BIOCHEMISTRY

Manual for international students

of medical faculty

Minsk BSMU 2018

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА БИОЛОГИЧЕСКОЙ ХИМИИ

БИОХИМИЯ

BIOCHEMISTRY

Рекомендовано Учебно-методическим объединением
по высшему медицинскому, фармацевтическому образованию
Республики Беларусь в качестве учебно-методического пособия
для студентов учреждений высшего образования, обучающихся
на английском языке по специальности 1-79 01 01 «Лечебное дело»

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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 клинико-диагностическое значение. Включены вопросы для подготовки к коллоквиумам, примерный перечень экзаменационных вопросов. Первое издание вышло в 2016 году.

Предназначено для иностранных студентов 2-го курса, обучающихся на английском языке по специальности 1-79 01 01 «Лечебное дело».

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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 must 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. Formulas for proteinogenic amino acids, terminology.
3. Peptides, their structure. Classification and biological role of peptides.
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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 13–28.
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.

Data for construction of a calibration graph:

C, g/l	20	40	60	80	100
E (extinction)					

Data obtained in testing of a serum sample:

Etest.= *Ctest.=*

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. Levels of organization of a protein molecule (concept, varieties and bonds stabilizing the structure).
2. Conformational changes in functioning of proteins. Interaction of proteins with ligands. Cooperativity effect.

3. 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).

4. Stability of protein solutions (the role of a protein charge, hydrate shell, molecular weight, molecule shape). Isoelectric state.

5. Denaturation. Reversibility of denaturation. Mechanisms of denaturing factors action.

6. 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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 28–35.
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, $(NH_4)_2SO_4$, $MgSO_4$.

In presence of high salt concentrations dehydration of protein molecules and partial elimination of their charge take place. 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 $(NH_4)_2SO_4$ and globulins — in a semi-saturated solution of $(NH_4)_2SO_4$, 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:

$SO_4^{2-} > Cl^- > Br^- > NO_3^- > I^- > CNS^-$

$Ca^{2+} > Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$

Separation of albumins and globulins of egg-white

Procedure. Add 20 drops of a saturated solution of $(NH_4)_2SO_4$ 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 undissolved. Then filter out the albumin deposit. Expose the filtrate to biuret reaction: add 2 drops of 1 % solution of $CuSO_4$ + 5 drops of 10 % solution of NaOH to the filtrate. A negative biuret reaction (blue staining) indicates the absence of protein in the final filtrate.

Result:

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.

2.1. 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	**1st test-tube**	**2nd test-tube**
Egg-white solution	5 drops	5 drops
1 % copper sulfate solution	1–2 drops	–
5 % silver nitrate solution	–	1–2 drops
Mark the formed precipitate		
1 % copper sulfate solution (excess)	5–10 drops	–
5 % silver nitrate solution (excess)	–	5–10 drops
Mark the dissolution of the precipitate		

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.

2.2. 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	**1st test-tube**	**2nd test-tube**
HNO_3 (concentrated)	10 drops	–
H_2SO_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_2SO_4 (concentrated)	–	10 drops
Mark the dissolution of the precipitate		

Conclusion:

3. METHODS OF SEPARATION, ISOLATION AND PURIFICATION OF PROTEINS. CONJUGATED 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 polyacrilamide 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.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 9–11.
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. Coenzymes, their classification and role in catalysis. Block-structures of the NAD^+, $NADP^+$, FAD and FMN.
4. The mechanism of enzyme action. Enzyme kinetics. The effect of substrate concentration, pH, temperature on the velocity of an enzyme catalyzed reaction (molecular mechanism, graphical relationship). Michaelis constant (K_m), usage of K_m for predicting the course of biochemical reactions.
5. Specificity of enzyme action. Types of specificity.
6. Units of enzyme activity.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 12–15.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 80–103.
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

1.1. 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: amylodextrines — violet, erythrodextrines — 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. 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 naitive 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_2O		

Conclusion:

1.2. 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)			

Conclusion:

1.3. 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 % $CuSO_4$ 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_2O)	2(NaCl)	3($CuSO_4$)
Reaction with compound iodine (color)			

Conclusion:

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			

Conclusion:

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. An active site of the enzyme, its organization. The theory explaining the work of the active site.
2. Structure peculiarities of allosteric enzymes, allosteric center. The concept of a "key enzyme".
3. 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.
4. Regulation of enzyme activity: covalent modification, activators and inhibitors (examples). Types of inhibition (irreversible and reversible, isosteric and allosteric), characteristic, examples.
5. Isoenzymes. Examples, their biological role.
6. Medical aspects of enzymology.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 15–18.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 91–93, 103–113.
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, brown 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									
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**	**10th**
Staining with iodine										
Conclusions										

Example of calculation. Having marked the test-tube with a complete starch hydrolysis with 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.

Calculation: ***x*** =

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:

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

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.

Result. *T* =

X_{unit} =

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. Be able to write formulas of peptides, determine their charge.
3. Proteins as a class of organic substances, their biological functions. Classification of proteins.
4. Quantitative determination of total protein in biological fluids (biuret method).
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. Compound (conjugated) proteins, their classification, structure and functions.
14. Enzymes as protein catalysts.
15. Modern classification of enzymes and terminology of enzymes (systemic and working names). Enzyme code. General characteristic of classes.
16. The structure of enzymes. Coenzymes, their classification and role in catalysis. Block-structures of the NAD^+, $NADP^+$, FAD and FMN.
17. 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.
18. Specificity of enzyme action. Types of specificity.
19. Units of enzyme activity.
20. An active site of the enzyme, its organization. The theory explaining the work of the active site.
21. Structure peculiarities of allosteric enzymes, allosteric center. The concept of "key enzymes".
22. 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.
23. Regulation of enzyme activity: covalent modification, activators and inhibitors (examples). Kinds of inhibition (irreversible and reversible, isosteric and allosteric), characteristic, examples.
24. Isoenzymes. Examples, their biological role.
25. Medical aspects of enzymology. Examples of enzymes and inhibitors usage in diagnosis and treatment.

7. INTRODUCTION TO METABOLISM. CENTRAL METABOLIC PATHWAYS (OXIDATIVE DECARBOXILATION 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 decarboxilation 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, linear and cyclic 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 B_2 in redox reactions. Block-structures of coenzymes NAD^+, $NADP^+$, FAD, FMN.
4. 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.
5. Oxidative decarboxylation of pyruvate. Pyruvate dehydrogenase complex (enzymes, co-enzymes, scheme of reactions).
6. Tricarboxylic acid cycle as a central metabolic pathway. Cellular localization, reactions, enzymes, co-enzymes.
7. 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 CO_2 formation mechanism that is an end product of carbonic compounds catabolism.
8. 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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 71–79, 114–123, 149–150.
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 catalyzes 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)
1	Phosphate buffer	2.0	2.0
2	Acetyl-CoA solution	0.5	0.5
3	Oxaloacetate solution	0.5	0.5
4	Malonic acid solution	1.0	–
5	Saline solution	–	1.0
6	Homogenate of the liver	0.5	0.5
	10 min incubation at room temperature		
7	Folin's reagent A	0.5	0.5
8	Folin's reagent B	0.5	0.5
Observed changes (color of solution):			

Conclusion:

Work 2. TCA cycle functioning manifested by the formation of CO_2

Principle of the method. When acetyl-CoA is oxidized in TCA cycle, CO_2 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)
1	Phosphate buffer ph = 7.4	2.0	2.0
2	Acetyl-CoA solution	0.5	0.5
3	Oxaloacetate solution	0.5	0.5
4	Malonic acid solution	1.0	–
5	Incubation solution	–	1.0
6	$Ca(OH)_2$ solution	1.0	1.0
7	Homogenate of the liver	0.5	0.5
	10 min incubation at room temperature		
8	0.1N solution of sulfuric acid	1.0	1.0
Observed changes (gas bubbles):			

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-dichlorphenolindophenol (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)
1	Phosphate buffer ph=7.4	2.0	2.0
2	Acetyl-CoA solution	–	0.5
3	Oxaloacetate solution	–	0.5
4	Distilled water	1.0	–
5	Homogenate of the liver	1.0	1.0
6	0.001N DCPI solution	1.0	1.0
	Incubation at room temperature		
Observed changes (color of solution):			

Conclusion:

8. BIOLOGICAL OXIDATION. PATHWAYS OF OXYGEN UTILIZATION BY CELLS. OXIDATIVE PHOSPHORYLATION

Object. To get the notion of the ways of oxygen utilization by cells; localization, structure and functioning of components of the respiratory chain and microsomal oxidation chain, oxidative 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. 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.
2. The structure of the respiratory chain components, enzyme complexes, co-enzymes, functioning mechanism.
3. The diagram of the respiratory chain, phosphorylation points, the mechanism of an electro-chemical gradient formation.
4. 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.
5. Regulation of the respiratory chain and H^+-ATP-synthase.
6. Causes for the hypoenergetic states development. Uncoupling of oxidative phosphorylation and tissue respiration (mechanism, uncoupling agents). Inhibitors of electron transport and oxidative phosphorylation.
7. Microsomal oxidation, its role for the cell.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 24–29.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 124–135, 408–411.
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 may be 0.5–1.5–2.5). 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	Test		
		1 (ml)	2 (ml)	3 (ml)	4 (ml)
1	Incubation mixture	1.0	1.0	1.0	1.0
2	Saline solution	0.5	–	–	–
3	Malate solution	–	0.5	–	–
4	Succinate solution	–	–	0.5	–
5	Ascorbate solution + Cytochrom c	–	–	–	0.5
6	Mitochondria suspension	0.5	0.5	0.5	0.5

№	Content of test-tubes	Control	Test		
		1 (ml)	2 (ml)	3 (ml)	4 (ml)
	10 min incubation at room temperature, then add:				
7	Trichloracetic acid (TCA)	1.0	1.0	1.0	1.0
8	Ammonia molybdate solution	0.5	0.5	0.5	0.5
9	Reducing solution of Fiske and Subarrow				
10	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)
1	Malate solution	0.5	0.5
2	2,4-DNP solution	–	0.5
3	Saline solution	0.5	–
4	Mitochondrium suspension	0.5	0.5
	10 min imcubation at room temperature		
5	TCA solution	1.0	1.0
6	Ammonia molibdate solution	0.5	0.5
7	Reducing solution	1.0	1.0
Observed changes (color of solution):			

Conclusion:

9. DIGESTION OF CARBOHYDRATES. GLYCOGENESIS AND GLYCOGENOLYSIS. GLYCOLYSIS

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. Carbohydrates digestion, end products. Digestion impairments, their molecular mechanisms, symptoms. The role of cellulose and pectin in the human diet.

2. Absorption of carbohydrates digestion products, molecular mechanisms. The fate of absorbed monosaccharides. Glucose transport to cells.

3. Glycogen synthesis, purpose, sequence of reactions, expenditure of energy and regulation. Aglycogenosis.

4. Degradation of glycogen in the liver and muscles, sequence of reactions, regulation. Glycogenoses.

5. Glycolysis, its biological role, subcellular localization, phases (preparatory or unoxidative, oxidative), reactions, energy yield and mechanism of ATP formation. Glycolysis regulation, key enzymes.

6. Alcoholic fermentation. The difference between glycolysis and alcoholic fermentation.

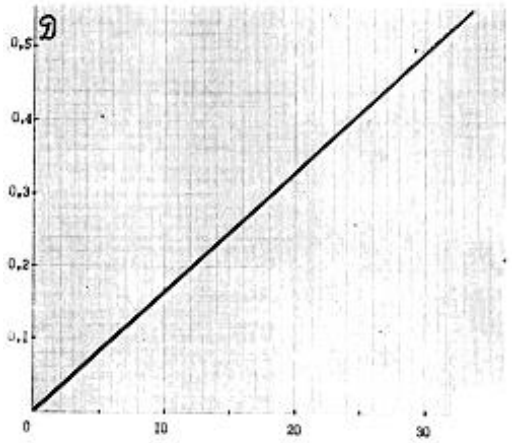
Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 30–38.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 46–56, 137–149, 156–167.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 136–153.
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 CO_2 and H_2O.



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

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-dinitrophenylhydrasine in alkaline medium forms 2,4-dinitrophenylhydrasone 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 H_2O 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-dinitrophenyl-hydrosine 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 (540 nm).

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.

Result: D = C =

Calculation:

Clinical and diagnostic value. In avitaminosis and hypovitaminosis of B_1 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.

Conclusion:

Work 2. Alcoholic fermentation

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

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

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 CO_2*. Remove the test tube from the thermostat and fill up to the top with 10 % sodium hydroxide. 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:

$$C_2H_5OH + 4I_2 + 6NaOH \rightarrow CHI_3 + 5NaI + HCOONa + 5H_2O.$$

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.

Results:

Conclusion:

10. METABOLIC PATHWAYS OF PYRUVATE. GLUCONEOGENESIS. AEROBIC OXIDATION OF GLUCOSE TO END-PRODUCTS (CO_2 AND H_2O). DETERMINATION OF BLOOD GLUCOSE

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. Pyruvate as a central metabolite. Pathways of pyruvate conversion depending on the energetic status and peculiarities of oxidative cellular metabolism.
2. Gluconeogenesis (biological role, substrates, key reactions and enzymes, regulation, expenditure of energy).
3. Oxidative decarboxylation of pyruvate (biological role, subcellular localization, reactions); pyruvate dehydrogenase complex (enzymes, coenzymes), regulation of pyruvate dehydrogenase complex activity.
4. Citric acid cycle (subcellular localization, reactions, energetic balance, enzymes, regulation, biological role).
5. Aerobic oxidation of glucose to CO_2 and H_2O (steps associated with oxidative phosphorylation, energy yield).

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 38–41, 21–24.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 149–150, 167–171, 118–123.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 136–145, 153–163.
4. Lecture material.

PRACTICAL PART

Determination of glucose concentration in serum by enzymatic method

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

$$\text{Glucose} + H_2O + O_2 \xrightarrow[\textbf{Glucose oxidase}]{} \text{Gluconolacton} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow[\textbf{Peroxidase}]{} \text{Chinonimine} + 4\ H_2O$$

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.

Calculation is done by the formula:

$$\mathbf{C_t = E_t \cdot C_s / E_s,}$$

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.

Results:

E_t = **E_s =** **C_t =**

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:

11. SECONDARY PATHWAYS OF GLUCOSE METABOLISM. EFFECT OF HORMONES ON THE 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).
2. Uronic acid pathway (tissue and subcellular localization, biological role).
3. Metabolism of fructose, galactose.
4. Regulation of blood glucose content. Mechanisms of hormonal regulation (insulin, epinephrine, glucagon, glucocorticoids etc.).

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 37, 41–46.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 150–155, 172–176.
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.

Procedure: see the preceding classes (p. 20). A control sample can be prepared one for the whole group.

Calculate glucose concentration according to the formula:

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

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

Conclusion:

12. COLLOQUIUM "INTRODUCTION TO METABOLISM, CENTRAL METABOLIC PATHWAYS, BIOLOGICAL OXIDATION, OXIDATIVE PHOSPHORYLATION, CARBOHYDRATE METABOLISM"

Questions for preparation:

1. Metabolism, catabolism and anabolism, their distinctions and interrelations. Linear and cyclic metabolic pathways, 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. 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.
7. Causes for the hypoenergetic states development. Uncoupling of oxidative phosphorylation (mechanism, uncoupling agents). Inhibitors of electron transport and oxidative phosphorylation.
8. Microsomal oxidation, enzymes, coenzymes, principle of reactions, biological role.
9. Carbohydrates, classification, main representatives.
10. Carbohydrates digestion, end products, digestion impairments. The role of cellulose and pectines in the human diet.
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. Aglycogenosis.
13. Degradation of glycogen in the liver and muscles, sequence of reactions, regulation. Glycogenoses.
14. Glycolysis, its biological role, subcellular localization, phases (preparative, 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. Gluconeogenesis, biological role, substrates, key reactions and enzymes, regulation, expenditure of energy.
17. Aerobic oxidation of glucose to CO_2 and H_2O (stages, energy yield, mechanisms of ATP formation). Oxidative decarboxylation of pyruvate and TCA cycle as stages of aerobic glucose oxidation (schemes of the pathways, enzymes, coenzymes, energy yield, regulation).
18. Pentose phosphate pathway, subcellular localization, steps, key enzymes, metabolites, biological role, regulation.
19. Uronic acid pathway (tissue and subcellular localization, biological role).
20. Peculiarities of fructose and galactose metabolism.
21. 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).

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 acylglyceroles and glycerophospholipids; block-structures of waxes, sphingophospholipids, glycolipids, sulfolipids). Biological role of lipids.
2. 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.
3. Hydrolysis of lipids (conversion patterns). Enzymes (place of formation, substrate specificity). Activation of pancreatic lipase. Absorption (mechanisms, micellar dissolution, fate of micelles).
4. 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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 57–70, 177–178.
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

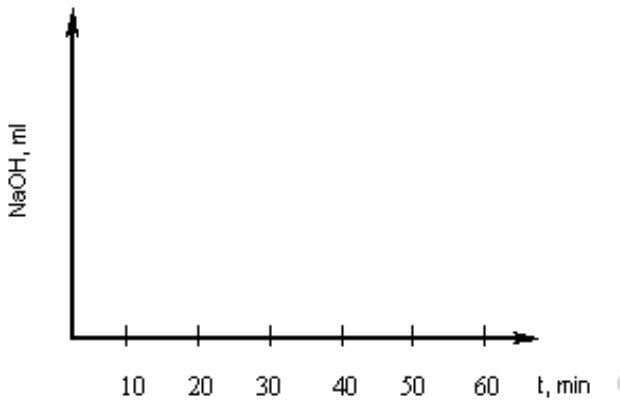
Principle of the method. The lipase action rate in separate portions of milk is evaluated by the amount of fatty acids formed in hydrolysis of milk fat for a definite interval. The amount of fatty acids is determined by alkaline titration.

Procedure. Prepare two test-tubes, each 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 per 1 ml of 10 % solution of pancreatin (pancreatic juice which contains lipase) into both test-tubes. Quickly stir the mixture in the test-tubes. Apply per 1 ml of the mixture from control and tested tubes into flasks for titration. Add per 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 per 1 ml of the mixture from control and tested 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. Fill in the table below with received data.

When constructing the graphs, subtract the value obtained in first titration ("reaction time "0") from each obtained value. In this case, the kinetic curves will pass through the point zero, because the result of the titration of the initial amount of fatty acids, present in milk, is subtracted. Plot two kinetic curves reflecting the process of fat hydrolysis by pancreatic lipase vs time, depending on the presence or absence of bile.

Results:

Test-tube	Result of titration	**Incubation time**					
		0 min	**10 min**	**20 min**	**30 min**	**40 min**	**50 min**
Control (with water)	NaOH volume, ml						
Tested (with bile)	NaOH volume, мл						



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.

Result:

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. 52–54, 60–61.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 179–182, 207–212.
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. Electrophorethic 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 depends on the age and sex.

Results:

$E_1 =$ $E_2 =$ $x\ (g/l) = (E_2 - E_1) \cdot 10 =$

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.

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. 54–60, 61–63.

2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 182–196, 346–349.

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-amino-phenazone in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\textit{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\textit{Cholesterol oxidase}} \text{Cholestene-3OH} + H_2O_2$$

$$2\ H_2O_2 + \text{4-aminophenazone} + \text{Phenol} \xrightarrow{\textit{Peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

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:

$$C_{\text{cholesterol}}\,(\text{mmol/l}) = 5{,}17 \times (E_t/E_s)$$

Results:

E_t = **E_s =**

$C_{cholesterol}$ =

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 and acetoacetic acid

Procedure.

2.1. Legal's test for acetone. In alkaline medium acetone and acetoacetic acid together with Sodium nitroprusside form orange-red staining. After acidation by acetic acid a compound of 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.

Result:

2.2. 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.

Result:

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, sulfolipids 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, products). Absorption (mechanisms, micellar dissolution, fate of micelles).
3. Re-synthesis of triacylglyceroles and glycerophospholipids in enterocytes (monoacylglycerol and glycerol phosphate pathways).
4. Transport forms of lipids in the blood. Structure and metabolism of chylomicrons.
5. Synthesis of TAG and glycerophospholipids in the liver and adipose tissue (role of lipotropic factors, sources of glycerol-3-phosphate and fatty acids).
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. Transport of free fatty acids in blood.
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 syntase 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.
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.

17. 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. Analysis of gastric juice.
6. 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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 213–216.
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 dimethyl-aminoazobenzole 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:

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

Objective. To learn the common routs 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 B_6. Evaluation of amino transferases activity in serum, clinical-diagnostic value.
2. Types of amino acid deamination. Oxidative deamination of glutamic acid (reactions, coenzymes), 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.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 67–70.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 216–222, 228–231.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 242–264.
4. Lecture material.

PRACTICAL PART

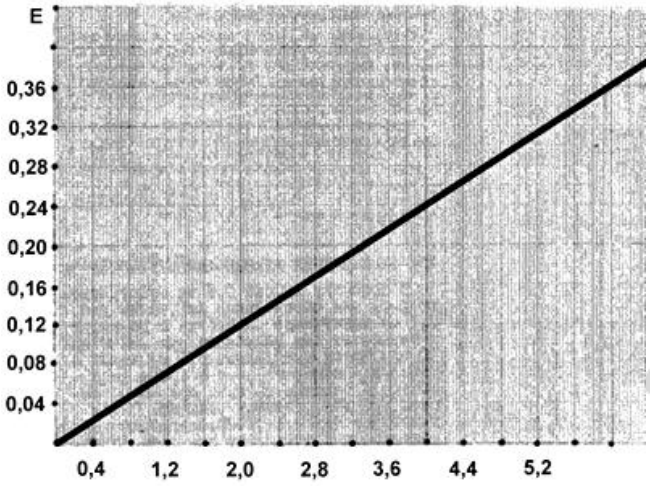
Determination of alanine aminotransferase (ALT) activity

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-dinitrophenylhydrasine 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 μM/h·ml for AST and 0.1–0.68 μM /h·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 dinitrophenyl-hydrasine 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.



Pyruvate content (microgram per sample)

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

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

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.

Result:

E = **a =** **ALT (μM/h·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:

19. 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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 223–227.
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 (400 nm) 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.

Results:

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 10^{th}–12^{th} day of life.

Principle of the method. Nonprotein blood nitrogen is determined in non-protein filtrate after blood proteins sedimentation by various agents (trichloracetic acid or volframate) 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 3d 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 (400 nm) 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.

Results:

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:

20. 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.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 77–85.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 36–45, 255–268.
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 = **Uric acid, mg/24h =** **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:

21. 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.
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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 270–299.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 303–415.
4. Lecture material.

PRACTICAL PART

Analysis of yeast nucleoprotein hydrolysis products

The acid hydrolysis of yeast is used for study nucleoproteins' structure as yeast is rich in nucleoproteins. Specific reactions are discovered8 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:

22. 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. Analysis of gastric juice.
6. Amino acid pool of the cell — its sources and utilization.
7. Transamination reactions, aminotransferases, co-enzyme function of vitamin B_6. Evaluation of amino transferases activity in serum, clinical-diagnostic value.
8. Types of amino acid deamination. Oxidative deamination of glutamic acid (reactions), the significance of a glutamate dehydrogenase reaction. Indirect deamination.
9. The fate of amino acid carbon skeletons. Glucogenic and ketogenic amino acids. Pathways of amino acid synthesis.
10. Decarboxylation of amino acids, enzymes, co-enzymes. Biogenic amines (tryptamine, serotonin, histamine, γ-aminobutyric acid), catecholamines (dopamine, norepinephrine, epinephrine). Reactions of biosynthesis, biological role.
11. Ways of ammonia binding in cells (reductive amination of α-ketoglutarate, synthesis of glutamine and asparagine, formation of carbamoyl phosphate). Transport forms of ammonia.
12. Ammonia salts formation in kidneys (source of ammonia, the role of glutaminase and glutamate dehydrogenase, the significance of renal glutaminase activation in acidosis).
13. Ornithine cycle of urea formation (cycle pattern, substrates, enzymes, energetic supply, relation to the citric acid cycle, regulation). Fate of urea.
14. Nonprotein blood nitrogen (main components and their relative content). Principle of determination and clinical-diagnostic significance.
15. Mononucleotides, structure, terminology, biological role.
16. Primary, secondary and tertiary structures of nucleic acids (peculiarities of the structure, varieties, types of stabilizing bonds).
17. Nucleoprotein metabolism. Digestion of nucleoproteins in the digestive tract (significance, steps, enzymes).
18. Degradation of purine nucleotides (reactions, uric acid as an end-product of catabolism). Disorders of purine metabolism (hyperuricemia and gout, urolithiasis).
19. 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.
20. Degradation of pyrimidine nucleotides (end products and their fate). Biosynthesis of pyrimidine nucleotides (substrates, process pattern, key enzyme, regulation, role of vitamins).
21. Synthesis of deoxyribonucleotides.
22. Replication, biological role, substrates, enzymes, molecular mechanism.
23. Transcription, biological role, substrates, enzymes, RNA processing.
24. Genetic code and its properties.
25. 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.
26. Modern understanding of protein biosynthesis. Regulation of protein biosynthesis in the cell at a genetic level.
27. Posttranslational modification of protein molecules, kinds, biological role.

23. 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.
2. Peculiarities of hormones 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, IP_3, Ca^{2+}, 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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 301–307.
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 thyroiodine 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 thyroiodine 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 thyrosine (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:

24. 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 glycemic 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, thyreoglobulin, 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 glycemic curves.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 96–100.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 308–342.
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, B_1 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 serum 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 endocrine glands 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 № 10 "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 fill in the table and construct a curve. Analyze the glycemic curve, put down your conclusions.

Results.

$E_{standard}$ =

		0 min	30 min	60 min	90 min	120 min	150 min
Patient 1	E_t						
	$C_{glucose}$ (mmol/l)						
Patient 2	E_t						
	$C_{glucose}$ (mmol/l)						

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 glycemic curves evaluation. In patients with ***various forms of diabetes*** the elevation of the glycemic 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.

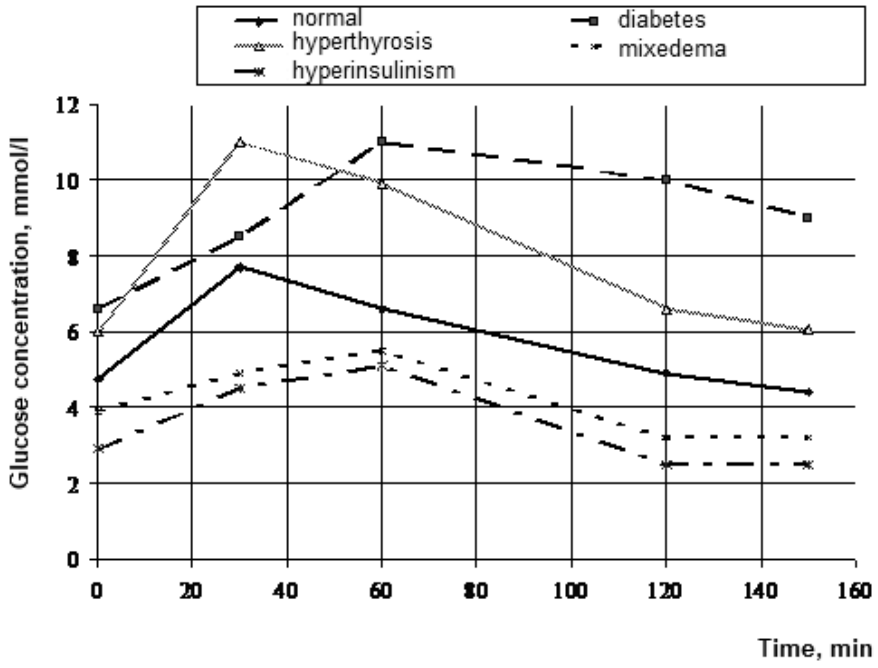
Thyroid gland diseases associated with its hyperfunction are characterized by glycemic curves with a steeper rise, which may be caused by more intensive metabolism and excitation of a sympathetic nervous system.

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.

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

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



Conclusion:

25. 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. Detoxificating 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. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 101–104.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 245–254, 398–414.
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 cab 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 (μmol/l) is 17,104.

Results.

E_t = E_s = C_t (mg per 100 ml) =

C_t (μmol/l) =

Normal total bilirubin concentration in plasma (serum) is 0.5–1.2 mg per 100 ml (8.55–20.52 μmol/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 μM/l. The blood of newborns, especially prematually born, has a higher bilirubin content (physiological jaundice). The increase of bilirubin concentration observed from 2^{nd}–3^{rd} to 7^{th}–10^{th} 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 μM/l; its level of 86–169 μM /l testifies to moderately severe, and over 170 μM /l — to a severe jaundice.

Conclusion:

26. 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 conjugated. 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 T_3 and T_4. 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. Pheochromocytoma.

17. Hormones of the adrenal cortex: glucocorticoids and mineralocorticoids. The chemical nature, structure of receptors, Mechanism of signal transduction, effects on metabolism. Cushing's syndrome, Addison's disease ("Bronze disease").

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. Porphyria.

29. Degradation of hemoglobin. Metabolism of bile pigments.

30. Jaundice. Types, causes, mechanisms of development. Laboratory diagnosis.

31. The biochemical methods of diagnosis of liver diseases. Which enzymes are specific for the hepatocytes?

32. 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).

33. 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.

34. Integration of metabolism, its principal components. Mechanisms of metabolic regulation.

35. Regulation of metabolic processes in the liver, muscle and adipose tissue after a meal and in fasting.

36. Inter-organ metabolism in different periods of fasting. The mechanism of overproduction of ketone bodies.

27. 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 main participants of gas transport in the blood (the role of hemoglobin and carbonic anhydrase). Reversible binding of oxygen and carbon dioxide as a means of transport (mechanisms of binding CO_2 and O_2 to hemoglobin, co-operative interaction of hemoglobin subunits). Hypoxia, forms, mechanisms of development.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 150–154.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 380–388.
3. *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.

Results:

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 ($AgNO_3$) is equivalent to the content of chloride-ions.

Titration of blood chloride-ions by silver nitrate is performed in the presence of indicator K_2CrO_4. On reaching an equivalent titration point the excess of silver ions and the indicator form a compound of a brick-red color (Ag_2CrO_4).

Procedure.

1. Sedimentation of serum proteins. Prepare a mixture of solutions in two test-tubes: 5 ml of 0.45 % $ZnSO_4$ + 1 ml of 0.1 N NaOH. Then apply 0.1 ml of serum into the 1st tube, 0.1 ml of $H_2O_{dist.}$ 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 K_2CrO_4. Add 2 drops of 1–2 % solution of K_2CrO_4 to the filtrate and titrate it with $AgNO_3$ till a yellow color of the solution changes to brick-red.

Calculation. Calculate the difference between the volume of $AgNO_3$ spent for titration of the tested solution (V_t, ml) and the volume of $AgNO_3$ 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).

Results:

V_t (ml) = V_c (ml) =

$C(mmol/l) = (V_t - V_c) \cdot 0.355 \cdot 1000 \cdot 0.282 =$

Conclusion:

28. BLOOD PLASMA PROTEINS. 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. Blood plasma proteins. Main protein fractions: albumins, globulins, fibrinogen (content, functions); albumin-globulin ratio and its diagnostic value.
2. Blood plasma enzymes (secretory, indicator, excretory). Diagnostic value of plasma enzymes activity determination.
3. Hemostasis (definition, structural-functional units and their biological role). Primary and secondary (coagulation) hemostasis. The notion of blood coagulation system functioning impairments.
4. Coagulating system (components and their origin), hemocoagulation (definition, phases and their duration, sources of phospholipid surfaces). Intrinsic and extrinsic pathways of blood coagulation.
5. Vitamin K (chemical structure, varieties, natural sources, role in coagulation).
6. Anticoagulant system, classification of physiological anticoagulants: primary and secondary (representatives, mechanism of action). Artificial anticoagulants of direct and indirect action.
7. 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, 154–156.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 389–397.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 580–607.
4. Lecture material.

PRACTICAL PART

Work 1. Separation of serum proteins by electrophoresis on acetylcellulose

In clinical laboratories, electrophoretic methods for studying the protein spectrum of blood plasma (serum) are most common. Electrophoresis of blood serum proteins is an objective method used in laboratory diagnostics of acute and chronic inflammatory diseases, malignant tumors, liver diseases, protein-deficient states, and antibody deficiencies.

Separation of the serum proteins on acetylcellulose films gives a clear fractionation and shortens the electrophoresis time to 25–30 minutes.

Principle of the method. The method is based on the fact, that under constant electric field, electrically charged serum proteins move on the acetylcellulose film moistured by buffer solution with the speed depending on the electric charge and molecular weight of particles. It results in separation of serum proteins into 5–7 fractions: albumins and globulins α_1, α_2, β and γ. The content of fractions can be evaluated by photometry. The relative content of protein fractions in the blood serum of a healthy adult person is: albumins — 52–65 %; globulins — 29–54 %; α_1-globulins — 2–5 %, α_2-globulins — 7–13 %, β -globulins — 8–14 %, γ-globulins — 12–22 %. Albumin-globulin ratio is the ratio of the number of albumins to the number of globulins in biological fluids. Normally, the albumin-globulin ratio in blood is relatively constant and is 1.5-1.7. Many pathological conditions are accompanied by the decrease in the albumin-globulin ratio. Such decrease can be associated with both an increase in the absolute number of globulins (in acute and chronic inflammatory processes) and a decrease in the absolute amount of albumins (in liver cirrhosis, hepatitis and other liver diseases).

1. Electrophoretic separation of blood serum proteins.

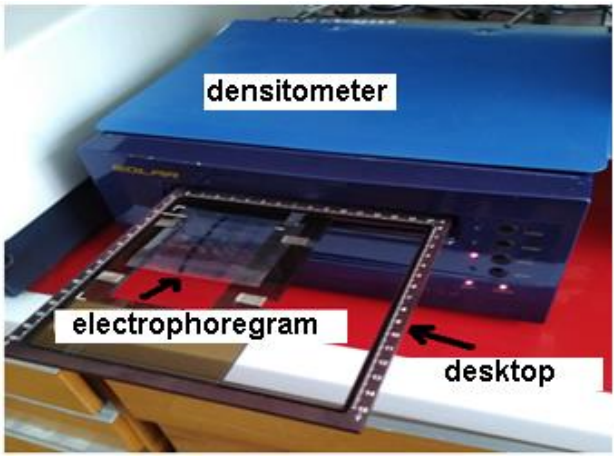
The cuvettes of the electrophoresis apparatus are filled with a buffer solution. Strips of acetylcellulose are wetted with a buffer and tightly stretched between the cuvette compartments. An electric current is passed through the strip for 5 minutes. Then, apparatus is turned off, and a sample of blood serum is applied on the start line on surface of the acetylcellulose film (closer to the cathode). The instrument is switched on again and electrophoretic separation of the proteins is carried out.

2. Staining of electrophoregrams.

After switching off the device, the films are removed and immediately placed in a dye solution (amidoblack 10 B) for 10–15 minutes. To remove excess of dye, the acetyl-cellulose strips are transferred to a cuvette with a 2 % acetic acid solution. After repeated washing with acetic acid, the blue spots corresponding to different protein fractions become clearly visible on the clear background of the acetylcellulose film.

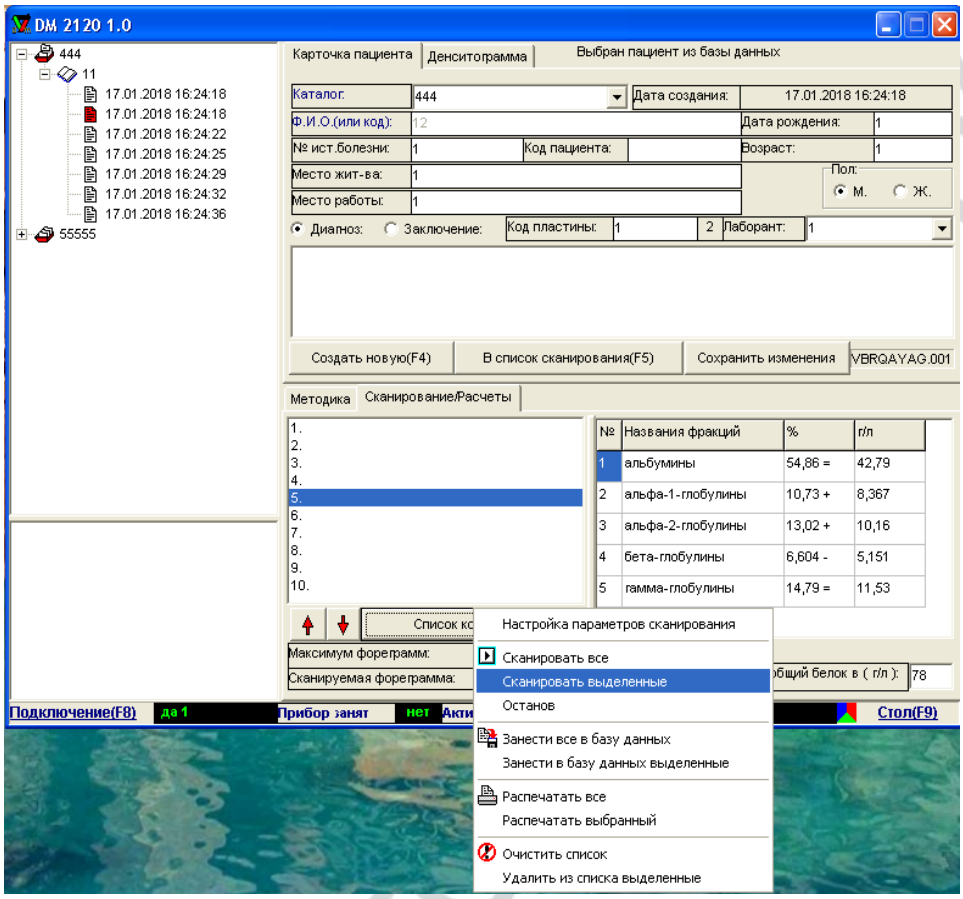
3. Quantitative evaluation of electrophoregrams is carried out by densitometry or photometry of the corresponding eluates.

3.1. Densitometry of electrophoregrams is performed using a densitometer. Densitometer is a photometric device that provides scanning of electrophoregrams. The purpose of the scannnig is to obtain a densitogram — a graphic representation of the distribution of the dye concentration along the electrophoregram. It looks like a series of individual peaks, and the peak areas and their ratio correspond to the relative content of each protein fraction. The peak areas are calculated by the integrator and the microprocessor of the device.



Procedure. Examine the densitometer device. Place the test electrophoregram on the instrument desktop so that the albumin fraction is on the left because the device measures the optical density from left to right.

In the program, which controls the device, select the electrophoregram track number. Choose the command “Scan selected”. The device automatically scans the selected electrophoregram, automatically detects the boundaries of the fractions and displays their percentage. If necessary, the boundaries of fractions can be set by pressing the left mouse button.



Fill in the table with the obtained results:

Fraction	albumins	α_1-globulins	α_2-globulins	β-globulins	γ-globulins
Relative content, %					

Determine the conformity of the fractions content to the normal values, and calculate the albumin-globulin ratio by the formula:

$$A / G = \frac{\text{albumin content,\%}}{\text{globulins content (the sum of all globulin fractions),\%}}$$

Calculation: A / G =

Conclusion:

3.2. Photometry of the eluates of protein fractions.

Procedure.

Apply per 5 ml of 0.1 N NaOH into 5 test-tubes. Cut each fraction of proteins from the paper electrophoregram with scissors, cut the sections into small pieces and place into numbered test-tubes with alkaline. Carefully stir up the tubes and leave them for 20–30 minutes for complete

extraction of the dye-stuff from the paper. Then determine the optical density of every solution versus water (control) by the photometer (a 10-mm cuvette, a red light filter with λ = 670 nm). Knowing optical densities of the solutions corresponding to every fraction, calculate the sum of all optical densities (∑) and the proportional protein content in every fraction, determine the albumin-globulin ratio.

Example.

Relative content of albumins (%) = (optical density of albumins eluate / ∑) · 100 %

Results.

$E_{alb.}$ =	$E_{\alpha 1\text{-glob}}$ =	$E_{\alpha 2\text{-glob}}$ =	$E_{\beta\text{-glob}}$ =	$E_{\gamma\text{-glob}}$ =	∑ =
% =	% =	% =	% =	% =	

Calculation: A / G =

Conclusion:

Work 2. 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 violet color. In titration of so stained solution with EDTA (double-substituted sodium salt ethylenediaminotetraacetic acid forming tight complexes with calcium ions) staining will change to a blue color in an equivalent point corresponding to binding of all calcium ions in the solution by EDTA.

Procedure. Apply 25 ml of H_2O 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 violet. Then titrate the solution with 0.002 M solution of EDTA to a blue color. Calculate the content of calcium in blood plasma by the volume of EDTA spent for titration:

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

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; Vt — volume of EDTA spent for titration.

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

Result:

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

C(mmol/l) =

Conclusion:

29. 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. Syndrome of malnutrition (forms, characteristic).
3. Vitamins of B group: a) thiamine (B_1); b) riboflavin (B_2); c) pantothenic acid; d) niacin; e) pyridoxine (B_6); f) folic acid (B_9); g) cobalamine (B_{12}). Chemical nature, co-enzyme forms, molecular mechanisms of action.
4. Biotin (vitamin H), vitamin C. Structure, role in metabolic processes.
5. Fat-soluble vitamins (A, E, D, K). Structure, role in metabolic processes.
6. Determination of the body's saturation with vitamins.
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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 350–378.
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 B_1

Vitamin B_1 consists of a pyrimidine and thiazole rings. Vitamin B_1 has got a name of thiamine as it contains sulfur and nitrogen.

1.1. 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.

1.2. 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.

Results:

Conclusion:

Work 2. Qualitative reaction for vitamin B_2

Principle of the method. The oxidized form of vitamin B_2 represents a yellow substance fluorescent in UV rays. The reaction for vitamin B_2 is based on its ability to be reduced easily. The solution of vitamin B_2 of a yellow color becomes at first rosy due to the formation of intermediate compounds and then decolorizes as a reduced form of vitamin B_2 is colorless.

Procedure. Apply 10 drops of vitamin B_2 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 B_2 (oxidized and reduced) by fluorescence.

Results:

Conclusion:

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.

Result:

Conclusion:

Work 4. Qualitative reaction for vitamin B_6

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 B_6 add the equal amount of 1 % solution of chlorous iron ($FeCl_3$) and stir. Red staining develops.

Result:

Conclusion:

Work 5. Qualitative reaction to vitamin B_{12}

B_{12} 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 (B_{12}) on the filter and heat again over the burner. Green staining appears, more often along the edge, it evidencing to the presence of cobalt.

Result:

Conclusion:

Work 6. Determination of vitamin C

Principle of the method. The method is based on the ability of vitamin C to reduce 2,6-dichlorphenolindophenol, which has red staining in acidic medium 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-dichlorphenolindophenol 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 deficiency 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 deficiency, 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-dichlorphenolindophenol 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-dichlorphenolindophenol; A — the result of titration by 0.001 N solution of 2,6-dichlorphenolindophenol, ml; B — an average diuresis (for men it is 1500 ml, for women — 1200 ml); C — the volume of urine taken for titration, ml.

Result.

A (ml) = **X =**

Conclusion:

30. BIOCHEMISTRY OF NUTRITION. MINERAL SUBSTANCES. REGULATION OF WATER AND MINERAL 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.
2. Hormonal regulation of salt and water balance. Renin-angiotensin system, the role of aldosterone, vasopressin, atrial natriuretic factor.
3. Hormones regulating calcium and phosphorus metabolism. Chemical nature, mechanism of signal transduction in target-cells, biological action.
4. The role of iron in the organism (absorption, transport, intracellular metabolism). Iron deficiency states and iron-deficient anemias.
5. The role of copper, zinc, manganese, cobalt, selenium, iodine and fluorine in tissue metabolism.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2017. P. 121–128.
2. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 342–346.
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 uranilacetate. Uranil-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.

Procedure. Take 2 centrifuge test-tubes. Measure 1.0 ml of reagent 1 into the 1st tube (tested sample) and then add 0.02 ml of serum. Into the 2nd tube (standard sample), introduce 1.0 ml of reagent 1 + 0.02 ml of the standard solution. **It is necessary to strictly follow the sequence of introducing reagents to the test-tubes (add serum to reagent)!** Close the test-tubes and stir up their content for 30 s. In 5 minutes do the same (stir up) and leave the samples for 30 minutes in the darkness. Then centrifuge them for 10 minutes (1000 turns/min).

Take out 0.02 ml of supernatant from every centrifuge test-tube into usual tubes and add per 2.0 ml of reagent 2 into each of them. Simultaneously prepare a control sample (0.02 ml of reagent 1 + 2.0 ml of reagent 2). Stir carefully the reaction mixture and incubate for 5 minutes at room temperature. Measure optical density of all samples vs water using cuvettes with a working width of 5 mm (wave length — 400 nm).

Calculation: the concentration of Na^+ (mmol/l) is calculated by the formula:

$$Ct = \frac{Ec - Et}{Ec - Es} * Cs$$

where Ec, Et and Es — extinctions of the control, tested and standard samples respectively; Cs — concentration of the standard solution (150 mmol/l).

Results.

Ec = **Et =** **Es =** **Ct =**

Clinical and diagnostic value. Normal content of Na^+ in serum is 135–150 mmol/l. Decrease of sodium concentration in blood serum results in appearance of apathy, loss of appetite, nausea, vomiting, impairment of reflexes, tachycardia, hypotension, psychoses.

Hypernatremia is associated with a poor general condition of patients, elevation of body temperature, tachycardia, hypertension, edema.

Conclusion:

Work 2. Determination of potassium content in serum by a turbidimetric method

Principle of the method. The method is based on the ability of potassium ions to form a stable suspension with ions of tetraphenylborate. Turbidity of the suspension is proportional to the concentration of potassium ions.

Procedure. Take 2 test-tubes. Apply 2.0 ml of reagent into the 1st tube and add 0.04 ml of blood serum (tested sample), introduce 2.0 ml of reagent + 0.04 ml of standard solution into the 2nd tube (standard sample) and add only reagent (2.0 ml) into the 3rd tube (control sample). **It is necessary to strictly follow the sequence of introducing reagents into test-tubes (serum should be added to reagent)!** Stir and incubate for 2 minutes. Again stir carefully and incubate

exactly 10 minutes at room temperature. Measure optical density of the test sample (Et) and standard solution (Es) versus the control sample. Use cuvettes with a working width of 5 mm, wave length — 590 nm. Stir up the samples before photometry.

Calculation: calculate the concentration of K^+ by the formula:

$$Ct = Cs \times Et / Es,$$

where Cs — concentration of K^+ in standard solution (5 mmol/l).

Results.

Et = **Es =** **Ct =**

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:

31. COLLOQUIUM "BIOCHEMISTRY OF NUTRITION. BIOCHEMISTRY OF THE BLOOD. WATER AND MINERAL METABOLISM"

Questions for preparation:

1. Hemoglobin (structure, molecular forms of hemoglobin, hemoglobin derivatives, abnormal forms of hemoglobin).
2. Mechanisms of transport of oxygen and carbon dioxide in blood. The ways of oxygen utilization in the cells. Types and causes of hypoxia.
3. Buffer systems of blood (components, mechanism of action, buffer capacity). Lactic acidosis, ketoacidosis (mechanisms of development).
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, α1-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 B_1 (thiamine), B_2 (riboflavin), pantothenic acid, PP (nicotinic acid), B_6 (pyridoxine), B_9 (folic acid), B_{12} (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 coenzymes derived from vitamin PP (NAD^+ and $NADP^+$) and vitamin B_2 (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. Vitamin K. The chemical nature. The biological role. Symptoms of hypovitaminosis. The main sources and the daily requirement. Vikasol. Antivitamins.

17. Vitamin D. The chemical nature, sources and daily requirement. The formation of the active form of vitamin D_3 in the body. The biological role. The symptoms of hypo- and hypervitaminosis.

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

19. Ways of ATP usage at rest. Contribution to the energy production of proteins, fats and carbohydrates.

20. Metabolic processes involved carbohydrates, fats and proteins to energy production (glycogenolysis, aerobic oxidation of glucose, mobilization of fats and β-oxidation of fatty acids).

21. Metabolic processes of dietary lipids and carbohydrates storage, ketogenesis.

22. Nutritional value of carbohydrates, lipids and proteins. Essential dietary factors. Role of dietary fibers.

23. Kwashiorkor and marasmus as clinical forms of malnutrition syndrome. Characteristics, similarities and differences.

24. The norms of water intake and excretion. The content and the role of water in the body.

25. Calcium and phosphorus. Daily requirement, food sources, role in metabolism, metabolic disorders. Hormonal regulation of calcium and phosphorus metabolism.

26. 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.

27. 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.

28. The role of copper, zinc, selenium, iodine, fluorine, manganese and cobalt in the metabolism. Daily requirement and food sources. Metabolic disorders.

29. The mechanism of action of hormones regulating water and mineral metabolism (vasopressin, natriuretic peptide, aldosterone). Conn's syndrome.

30. The renin-angiotensin system and its role in the regulation of water and mineral metabolism.

32. BIOCHEMISTRY OF THE URINE. PHYSIOLOGICAL AND PATHOLOGICAL COMPONENTS OF THE URINE

Objective. To know how to use knowledge of physiological and pathological components of the urine to solve questions of diagnosis, prevention and prognosis of diseases associated with renal and extrarenal pathology.

Problems for discussion:

1. Normal characteristics of urine volume, density, color, transparency, pH.
2. Inorganic and organic components of the urine.
3. 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. *Biochemistry*. Manual for international students of medical faculty / A. D. Tahanovich [et al.]. Minsk : BSMU, 2018. P. 58–61.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003.

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.

Procedure. Pour urine into cylinder and carefully immerse an urometer there. The determination is made by the urometer scale corresponding to a lower meniscus of the fluid.

Result:

Work 2. Determination of urine acidity

Normal urine reaction is mildly acidic.

Procedure. Apply a drop of urine on litmus paper and evaluate its reaction:

1) the blue litmus paper becomes red, the red one doesn't change the color — acidic reaction;
2) the red paper becomes blue, the blue paper doesn't change the color — alkaline reaction;
3) both papers don't change the color — neutral reaction.

One can use other indicator papers.

Result:

Work 3. Qualitative determination of inorganic urine components

The amount of sodium chloride — 8–15 g/24 h — is the greatest among all mineral salts excreted with urine.

3.1. Revealing of chlorides. Chlorine ions react with silver nitrate, the deposit of silver nitrate being formed that is not dissolved in nitric acid.

Procedure. Add 3–5 drops of 1 % $AgNO_3$ and 2 drops of 10 % solution of nitric acid to 1 ml of urine (20 drops). A white deposit of AgCl precipitates. It becomes dark in the light, is insoluble in nitric acid.

3.2. Revealing of sulfates. In acidic medium sulfates with chloric barium form a white sediment of $BaSO_4$.

Procedure. Add 5 drops of 10 % solution of HCl to 20 drops of urine. Then slowly, drop by drop, add the solution of $BaCl_2$ till the sediment forms. Filter out the formed sediment. You will

reveal salts of ether-sulfuric acids while heating the filtrate in boiling water bath (5–10 minutes). Again turbidity will appear as sulfuric acid is released from ether-sulphuric acids.

3.3. Revealing of phosphates. Phosphates of urine coming into reaction with molybdenum reagent form a yellow crystalline sediment of ammonia phosphomolybdate.

Procedure. Apply 20–30 drops of molybdenum reagent into the test-tube and heat the solution till it boils (but don't boil). Then add some drops of urine. A yellow sediment of ammonia phosphomolibdate will appear.

3.4. Revealing of calcium. Calcium of urine forms a sediment, when ammonia oxalate is added.

Procedure. Add 1–2 drops of 10 % solution of acetic acid and 2–3 drops of 5 % solution of ammonia oxalate to 20 drops of urine. A crystalline calcium oxalate precipitates.

Results:

Work 4. Qualitative determination of organic urine components

4.1. Revealing of protein. There are protein traces in urine normally.

4.1.1. Geller's test. Under the action of nitric acid the protein forms an insoluble precipitate.

Procedure. Very carefully apply about 1 ml of concentrated HNO_3 and apply in layers 1 ml of urine. If protein is present in urine, a turbid whitish spot appears on the border between the fluids.

4.1.2. Protein precipitation by sulfosalycilic acid. This test is the most sensitive reaction for protein.

Procedure. Add 5 drops of 20 % solution of sulphosalycilic acid to 20 drops of urine. Protein precipitates (the solution becomes turbid).

4.2. Revealing of glucose. Normally glucose in urine is not detectable. For qualitative revealing of glucose in urine use the following reactions:

4.2.1. *Trommer's reaction*. In alkaline medium in the presence of glucose and addition of $CuSO_4$ a yellow sediment of copper protoxide hydrate or a red sediment of copper protoxide forms.

Procedure. Add 5 drops of 10 % solution of NaOH and 5 drops of 1 % solution of copper sulfate to 5 drops of studied urine. Heat up.

4.2.2. *Feling's reaction*. The test is based on the same principle as Trommer's reaction. The advantage of this reaction is due to the fact that Feling's reagent binds the excess of copper hydroxide which interferes with the reaction and may give a black color.

Procedure. Add 20 drops of Feling's reagent to the same volume of studied urine and heat till it starts to boil.

4.2.3. *Nilander's reaction*. Nilander's reagent includes bismuth nitrate. In alkaline medium bismuth hydroxide is formed which is reduced in the presence of glucose to metallic bismuth and stains the fluid into a black color.

Procedure. Add 10–20 drops of Nilander's reagent to 20 drops of studied urine and boil for 1–2 minutes. The fluid becomes brown, then a black sediment of metallic bismuth appears.

4.3. Revealing blood pigments. Norm urine has no blood pigments.

Guaiacum test. Guaiacum resin in the presence of hydrogen peroxide is reduced by blood peroxidase into azonide of guaiacum resin of a blue color.

Procedure. Apply 20 drops of urine, 5 drops of guaiacum resin and some drops of H_2O_2. Blue staining appears in the presence of blood.

4.4. Revealing ketone bodies. Ketone bodies are revealed in urine in diabetes, fasting and other acidotic states. The method is based on color reaction that ketone bodies give with sodium nitroprusside.

Procedure. Add 2 drops of 10 % NaOH and 2 drops of sodium nitroprusside to 2 drops of urine. Add 6 drops of concentrated acetic acid — cherry staining appears.

Results:

Work 5. Quantitative determination of organic urine components

5.1. Determination of glucose in urine by the enzymatic (glucose oxidase) method. Normally only traces of sugar are present in urine, and usual methods can't reveal them.

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

$$\text{Glucose} + H_2O + O_2 \xrightarrow[\textbf{Glucose oxidase}]{} \text{Gluconolacton} + H_2O$$

$$H_2O_2 + \text{4-aminoantipyrin} + \text{phenol} \xrightarrow[\textbf{Peroxidase}]{} \text{Chinonimine} + 4H_2O$$

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

Procedure

	Tested sample, ml	**Standard sample, ml`**
Introduce into centrifuge test-tubes:		
Urine	0.1	–
Standard glucose solution	–	0.1
Distilled water	1.0	1.0
Stir up		
Take out the received solution into dry test-tubes	0.2	0.2
Working enzyme solution	2.0	2.0
Stir and incubate the reaction mixture for 10 minutes at 37 °C or 30 minutes at room temperature		

On completion of the incubation measure optical density of the tested and standard samples by PEC (wave length 490–540 nm) in 5 mm thick cuvettes vs the control.

The control sample contains 0.2 ml of water and 2.0 ml of working enzyme solution. The control sample can be prepared one for the group.

Calculate by the formula:

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

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

Calculate daily glucose excretion with urine (taking into consideration the diuresis of 1200–1500 ml).

Results.

E_t = **E_s =** **C_t =**

Daily glucose excretion =

5.2. Determination of protein concentration in urine.

Principle of the method. The method is based on the ability of sulfosalicylic acid to come into reaction with protein causing turbidity, the intensity of which is proportional to the protein content in urine.

Procedure. Add 3 ml of 3 % solution of sulphosalycilic acid to 1 ml of transparent urine, stir up the mixture and in 5 minutes measure the optical density of the test-tube content by PEC under a red light filter (wave length 630–650 nm) in a 10 mm wide cuvette vs the control sample (control sample: add 3 ml of NaCl isotonic solution to 1 ml of urine).

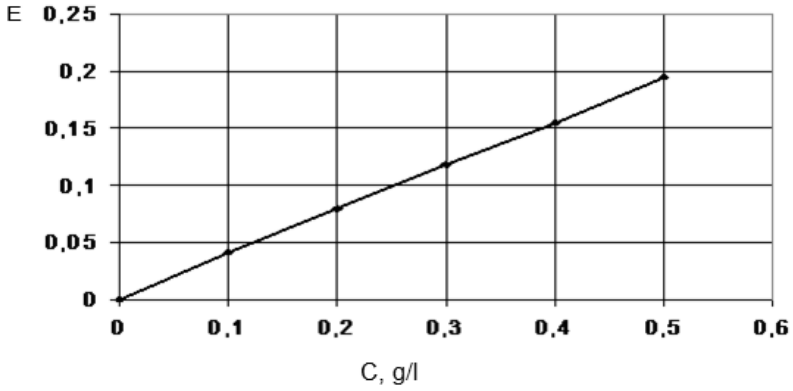
Make the calculation by a calibration graph. Take into consideration the diuresis (1200–1500 ml) while making calculations of daily loss of protein.

Results.

E = **C (g/l) =**

Daily loss of protein =

Clinical and diagnostic value of protein determination in urine. Normally there are only "protein traces" in urine (albumin and globulins, no more than 0.15 g/24 h). The elevation of protein in urine — proteinuria — reflects the dysbalance between the processes of its filtration and reabsorption and is noted in diseases of kidneys, urinary tract, accelerated breakdown of tissue proteins. Functional renal proteinurias are associated with increased permeability of the renal filter or by slowing down the blood flow in glomeruli (under the influence of exposure to excessive cold, physical and psychic overstrain).



Calibration graph for determination total protein content in urine

Conclusion:

33. CONTROL OVER PRACTICAL SKILLS OF BIOCHEMICAL ANALYSIS. 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.

Reaction	Principle of the reaction	Procedure	Notes	Results
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 shiff base is formed that is re-grouped, decarboxylated and split into aldehyde and aminodiketohydrinden	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, polypep-tides and free amino acids	
Xantho-protein reaction	While processing the protein solution with concentrated nitric acid a yellow staining appears. Aromatic amino acids 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 for tyrosine (Millon's)	Tyrosine interacting with Millon's reagent while boiling forms bloody-red compound 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)	

Conclusion:

Work 2. Determination of total protein in serum by biuret method

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

Procedure. Every student receives a test-tube containing 0.1 ml of studied serum (tested sample, 1st test-tube). Apply 0.1 ml of standard protein solution with concentration 100 g/l into the 2nd test-tube (standard sample). Measure 0.1 ml of 0.9 % solution of Sodium chloride into the 3rd test-tube (control sample, it can be one for the whole group). Add 5 ml of biuret reagent into all test-tubes. Carefully stir the content of the test-tubes avoiding the foam formation, and in 30 minutes perform photometry of the studied and standard samples in 10 mm cuvettes under a green light filter (wave length — 540 nm) versus the control sample. Having evaluated the optical

densities of the samples, the protein concentration is calculated (g/l) in the studied serum by the formula:

$$\mathbf{C_t\,(g/l) = (E_t / E_s) \cdot C_s,}$$

where C_t — protein concentration in tested serum; C_s — concentration of protein in a standard solution (100 g/l); E_t — extinction of a tested sample; E_s — extinction of a standard sample.

Results:

E_t = **E_s =** **C_t =**

Conclusion:

34. CONTROL OVER PRACTICAL SKILLS OF BIOCHEMICAL ANALYSIS: ANALYSIS OF GASTRIC JUICE AND URINE

Objective. To check: 1) the skills of the students in performing a qualitative and quantitative analysis of biological fluids; 2) their ability to interpret the findings of the analysis and give them a correct assessment; 3) understanding of the origin and diagnostic significance of pathologic components of analyzed biological fluids.

Having received individual control samples of gastric juice and urine the students start their analysis using methodic recommendations of class № 17 (Analysis of gastric juice) and № 32 (Physiological and pathological components of urine).

1. Determination of gastric juice acidity.

Results:

	Free HCl	**Bound HCl**	**Total acidity**
Gastric juice №	A_1 =	A_2 =	A_3 =
	X (mmol/l) =	X (mmol/l) =	X (mmol/l) =

Conclusion:

2. Evaluation of urine content.

In the studied urine sample, determine the presence of pathological impurities (glucose, ketone bodies, protein and erythrocytes) by qualitative reactions. *If glucose or protein is present in the urine*, perform quantitative determination.

Results:

	Qualitative reactions	**Quantitative determination**		
Urine №	Protein:	Protein:	E_t =	C_t =
	Glucose:	Glucose:	E_t =	E_{st} =
	Ketone bodies:		C_t =	
	Blood:			

Conclusion:

Supplement 1

EXAMINATION QUESTIONS
FOR INTERNATIONAL MEDICAL STUDENTS (2017/18)

CHEMISTRY OF PROTEINS

1. Amino acids. Classification, properties and structure.
2. Bonds between amino acid residues in protein molecules. Properties of peptide bond.
3. Physical and chemical properties of proteins. Solubility of proteins, factors of protein solution stability. Salt fractionation.
4. Functions of proteins.
5. An application of blot-analysis for protein resolution.
6. Primary, secondary and super-secondary structures of proteins. Kinds of bonds between amino acid residues, specific for those structures. Protein domains.
7. Tertiary structure of protein molecule. Kinds of bonds between amino acid residues, which are specific for tertiary structure. Denaturation of proteins. Denaturing agents.
8. Quaternary structure of protein molecule. Examples of functioning of proteins with quaternary structure.
9. Principles of protein classification. Simple proteins and their role in organism.
10. Compound proteins. The structure of prosthetic groups in compound proteins. Functions of compound proteins.

ENZYMES

11. The biological role of enzymes. Enzyme nomenclature and classification.
12. The origin and common properties of enzymes.
13. Structure of enzymes. An enzyme active site.
14. Coenzymes. Their classification and role.
15. The mechanism of enzymatic action. Enzyme kinetics.
16. Isoenzymes, their molecular forms and biological role.
17. Common principles of regulating catalytic activity.
18. The mechanism of isosteric regulation of enzyme activity.
19. The mechanism of allosteric regulation of enzyme activity.
20. Covalent modification of enzyme structure as a mechanism for regulating catalytic activity.
21. Enzyme inhibitors, classification and characteristics.
22. An application of enzymes in medicine.

BIOENERGETICS

23. The term “metabolism”. Interrelationship between catabolism and anabolism. Central metabolic pathways.
24. Biological oxidation. Pathways of oxygen utilization.
25. Adenilate system and its biological relevance. Mechanisms of ATP synthesis and utilization.
26. Tissue respiration. The mitochondrial respiratory chain and its components. $NADH{\cdot}H^+$ dehydrogenase and flavoproteins. Ubiquinone (coenzyme Q), cytochromes. Their chemical structure and role in biological oxidation.
27. Oxidative phosphorylation. Chemiosmotic theory of coupling between oxidative phosphorylation and tissue respiration.
28. Causes of cell hypoenergetic states. Inhibitors and uncouplers of the tissue respiration and oxidative phosphorylation, mechanisms of their action.

CARBOHYDRATE METABOLISM

29. Carbohydrates. Classification. Physiologic significance.
30. Nutritive value of carbohydrates. Digestion and absorption of carbohydrates. Biological role of cellulose.
31. Glycogenesis and glycogenolysis. Mechanisms of their regulation. The difference of glycogenolysis in liver and muscle.
32. Anaerobic conversion of glucose. Energetic balance and mechanisms of ATP formation.
33. Aerobic conversion of glucose. Stages, end products. Energetic balance and mechanisms of ATP formation.
34. The fate of glycolysis products — pyruvate and lactate. Gluconeogenesis, enzymes and regulation of the process.
35. Oxidative decarboxylation of pyruvate and other α-ketoacids, enzymes, coenzymes, biological significance.
36. The citric acid cycle, its intermediate stages, enzymes, biological significance. Connection with oxidative phosphorylation.
37. The pentose phosphate pathway of glucose oxidation and its biological significance.
38. Uronic acid pathway of glucose metabolism, its biological role.
39. Regulation of blood glucose. Hormonal mechanisms which regulate the concentration of blood glucose (insulin, glucagon, epinephrine, glucocorticoids).

LIPID METABOLISM

40. Lipids, their basic properties. Biological role. Lipid classification.
41. Classification of fatty acids. Polyunsaturated fatty acids. Arachidonic acid derivatives — eicosanoids (prostaglandins, prostacyclins, tromboxanes, leukotriens) and their biological role.
42. Glycerophospholipids. Chemical structure, properties and biological role.
43. Cholesterol, its biosynthesis and biological role. Disorders of cholesterol metabolism (atherosclerosis, cholelithiasis).
44. Digestion of lipids in gastrointestinal tract: emulsification, enzymes, products of hydrolysis, micelle formation. Significance of bile acids in lipid digestion.
45. Resynthesis of triacylglycerols and phospholipids in enterocytes. Formation of chylomicrons, their composition and structure.
46. Serum lipoproteins, their classification, composition, the place of formation, interconversion. The role of lipoproteinlipase, lecithin:cholesterol acyltransferase (LCAT).
47. Synthesis and degradation of triacylglycerols in adipocytes. Hormone-sensitive lipase.
48. Synthesis and secretion of lipids in the liver. The role of lipotropic factors.
49. The central role of acetyl-CoA in cell metabolism.
50. Cell localization and reactions of β-oxidation of fatty acids. The role of CoA-SH and ATP. Connection with oxidative phosphorylation. Energetic balance.
51. Ketone bodies, their biological role. The mechanism of ketogenesis. Ketosis in diabetes mellitus and starvation. Determination of ketone bodies in urine.
52. Fatty acid synthesis. Connection with glycolysis, pentose phosphate pathway of glucose metabolism, Krebs cycle. The importance of CO_2, ATP, $NADPH{\cdot}H^+$, biotin. The multienzyme complex for fatty acid synthesis. Activators and inhibitors of fatty acid synthesis.
53. Hormonal regulation of lipid metabolism.

METABOLISM OF PROTEINS AND AMINO ACIDS

54. The nitrogen balance. Protein requirement. Nutritional value of proteins.
55. Characteristics of proteases (peptidases). Biological role of selective proteolysis.
56. Digestion of proteins. The role of HCl. The analysis of gastric juice.

57. Proteases and peptidases of pancreatic juice, the mechanism of their action. Inhibitors of peptidases and their usage for treatment of pancreatitis.
58. Amino acid pool of the cell. Its utilization and sources of replenishment.
59. Transamination. Enzymes, coenzymes. Biological role of the process. Diagnostic value of the determination of serum transaminase activity.
60. Kinds of amino acid deamination. Enzymes and coenzymes of oxidative deamination. Biological importance of the L-glutamate dehydrogenase reaction.
61. Fate of carbon skeletons of amino acids. Glucogenic and ketogenic amino acids.
62. Ways of ammonia detoxification. Formation of glutamine and asparagine, their role in ammonia transfer.
63. Urea production. The role of liver in urea production. Medical importance of the determination of urea and nonprotein nitrogen in blood.
64. Decarboxylation of amino acids. Formation of biogenic amines and their biological role.

CHEMISTRY AND METABOLISM OF NUCLEIC ACIDS

65. Nucleic acids. DNA and RNA, their structure, cell localization and functions.
66. Primary and secondary structure of DNA and RNA. Binding of nucleic acids to proteins. Structure of nucleoproteins.
67. End products of purine and pyrimidine nucleotides catabolism. The medical importance of the determination of uric acid in serum and urine.
68. Purine nucleotide biosynthesis. Substrates and regulation of the process.
69. Pyrimidine nucleotide biosynthesis. Substrates and regulation of the process.
70. Nominate deoxyribonucleotides which are used for DNA synthesis, and specify the ways of their formation.
71. DNA replication. Enzymes and substrates. Characteristic of the process in eukaryotes.
72. RNA synthesis. Enzymes and substrates. Characteristic of the process in eukaryotes.
73. Genetic code and its properties.
74. The role of tRNA in protein biosynthesis. Aminoacyl-tRNA synthetase specificity. The adapter function of tRNA.
75. Modern conception of protein biosynthesis.

HORMONES

76. Hormones. Their chemical structure and classification. Connection of the hormone structure and its mechanism of action.
77. Mechanisms of hormonal action on cells. Role of G-proteins, second messengers, protein kinases.
78. Hormones of hypophysis, chemical structure. Hormones of adenohypophysis Their role in regulation of peripheral gland functions. Growth hormone, molecular mechanism of signal transmission in target cells, the influence on metabolism.
79. Hormones of neurohypophysis. Vasopressin, the mechanism of signal transmission in target cells, the influence on metabolism.
80. Hormones of thyroid gland. Their structure, the mechanism of action, the influence on metabolism. Hypo- and hyperthyroidism.
81. Hormones which regulate calcium and phosphorus metabolism. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
82. Insulin. Chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism. Diabetes mellitus.
83. Glucagon. Chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.

84. Glucocorticosteroids. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
85. Mineralocorticosteroids. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
86. Hormones of adrenal medulla. Catecholamines: dopamine, epinephrine, norepinephrine. Their structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
87. Male and female sex hormones. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.

BIOCHEMISTRY OF NUTRITION AND INTEGRATION OF METABOLISM

Water-soluble vitamins

88. General characteristic and classification of vitamins. Evaluation of the body vitamin saturation.
89. Biotin. Coenzyme form. Biological role. Specific symptoms of deficiency. Food resources, daily requirement.
90. Vitamin B_1. Participation in coenzyme arrangement. The role in metabolism. Specific syndromes of deficiency. Food resources, daily requirement.
91. Vitamin B_2. Structure, participation in flavin coenzymes arrangement. Biological role. Specific symptoms of deficiency. Food resources, daily requirement.
92. Vitamin B_6. Its participation in coenzyme arrangement. The role in metabolism. Specific symptoms of deficiency. Food resources, daily requirement.
93. Vitamin B_{12}. Cobalamins. The role in metabolism. Specific symptoms of deficiency. Food resources, daily requirement.
94. Vitamin C. Biological importance. Specific symptoms of deficiency. Food resources, daily requirement.
95. Pantothenic acid. Coenzymes which contain pantothenic acid. Biological importance. Specific symptoms of deficiency. Food resources, daily requirement.
96. Vitamin PP. Structure, participation in nicotinamide coenzymes arrangement. Biological role. Specific symptoms of deficiency. Food resources, daily requirement.
97. Folic acid. Structure, participation in coenzymes arrangement. Biological role. Specific symptoms of deficiency. Food resources, daily requirement.
98. Vitamin-like substances: bioflavonoids (vitamin P), para-aminobenzoic acid, inositol, pangamic acid, lipoic acid, choline, vitamin U. Biological role.

Lipid-soluble vitamins

99. Forms of vitamin A. Biological role. Vitamin A deficiency and toxicity. Food resources, daily requirement.
100. Vitamin E. Biological role. Vitamin E deficiency. Food resources, daily requirement.
101. Vitamin D. Its structure, biological role. Vitamin D deficiency and toxicity. Food resources, daily requirement.
102. Vitamin K. Biological role. Vitamin K deficiency. Food resources, daily requirement.

Water and minerals

103. Water, its biological significance. The biological role of sodium, potassium, chlorine. Regulation of the water and salt balance, mechanisms.
104. Macroelements (calcium, phosphorus, magnesium). The biological role.
105. Microelements, their significance. The biological role of manganese, copper, zinc, selenium, iodine, cobalt, fluoride.
106. The biological role of sulfur. The role of thiol and disulfide groups in the formation of specific structure and properties of proteins and hormones. Glutathione, sulfolipids, thiamine, biotin, participation in detoxification.
107. Mechanisms of iron absorption, transport and storage. The role of iron in metabolism.

The integration of metabolism and malnutrition

108. Interorgan metabolism and fuel supply in the well fed state.
109. Interorgan metabolism and fuel supply between meals and in fasting.
110. Clinical forms of malnutrition. Their origin and typical abnormalities.

BIOCHEMISTRY OF THE BLOOD

111. Chemical content of blood plasma. Plasma proteins and their role. Clinical importance of the total plasma protein and its fractions determination.
112. The origin of plasma enzymes. Clinical importance of the determination of enzyme activity in plasma.
113. Blood buffer systems and their value.
114. Mechanisms of oxygen and carbon dioxide transport in blood. Development of hypoxic states.
115. Blood clotting. Phases of hemostasis. Factors and mechanisms involved in blood coagulation.
116. The role of Ca^{2+} and vitamin K in blood clotting.
117. Anticoagulant system.
118. Fibrinolysis. Its biological value. Plasmin system.

BIOCHEMISTRY OF THE LIVER

119. The role of liver in metabolic processes. Antitoxic function of liver. Biochemical methods of the diagnosis of liver damage.
120. Synthesis and breakdown of blood pigments. Metabolism of bile pigments.

BIOCHEMISTRY OF MUSCLE TISSUE

121. Chemical content of muscle tissue. Structure and role of contractile proteins.
122. Molecular mechanisms of muscle contraction and relaxation. Fuel resources for muscle contraction.

BIOCHEMISTRY OF CONNECTIVE TISSUE

123. Protein-carbohydrate complexes. Classification and role in the body.
124. Structural and specialized proteins of extracellular matrix (collagen, elastin, fibronectin). Their molecular features and functions.
125. Biosynthesis of collagen and elastin. The role of vitamin C in collagen biosynthesis.

BIOCHEMISTRY OF URINE

126. Normal characteristics of the urine.
127. Pathological urine components and their determination.

BIOCHEMISTRY OF NERVOUS TISSUE

128. Metabolism of lipids, carbohydrates and amino acids in nervous tissue.
129. The origin of the most important neurotransmitters (acetylcholine, glutamate, glycine, serotonin, catecholamines, GABA). Their biological role and catabolism.

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 μmol/l
Uric acid:	men women	0.20–0.42 mmol/l 0.12–0.34
Hemoglobin:	men women	130–170 g/l 120–150 g/l
Bilirubin:	total direct indirect	8.5–20.5 μmol/l 2.2–5.1 μmol/l 1.7–17.1 μmol/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

On the 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 following 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 second messengers (cAMP, inositol triphosphate, diacylglycerol);
– via intracellular receptors.

CONTENTS

Учебное издание

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БИОХИМИЯ

BIOCHEMISTRY

Учебно-методическое пособие

На английском языке

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