical and diagnostic value. In gastric diseases the acidity can be zero, decreased and increased. In ulcers and hyperacidic gastritis the content of free hydrochloric acid and total acidity increase (hyperchlorhydria). In hypoacidic gastritis or gastric cancer the decrease of free hydrochloric acid and total acidity occurs (hypochlorhydria). Sometimes in gastric cancer and chronic gastritis a complete absence of hydrochloric acid is observed (achlorhydria). In malignant anemia, gastric cancer a complete absence of hydrochloric acid and pepsin (achylia) are noted.

Conclusion:

19. INTRACELLULAR AMINO ACID METABOLISM. DETERMINATION OF AMINO TRANSFERASE ACTIVITY IN SERUM

Objective. To learn the common routes of amino acids metabolism. To get notion of the fate of amino acid carbon skeletons, the role of amino acids in the formation of important biologically active compounds. To show the significance of indicator enzymes in diagnosis and prognosis of diseases by the example of determination of amino transferases activity in serum.

Problems for discussion:

1. Transamination, aminotransferases, co-enzyme function of vitamin B₆. Evaluation of amino transferases activity in serum, clinical-diagnostic value.
2. Types of amino acid deamination. Oxidative deamination of glutamic acid (reactions), the significance of a glutamate dehydrogenase reaction. Indirect deamination.
3. The fate of carbon skeletons of amino acids. Glucogenic and ketogenic amino acids. Pathways for amino acid synthesis.
4. Decarboxylation of amino acids, enzymes, co-enzymes. Biogenic amines (tryptamine, serotonin, histamine, γ -aminobutyric acid), catecholamines (dopamine, norepinephrine, epinephrine). Reactions of biosynthesis, biological role. Oxidation of biogenic amines by mono- and diamino oxidases. Drugs that are inhibitors of aminoxidases.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 67–70.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 155–161.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed, 2003. P. 242–264.
4. Lecture material.

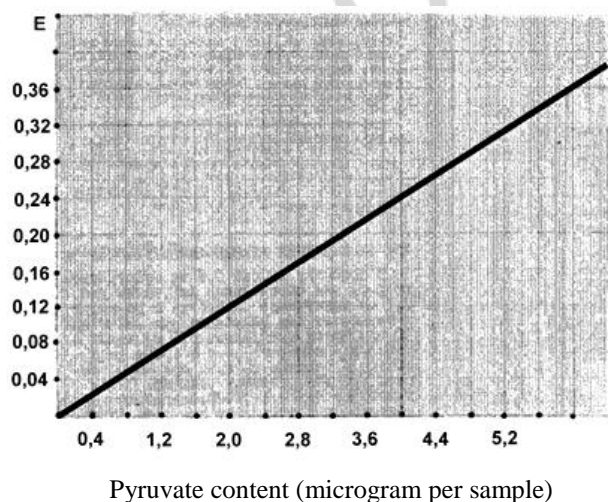
PRACTICAL PART

Determination of alanine aminotransferase (ALT) activity

Aminotransferases (transaminases) are enzymes that use phosphopyridoxal as a co-enzyme and catalyze a reversible amino group transfer from amino acids to ketoacids. Evaluation of formed α -ketoacids concentration underlies transaminase activity determination methods.

Principle of the method. Alanine is converted to pyruvate after transamination. Addition of acidic 2,4-dinitrophenylhydrazine stops the enzymatic process. In alkaline medium the formed hydrosone of pyruvate gives brown-red staining, the intensity of which is proportional to the amount of produced pyruvate.

Aminotransferase activity is expressed in micromoles of pyruvate produced in 1 incubation hour at 37 °C by 1 ml of blood serum. Normal aminotransferase activity in the blood is not high and is from 0.1 to 0.45 $\mu\text{M}/\text{h}\cdot\text{ml}$ for AST and 0.1–0.68 $\mu\text{M}/\text{h}\cdot\text{ml}$ for ALT.



Procedure. Apply 0.5 ml of substrate solution into a test-tube, then add 0.1 ml of studied serum and incubate it in the thermostat at 37 °C for 30 minutes. Then add 0.5 ml of dinitrophenylhydrazine solution and leave the samples for 20 minutes at room temperature. Then add 5 ml of 0.4 N NaOH, carefully stir and leave to stay for 10 minutes at room temperature for staining development. Measure optical density by photometrically under a green light filter (530 nm) in a 10 mm thick cuvette versus a control sample for reagents. The control sample contains all ingredients of the tested sample excluding serum, instead of serum add 0.1 ml of distilled water.

Fix the pyruvate concentration in the serum sample by a readymade calibration graph. Calculate enzyme activity by the following formula:

$$\text{ALT } (\mu\text{M/h}\cdot\text{ml}) = a \cdot 10 \cdot 2 / 88,$$

where a — the amount of pyruvate in 0.1 ml of serum found by the calibration graph, in μg ; 88 — the weight of 1 μM of pyruvate in μg ; 2 — conversion factor to 1 incubation hour; 10 — conversion factor to 1 ml of serum.

E = **a =** **ALT ($\mu\text{M/h}\cdot\text{ml}$) =**

Clinical and diagnostic value. Aminotransferases belong to indicator enzymes and their activity evaluation is widely spread in diagnosing heart and liver diseases. In myocardial infarction the increase of serum AST level is observed in 4–6 hours, its maximum activity — in 24–36 hours. The serum activity of both aminotransferases, especially that of ALT, elevates in hepatitis. The diagnostic value of ALT evaluation in anicteric form of infectious hepatitis and during the incubation period is of particular importance.

Conclusion:

20. DETOXIFICATION OF AMMONIA. DETERMINATION OF NONPROTEIN NITROGEN IN BLOOD AND UREA IN URINE

Objective. To study processes of ammonia detoxification in the organism for understanding mechanisms of hyperammoniemia development. To acquire skills of nonprotein blood nitrogen and urine urea determination and to learn the diagnostic value of these tests.

Problems for discussion:

1. Ways of ammonia binding in cells (reductive amination of α -ketoglutarate, synthesis of glutamine and asparagine, formation of carbamoyl phosphate). Transport forms of ammonia.
2. Ammonia salts formation in kidneys (source of ammonia, the role of glutaminase and glutamate dehydrogenase, the significance of renal glutaminase activation in acidosis).
3. The role of hepatic cells in detoxification of ammonia. Ornithine cycle of urea formation (cycle pattern, substrates, enzymes, energetic supply, relation to the citric acid cycle, regulation). Fate of urea.
4. Nonprotein blood nitrogen (main components and their relative content). Principle of determination and clinical-diagnostic significance.

Recommended literature

1. *Biological chemistry*. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 70–72.
2. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 162–169.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 242–264.
4. Lecture material.

PRACTICAL WORK

Work 1. Determination of urea in urine

In a healthy person about 20–35 g or 333–583 mmol of urea are excreted with urine for 24 hours.

The principle of the method. The method is based on the ability of urea containing amino groups to form with paradimethylaminobenzaldehyde a complex compound in acid medium that is stained yellow. The staining intensity is proportional to urea concentration in the studied urine and is measured photometrically.

Procedure. Pipettes and test-tube must be dry. Apply per 0.2 ml of urine (test sample), 25 mg/l urea solution (standard sample) and water (control sample) respectively into 3 test-tubes, add per 1.2 ml of 2 % solution of paradimethylaminobenzaldehyde into each of them and carefully stir. In 15 minutes perform photometry of the test and standard samples in dry 3 mm thick cuvettes under a blue light filter versus a control sample.

Calculation. Calculate the urea content in the test sample according to a standard urea solution by the formula:

$$C_t = C_s \cdot E_t / E_s,$$

where C_t — urea concentration in the urine sample, mg/ml; C_s — urea concentration in the standard sample, 25 mg/ml; E_t — optical density of the sample; E_s — optical density of the standard urea solution.

Multiply the received value by diuresis (1200-1500 ml) and get the daily content of urea in the urine. Conversion factor to SI units (mmol/24 hours) is 0.0167.

$E_t =$ $E_s =$ $C_t =$ **Urea content in daily urine =**

Clinical and diagnostic value. The decreased urea content in urine is noted in nephritis, acidosis, hepatocellular jaundice, liver cirrhosis, uremia, while the elevated one — in fasting, malignant anemia, fever, intensive break-down of proteins in the organism, after taking salicylates, in phosphorus poisoning.

Conclusion:

Work 2. Determination of nonprotein blood nitrogen

Nitrogen-containing non-protein substances compose a fraction of nonprotein blood nitrogen (intermediate or end products of protein metabolism). They are: urea, uric acid, creatine, ammonia, indican, bilirubin, polypeptides, amino acids, etc. Nitrogen of these substances is called nonprotein as it stays in filtrate after sedimentation of serum proteins.

The main part of nonprotein blood nitrogen is urea nitrogen — 50 %, then nitrogen of amino acids — 25 % and nitrogen of other nitrogen-containing components. Normal values for blood nitrogen are 14.3–25.0 mmol/l (20–40 mg per 100 ml); in newborns — 42.84–71.40 mmol/l (60–100 mg per 100 ml); it decreases to the level found in adults by 10th–12th day of life.

Principle of the method. Nonprotein blood nitrogen is determined in non-protein filtrate after blood proteins sedimentation by various agents (trichloroacetic acid or wolframate) with further mineralization of non-protein filtrate by concentrated sulfuric acid forming ammonia sulfate that interacts with Nessler's reagent (alkaline solution of complex mercury salt $K_2(HgI_4)$) giving a compound of a yellow-orange color. The staining intensity is proportional to ammonia concentration, consequently to that of nitrogen.

Procedure. Prepare 3 usual test-tubes. Apply 1 ml of ready mineralizate and 9 ml of water into the 1st one (test sample), 1 ml of standard solution of ammonia sulfate and 9 ml of water into the 2nd tube (standard sample) and 10 ml of water into the 3^d one (control). Then apply per 0.5 ml of Nessler's reagent into all tubes. Perform photometry of the tested and the standard samples versus the control one under a blue light filter in 5 mm thick cuvettes.

Calculation. Calculate the nonprotein nitrogen content in the tested sample by the formula:

$$C_t = (C_s \cdot E_t / E_s) \cdot 100,$$

where C_t — nonprotein blood nitrogen concentration in the blood, mg per 100 ml; C_s — nitrogen concentration in the standard sample (0.1 mg per 1 ml); E_t — extinction of the tested sample (mineralizate); E_s — extinction of the standard sample (ammonia sulfate).

Conversion factor to SI units (mmol/l) is 0.714.

$E_t =$

$E_s =$

$C_t =$

Clinical and diagnostic value. Evaluation of nonprotein nitrogen and its fractions is used for diagnosing the impairment of renal excretory function and urea-formation function of the liver. The increase of blood nonprotein nitrogen is observed in cachexia of uncancerous origin caused by tuberculosis, diabetes and liver cirrhosis, in cardiac insufficiency, infectious diseases (scarlet fever, diphtheria). In prematurely born infants it can be associated with renal insufficiency and accelerated break-down of tissue proteins. The decrease of nonprotein blood nitrogen is observed in malnutrition and sometimes in pregnancy.

Conclusion:

21. NUCLEOPROTEINS CHEMISTRY AND METABOLISM. DETERMINATION OF URIC ACID IN URINE

Objective. To get the notion of nucleoprotein catabolism in tissues and alimentary tract, mechanisms of biosynthesis and break-down of nucleotides and regulation of these processes. To get acquainted with examples of using this knowledge in diagnosing and treatment of diseases. To perform a laboratory work on determination of uric acid in urine for consolidation of the theoretical material.

Problems for discussion:

1. Mononucleotides, structure, terminology, biological role.
2. Primary, secondary and tertiary structures of nucleic acids (peculiarities of the structure, varieties, types of stabilizing bonds).
3. Nucleoprotein metabolism. Digestion of nucleoproteins in the gastrointestinal tract (significance, steps, enzymes).
4. Degradation of purine nucleotides (reactions, uric acid as an end-product of catabolism). Disorders of purine metabolism (hyperuricemia and gout, urolithiasis).
5. Biosynthesis of purine nucleotides *de novo* (sources of nitrogen and carbon of a purine ring, participation of folic acid, main intermediate products, key enzyme, regulation). The notion of nucleotide synthesis from free nitrogenous bases and nucleosides.
6. Degradation of pyrimidine nucleotides (end products and their fate).
7. Biosynthesis of pyrimidine nucleotides (substrates, process pattern, key enzyme, regulation, role of vitamins).
8. Synthesis of deoxyribonucleotides.
9. Use of synthetic structural analogues of nucleosides and folic acid in medicine.

Recommended literature

1. *Biological chemistry*. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 77–85.
2. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 175–180, 185.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 286–303.
4. Lecture material.

PRACTICAL PART

Determination of uric acid in the urine

Uric acid is an end-product of purine catabolism in humans. About 1.6–3.54 mmol/24 h (270–600 mg/24 h) are usually excreted in healthy human in urine.

Principle of the method. The method is based on the ability of uric acid to reduce phosphorous-tungsten reagent into phosphorous-tungsten blue, the staining intensity of which is proportional to the content of uric acid. The amount of phosphorous-tungsten blue is determined by the red blood salt ($K_2[Fe(CN)_6]$) titration. The last one oxidizes the phosphorous-tungsten blue and blue staining disappears.

Procedure. Apply 1 ml of 20 % solution of Sodium carbonate and 1 ml of phosphorous-tungstic Folin reagent to 1.5 ml of urine and titrate it by 0.01 N solution of $K_2[Fe(CN)_6]$ until blue staining disappears.

Calculation. Calculate the content of uric acid (in mg) in daily urine by the formula:

$$\text{Uric acid, mg/24h} = 0.8 \cdot a \cdot b / 1.5,$$

where 0.8 mg of uric acid corresponds to 1 ml of $K_2[Fe(CN)_6]$; a — the amount of $K_2[Fe(CN)_6]$ used for titration, ml; b — diuresis (1200–1500 ml); 1.5 — the sample volume, ml.

Conversion factor to SI units (mmol/24 h) is 0.0059.

Results.

$$a = \quad \quad \quad \text{Uric acid, mg/24h} = \quad \quad \quad \text{Uric acid, mmol/24h} =$$

Clinical and diagnostic value. Hypouricuria (decrease of uric acid excretion with urine) is noted in gout, nephritis, renal insufficiency; hyperuricuria (increase of uric acid excretion with urine) — in leukemia, accelerated breakdown of nucleoproteins. Children excrete relatively more uric acid than adults. Uric acid excretion depends on the purines content in food and intensity of nucleoproteins metabolism.

In gout uric acid salts (urates) precipitate in cartilages, muscles and joints. The content of uric acid in the blood can be increased while in the urine — decreased.

Conclusion:

22. MATRIX BIOSYNTHESES (SYNTHESIS OF DNA, RNA, PROTEIN)

Objective. To learn molecular mechanisms of replication, reparation, transcription, translation and mechanisms of their regulation. To discuss possible impairments of genetic information realization for understanding consequences and approaches to treatment of these impairments.

Problems for discussion:

1. Replication, biological role, substrates, enzymes, molecular mechanism.
2. Transcription, biological role, substrates, enzymes, RNA processing.
3. Genetic code and its properties.
4. Recognition and translation as steps of genetic information realization in cells. Substrate specificity of aminoacyl-tRNA synthetases. tRNA and its role in protein biosynthesis.
5. Modern understanding of protein biosynthesis. Regulation of protein biosynthesis in cells at a genetic level. Inhibitors of protein biosynthesis (drugs, toxins).
6. Posttranslational modification of protein molecules (hydroxylation, glycosylation, limited proteolysis, phosphorylation, carboxylation).

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 85–90.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 180–184, 186–202.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 303–415.
4. Lecture material.

PRACTICAL PART

Analysis of yeast hydrolysis nucleoprotein products

The acid hydrolysis of yeast is used for study nucleoproteins' structure as yeast is rich in nucleoproteins. Specific reactions are discovered hydrolysis products — polypeptides, purine bases, phosphoric acid and sugar.

The principle of the method: Baker's yeast was hydrolyzed by the action of dilute sulfuric acid. The resulting hydrolyzate is used for further work.

Work 1. Biuret reaction for the polypeptides. Add 10 drops of 10 % NaOH solution to 5 drops of yeast hydrolyzate, and then add 2 drops of 1 % copper sulfate solution. Mark the pink-violet staining.

Work 2. Silver test for purine bases. Add 10 drops of concentrated ammonia solution to 10 drops of yeast hydrolyzate, and then add 10 drops of 2 % ammonium silver nitrate solution. The light brown of purine bases' silver salts precipitation is formed in 3–5 minutes (it is not necessary to stir content of the tube during this time).

Work 3. Qualitative reaction for pentose (Molisch). Add 3 drops of a 1 % alcoholic solution of thymol to 10 drops of yeast hydrolyzate, stir and then add with caution 20–30 drops of concentrated sulfuric acid (the reagent should run down the inner wall of the test-tube). During the shaking of the solution the red-colored product appears.

Work 4. Molybdenum test for phosphoric acid. Add 20 drops of a molybdenum reagent to 10 drops of yeast hydrolyzate, heat the solution. You will see the lemon staining. The test-tube should be immediately cooled in a stream of cold water. A lemon-yellow, crystalline precipitate of phosphomolybdic acid appears.

Conclusion:

23. COLLOQUIUM “METABOLISM OF SIMPLE PROTEINS AND NUCLEOPROTEINS. SYNTHESIS OF DNA, RNA AND PROTEIN”

Questions for preparation:

1. Nitrogen balance. Types of nitrogen balance under physiologic conditions and in pathology.
2. Requirements in proteins. The biological value of food proteins.
3. Proteolysis. Kinds, role.
4. Digestion of proteins. General characteristic of proteases, their substrate specificity.
5. Role of hydrochloric acid in digesting proteins. Механизмы образования соляной кислоты в желудке. Analysis of gastric juice.
6. Putrefaction of proteins. The origin of phenol, cresol, skatole, indole, cadaverine, putrescine. Mechanisms of detoxification of products of putrefaction of proteins.
7. Amino acid pool of the cell — its sources and utilization.
8. Transamination reactions, aminotransferases, co-enzyme function of vitamin B₆. Evaluation of amino transferases activity in serum, clinical-diagnostic value.
9. Types of amino acid deamination. Oxidative deamination of glutamic acid (reactions), the significance of a glutamate dehydrogenase reaction. Indirect deamination.
10. The fate of amino acid carbon skeletons. Glucogenic and ketogenic amino acids. Pathways of amino acid synthesis.
11. Decarboxylation of amino acids, enzymes, co-enzymes. Biogenic amines (tryptamine, serotonin, histamine, γ -aminobutyric acid), catecholamines (dopamine, norepinephrine, epinephrine). Reactions of biosynthesis, biological role.
12. Ways of ammonia binding in cells (reductive amination of α -ketoglutarate, synthesis of glutamine and asparagine, formation of carbamoyl phosphate). Transport forms of ammonia.

13. Ammonia salts formation in kidneys (source of ammonia, the role of glutaminase and glutamate dehydrogenase, the significance of renal glutaminase activation in acidosis).
14. Ornithine cycle of urea formation (cycle pattern, substrates, enzymes, energetic supply, relation to the citric acid cycle, regulation). Fate of urea.
15. Nonprotein blood nitrogen (main components and their relative content). Principle of determination and clinical-diagnostic significance.
16. Mononucleotides, structure, terminology, biological role.
17. Primary, secondary and tertiary structures of nucleic acids (peculiarities of the structure, varieties, types of stabilizing bonds).
18. Nucleoprotein metabolism. Digestion of nucleoproteins in the digestive tract (significance, steps, enzymes).
19. Degradation of purine nucleotides (reactions, uric acid as an end-product of catabolism). Disorders of purine metabolism (hyperuricemia and gout, urolithiasis).
20. Biosynthesis of purine nucleotides *de novo* (sources of nitrogen and carbon of a purine ring, participation of folic acid, main intermediate products, key enzyme, regulation). The notion of nucleotide re-synthesis from free nitrogenous bases and nucleosides.
21. Degradation of pyrimidine nucleotides (end products and their fate). Biosynthesis of pyrimidine nucleotides (substrates, process pattern, key enzyme, regulation, role of vitamins).
22. Synthesis of deoxyribonucleotides.
23. Use in medicine of synthetic structural analogues of nucleosides and folic acid.
24. Replication, biological role, substrates, enzymes, molecular mechanism.
25. Transcription, biological role, substrates, enzymes, RNA processing.
26. Genetic code and its properties.
27. Recognition and translation as steps of genetic information realization in the cell. Substrate specificity of aminoacyl-tRNA synthetases. tRNA and its role in protein biosynthesis.
28. Modern understanding of protein biosynthesis. Regulation of protein biosynthesis in the cell at a genetic level. Inhibitors of protein biosynthesis (drugs, toxins).
29. Posttranslational modification of protein molecules, kinds, biological role.

24. HORMONES. GENERAL CHARACTERISTIC AND PECULIARITIES OF BIOLOGICAL ACTION. QUALITATIVE REACTIONS FOR HORMONES

Objective. To learn how to apply knowledge of hormone classification, types of hormonal receptors, G-proteins and a further cascade of intracellular transmitters for understanding specific mechanisms of hormones action. To understand mechanism of metabolic disorders development in case of insufficient or excessive hormone production.

Problems for discussion:

1. Terminology and classification of hormones by the site of synthesis, chemical structure. Hormones as medications.
2. Peculiarities of hormones synthesis, their transport in blood and biological action.
3. Concept "hormone receptor". Classification and structure of receptors: intracellular receptors (nuclear and cytosolic), receptors of a plasma membrane (canal-forming receptors, 1-TMS and 7-TMS receptors).
4. Mechanisms of steroid, amino acid-derived, protein-peptide hormones action. The role of G-proteins, secondary messengers (cyclic nucleotides, IP₃, Ca²⁺, diacylglycerol), proteinkinases. Peculiarities of signal transduction from intracellular and 1-TMS-receptors.

Recommended literature

1. *Biological chemistry. Lecture notes / A. D. Tahanovich [et al.].* Minsk : BSMU, 2017. P. 91–96.
2. *Konevalova, N. Yu. Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova.* Vitebsk, 2008. P. 203–208.

3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 434–473.
4. Lecture material.

PRACTICAL PART

Qualitative reactions for hormones

Hormones of the thyroid gland

The thyroid gland synthesizes and secretes iodine-containing thyroid hormones of high activity: thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3), as well as noniodized hormone (polypeptide) thyreocalcitonine, the function of which is associated with regulation of calcium and phosphor levels in blood.

Work 1. Qualitative reaction for thyroxine

Principle of the method. When thyriodine is broken-down, potassium iodide is formed, from which iodine is easily forced out by potassium iodate. Forcing iodine out of the salt of hydroiodic acid is a redox reaction where potassium iodide is a reductant and potassium iodate (residue of iodic acid) is an oxidizer. The released iodine is revealed by starch (blue staining) in acidic medium.

Procedure. Apply 24 drops of thyriodine hydrolyzate into a test-tube, add 3 drops of 1 % starch solution, 1 drop of phenolphthalein and then 4 drops of potassium iodate and about 10–15 drops of 10 % solution of sulfuric acid until decolorization and appearance of blue staining.

Hormones of the pancreas

The pancreas produces insulin and glucagon. Insulin is produced by β -cells of Langerhans islets (from Latin *insula* — islet, island), so it got its name. Insulin is a protein consisting of two polypeptide chains connected to each other by disulfide bonds.

The primary structure of insulin is completely decoded and chemical synthesis of insulin is accomplished. Target-organs for insulin are: the liver, muscular tissue, adipose tissue. Insulin acts in a variety of ways. It plays an important role in metabolism of carbohydrates. It decreases glucose content in the blood, increases biosynthesis of glycogen in the liver and muscles, enhances lipogenesis, i.e. formation of lipids from carbohydrates, stimulates synthesis of proteins. Insulin is an antagonist of glucagon and epinephrine in regulation of metabolism.

Work 2. Color reactions for insulin

Insulin gives characteristic reactions for protein: biuret, ninhydrine, Millon's, etc.

Biuret reaction

Procedure. Add 5 drops of 10 % solution of NaOH, 2 drops of 1 % solution of copper sulfate to 5 drops of 1 % solution of insulin, stir the mixture; the content of the test-tube acquires a violet staining.

Ninhydrine reaction

Procedure. Add 5 drops of 0.5 % water solution of ninhydrine to 5 drops of 1 % solution of insulin and boil for 1–2 minutes. A rose-violet staining appears in the test-tube, and with time the solution becomes blue.

Xanthoprotein reaction

Procedure. Apply 5 drops of 1 % solution of insulin into a test-tube, then add 3 drops of concentrated nitric acid and carefully boil. Yellow precipitate appears in the tube.

Reaction for tyrosine (Millon's)

Procedure. Apply 5 drops of 1 % solution of insulin into a test-tube, add 3 drops of Millon's reagent and carefully boil. Dark-red precipitate appears in the tube.

Reaction for amino acids containing loosely bound sulfur (Foll's reaction)

Procedure. Apply 5 drops of 1 % solution of insulin into a test-tube and add 5 drops of Foll's reagent, boil intensely and leave to stay for 1–2 minutes. A black or brown precipitate of lead sulfide will appear.

Hormones of the adrenal medulla

Catecholamines having a pyrocatechous nuclear and an amino group are synthesized in the adrenal medulla.

Work 3. Qualitative reactions for epinephrine

Reaction with chlorous iron

Principle of the method. Epinephrine has a low-alkaline reaction, is easily oxidized in the air with formation of adrenochrome associated with staining of the solution into a red color. In interacting with nitrite yellow-orange staining is observed, with diazoreagent — a red one and with chlorous iron — a green one. The reaction with chlorous iron is characteristic of the pyrocachetinous ring included into a molecule of epinephrine and norepinephrine.

Procedure. Apply 10 drops of epinephrine solution into a test-tube and add 1 drop of chlorous iron. Green staining appears due to the presence of pyrocatechine in epinephrine molecule. Add 3 drops of 10 % solution of NaOH and observe modification of staining (for cherry-red).

Diazoreaction

Principle of the method. When diazoreagent interacts with epinephrine, the fluid is stained red due to the formation of a complex compound, kind of an azostain.

Procedure. Add 6 drops of 0.5 % solution of Sodium nitrite (diazoreagent mixture), 10 drops of epinephrine solution and 3 drops of 10 % solution of NaOH to 6 drops of 0.5 % solution of sulfonic acid. The fluid is stained red.

Work 4. Fluorescence of epinephrine oxidation products

Principle of the method. Epinephrine oxidizing with air oxygen gives fluorescent products, if alkaline is added.

Procedure. Add 6 drops of 10 % solution of NaOH and 6 drops of epinephrine solution to 10 drops of water. Placing the test-tube in front of a switched on fluorimeter observe green fluorescence of epinephrine oxidation products.

Hormones of sex glands

Sex hormones are synthesized in testicles, ovaries, placenta and adrenal glands.

Female sex hormones — estrogens — can be considered as derivatives of estran (a hydrocarbon with 18 atoms of carbon). The main natural estrogens are estradiol, estron and a hormone of corpus luteum — progesteron.

Male sex hormones — androgens — can be considered as derivatives of androstan (a hydrocarbon with 19 atoms of carbon). Testosteron and androsteron are male sex hormones.

Work 5. Qualitative reactions for folliculin

Principle of the method. A qualitative reaction for folliculin (estron) is performed with concentrated sulfuric acid and is due to formation of an ether compound of a straw-yellow color with green fluorescence.

Procedure. The reaction with oil solution of folliculin is conducted at room temperature. Add 30 drops of concentrated sulfuric acid to 2 drops of oil folliculin solution. Gradually a straw-yellow staining develops.

Conclusions:

25. BIOCHEMISTRY OF HORMONES. GLUCOSE TOLERANCE TEST

Objective. To consolidate knowledge of chemical structure and mechanisms of individual hormones action. Special attention should be paid to endocrine pathology of the pancreas. To learn how to construct and interpret various types of a sugar curves.

Problems for discussion:

1. Hypothalamic hormones: chemical structure, type of receptor in target cells and mechanism of a hormonal signal transduction, response of hypophyseal cells to the liberins and statins action.

2. Adenohypophyseal hormones: chemical structure, types of receptors in target-tissues and mechanism of a hormonal signal transduction, realization of hormonal effect at a target-tissue level. The role of excessive and insufficient secretion of hormones.

3. Neurohypophyseal hormones: chemical structure, type of receptor in target-tissue and mechanism of a hormonal signal transduction, realization of oxytocin and vasopressin effects at the level of target-tissues. The role of excessive and insufficient secretion of hormones. Diabetes insipidus.

4. Thyroxine and triiodothyronine: chemical structure, precursor, thyroglobulin, type of receptor in target-tissue, realization of thyroidal hormones effects at a cellular level. The role of peroxidase and deiodase in hormones metabolism. Manifestations of hypo- and hyperthyroidism.

5. Hormones of the adrenal cortex: chemical structure, precursor, type of receptor in target-tissue, realization of glucocorticoids and mineralocorticoids effect at a cellular level. Cushing's syndrome. "Bronze disease".

6. Hormones of the adrenal medulla: chemical structure, precursor, type of receptor in target-tissue, realization of epinephrine and norepinephrine effect at a cellular level.

7. Sex hormones: chemical structure, precursor, realization of the effect of estrogens, progesterone and male sex hormones at a cellular level. Excessive and insufficient secretion of sex hormones.

8. Insulin and glucagon: chemical structure, insulin synthesis, types of receptors in target-tissues for glucagon and insulin, realization of pancreatic hormone effect at a cellular level. Diabetes mellitus. Diagnostic value of sugar curves.

Recommended literature

1. *Biological chemistry*. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 96–100.
2. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 123–125, 209–276.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 434–455.
4. Lecture material.

PRACTICAL PART

Studying of carbohydrate metabolism by glucose loading

To diagnose diabetes mellitus and some pathologic states (insufficiency of the liver and kidneys function, some endocrine diseases, neoplasms of the brain, pancreas and adrenal glands, B₁ hypovitaminosis, some hereditary enzymopathies) it is important to realize the state of carbohydrate metabolism in patients. The blood glucose level is one of most significant parameters. Normal blood glucose concentration in adults is **3.9–6.1 mmol/l**.

The peroral test for glucose tolerance (glucose loading) makes it possible to reveal pathology in those cases, when examination of blood glucose content on an empty stomach doesn't reveal metabolic impairments.

Indications for performing a glucose tolerance test

- Unambiguous results of a single blood analysis on an empty stomach.
- Glucosuria: pancreatic and non-pancreatic (the first one is associated with insufficient secretion or insufficiency of insulin itself; extrapancreatic glucosuria develops, when other organs of internal secretion are impaired, in emotional stress, kidney and liver diseases, excess of carbohydrates in the diet, in pregnancy).
- Clinical signs of diabetes mellitus and its complications in normal blood glucose concentration on an empty stomach (latent forms of diabetes).

Loading. Blood is taken in the morning on an empty stomach from the patient's finger to determine the glucose content, then he is given 200 ml of glucose to drink (calculated as 1 g of glucose per 1 kg of body weight) during 5 minutes. Then the patient is taken blood from his finger every 30 minutes (in the range of 2.5–3 hours), and the results of glucose determination in these samples are used for constructing sugar curves, marking values of glucose concentration in every sample on a vertical axis and those for the time (min or h) on a horizontal axis.

Procedure. Evaluate glucose content in analysis samples № 1–6 (See Instruction for practical class № 11 "Determination of glucose concentration in serum by an enzymatic method"). Test-tube

№ 1 contains the serum taken before, and test-tubes № 2–6 — taken every 30 minutes after glucose loading. On the basis of received data construct a curve. Analyze the glyceimic curve, put down your conclusions.

Results.

Sample	Optical density (E)	Glucose concentration (mmol/l)
1		
2		
3		
4		
5		
6		
Standard		

Normally the blood glucose concentration increases after loading during the first hour by 50–80 %, in 2 hours its level decreases (often it becomes lower than initial) and in 2.5–3 hours it returns to the initial one. In cases, when glucose tolerance is impaired, a considerable elevation of glucose concentration (up to 10.0 mmol/l) stays after loading over 3 hours.

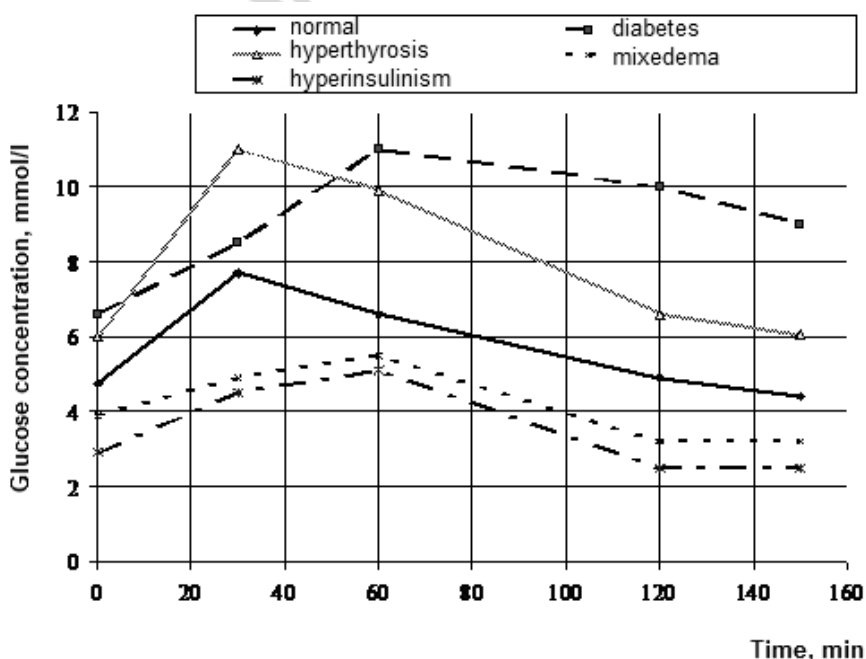
Glycemic curves for children have the same character as for adults with the only difference, that the elevation of blood glucose concentration in children is less.

Clinical and diagnostic value of glyceimic curves evaluation. In patients with various forms of diabetes the elevation of the glyceimic curve occurs slower reaching a considerable value in 60–150 minutes (more than 1.8-fold exceeding the initial value), in the majority of cases glucosuria is noted. The more severe is the disease, the later glycemia maximum is reached and the higher it is. The decrease of the curve occurs very slowly, often it prolongs for 3–4 hours.

Thyroid gland diseases associated with its hyperfunction are characterized by glyceimic curves with a steeper rise, which may be caused by more intensive metabolism and excitation of a sympathetic nervous system.

Patients with adenoma of Langerhans islets, hypothyroidism (mixedema), Addison disease have a low initial level of the curve, its low peak and a high postglycemic ratio.

Glycemic curves in single glucose loading (normal and in some pathologic states)



WHO criteria for diagnosing “diabetes mellitus” and “impaired glucose tolerance”

Diagnosis	Time of taking a blood sample	Venous whole blood, mmol/l
Diabetes mellitus	After night fasting	> 6.7
	2 hours after glucose loading	> 10.0
Impaired glucose tolerance	After night fasting	< 6.7
	2 hours after glucose loading	6.7–10.0

Conclusion:

26. BIOCHEMISTRY OF THE LIVER. INTEGRATION OF METABOLISM

Objective. To be able to use knowledge of homeostatic and integrating role of the liver in carbohydrate, lipid and amino acid metabolism for explaining mechanisms of metabolic disorders development in diseases of the liver and bile ducts. To be able to use knowledge of existing conversion patterns for xenobiotics in the liver to understand biochemical aspects of pharmacology and toxicology. To understand principles and mechanisms of interaction of various metabolic pathways for maintaining vital activity and adaptation.

Problems for discussion:

1. Basic functions and chemical composition of the liver.
2. The role of the liver in carbohydrate metabolism.
3. The role of the liver in lipid metabolism.
4. The role of the liver in protein metabolism.
5. Detoxifying function of the liver, mechanisms: (protective syntheses, acylation, microsomal oxidation, conjugation).
6. The role of the liver in pigment exchange. Synthesis and degradation of hemoglobin (schemes). Normal bilirubin metabolism and its disorders.
7. Biochemical methods of diagnosing liver disturbances.
8. The necessity of metabolism integration, its principal components. Mechanisms of metabolic regulation.
9. Peculiarities of metabolism in the liver in fed state and between meals.
10. Inter-organ metabolism in fasting.

Recommended literature

1. *Tahanovich, A.D. et al.* Biological chemistry. Lecture notes / Minsk, BSMU, 2017. P. 101–107.
2. *Konevalova, N. Yu.* Clinical Biochemistry. Materials for the state examination in biochemistry / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 4–10, 19–31.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 270–285, 231–236.
4. Lecture material.

PRACTICAL PART

Determination of total bilirubin in serum

Principle of the method. Diazo reagent forms azobilirubin stained in a rose color with soluble bilirubin. Staining intensity of azobilirubin solution is proportional to bilirubin concentration and can be evaluated colorimetrically. Conjugated (direct) bilirubin gives a direct reaction with diazo reagent. Unconjugated (indirect) bilirubin can be soluble after adding ethyl alcohol to blood serum.

Procedure. Measure 1 ml of blood serum to a centrifuge test-tube, 2 ml of ethyl alcohol, carefully stir the content with a glass stick and centrifuge for 15 minutes at the speed of 3000 turns/min. Then pour off the supernatant into another test-tube and add 0.25 ml of diazo reagent. Red-rose staining appears. In 10 minutes determine its intensity measuring the sample optical density (E_t) versus water in a 5 mm thick cuvette under a green light filter (500–560 nm).

Perform the photometry of azobilirubin standard solution corresponding to bilirubin concentration 0.4 mg per 100 ml (C_s), versus water (E_s for the calculation below).

Calculate by the formula:

$$C_t \text{ (mg per 100 ml)} = E_t \cdot C_s / E_s.$$

Conversion factor to SI units ($\mu\text{mol/l}$) is 17.104.

Results.

$$E_t = \quad \quad \quad E_s = \quad \quad \quad C_t \text{ (mg per 100 ml)} =$$

$$C_t \text{ (}\mu\text{mol/l)}$$

Normal total bilirubin concentration in plasma (serum) is 0.5–1.2 mg per 100 ml (8.55–20.52 $\mu\text{mol/l}$); indirect bilirubin forms 75 % of its amount.

Clinical and diagnostic value of studying pigment metabolism. Jaundice is one of important signs of pigment metabolism impairment, it is usually noted at bilirubin level in the blood over 27–34 $\mu\text{M/l}$. The blood of newborns, especially prematurely born, has a higher bilirubin content (physiological jaundice). The increase of bilirubin concentration observed from 2nd–3rd to 7th–10th days mainly due to indirect bilirubin is associated with functional insufficiency of the liver, in particular with low activity of enzyme UDP-glucuronyl transferase, which is necessary for the formation of direct bilirubin.

Hemolytical jaundice (pre-hepatic) is caused by *the enhanced erythrocyte hemolysis* that results in enhanced formation of unconjugated (indirect) bilirubin. Excess direct bilirubin is produced in the liver and is excreted into the intestines with the bile.

Parenchymous jaundice (hepatic) is caused by *the impairment of hepatic cells function*. It may be also caused by hereditary defects of bilirubin excretion and bilirubin biglucuronide formation.

Mechanic jaundice (post-hepatic, obstructive) results from *the bile outflow troubles*. It occurs due to the obstruction of bile ducts, their rupture and consequent entrance of bile into the blood.

The severity of jaundice usually corresponds to the level of bilirubinemia. It is considered that jaundice has a mild form if the content of bilirubin in plasma (serum) does not exceed 85 $\mu\text{M/l}$; its level of 86–169 $\mu\text{M/l}$ testifies to moderately severe, and over 170 $\mu\text{M/l}$ — to a severe jaundice.

Conclusion:

27. COLLOQUIUM “HORMONES. BIOCHEMISTRY OF THE LIVER. INTEGRATION OF METABOLISM”

Questions for preparation:

1. Properties of hormones. Peculiarities of biological action. Examples of hormones which realized their effect via different types of receptors.
2. Hormone receptors, classification, structure of receptors.
3. G-proteins, their types and role in the mechanism of action of hormones that bind to the membrane receptors.
4. Effector systems and second messengers of hormonal signal into the cell. Mechanisms of second messengers' formation and activation.
5. Role of calcium in the hormonal signal transduction mechanism.
6. The soluble and membrane-bound guanylate cyclase, the activation mechanism, realization of effect.
7. Mechanisms of hormonal signal amplification.
8. Hormones — proteins: simple and complex. Place of synthesis, example of molecular action.

9. Hormones — amino acid derivatives. Place of synthesis, mechanisms of hormonal signal transduction.
10. Basic principles of peptide/protein hormones biosynthesis.
11. Basic principles of steroid hormones biosynthesis.
12. Vasopressin, chemical nature, hormonal signal transduction mechanism, effects. Diabetes insipidus.
13. Growth hormone, receptor, hormonal signal transduction mechanism, the effect on the metabolism. Excess and insufficiency of the hormone.
14. Iodine-containing thyroid hormones. The chemical nature, the synthesis of T₃ and T₄. Mechanism of signal transduction, effects on metabolism. Hypo- and hyperthyroidism.
15. Glucagon. The chemical nature, place of synthesis. Mechanism of signal transduction, effects on metabolism.
16. Hormones of the adrenal medulla. The chemical nature, scheme of synthesis, Mechanism of signal transduction, effects on metabolism.
17. Hormones of the adrenal cortex: glucocorticoids and mineralocorticoids. The chemical nature, structure of receptors, Mechanism of signal transduction, effects on metabolism.
18. Insulin. The chemical nature, synthesis, insulin receptor structure. Mechanisms of signal transduction. Effects on carbohydrate, lipid, protein metabolism.
19. Sex hormones. The chemical nature, structure of receptors. Mechanisms of signal transduction, effects on metabolism.
20. Diabetes mellitus. Types, causes. Disorders of carbohydrate, lipid, protein metabolism. Biochemical diagnosis of diabetes. Construction of sugar curves.
21. Diabetes mellitus: mechanism of ketonemia. Glucose metabolism in insulin-independent tissues. Glucose reduction pathway.
22. The liver as the main body organ homeostasis. Liver function.
23. The role of the liver in the metabolism of carbohydrates. It is essential to know the schemes of basic metabolic pathways of carbohydrate metabolism, key enzymes and their regulators. Pentose phosphate pathway, uronic acid pathway and their biological role. Metabolism of exogenous ethanol in the liver.
24. The role of the liver in protein metabolism. It is essential to know the conversion reaction of amino acids, the methods of detoxification of products of putrefaction of proteins and ammonia.
25. The role of the liver in lipid metabolism. It is essential to know the basic schemes of lipid metabolism pathways. Mechanisms of development of fatty liver disease. Lipotropic factors.
26. The central role of acetyl-CoA in lipid metabolism. The scheme of the formation of ketone bodies and reactions of fatty acids and cholesterol synthesis.
27. Antitoxic liver function. Neutralization of the toxic substances, the normal metabolites and drugs in the liver. Microsomal oxidation and oxidation of ethanol and acetaldehyde in liver cells.
28. The role of the liver in the pigment metabolism. Heme biosynthesis, regulation. The proteins containing heme as a prosthetic group, and their functions in the body. Porphyrin.
29. Degradation of hemoglobin. Metabolism of bile pigments.
30. Metabolic processes of utilization of carbohydrates, fats and proteins as energy sources (glycogenolysis, aerobic oxidation of glucose and subsequent oxidation of pyruvate, mobilization of fats and β -oxidation of fatty acids).
31. The metabolic processes of dietary carbohydrates and lipids storage. The schemes for the synthesis of glycogen re-synthesis and synthesis triacylglycerols, phospholipids. The difference of triacylglycerol synthesis in the liver and adipose tissue.
32. Integration of metabolism, its principal components. Mechanisms of metabolic regulation.
33. Regulation of metabolic processes in the liver, muscle and adipose tissue after a meal and in fasting.
34. Inter-organ metabolism in different periods of fasting. The mechanism of overproduction of ketone bodies.

28. PHYSICAL AND CHEMICAL PROPERTIES OF THE BLOOD. HEMOGLOBINOSES

Objective. To study physical and chemical properties of the blood, to consolidate knowledge of the origin of plasma components and their physiological concentrations, buffer blood systems, structure and functioning of hemoglobin, gas transport in the blood and mechanisms of hypoxia development, diagnostic significance of the most important biochemical blood components.

Problems for discussion:

1. Chemical composition of plasma (physiological concentrations of the most important plasma components and their origin).
2. The most important blood buffer systems: bicarbonate, hemoglobin, phosphate, protein (components and their proportion, mechanism of action, capacity). The notion of acid-base disturbances (acidosis, alkalosis).
3. Proteins of red blood cells. The structure of hemoglobin, heme, globin; varieties (normal and abnormal) and derivatives of hemoglobin. Metabolism of red blood cells.
4. Respiratory function of the blood. Red blood cells as a main participants of gas transport by the blood (the role of hemoglobin and carbonic anhydrase). Reversible binding of oxygen and carbon dioxide as a means of transport (mechanisms of binding CO₂ and O₂ to hemoglobin). Hypoxia, mechanisms of development.
5. Blood plasma proteins. Main protein fractions: albumins, globulins, fibrinogen (content, functions); albumin-globulin ratio and its diagnostic value.
6. Blood plasma enzymes (secretory, indicator, excretory). Diagnostic value of plasma enzymes activity determination.

Recommended literature

1. *Tahanovich, A.D. et al.* Biological chemistry. Lecture notes / Minsk, BSMU, 2017. P. 150-156.
2. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 580–597, 609–625.

PRACTICAL PART

Work 1. Buffer properties of serum

The bicarbonate, protein and phosphate buffer systems function in serum.

Principle of the method. Titrate 1 ml of serum (the 1st test-tube) and 1 ml of water (the 2nd test-tube) with 0.1 N solution of HCl in the presence of the blue bromphenol indicator (per 1 drop into every test-tube) till yellow staining appears. Compare the results of titration.

V_{water} =

V_{serum} =

Conclusion:

Work 2. Determination of chlorides in serum according to Levinson

Chlorine is present in the organism mainly in the form of ions. A chloride-ion is the main plasma anion. Chloride anions are the important osmotic active components of blood, lymph, cerebrospinal fluid. The content of chlorine (chloride-ions) in serum of practically healthy adult people is 95–105 mmol/l. In newborns the normal concentration of serum chloride-ions is 80–140 mmol/l.

Principle of the method. The argentometric method is based on the ability of silver ions to form insoluble salts with ions of chlorine. The amount of depositing substance (AgNO₃) is equivalent to the content of chloride-ions.

Titration of blood chloride-ions by silver nitrate is performed in the presence of indicator K₂CrO₄. On reaching an equivalent titration point the excess of silver ions and the indicator form a compound of a brick-red color (Ag₂CrO₄).

Procedure.

1. Sedimentation of serum proteins. Prepare a mixture of solutions in two test-tubes: 5 ml of 0.45 % ZnSO₄ + 1 ml of 0.1 N NaOH. Then apply 0.1 ml of serum into the 1st tube, 0.1 ml of H₂O_{dist.} into the 2nd tube. Heat the test-tubes for 3 minutes (boiling). Then filter the content of the test-tubes into flasks through the cotton-wool. Rinse the residue on the cotton-wool filter twice with water (per 3 ml).

2. Sedimentation of chloride ions in the presence of K₂CrO₄. Add 2 drops of 1–2 % solution of K₂CrO₄ to the filtrate and titrate it with AgNO₃ till a yellow color of the solution changes to brick-red.

Calculation. Calculate the difference between the volume of AgNO₃ spent for titration of the tested solution (V_t, ml) and the volume of AgNO₃ spent for titration of the control solution (V_c, ml), multiply the received difference by 0.355. The result corresponds to the mg of chloride per 0.1 ml of serum. Multiply the received value by 1000 (get the mg of chloride per 100 ml) and by 0.282 (the conversion factor to SI units, mmol/l).

V_t (ml) =

V_c (ml) =

C(mmol/l) = (V_t – V_c) · 0.355 · 1000 · 0.282 =

Conclusion:

29. BLOOD CLOTTING SYSTEM

Objective. To get acquainted with the principles of determination of total plasma protein, protein fractions and individual proteins, to understand their diagnostic value. To get the notion of hemostasis and to study functioning of the blood clotting, anticoagulant and fibrinolysis systems.

Problems for discussion:

1. Understanding of hemostasis (definition, structural-functional units and their biological role). Primary and secondary (coagulation) hemostasis. The notion of blood coagulation system functioning impairments.

2. Coagulating system (components and their origin), hemocoagulation (definition, phases and their duration, sources of phospholipid surfaces). Intrinsic and extrinsic pathways of blood coagulation.

3. Vitamin K (chemical structure, varieties, natural sources, role in coagulation).

4. Anticoagulant system, classification of physiological anticoagulants: primary and secondary (representatives, mechanism of action). Artificial anticoagulants of direct and indirect action.

5. Fibrinolytic system, mechanisms of fibrinolysis. Plasmin system (components and their origin, mechanism of action).

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 73–76.
2. *Konevalova, N. Yu. Clinical Biochemistry. Materials for the state examination in biochemistry* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 11–18.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 580–607.
4. Lecture material.

PRACTICAL PART

Work 1. Determination of calcium in plasma

Calcium plays an important role in realization of vital processes. It influences the permeability of biological membranes, excitability of nerves and muscles, participates in neuromuscular conductivity, constriction and relaxation of musculature (including cardiac muscles), secretory

processes, formation of bones and cartilages; produces effect on metabolism in cells, is an important factor of hemostasis and is a mediator of hormones action in the cell. Determination of plasma total calcium is of great importance for diagnosing a number of diseases and managing the treatment.

Normal total calcium concentration in blood plasma is 2.2–2.7 mmol/l (9–11 mg%).

Principle of the method. The indicator, chromogen black ET-00, forms with calcium a compound of a rose-violet color. In titration of so stained solution with EDTA (double-substituted sodium salt ethylenediaminetetraacetic acid forming tight complexes with calcium ions) staining will change to a blue-rose color in an equivalent point corresponding to binding of all calcium ions in the solution by EDTA.

Procedure. Apply 25 ml of H₂O into a flask and add 1 ml of ammonia buffer solution. Then add 1 ml of studied blood plasma and 2 drops of indicator chromogen black. The solution becomes rose-violet. Then titrate the solution with 0.002 M solution of EDTA to a blue-rose color. Calculate the content of calcium in blood plasma by the volume of EDTA spent for titration:

$$X \text{ (mg per 100 ml)} = \frac{0,002 * 40,8 * 100 * V_t}{1}$$

where 0.002 — molar concentration of EDTA solution; 40.8 — molecular weight of Ca; 100 — conversion factor to mg per 100 ml; 1 — the serum volume taken for analysis; V_t — volume of EDTA spent for titration.

The conversion factor to SI units (mmol/l) — 0.245.

V_t (ml) = X (mg per 100 ml) =

C(mmol/l) =

Conclusion:

30. BIOCHEMISTRY OF NUTRITION. ROLE OF PROTEINS, FATS, CARBOHYDRATES, VITAMINS

Objective. To consolidate knowledge of the chemical structure and molecular mechanisms of biological action of co-enzyme forms of vitamins, involvement of other essential factors of nutrition into metabolism. To form the notion of biochemical mechanisms of utilizing food components for maintaining normal vital activity of the organism, pathological states of insufficient nutrition. To acquaint students with methods of vitamins detection and their determination in food products.

Problems for discussion:

1. The nutritive value of proteins, fats, carbohydrates. The role of fibrous polysaccharides for functioning of the digestive tract and metabolic processes in the organism. Essential nutritive factors.

2. Vitamins of B group: a) thiamine (B₁); b) riboflavin (B₂); c) pantothenic acid; d) niacin; e) pyridoxine (B₆); f) folic acid (B₉); g) cobalamine (B₁₂). Chemical nature, co-enzyme forms, molecular mechanisms of action.

3. Biotin (vitamin H), vitamin C. Structure, role in metabolic processes.

4. Fat-soluble vitamins (A, E, D, K). Structure, role in metabolic processes.

5. Determination of the body's saturation with vitamins.

6. The concept of free radicals and active forms of oxygen, their physiological and damaging effect. Enzymatic and non-enzymatic antioxidant protection. Antioxidant effects of vitamins C, A and E.

7. Vitamin-like substances: bioflavonoids (vit. P), choline, lipoic acid, inositol, paraaminobenzoic acid, vit. U, etc. Biological role.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 110–120.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 285–308.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 474–497.
4. Lecture material.

PRACTICAL PART

To reveal vitamins and evaluate their amount in various substances and biological fluids color reactions are used, as they are specific for this or that group of vitamins.

Work 1. Qualitative reactions for vitamin B₁

Vitamin B₁ consists of a pyrimidine and thiazole rings. Vitamin B₁ has got a name of thiamine as it contains sulfur and nitrogen.

1. Oxidation reaction.

Principle of the method. In alkaline medium thiamine is oxidized by potassium ferricyanide and gives thiochrome. Thiochrome has blue fluorescence, when the solution is UV radiated on the fluoroscope.

Procedure. Add 5–10 drops of 10 % solution of NaOH, 1–2 drops of 5 % solution of potassium ferricyanide to 1 drop of 5 % solution of thiamine and stir up the mixture. After the fluoroscope has got warmed you'll observe blue fluorescence while radiating the solution with UV rays.

2. Diazoreaction.

Principle of the method. In alkaline medium thiamine with diazoreagent forms a complex compound of an orange color.

Procedure. To diazoreagent containing 5 drops of 1 % solution of sulphanic acid and 5 drops of sodium nitrate add 1–2 drops of 5 % solution of thiamine and then very carefully, by the wall of the test tube add 5–7 drops of 10 % solution of sodium bicarbonate. On the border of two fluids an orange ring appears.

Work 2. Qualitative reaction for vitamin B₂

Principle of the method. The oxidized form of vitamin B₂ represents a yellow substance fluorescent in UV rays. The reaction for vitamin B₂ is based on its ability to be reduced easily. The solution of vitamin B₂ of a yellow color becomes at first rosy due to the formation of intermediate compounds and then decolorizes as a reduced form of vitamin B₂ is colorless.

Procedure. Apply 10 drops of vitamin B₂ into a test-tube, add 5 drops of concentrated hydrochloric acid and dip a granule of metal zinc. The release of hydrogen vesicles starts, the fluid gradually becomes rosy, then decolorizes. Compare both forms of vitamin B₂ by fluorescence.

Work 3. Qualitative reaction to vitamin PP

Principle of the method. Vitamin PP being heated with the solution of copper acetate forms a poorly dissolved blue deposit of copper nicotinate.

Procedure. Apply 20 drops of 3 % solution of vitamin PP (before evaluation stir up the solution) and heat it till boiling; the solution transforms from turbid into transparent. Stir up 5 % solution of copper acetate, add 20 drops of it to the heated solution of vitamin PP. Bring the content of the test-tube to boiling and immediately cool it under cold running water. A blue deposit of copper nicotinate appears.

Work 4. Qualitative reaction for vitamin B₆

Principle of the method. Vitamin B₆ in interacting with the solution of chlorous iron forms a complex salt kind of iron phenolat of red color.

Procedure. To 5 drops of vitamin B₆ add the equal amount of 1 % solution of chlorous iron and stir. Red staining develops.

Work 5. Qualitative reaction to vitamin B₁₂

B₁₂ is the only vitamin containing a metal (cobalt) in its structure.

Principle of the method. When cobalt ions interact with thiourea cobalt rodanide of a green color is formed during heating.

Procedure. Apply 2–3 drops of thiourea solution on a paper filter and dry over the burner. Then apply 1–2 drops of mineralizate (B₁₂) on the filter and heat again over the burner. Green staining appears, more often along the edge, it evidencing to the presence of cobalt.

Fill in the table with the results:

№	Experiment	Observed changes	Conclusions

Work 6. Determination of Vitamin C

Principle of the method. The method is based on the ability of vitamin C to reduce 2,6-dichlorophenolindophenol, which in acidic medium has red staining and decolorizes after reduction; in alkaline medium it has blue staining. To preserve vitamin C from destruction the studied solution is titrated in acidic medium by alkaline solution of 2,6-dichlorophenolindophenol till rose staining appears.

Determination of vitamin C content in urine

Evaluation of vitamin C content in urine gives the notion about pools of this vitamin in the organism, because there is correlation between blood concentration of this vitamin and its amount excreted with urine. However, in C hypovitaminosis the content of ascorbic acid in urine is not always diminished. Often it is normal despite considerable insufficiency of this vitamin in tissues and organs.

In healthy people taking 100 mg of vitamin C *per os* quickly results in its elevation in blood and urine. In C hypovitaminosis the tissues, suffering from its insufficiency, catch the given vitamin C and its concentration in urine does not increase. The urine of a healthy person contains 20–30 mg/24 h of vitamin C or 113.55–170.33 μM/24 h. In children the level of this vitamin decreases in scurvy as well as in acute and chronic infectious diseases.

Procedure. Measure 10 ml of urine and 10 ml of distilled water into a flask, stir, acidize with 20 drops of 10 % solution of hydrochloric acid and titrate with 0.001 N solution of

2,6-dichlorophenolindophenol till a rose staining appears. To calculate the content of ascorbic acid in urine use the formula:

$$X = \frac{0,088 * A * B}{C}$$

where X — the content of ascorbic acid in mg/24 h; 0.088 — the amount of ascorbic acid (mg) corresponding to 1 ml of 2,6-dichlorophenolindophenol; A — the result of titration by 0.001 N solution of 2,6-dichlorophenolindophenol, ml; B — an average diuresis (for men it is 1500 ml, for women — 1200 ml); C — the volume of urine taken for titration, ml.

Results.

A (ml) = **X =**

Conclusions:

31. BIOCHEMISTRY OF NUTRITION. MINERAL SUBSTANCES. REGULATION OF WATER-ELECTROLYTE BALANCE

Objective. To consolidate knowledge of electrolyte composition of fluids of the organism, role of macro- and trace elements in cells and extracellular fluids, mechanisms of regulation of water and mineral exchange.

Problems for discussion:

1. Sodium, potassium, chlorine, calcium, phosphorus, magnesium, sulfur. The role in metabolism. Drugs acting on ion channels and Na^+/K^+ - ATPase.
2. Hormonal regulation of exchange of calcium and phosphorus (calcitonin, parathyroid hormone, calcitriol). Mechanisms of hormonal signal transmission in the cell, effects..
3. Hormonal regulation of salt and water balance. Renin-angiotensin system, the role of aldosterone, vasopressin, atrial natriuretic factor. Angiotensin converting enzyme inhibitors.
4. Hormones regulating calcium and phosphorus metabolism. Chemical nature, mechanism of signal transduction in target-cells, biological action.
5. The role of iron in the organism (absorption, transport, intracellular metabolism). Iron deficiency states and iron-deficient anemias.
6. The role of copper, zinc, manganese, cobalt, selenium, iodine and fluorine in tissue metabolism.
7. Biochemical approaches to the creation of vitamin-mineral complexes.

Recommended literature

1. *Biological chemistry. Lecture notes* / A. D. Tahanovich [et al.]. Minsk, BSMU, 2017. P. 121–128.
2. *Konevalova, N. Yu. Biochemistry lecture course* / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2008. P. 277–284.
3. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003. P. 481–497.
4. Lecture material.

PRACTICAL PART

Work 1. Determination of sodium content in serum by a photometric method

Principle of the method. The method is based on the fact that sodium contained in the sample is deposited by manganese uranylacetate. Uranyl-anions left in the solution are capable to form a stained complex with thioglycolate. Sodium concentration is proportional to the difference between control (without precipitation) and tested sample.

Clinical and diagnostic value. Normal content of K⁺ in serum is 3.4–5.6 mmol/l.

Decrease of potassium concentration in blood serum results in severe impairments — up to the diastolic cardiac arrest. Psychic and mental activity becomes worse, muscle hypotonia, intestinal atonia, arrhythmia develop.

Hyperkalemia is associated with a sensation of tingles, cardiac rhythm impairments: transverse blockade, fibrillation, cardiac arrest.

Conclusion:

32. PHARMACEUTICAL BIOCHEMISTRY. BIOTRANSFORMATION OF SUBSTANCES AND THEIR DISTRIBUTION FROM THE ORGANISM. BIOCHEMISTRY OF URINE

Objective. Learn how to apply knowledge about the chemical composition of living organisms, the laws of metabolism and energy, the main ways of entering, converting and removing drugs and endogenous compounds from the body to assimilate the basics of biopharmacy, pharmacokinetics, methods of standardization and quality control of drugs. Learn to evaluate the content of drugs and their metabolites in the urine.

Problems for discussion:

1. Pharmaceutical biochemistry. Absorption and transport of drugs. Specific and nonspecific transport systems of blood. Distribution of medicinal substances in tissues. Factors affecting the pharmacokinetics processes.

2. Biotransformation phases of substances. Changes in the activity and toxicity of substances in the process of metabolism. Factors affecting the metabolism of drugs: genetic, physiological and environmental media.

3. Reactions of oxidation, reduction, hydrolysis. Microsomal oxidation system, the role of cytochrome P450. Metabolism of endogenous ethanol.

4. Phase conjugation in the system of neutralizing drugs. Types of conjugation (glucuronidation, acetylation, methylation, sulfation and hydration), co-substrates and enzymes (examples of reactions).

5. Removal of medicinal substances from the body. The main ways of excretion, their mechanisms.

6. Normal characteristics of urine volume, density, color, transparency, pH.

7. Inorganic and organic components of the urine.

8. Diagnostic significance of the urine pathologic components and their determination:

- a. Renal and extrarenal proteinuria;
- b. Glucosuria in diabetes mellitus, renal glucosuria;
- c. Renal and extrarenal hematuria;
- d. Ketonuria in fasting, diabetes.

Recommended literature

1. *Biological chemistry*. Lecture notes / A. D. Tahanovich [et al.]. Minsk, BSMU, 2017. P. 157–159.
2. *Harper's biochemistry* / R. K. Murray [et al.]. 26th ed. 2003.
3. Lecture material.

PRACTICAL PART

Work 1. Determination of urine density

As urine portions differ in their specific weight and chemical composition, the density is evaluated for daily urine. Normally, density of urine correlates with diuresis and is 1.010–1.025. In various states it may vary from 1.000 to 1.060.

4. Enzymes of blood plasma: secretory, excretory, indicator (examples of enzymes, their place of synthesis). Diagnostic value of the indicator enzymes of blood plasma.
5. Proteins of blood plasma. Basic protein fraction: albumin, globulins, fibrinogen (normal content, functions); albumin-globulin ratio, and its diagnostic value. The biological role and the diagnostic significance of certain plasma proteins (haptoglobin, transferrin, ceruloplasmin, α 1-protease inhibitor, C-reactive protein, interferon, cryoglobulins).
6. Metabolism of red blood cells.
7. Hemostasis. Hemostatic mechanisms. Three basic structural and functional components of hemostasis. Pathological conditions caused by disturbances in the hemostatic system.
8. Molecular mechanisms of primary hemostasis.
9. The blood coagulation system (components and their origin). Coagulation (phases and their duration). Intrinsic and extrinsic pathways of blood coagulation (scheme), general steps and differences. The role of platelets in the blood coagulation. Physiological concentrations of fibrinogen in the blood. Conversion of fibrinogen to fibrin. Physiological calcium concentration in blood and participation of calcium in blood coagulation. Role of vitamin K in blood coagulation.
10. Anticoagulant system. Classification of natural anticoagulants. The most significant natural anticoagulants, their mechanism of action. Artificial anticoagulants (examples). The mechanism of action of heparin and dicumarol.
11. The fibrinolytic system, the mechanisms of fibrinolysis. Plasmin system (components and their origin, mechanism of action).
12. Vitamin B₁ (thiamine), B₂ (riboflavin), pantothenic acid, PP (nicotinic acid), B₆ (pyridoxine), B₉ (folic acid), B₁₂ (cobalamin), H (biotin), C (ascorbic acid). Coenzyme forms and role in metabolism, signs of vitamins' deficiencies, the daily requirement and food sources. Block-structures of coenzyme form of vitamin PP (NAD and NADP) and vitamin B₂ (FMN and FAD).
13. Vitamin-like substances: bioflavonoids (vitamin E), choline, lipoic acid, para-aminobenzoic acid, vitamin U, carnitine, inositol, pangamic acid. Biological role.
14. Tocopherol. The chemical nature, role in the metabolism, signs of vitamin deficiency, daily requirement, the main sources of vitamin E.
15. Retinol. The chemical nature, role in the metabolism, signs of hypo- and hypervitaminosis, the daily requirement, the main sources of vitamin A.
16. Education and physiological role of active forms of oxygen and nitrogen. Damaging effect of free radicals and reactive oxygen species. Antioxidant protection.
17. Vitamin K. The chemical nature. The biological role. Symptoms of hypovitaminosis. The main sources and the daily requirement. Vikasol. Antivitamins.
18. Vitamin D. The chemical nature, sources and daily requirement. The formation of the active form of vitamin D₃ in the body. The biological role. The symptoms of hypo- and hypervitaminosis.
19. Determination of the body's saturation with vitamins. Biochemical foundations for the creation of multivitamin and vitamin-mineral complexes.
20. Ways of usage ATP in a state of rest. Contribution to the energy production of proteins, fats and carbohydrates.
21. Nutritional value of carbohydrates, lipids and proteins. Essential dietary factors. Role of dietary fibers.
22. The norms of water requirement and excretion. The content and the role of water in the body.
23. Calcium and phosphorus. Daily requirement, food sources, role in metabolism, metabolic disorders. Hormonal regulation of calcium and phosphorus metabolism.
24. Sodium, potassium, chlorine, magnesium and sulfur. Daily requirement, dietary sources, distribution in the body and their role in the metabolism of macronutrients. Na⁺/K⁺-ATPase. Metabolic disorders, cystic fibrosis.
25. The biological role of iron. Requirements. Metabolism of iron in the body (absorption, transport blood, intracellular metabolism). Storage of iron. The symptoms of iron excess in the body.

26. The role of copper, zinc, selenium, iodine, fluorine, manganese and cobalt in the metabolism. Daily requirement and food sources. Metabolic disorders.
27. The mechanism of action of hormones regulating water and mineral metabolism (vasopressin, natriuretic peptide, aldosterone).
28. The renin-angiotensin system and its role in the regulation of water-mineral metabolism.
29. Pharmaceutical Biochemistry. Tasks and biochemical methods used in the analysis and quality control of drugs.
30. Fundamentals of pharmacokinetics. Ways of intake of medicinal substances into the body and mechanisms of absorption. Factors affecting suction.
31. Transport of medicinal substances. Specific and nonspecific transport systems of blood. Distribution of medicinal substances in tissues. Factors affecting the pharmacokinetics processes.
32. Phases of biotransformation of substances. Changes in the activity and toxicity of substances in the process of metabolism. Prodrugs. Factors affecting the metabolism of drugs: genetic, physiological and environmental media.
33. Reactions of oxidation, reduction, hydrolysis. Microsomal oxidation system, the role of cytochrome p450 (oxidation scheme). Induction of cytochrome p450. Metabolism of exogenous ethanol.
34. Phase conjugation in the system of neutralization of drugs. Types of conjugation (glucuronidation, acetylation, methylation, sulfation), co-substrates and enzymes. Examples of reactions.
35. Removal of medicinal substances from the body (pathways and mechanisms) Role of P-glycoprotein, metallothionenin.

EXAMINATION QUESTIONS

FIRST QUESTIONS

1. Amino acids. Structure, classification, properties. Application as medicines.
2. Peptide theory of the structure of protein molecules (Fisher's theory). Principles of the classification of proteins. Similarities and differences between proteins and peptides.
3. Physico-chemical properties of proteins. Solubility of proteins in water. Stability factors of protein solutions. General reactions to proteins: color and precipitation. Use of these reactions in pharmaceutical practice.
4. Methods of separation of proteins, peptides and amino acids (electrophoresis, adsorption, ion exchange, distribution chromatography). Using Western blot analysis to identify proteins.
5. Stages of studying the primary structure of proteins and peptides. Methods for purification, separation and determination of the molecular weight of proteins and peptides (dialysis, gel chromatography, gel electrophoresis, isoelectrofocusing, affinity chromatography).
6. Methods for studying the amino acid composition (ion exchange chromatography) and the amino acid sequence of proteins and peptides (Sanger, Edman, Akabori).
7. Methods and approaches to the artificial synthesis of proteins and peptides for the preparation of drugs.
8. Primary and secondary structure of the protein molecule. Bonds that stabilize them. Peculiarities of the structure of the peptide bond and their role in the formation of the spatial structure of the protein (Pauling-Corey postulates). Types of secondary structure.
9. The concept of a supra-secondary protein structure. Structural and functional domains. The causes of the formation of the tertiary structure of the protein molecule.
10. Tertiary structure of the protein. Relations that stabilize the tertiary structure. Conformational changes in the functioning of proteins. Denaturation of the protein and the factors that cause it. Use of the phenomenon of denaturation in medical practice.
11. Quaternary structure of proteins. Advantages of the existence of proteins with a quaternary structure. Co-operative changes in the conformation of polypeptide chains in the functioning of proteins with a quaternary structure on the example of hemoglobin.
12. Protein-ligand interaction. Complex proteins. Types of bonds between the protein and non-protein parts of the molecule. Functions of complex proteins in the body.
13. Mononucleotides, their structure and role in the cell. The role of cyclic nucleotides. Primary structure of nucleic acids. Features of the structure, function and distribution in the cell of DNA and RNA. Method for analyzing the primary structure of DNA (Sanger).
14. Secondary structure of DNA and RNA. Types of RNA and their functions. Interaction of nucleic acids with proteins. The structure of nucleoproteins. Features of the structure of chromosomes and ribosomes.
15. Blot analysis of DNA (Southern blot) and the method of "fingerprints" of DNA. Basic stages and application in medical practice.
16. Lipids, classification of lipids. Functions of acylglycerols, phospho- and glycolipids in the body.
17. Complex lipids. Representatives. Structure, polarity, biological role.
18. Fatty acids, classification and nomenclature. Highly limiting fatty acids. The origin and biological role of prostaglandins, thromboxanes, leukotrienes.
19. Carbohydrates. Classification. Biological role of individual groups of carbohydrates (monosaccharides, disaccharides, homo- and heteropolysaccharides).
20. The role of enzymes in life processes. Principles of nomenclature and classification of enzymes. Units of activity.

21. Chemical nature and general properties of enzymes. Immobilized enzymes, their characteristics and use. Enzymes as analytical reagents in laboratory studies.
22. Coenzymes, classification and role.
23. Mechanism of action of enzymes and enzymatic kinetics. Equations Michaelis–Menten and Lineweaver–Burke.
24. Isozymes, their molecular species, meaning in the cell.
25. The concept of the active and allosteric centers of enzymes. The role of spatial structural organization in their formation.
26. General characteristics and classification of vitamins. Hyper-, hypo- and avitaminosis. Antivitamins. Assessment of the body's supply of vitamins. Biochemical approaches to the creation of multivitamin complexes.
27. Vitamins of group A. Provitamins (carotenes). Structure, properties and biological role. Absorption in the intestine. The phenomena of hypo- and hypervitaminosis. Food sources. Daily requirement.
28. Vitamins of the group D. Provitamins. Structure and properties. Biological role. The phenomena of hypo- and hypervitaminosis. Food sources. Daily requirement.
29. Vitamins of group E. Structure and properties. Biological role. Phenomena of insufficiency. Food sources. Daily requirement.
30. Vitamins of group K. Structure and properties. Biological role. Hypovitaminosis. Food sources. Daily requirement. Vikasol. Antagonists of vitamin K.
31. Biotin. Structure and properties, coenzyme form. Biological role. The biotin-avidin complex. Phenomena of insufficiency. Food sources. Daily requirement.
32. Vitamin B₁. Structure and properties. Participation in the construction of coenzymes. Role in metabolism. Phenomena of insufficiency. Food sources. Daily requirement.
33. Vitamin B₂. Structure and properties. Participation in the formation of flavin coenzymes. Biological role. Food sources. Daily requirement.
34. Vitamin B₆. Structure and properties, participation in the formation of coenzymes. Role in metabolism. The phenomena of hypovitaminosis. Food sources. Daily requirement.
35. Vitamin B₁₂. Structure and properties. Cobamide coenzymes. Participation in the metabolism. The internal factor. The phenomena of hypovitaminosis. Food sources. Daily requirement.
36. Vitamin C. Structure and properties. Biological significance. Signs of hypo- and hypervitaminosis. Food sources. Daily requirement.
37. Pantothenic acid. Structure and properties. Coenzymes containing pantothenic acid. Biological role. Food sources. Daily requirement.
38. Vitamin PP. Structure and properties. Participation in the formation of nicotinamide coenzymes. Biological significance. Manifestations of hypovitaminosis. Food sources. Daily requirement.
39. Folic acid, structure and properties, participation in the formation of coenzymes. Role in metabolism. The main manifestations of insufficiency. Food sources. Daily requirement.
40. Vitamin-like substances: bioflavonoids (vitamin P), paraaminobenzoic acid, inositol, pangamic acid, lipoic acid, choline, vitamin U, carnitine. Biological role.

SECOND QUESTIONS

1. Exchange of substances and energy, as the most important sign of life. Metabolism. Catabolic and anabolic pathways. Central pathways of metabolism. The unity of the processes of assimilation and dissimilation. Communication at the level of substrates, reduced coenzymes, energy, exchange regulators.
2. Adenyl system and its biological significance. The energy charge of a cell. Other macroergic compounds. Mechanisms of ATP synthesis.

3. Oxidation-reduction processes in tissues. Oxidoreductases, coenzymes of oxidoreductases. The role of oxygen in the processes of biological oxidation. The involvement of mitochondria in the processes of biological oxidation.
4. Modern idea of tissue respiration. Substrates of tissue respiration. Respiratory chain of mitochondria and its characteristic: pyridine-dependent and flavin-dependent dehydrogenases, ubiquinone (coenzyme Q), cytochromes. Chemical structure, participation in the transport of electrons to oxygen.
5. Mitochondria, features of the structure of mitochondrial membranes. Complexes of the respiratory chain: composition, topology, participation in the processes of biological oxidation. Mitochondrial synthesis of ATP. ATP-synthase. Conjugation of the processes of tissue respiration and phosphorylation.
6. Oxidative phosphorylation as the main mechanism of ATP synthesis in animal cells. Stages, regulation. Causes of hypoenergetic states. Dissociators and inhibitors of tissue respiration and oxidative phosphorylation, the mechanism of their action.
7. Photosynthesis, the total equation. The importance of photosynthesis for life. Characteristics of photosynthetic structures. Pigments of photosynthesis.
8. Stages of photosynthesis. The mechanism of the light stage. The final products of the light stage.
9. Formation of a proton gradient in thylakoids. ATP synthase, its characteristic. Mechanism of photophosphorylation. Noncyclic and cyclic electron transfer in photosystems.
10. Dark phase of photosynthesis, the total equation. Photosynthetic formation of hexoses (the Calvin cycle), its significance for life.
11. Dehydrogenases, oxidases, oxygenases. Biological role in the cell. System of microsomal oxidation.
12. Transport of glucose into cells of various organs and tissues. Ways of glucose metabolism, their importance and interrelation.
13. Metabolism of glycogen: glycogenesis and glycogenolysis, purpose. Sequence of reactions. Mechanisms of regulation.
14. Phosphorolysis and hydrolysis of glycogen in the liver and muscles. The influence of adrenaline, glucagon and insulin on glycogenolysis.
15. Anaerobic and aerobic oxidation of glucose, stages, final products. Energy output.
16. Glycolysis. Stages, reactions, regulation, biological role. Energy output and mechanism of ATP formation under anaerobic conditions. The connection of glycolysis with other metabolic processes.
17. Alcoholic fermentation of carbohydrates. Common reactions for alcohol fermentation and glycolysis, the differences between these two processes. Exogenous ethanol exchange.
18. Pyruvate as a central metabolite. The ways of the transformation of pyruvate depending on the energy status and the characteristics of the oxidative metabolism of the cell.
19. Gluconeogenesis. Substrates, enzymes, energy inputs, biological role. Regulation of gluconeogenesis.
20. Oxidative decarboxylation of pyruvic acid and other α -keto acids, enzymes, coenzymes, biological significance. Difference from simple decarboxylation.
21. Citrate cycle as the central metabolic pathway (localization, the sequence of chemical transformations). Biological significance of the cycle. Relationship with the process of oxidative phosphorylation.
22. Pentosophosphate pathway of glucose breakdown, stages, purpose.
23. Glucuronic acid pathway, stages, purpose.
24. Mechanisms of the formation of carbon dioxide and water — the final products of metabolism.
25. Lipid resynthesis in the cells of the small intestinal mucosa. The ways of resynthesis of triacylglycerols, phospholipids, cholesterol esters.

26. Transport of lipids in the blood. Structure, formation and metabolism of chylomicrons. The role of lipoprotein lipase in the exchange of chylomicrons and other lipoproteins.
27. Preparation of liposomes and their use for delivery of drugs to organs and tissues.
28. Transport of lipids in the blood. Structure, formation and metabolism of VLDL, LDL and IDL. The role of lipoprotein lipase, hepatic lipase and cell surface receptors.
29. Delivery of lipids to cells of organs and tissues. Transport of cholesterol, fatty acids. The mechanism of maintaining the balance of cholesterol in the cells of the body.
30. Transport of lipids. Formation and subsequent metabolism of HDL in the body. The role of LCAT. Factors affecting the level of plasma lipoproteins.
31. Deposition of lipids in adipose tissue and mobilization of fat from the depot. The role of hormones, leptin. Sources of substrates for the synthesis of triacylglycerols in adipose tissue.
32. Transport, entry into the cell and use of fatty acids as energy sources. Oxidation of fatty acids in mitochondria and peroxisomes. Energy output.
33. Biosynthesis of fatty acids. The origin of substrates. Polyenzyme complex, which synthesizes fatty acids in a eukaryotic cell. The value of biotin, NADPH:H⁺. Activators and inhibitors of the synthesis of fatty acids.
34. Biosynthesis of cholesterol. Regulation of cholesterol levels in cells. Derivatives of cholesterol. Relationship of lipid metabolism disorders with the development of diseases (atherosclerosis, cholelithiasis, fatty liver degeneration).
35. Ketone bodies. Formation of ketone bodies. Ways of catabolism. Causes and effects of increasing the formation of ketone bodies.
36. Biosynthesis of phospholipids. Lipotropic factors as medicines.
37. Transamination. Enzymes. Coenzymes. The role of this process for the cell. Diagnostic value of the determination of transaminase activity in serum.
38. Ways of deamidation of amino acids. Enzymes and coenzymes of oxidative deamination. The biological significance of the glutamate dehydrogenase reaction.
39. Ways of conversion of a nitrogen-free residue of amino acids. Glycogen and ketogenic amino acids.
40. Ammonia detoxification in the body. Transport forms of ammonia in blood.
41. Amino acid cell fund. Sources of replenishment. Ways of using an amino acid fund. Nonessential and essential amino acids. Mechanisms for the synthesis of amino acids.
42. Formation of urea. The role of the liver in the formation of urea. The importance of studying the level of urea and residual nitrogen in clinical practice.
43. Decarboxylation of amino acids. The formation of biogenic amines and their role in the body. Oxidation of biogenic amines. Drugs as inhibitors of aminoksidases. Antihistamines.
44. Relationship between lipid and carbohydrate metabolism. Their interconversions.
45. Formation and use in the cell of acyl-CoA and acetyl-CoA.
46. Formation and physiological role of reactive oxygen species. Damaging effect of free radicals and reactive oxygen species.
47. Enzymatic and non-enzymatic systems of antioxidant protection in the cell. Antioxidants as medicines.

THIRD QUESTIONS

1. Hormones. Chemical nature. Classification. Relationship of the structure of hormones with the mechanism of their action. Hormones as medications, sources of reception and their use in medicine.
2. Hormones of adenohipofysis. Connection with the hypothalamus. Role in the regulation of peripheral endocrine glands. Somatotropin hormone, chemical nature, mechanism of action, influence on metabolism.
3. Hormones of the posterior lobe of the pituitary: vasopressin, oxytocin. Their chemical nature, the molecular mechanism of the signal to the cell, the effect on metabolism.

4. Thyroid hormones. Their structure and education. The mechanism of action, the effect on metabolism. Hypo- and hyperthyroidism.
5. Hormones that regulate the exchange of calcium and phosphorus. Chemical nature, mechanism of action, effects.
6. Insulin. The chemical nature and mechanism of action. The role of insulin in the regulation of the metabolism of carbohydrates, lipids and proteins.
7. Metabolic disorders in diabetes mellitus. Role of glycosylation of proteins, glucose reduction pathway.
8. Glucagon. The chemical nature, receptors, the mechanism of signal transmission in target cells, the effect on metabolism.
9. Glucocorticoids, structure, receptors, the mechanism of signal transmission in target cells. Influence on metabolism.
10. Mineralocorticoids, their structure, the mechanism of signal transmission in target cells. Influence on metabolism.
11. Hormones of the adrenal medulla: adrenaline, norepinephrine. Structure, synthesis, the mechanism of the signal in the target cells, the effect on metabolism.
12. Sex hormones, chemical nature, the mechanism of signal transmission in target cells.
13. Biosynthesis of purine nucleotides. The starting substrates, regulation of synthesis. The role of vitamins in the mechanisms of synthesis.
14. Biosynthesis of pyrimidine nucleotides. The starting substrates of synthesis. Orotic acid. Sources of pentoses. Regulation of synthesis. The role of vitamins in the synthesis of pyrimidine nucleotides.
15. Matric mechanism of DNA synthesis. Enzymes and synthesis substrates. Features of synthesis in eukaryotes.
16. Polymerase chain reaction and cloning as methods of artificial DNA replication. The main stages and application in pharmaceutical practice and in the diagnosis of diseases.
17. Synthesis of RNA. Enzymes and synthesis substrates. Features of synthesis in eukaryotes. Regulation of synthesis.
18. Genetic code and its properties.
19. Role of tRNA in protein synthesis. Specificity of ARSase. Adaptive function of tRNA.
20. Recognition and translation as the stages of realization of genetic information in the cell. Substrates, enzymes, mechanism.
21. Regulation of protein biosynthesis in a cell at the genetic level. The role of histones, hormones, fat-soluble vitamins, antibiotics.
22. Post-translational modification of protein molecules. Hydroxylation, glycosylation, limited proteolysis. Other mechanisms of posttranslational modifications.
23. Convertible and irreversible regulation of biochemical reactions. The mechanism of isosteric regulation. Use of the principles of isosteric regulation in medical practice.
24. Mechanism of allosteric regulation of biochemical reactions. Allosteric effectors. Types of allosteric regulation.
25. Covalent modification of the enzyme structure as a mechanism for regulating biochemical reactions. The role of phosphorylation reactions in covalent modification. Regulators of phosphorylation of enzymes.
26. Humoral regulation of lipid metabolism. The role of individual hormones in the mechanisms of regulation of lipid metabolism (insulin, adrenaline, glucagon, steroid hormones).
27. Humoral regulation of carbohydrate metabolism. The role of individual hormones in the mechanisms of regulation of carbohydrate metabolism (insulin, adrenaline, glucagon, glucocorticoids).
28. Humoral regulation of blood glucose. Mechanisms of the regulatory action of hormones.
29. Molecular organization of biological membranes. The involvement of structural membrane components in intercellular signaling. Receptors, classification of receptors.

30. Mechanisms of information transfer from external signals to intracellular processes. Mechanisms of signal amplification. Secondary intermediaries and mechanisms of their formation.
31. Mechanisms of information transfer from external signals to intracellular processes. Mechanisms of signal amplification. The role of G proteins in these processes.
32. Adenylate cyclase mechanism of information transfer from external signals to intracellular processes. The role of phosphodiesterase. The value of the level of cAMP for the cell.
33. Mechanisms of information transfer from external signals to intracellular processes involving phospholipase C.
34. Inositolphospholipid pathway of intracellular signaling. Inositoltriphosphate and diacylglycerol, mechanisms of formation and action.
35. The role of calcium ions in the mechanisms of transformation of external signals. Calmodulin.
36. The role of limited proteolysis in the mechanisms of regulation of vital processes. Blood coagulation. Factors and mechanisms of coagulation. The importance of calcium ions and vitamin K in the processes of blood clotting.
37. The role of limited proteolysis in the mechanisms of regulation of vital processes. Fibrinolysis. Biological role of fibrinolysis. Plasmin system.
38. Anticoagulant system. Primary and secondary anticoagulants.
39. The main characteristics and components of the integration of metabolism.
40. Features of metabolism in the liver after eating.
41. Features of metabolism in the liver on an empty stomach.

THE FOURTH QUESTIONS

1. Pharmaceutical biochemistry. Subject and tasks. Biogenic and synthetic medicines. Biochemical methods used in the analysis and quality control of medicines.
2. Biochemical basis of pharmacokinetics of medicines. Ways of receipt of drugs in the body and the mechanisms of their absorption. Factors affecting suction.
3. Transport of medicinal substances. Specific and nonspecific transport systems of blood. Distribution of medicinal substances in tissues. Ways and mechanisms of excretion of medicinal substances from the body.
4. Biotransformation of medicinal substances. Biotransformation phases, their characteristics. Factors affecting the metabolism of medicinal substances.
5. The role of cytochrome P₄₅₀ in the metabolism of endogenous compounds and drugs. Scheme of the oxidation process in the cytochrome P₄₅₀ system. Induction of the cytochrome P₄₅₀ system.
6. Metabolic transformation as the I phase of neutralizing drugs. Types and enzymes.
7. The phase of conjugation in the system of neutralization of endogenous compounds and drugs. Types of conjugation (glucuronidation, acetylation, methylation, sulfation). Coenzymes and enzymes of conjugation. Examples of reactions.
8. Digestion of nucleoproteins in the gastrointestinal tract. Final products of the decomposition of pyrimidine and purine nucleotides. Hyperuricemia, gout, approaches to diagnosis, prevention and treatment.
9. Nitrogen balance. The norms of proteins in nutrition. Biological value of proteins.
10. Nutritional value of proteins, carbohydrates, lipids, digestibility in the gastrointestinal tract. Nutritional factors. Energy - the need, origin and expenditure in the body.
11. The use of enzymes and their inhibitors in medical practice for the treatment and diagnosis of diseases. Enzymes as analytical reagents in laboratory studies.
12. The role of the liver in the metabolism of proteins, carbohydrates, lipids.
13. Antitoxic function of the liver. Neutralization in the liver of toxic substances, normal metabolites, drugs.

14. Synthesis and decomposition of blood pigments. The role of the liver in the formation of bile pigments. Metabolism of bile pigments. Icterus: types and causes of its occurrence.
15. Origin of plasma enzymes. The significance of determining the activity of enzymes in the blood plasma for diagnostic purposes and for monitoring the effectiveness of treatment.
16. Chemical composition of blood plasma. Methods for studying the chemical composition of blood plasma, used in clinical practice.
17. Buffer systems and their meaning. Proof of the buffer properties of blood serum. Regulation of the acid-base state.
18. Mechanisms of transfer of carbon dioxide and oxygen by blood. Mechanisms of development of hypoxic conditions.
19. Proteins of blood plasma. Functions. Clinical and biochemical value of the determination of the total protein of blood plasma and protein fractions.
20. Methods for detection and quantification of proteins in biological fluids (urine, blood plasma) and drugs.
21. The main indicators of urine analysis of a healthy person.
22. Pathological components of urine and their definition.
23. Digestion of proteins in the gastrointestinal tract. Qualitative and quantitative analysis of gastric juice. The role of hydrochloric acid.
24. Pancreatic juice. Participation in the digestion of carbohydrates and lipids. Principles and clinical significance of the determination of amylase activity in urine.
25. Classification and properties of proteases. Participation in the digestion of proteins. Substrate specificity. Inhibitors of proteases and their use in clinical practice.
26. Nutritional value of carbohydrates. Digestion and absorption of carbohydrates. The role of fiber and pectins in nutrition. Carbohydrates as medicines.
27. Stages of digestion of food lipids in the gastrointestinal tract. The role of bile acids, enzymes. Hepatic intestinal recirculation of bile acids. Mechanisms of absorption of products of enzymatic hydrolysis of fat.
28. Chemical reactions underlying the putrefaction of proteins in the intestine. Mechanisms of their neutralization in the body.
29. Water. The meaning of water. The biological role of sodium, potassium, and chlorine. Mechanisms of regulation of water-mineral metabolism.
30. Macroelements (calcium, phosphorus, magnesium). Biological role.
31. The role of sulfur in the metabolism (thiol and disulfide groups of proteins and hormones, their participation in the formation of the structure and specific properties of the protein, glutathione, sulfolipids, thiamine, biotin, sulfur participation in detoxification processes).
32. Trace elements. Their meaning. The role of ions of manganese, copper, zinc, selenium, cobalt, iodine, fluorine. Biochemical basis for the creation of balanced vitamin-mineral complexes.
33. Mechanisms of absorption, transport and deposition of iron. The role of iron in the body.

SOME BIOCHEMICAL PARAMETERS AND CONSTANTS OF BLOOD SERUM

Blood density	1.05–1.06
pH	7.37–7.44
Osmotic pressure	7.6–8.1 atm
Oncotic pressure	0.03–0.04 atm
Glucose	3.9–6.1 mmol/l
Total lipids	3.5–6.5 g/l
Triacylglycerols	0.5–1.8 mmol/l
Cholesterol	3.65–5.2 mmol/l
Total protein	68–85 g/l
Albumins	35–50 g/l
Globulins	20–35 g/l
Fibrinogen	2–4 g/l
Nonprotein blood nitrogen	14.3–25 mmol/l
Urea	2.5–8.3 mmol/l
Uric acid	0.15–0.5 mmol/l
Ammonia	6–65 μ mol/l
Uric acid: men	0.20–0.42 mmol/l
women	0.12–0.34
Hemoglobin: men	130–170 g/l
women	120–150 g/l
Bilirubin: total	8.5–20.5 μ mol/l
direct	2.2–5.1 μ mol/l
indirect	1.7–17.1 μ mol/l
Plasma Na ⁺	130–150 mmol/l
Plasma K ⁺	3.5–5.6 mmol/l
Plasma Ca ²⁺ (total)	2.2–2.7 mmol/l

In exam each student should be able to:

1. Write formulas of amino acids and nucleotides.

2. Write reactions using chemical formulas of:

- glucose anaerobic oxidation (glycolysis) and gluconeogenesis;
- oxidative decarboxylation of pyruvate;
- β -oxidation and synthesis of fatty acids;
- re-synthesis of lipids (triacylglycerols, phospholipids);
- ketone bodies synthesis and utilization;
- synthesis of mevalonic acid;
- lipid digestion (neutral fats and phospholipids);
- metabolism of amino acids (transamination, deamination, decarboxylation, reductive amination);
- degradation of purine nucleotides.

3. Write schemes of the next processes:

- glycogen synthesis and degradation; citric acid cycle; urea synthesis; nucleotide *de novo* synthesis; electron transport chain.

4. Write the schemes of hormonal signal transduction:

- with participation of seconds messengers (cAMP, inositol triphosphate, diacylglycerol) and via intracellular receptors also.

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