МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА БИОЛОГИЧЕСКОЙ ХИМИИ

ПРАКТИКУМ ПО БИОЛОГИЧЕСКОЙ ХИМИИ

ДЛЯ ИНОСТРАННЫХ УЧАЩИХСЯ СТОМАТОЛОГИЧЕСКОГО ФАКУЛЬТЕТА

MANUAL ON BIOCHEMISTRY

FOR INTERNATIONAL STUDENTS OF DENTAL FACULTY

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 07 «Стоматология»

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А в т о р ы: проф. А. Д. Таганович; доц. И. Л. Котович; доц. А. В. Колб; доц. Н. Н. Ковганко; доц. Т. Ю. Принькова; доц. А. Г. Кадушкин

Р е ц е н з е н т ы: д-р биол. наук, проф. каф. биохимии, проректор по учебной работе и международным связям Витебского государственного ордена Дружбы народов медицинского университета Н. Ю. Коневалова; каф. биологической химии Гродненского государственного медицинского университета

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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.

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Таганович Анатолий Дмитриевич
Котович Ирина Леонидовна
Колб Александр Владимирович и др.

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CONTENTS

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 pay full attention to all instructions, written or spoken, before and during experimental work in the student laboratory. No unauthorized experiments are allowed. In case of any unclear instruction student has to ask the teacher for further information.
2. Eating, drinking and smoking in the laboratory is strictly forbidden. Bringing food and beverages to the laboratory is not allowed either.
3. Students must use adequate footwear for the laboratory and always wear a laboratory coat, which must be kept button-down.
4. Every student works at his/her working place, where he/she is obliged to keep everything clean and in order. Every student is responsible for the condition of used equipment and reagents. Any loss or damage must be immediately reported to the teacher or technician.
5. Any heated or chemically contaminated tools can only be put aside on a specified place. Any reagent bottle should be returned to its place immediately after use.
6. In case of spillage or leakage of any reagent or biological material or any other unexpected event, every student is obliged to perform all possible measures to minimize harm to human health, equipment or environment, and notify the instructor immediately.
7. Spilled acid is immediately diluted with water, and washed out with water or removed by absorption into an inert material. Likewise, spilled alkali is washed out with water or removed by absorption into an inert material, and disposed as a dangerous waste.
8. For work with biological material and some hazardous substances the single-use latex gloves are used. Contaminated gloves should be changed immediately.
9. When heating and observing a sample in a test tube, the tube orifice must always be directed out from the student's face, as well as faces of any other persons around.
10. In case of any injury every student is obliged to provide an adequate first aid and notify the teacher or technician immediately.
11. After finishing the experiments every student is supposed to clean his/her working place, rinse used glassware and put all tools to their appropriate places. Only if the teacher or technician checks the working place and finds it in an acceptable condition, the student is allowed to leave.

First Aid in Laboratory Accidents

Chemicals in the eye

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

Corrosion of the skin

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

Burns

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

Open wound

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

Operation on photoelectrocolorimeter:

1. Turn on the device.
2. Put two cells with the control and test solutions so that the light beam passing through the cell with control solution, click on the start button «ПУСК».
3. When the cover is open press the «Ш(0)» button.
4. Close the cover and press the «К(1)» button.
5. Place the cell with the test solution in a beam of light and click on Д(5) button.
6. Read the numbers on the screen.

Operation on clinical laboratory centrifuge:

1. Turn on the device.
2. Open the cover.
3. The tubes were placed in slots opposite each other — even tubes.
4. Close the cover.
5. Set the desired speed.
6. Turn on the centrifuge by pressing the power switch located on the control panel.
7. At the end of the required time turn off the centrifuge by pressing the power switch.
8. Remove the cover and the tubes after a full stop of the centrifuge.
9. Close the cover.

Rules for working with the pipetator:

1. Carefully insert the pipette into the silicone holder of the pipetator.
2. Place the pipette into the liquid and rotate the wheel on the top of the pump with your finger.
3. In order to release the liquid, rotate in the opposite direction.
4. After finishing the work, remove the pipette from the silicone holder of the pipetator.

1. INTRODUCTION TO BIOCHEMISTRY. THE STRUCTURE OF PROTEINS. PHYSICAL AND CHEMICAL PROPERTIES OF PROTEINS. MECHANISMS OF PROTEINS SEDIMENTATION

Objective

To study the levels of structural organization of the protein molecule. To form the notion of conformational states of a protein molecule and the significance of spatial structure in protein functioning. To acquaint with the precipitation reactions of proteins.

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. Sedimentation of proteins (reversible — "salting-out", irreversible).

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 5–9.
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. 14–20, 30–48.
4. *Konevalova, N. Yu*. Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 6–9.
5. 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: hydrophylity 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 make precipitates in a saturated solution of $(NH_4)_2SO_4$ and globulins — in a semi-saturated solution of $(NH_4)_2SO_4$, because globulins have a high molecular mass and a smaller charge than those of 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 have another protein — egg-albumin. Add the fine powder of ammonia sulfate to the filtrate till complete saturation, i. e. till a new portion of the powder stays unsolved. Then filter out the albumin deposit. Expose the filtrate to biuret reaction: add 2 drops of 1 % solution of $CuSO_4$ + 5 drops of 10 % solution of NaOH to the filtrate. A negative biuret reaction (blue staining) indicates to the absence of protein in the tested solution.

Conclusion:

Laboratory work 2. Irreversible sedimentation of proteins

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

Protein sedimentation by salts of heavy metals, unlike salting-out, occurs in low salt concentrations. Proteins interacting with salts of heavy metals (lead, copper, silver, mercury etc.) adsorb them forming salt-like and complex compounds soluble in the excess of these salts (excluding the salts of silver nitrate and mercury chloride), but insoluble in water. Dissolution of the precipitate in the excess of salts is called ***adsorption peptisation***. It occurs as a result of acquiring the same positive charge by protein particles

Procedure

Reagents	**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.

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:

2. 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 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 characteristics 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 influence of conformational changes on enzyme activity.
5. The mechanism of enzyme action. Enzyme kinetics. The effect of substrate concentration, pH, temperature on enzyme reaction velocity (molecular mechanism, graphical relationship). Michaelis's constant (K_m), usage of K_m for predicting the course of biochemical reactions.
6. Specificity of enzyme action. Types of specificity.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. 49–71.
4. *Konevalova, N. Yu*. Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 18–26, 69–76.
5. Lecture material.

PRACTICAL PART

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

1. Evaluation of saliva amylase activity and its thermolability.

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

Hydrolysis of starch under the action of saliva α-amilase occurs untill the stage of dextrines formation. Starch together with iodine gives blue staining. Dextrines, depending on their size, together with iodine give various staining: amilodextrines — 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 native saliva diluted 1 : 10 into the 1st test-tube; add 10 drops of boiled saliva into the 2nd tube and 10 drops of water as a control to the 3rd tube. All test-tubes are placed into the thermostat at 38 °C for 10 minutes. Then the content of the test-tubes is exposed to qualitative reactions for starch and products of its disintegration.

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

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

Fill in the table with the results of the experiment.

Test-tube №	Reaction with compound iodine	Trommer's reaction
1. Native saliva		
2. Boiled saliva		
3. H_2O		

2. Effect of pH on the enzyme activity.

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

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

Fill in the table with the results of the experiment:

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

3. Activators and inhibitors of the saliva amylase activity.

Procedure. Add 1 ml of saliva diluted 1 : 40 into 3 test-tubes. Add 2 drops of water into the 1st tube, 2 drops of 1 % NaCl into the 2nd tube and 2 drops of 1 % $CuSO_4$ 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)			

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

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

Fill in the table with the results of the experiment:

Test tube №	Enzyme	Substrate	Feling's reaction
1			
2			

Conclusions:

3. 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 which regulate enzyme activity, to get acquainted with the role of enzymes in diseases diagnosis and treatment monitoring.

Problems for discussion:

1. The mechanism of enzymatic catalysis. The theory of the intermediate enzyme-substrate complexes, the types of relationships.
2. An active site of the enzyme, its organization. The theory explaining the work of the active site.
3. Structure peculiarities of allosteric enzymes, allosteric center. The concept of a "key enzyme".
4. Regulation mechanisms of the enzyme-catalyzed processes rate: regulation of the enzymes amount (synthesis, break-down), enzyme activity, modification of the substrate amount, the presence of isoenzymes, joining enzymes into multienzyme complexes, compartmentation of processes.
5. Key enzymes.
6. Regulation of enzyme activity: covalent modification, activators and inhibitors (examples). Types of inhibition (irreversible and reversible, isosteric and allosteric), characteristic, examples.
7. Multiple forms of enzymes (isoenzymes and true multiple forms), examples, their biological role.
8. Medical aspects of enzymology.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. 72–79.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 21–22, 27–37.
5. Lecture material.

PRACTICAL PART

Determination of saliva α-amilase activity

The method is based on evaluation of the least amount of amylase (at maximum saliva dilution) that completely digests the whole added starch. Amilase 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 (1:10) into the first one. Stir the content of this tube by pipetting it several times. Take into the pipette 1 ml of the mixture and put it into the 2nd test-tube. Stir the content of this test- tube and put 1 ml of mixture from the second test-tube 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 2 ml of 0.1 % of starch solution in each test-tubes with diluted saliva, stir it shaking the test-tubes and place them into the thermostat at 38 °C for 30 minutes. Cool the test-tubes after incubation under running water, add 1 drop of 0.1 % iodine solution into each tube and stir. The fluid in the tubes is stained in yellow, rose and violet color. Mark the last tube with yellow staining where the hydrolysis has been completed and make calculations. Put down the results into the table:

Starch hydrolysis in the presence of saliva enzymes in various dilutions

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

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

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

Hence, amylase activity is 320.

Conclusion:

4. INTRODUCTION TO METABOLISM. CENTRAL METABOLIC PATHWAY — THE KREBS 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. Adenilate 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. Tricarboxylic acid cycle as a central metabolic pathway. Cellular localization, reactions, enzymes, co-enzymes.
6. Dehydrogenase reactions of TCA cycle as a source of hydrogen for the system of tissue respiration. Decarboxylation in the citric acid cycle as a cellular CO_2 formation mechanism that is an end product of carbonic compounds catabolism.
7. The functions of TCA cycle: integrative, catabolic, anabolic, energetic, hydrogen-donor. Regulation. Anaplerotic reactions.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. 80–91, 122–135.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 49–68, 86–92.
5. 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 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	Liver homogenate	0.5	0.5
	10 min incubation at room temperature		
7	Folin reagent A	0.5	0.5
8	Folin reagent B	0.5	0.5

Observed changes:

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 with calcium hydroxide and is revealed, after sulfuric acid is added, by gas vesicles.

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:

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:

Conclusion:

5. 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 of water formation in the process of tissue respiration from a 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 (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, 2015. 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. 86–101.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 69–85.
5. 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 = 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
	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	0.5	0.5	0.5	0.5
10	Dilute (1:8) the content of all test-tubes with water, 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.

Procedure

№	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):

Conclusion:

6. COLLOQUIUM "ENZYMES, INTRODUCTION TO METABOLISM. CENTRAL METABOLIC PATHWAY, BIOLOGICAL OXIDATION. OXIDATIVE PHOSPHORYLATION"

Questions for preparation:

1. Enzymes as protein catalysts.
2. Modern classification of enzymes and terminology of enzymes (systemic and working names). Enzyme code. General characteristic of classes.
3. 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.
4. Enzyme active site and its structure.
5. Coenzymes, their classification and role in catalysis. Block-structures of the NAD^+, $NADP^+$, FAD and FMN.
6. Mechanisms of regulation of enzyme activity: reversible and irreversible regulation, isosteric and allosteric regulation, covalent modification of the structure of the enzyme.
7. Multiple forms of enzymes (isoenzymes and true multiple forms), examples, their biological role.
8. Medical aspects of enzymology. Examples of enzymes and inhibitors usage in diagnosis and treatment (including dentistry).
9. Metabolism, catabolism and anabolism, their distinctions and interrelations. Linear and cyclic metabolic pathways, regulatory (key) enzymes.
10. Adenilate system of the cell, its participation in energy exchange. Ways of ATP synthesis: substrate-level, oxidative and photosynthetic phosphorylation.
11. Tricarboxylic acid cycle as a central metabolic pathway. Cellular localization of TCA cycle, reactions, enzymes, co-enzymes. Functions of TCA cycle. Anaplerotic reactions.
12. Pyridine-dependent and flavin-dependent dehydrogenases. Block-structures of co-enzymes NAD^+, $NADP^+$, FAD, FMN.
13. 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.
14. Oxidative phosphorylation. 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.
15. Microsomal oxidation, its role for the cell.
16. Causes for the hypoenergetic states development. Inhibitors of electron transport and oxidative phosphorylation.

7. DIGESTION OF CARBOHYDRATES. METABOLISM OF GLYCOGEN. 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. Classification of carbohydrates. Carbohydrates digestion, final products. 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.
4. Degradation of glycogen in the liver and muscles, sequence of reactions, regulation.
5. Glycolysis, its biological role, subcellular localization, phases (preparatory or unoxidative, oxidative), reactions, energy yield and mechanism of ATP formation. Glycolysis regulation, key enzymes.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. 102–110, 136–152.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 93–98, 112–119.
5. Lecture material.

PRACTICAL PART

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.

Conclusion:

8. METABOLIC PATHWAYS OF PYRUVATE. GLUCONEOGENESIS. AEROBIC OXIDATION OF GLUCOSE TO FINAL PRODUCTS (CO_2 AND H_2O)

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. Reduction of pyruvate to lactate (reaction, LDH isoenzymes, the appointment of reactions), Cori cycle. Disposal of lactate by cells.
3. Gluconeogenesis (purpose, substrates, key reactions and enzymes, regulation, expenditure of energy).
4. Oxidative decarboxylation of pyruvate (biological role, subcellular localization, reactions); pyruvate dehydrogenase complex (enzymes, coenzymes), regulation of pyruvate dehydrogenase activity.
5. Citric acid cycle (subcellular localization, reactions, energetic balance, enzymes, regulation, biological role).
6. 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, 2015. P. 21–22, 38–41.
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. 136–144, 153–162.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 98–108.
5. Lecture material.

PRACTICAL PART

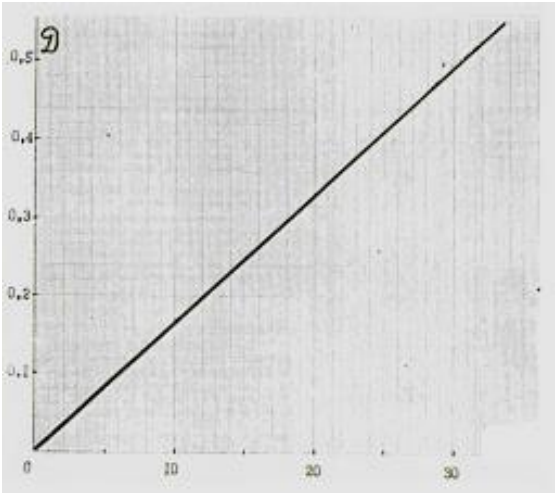
Determination of pyruvate in the urine

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.

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-dinitrophenylhydrosine solution and leave them for 20 minutes at room temperature. Then add 5 ml of 0.4N NaOH into each tube and in 10 minutes determine the optical density of the test sample versus the control sample using 10 mm cuvettes with a green light filter.



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

Calculation is performed according to a ready calibration graph. Recount the found value by daily diuresis (1500 ml for men and 1200 ml for women) and get the content of pyruvate in daily urine.

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 hypophysis-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:

9. SECONDARY PATHWAYS OF GLUCOSE METABOLISM. EFFECT OF HORMONES ON THE BLOOD GLUCOSE LEVEL. FEATURES OF CARBOHYDRATE UTILIZATION BY ORAL MICROFLORA

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. To understand the pathogenetic relationship of carbohydrate foods and tooth decay.

Problems for discussion:

1. Pentose phosphate pathway (subcellular localization, steps, key enzymes, metabolites, biological role).
2. Glucuronic pathway (tissue and subcellular localization, biological role).
3. Regulation of blood glucose content. Mechanisms of hormonal regulation (insulin, epinephrine, glucagon, glucocorticoids etc.).
4. Features of carbohydrate utilization by oral microflora.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 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. 153–172.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 108–111, 119–126.
5. 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 each sample.
2. According to the received data make a conclusion which of the samples corresponds to the mentioned states.

Determination of glucose concentration in blood serum by enzymatic method

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

$$\text{Glucose} + H_2O + O_2 \xrightarrow[\textit{glucose oxidase}]{} \text{Gluconolactone} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow[\textit{peroxidase}]{} \text{Quinoneimine} + 4H_2O$$

The resulting product has pink color. The color intensity is proportional to the 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 (5.55 mmol/l); E_t — extinction of the tested sample; E_s — extinction of the standard sample.

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

Results:

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

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, hypophysis; overdosage of insulin while treating diabetes mellitus, impairment of carbohydrate absorption, poisonings by phosphor, benzole, chlorophorm, in insufficient taking of carbohydrates with food, after considerable losses of blood.

Conclusion:

10. COLLOQUIUM "CARBOHYDRATE METABOLISM"

Questions for preparation:

1. Carbohydrates digestion, end products, digestion impairments. The role of cellulose and pectines 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.
5. Glycolysis, its biological role, subcellular localization, phases (unoxidative, oxidative), reactions, energy yield and mechanism of ATP formation. Glycolysis regulation, key enzymes.
6. Oxidative decarboxylation of pyruvate as a central metabolic pathway. Pyruvate dehydrogenase complex (enzymes, co-enzymes, scheme of reactions).
7. Pyruvate as a central metabolite. Pathways of pyruvate conversion depending on the energetic status and peculiarities of oxidative cellular metabolism.
8. Tricarboxylic acid cycle as a central metabolic pathway. Cellular localization of TCA cycle, reactions, enzymes, co-enzymes. Functions of TCA cycle. Anaplerotic reactions.
9. Gluconeogenesis (biological role, substrates, key reactions and enzymes, regulation, expenditure of energy).
10. Aerobic oxidation of glucose to CO_2 and H_2O (stages, energy yield, mechanisms of ATP formation).
11. Pentose phosphate pathway (subcellular localization, steps, key enzymes, metabolites, biological role, regulation).
12. Uronic acid pathway (tissue and subcellular localization, biological role).
13. Physiological concentrations of glucose in the blood. Hormonal regulation of blood glucose level.
14. Carbohydrate utilization by oral bacteria. Differences in the synthesis of glycogen in humans and bacteria. Chemical-parasitic theory of tooth decay. Role of sugar alcohols (sorbitol, xylitol) in the prevention of caries.
15. Substrate level phosphorylation and oxidative phosphorylation. The diagram of the respiratory chain.

11. 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.

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

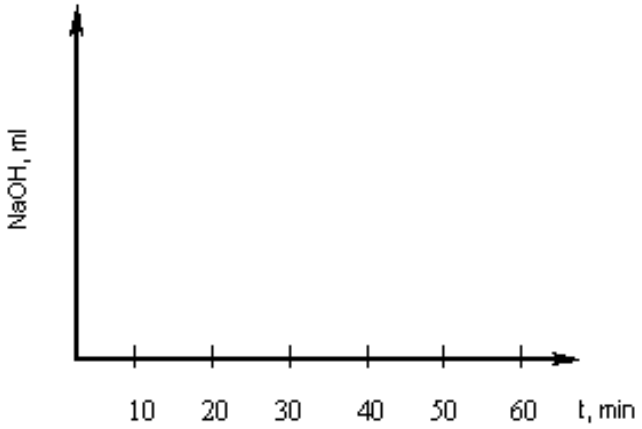
1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 47–51.
2. *Gubsky, Yu*. Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 57–70, 177–178.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 111–121, 197–218.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 127–129, 134–135.
5. 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. Add 5 ml of milk and 1 ml of 5 % pancreatine (pancreas juice) into the test-tube. Then add 1 ml of of bile and quickly stir the fluid in the test-tube. Take 1 ml of the mixture from test-tube and apply into flasks, add 1–2 drops of 0.5 % phenolphthalein solution and titrate by 0.05N solution of NaOH to a light-rose color, which doesn't disappear for 30 seconds. Place test-tube with remaining mixture into the thermostat at 38 °C. Every 10 minutes take out 1 ml of the mixture and titrate by 0.05N solution of NaOH in the presence of phenolphthalein to a light-rose color. Perform 5 such determinations and on the basis of received data construct the line, that will reflect the process of fat hydrolysis by lipase vs time.



Conclusion:

Work 2. Action of the pancreatic phospholipases

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

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

Conclusion:

12. LIPIDS BLOOD TRANSPORT. METABOLISM OF CHOLESTEROL. DEPOSITING AND MOBILIZATION OF LIPIDS. DETERMINATION OF PLASMA β-LIPOPROTEINS

Objective

Learn the molecular mechanisms of transport of exogenous and endogenous lipids by the bloodstream for subsequent analysis of biochemical aspects of violations of these processes. Learn the process and regulation of cholesterol biosynthesis. To acquire skills of β-lipoproteins determination in serum.

Problems for discussion:

1. Lipid transport forms (lipoproteins), structure and classification.
2. Cholesterol, the biological role, food sources. Elimination of cholesterol from the organism, bile acids as a major end product of cholesterol metabolism, cholelithiasis.
3. Cholesterol biosynthesis (tissue and subcellular localization, substrates, phases, reactions of the 1st phase, regulation).
4. Mechanisms for maintaining balance cholesterol in cells. Transport of cholesterol in extrahepatic cells, the role of apoB_{100}. The role of HDL and LCAT in the unloading of the cells from of excess cholesterol. Cholesterol ester metabolism, the role of ACAT, cholesterol esterase.
5. Transport of cholesterol in blood. Hypercholesterolemia and its causes. Biochemistry of atherosclerosis. Hypercholesterolemia as a risk factor, other risk factors. Fundamentals of prevention and diagnosis of hypercholesterolemia, atherosclerosis (atherogenic index).
6. The mobilization of lipids from adipose tissue (scheme, cAMP-dependent mechanism of activation of hormone-sensitive lipase, hormone regulation). The role of the deposition and mobilization of fat, violations of these processes in obesity.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 52–53, 54, 60–61, 62–63.
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. 205–230.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 134–136, 143–153.
5. Lecture material.

PRACTICAL PART

Determination of plasma β-lipoproteins (low density lipoproteins)

The most 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 0.025M 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 — 3–4.5 g/l. The content of β-lipoproteins varies depending on the age and sex.

$E_{1=}$ $E_{2=}$ x (g/l) = $(E_2 – E_1)\cdot 10$ =

Conclusion:

13. FATTY ACIDS METABOLISM. METABOLISM OF KETONE BODIES. DETERMINATION OF TOTAL CHOLESTEROL CONCENTRATION IN SERUM

Objective

To study the oxidation of fatty acids. To form a notion of ketone bodies and their role in physiological and pathological states. Acquire skills of qualitative determination of ketone bodies in urine.

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. Fatty acid synthase complex. 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, chemistry. Synthesis of ketone bodies. Molecular mechanisms of ketonemia in diabetes, insufficient carbohydrate diet, starvation. Utilization of ketone bodies (interconversion, activation, including in metabolism, energy oxidation).
6. Acetyl-CoA as a central metabolite.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 53–62.
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. 173–196.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 129–153.
5. Lecture material.

PRACTICAL PART

Determination of serum cholesterol concentration by enzymatic method

Principle of the method. Determination of cholesterol after its enzymatic hydrolysis and oxidation. The indicator is quinoneimine, formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The resulting product has a pink color. The color intensity is directly proportional to the concentration of cholesterol and measured photometrically.

Progress. Cholesterol is detected in serum. Reagents are added according to the following scheme:

Reagents	Test sample, ml	Standard sample, ml
Blood serum	0,02	–
Standard cholesterol solution	–	0,02
Enzymes solution	2,0	2,0
Stir and incubate the reaction mixture for 5 minutes at 37 °C or 10 min at room temperature		

At the end of the incubation, the optical density of the test and standard samples is measured on a colorimeter (wavelength 540 nm) in cuvettes with a layer thickness of 5 mm against the control.

The control sample contains 2.0 ml of a working solution of enzymes. A control sample can be prepared one per group.

To calculate the concentration of cholesterol use the formula:

Results: E t. = E std. =

Calculation:

C cholest. (mmol / L) = 5.17 × (E experiment. / E std.) =

The normal values of cholesterol content in serum are 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:

Qualitative reactions for acetone and acetoacetic acid

Procedure:

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.

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

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

Conclusion:

14. COLLOQUIUM "LIPID METABOLISM"

Questions for preparation:

1. Lipids, general characteristics, classification. Characteristic and biological role of lipid groups (chemical formulas and terminology of acylglyceroles and glycerophospholipids; block-structures of waxes, sphingophospholipids, glycolipids, sulfolipid structures).
2. Digestion of lipids, phases. Emulsification (purpose, factors, stabilization of fatty emulsion). Bile, bile acids (primary, conjugated, secondary). Enterohepatic re-circulation of bile acids. Hydrolysis of diet lipids (enzymes, conversion patterns). Absorption (mechanisms, micellar dissolution, fate of micelles).
3. Re-synthesis of triacylglyceroles and glycerophospholipids in enterocytes.
4. Synthesis of TAG and glycerophospholipids in the liver and fatty tissue (role of lipotropic factors).
5. 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, atherogeneity index.
6. Cholesterol, biological role, biosynthesis (tissue and subcellular localization, substrates, phases, reactions of the 1^{st} phase, regulation). Mechanisms of maintaining cholesterol balance in cells.
7. Mobilization of lipids from the adipose tissue. Hormone-sensitive lipase.
8. 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.
9. Biosynthesis of fatty acids. Subcellular localization, substrates, reactions, regulation. Peculiarities of the fatty acid syntase structure. The malic-enzyme role.
10. Polyunsaturated fatty acids as essential nutritive factors: representatives, biological role.
11. Metabolism of arachidonic acid. Biosynthesis of eicosanoids (prostaglandins, prostacyclins, leukotriens, thromboxans) and their biological role.
12. 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).
13. Acetyl-CoA as a central metabolite.
14. Hormonal regulation of lipid metabolism.

15. 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 erythrocytes. The structure of hemoglobin, heme, globin; varieties (normal and abnormal) and derivatives of hemoglobin.

4. Respiratory function of the blood. Erythrocytes as a main participant of gas transport by the blood (the role of hemoglobin and carbanhydrase). Reversible binding of oxygen and carbon dioxide as a means of transport (binding mechanisms of CO_2 and O_2 with hemoglobin, co-operative interaction of hemoglobin subunits). Hypoxia, forms, mechanisms of development.

Recommended literature

1. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 580–597, 609–625.
2. *Gubsky, Yu*. Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 380–388.
3. Lecture material.

PRACTICAL PART

Work 1. Buffer properties of serum

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

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

Conclusion:

Work 2. Determination of chlorides in blood according to Levinson

Chlorine is present in the organism mainly in the form of ions. A chloride-ion is the main source of anions. Chlorine anions are the most 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 blood proteins. Prepare a mixture of solutions in two test-tubes: 5 ml of 0.45 % $ZnSO_4$ + 1 ml of 0.1N 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 over the spirit-burner for 3 minutes. Then filter the content of the test-tubes into flasks through cotton wool. Rinse the residue on the cotton wool filter twice with water (per 3 ml).

2. Sedimentation of chlorine 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. Subtract from the volume of $AgNO_3$ spent for titration of the tested solution (V_t, ml) the volume of $AgNO_3$ spent for titration of the control solution (V_c, ml), multiply the received difference by 100,11.

V_t (ml) =

V_c (ml) =

$C(mmol/l) = (V_t - V_c) \cdot 100,11 =$

Conclusion:

16. BLOOD PLASMA PROTEINS. BLOOD CLOTTING SYSTEM

Objective

To get acquainted with principles of blood protein composition examination, to understand a diagnostic role of protein fractions and individual plasma proteins determination. To get the notion of hemostasis mechanisms and to study functioning of the blood clotting system.

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). Vascular-thrombocytic and 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 origin, 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, 2015. P. 73–76.
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. 580–608.
4. *Konevalova, N. Yu.* Materials for the state examination in biochemistry / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 11–18.
5. Lecture material.

PRACTICAL PART

Work 1. Determination of calcium in plasma

Calcium plays an important role in realization of vital processes. It influences the permeability of biological membranes, excitability of nerves and muscles, participates in neuromuscular conductivity, constriction and relaxation of musculature (including cardiac muscles), secretory processes, formation of bones and cartilages; produces effect on metabolism in cells, is an important factor of hemostasis and is a mediator of hormones action in the cell. Determination of plasma total calcium is of great importance for diagnosing a number of diseases and managing the treatment.

Normal total calcium concentration in blood plasma is 2.2–2.7 mmol/l.

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

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

$$C\ (mmol/l) = 1.992 * Vt$$

Vt (ml) = C (mmol/l) =

Conclusion:

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, 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. Absorption of amino acids, amino acid transport into cells.
7. Amino acid pool of the cell — its sources and utilization.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. 237–243.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 154–156.
5. Lecture material.

PRACTICAL PART

Determination of gastric juice acidity

Principle of the method. Total acidity of gastric juice is measured in milliliters of 0.1N 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 0.1N 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, total content of hydrochloric acid and bound hydrochloric acid is done on one portion of gastric juice. Titration is performed with two indicators: dimethylaminoazobenzole and phenolphthalein.

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

Titrate free hydrochloric acid by 0.1N 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), 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, 2015. 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. 242–263.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 156–161.
5. Lecture material.

PRACTICAL PART

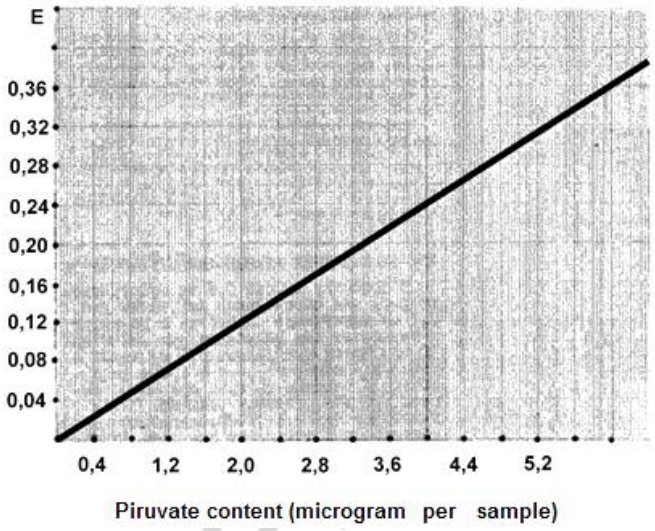
Determination of alanine aminotransferase (ALT) activity

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 dinitrophenylhydrasine solution and leave the samples for 20 minutes at room temperature. Then add 5 ml of 0.4N NaOH, carefully stir and leave to stay for 10 minutes at room temperature for staining development. Measure optical density by photoelectrocolorimeter under a green light filter (530 nm) in a 10 mm wide cuvette versus a control sample for reagents. The control sample contains all ingredients of the tested sample excluding serum, it being substituted by 0.1 ml of distilled water.



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

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

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.

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 jaundiceless 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, 2015. 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. 237–248.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 162–169.
5. 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 wide cuvettes under a blue light filter versus a control sample.

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

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

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

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

Clinical and diagnostic value. The decreased urea content in urine is noted in nephritis, acidosis, parenchymatose 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; in newborns — 42.84–71.40 mmol/l; 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 1^{st} one (test sample), 1 ml of standard solution of ammonia sulfate and 9 ml of water into the second tube (standard sample) and 10 ml of water into the third one (control). Then apply per 0.5 ml of Nessler's reagent into all tubes. Perform photometry of the tested and the standard sample versus the control under a blue light filter in 5 mm thick cuvettes.

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

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

where C_t — nonprotein blood nitrogen concentration in the blood, mmol/l; C_s — nitrogen concentration in the standard sample (0.0714); E_t — extinction of the tested sample (mineralizate); E_s — extinction of the standard sample (ammonia sulfate).

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. COLLOQUIUM: "METABOLISM OF SIMPLE PROTEINS", "BLOOD BIOCHEMISTRY"

Questions for preparation:

1. Nitrogen balance. Dietary protein requirement. The biological value of proteins.
2. Total and selective proteolysis, examples, biological role. Characteristics of proteases produced in gastrointestinal tract.
3. Digesting the proteins in the gastrointestinal tract – enzymes, their origin and mechanism of activation, specificity. Role of hydrochloric acid.
4. Amino acid pool of the cell — sources and utilization.
5. Transamination. Enzymes. Coenzyme. The role of this process in the life of the cell. The diagnostic value of the determination of transaminases (ALT and AST) activity in serum. Write down the reactions catalyzed by ALT and AST.

6. Ways of amino acid deamination. Enzymes and coenzymes of oxidative deamination. Write down the reaction catalyzed by glutamate dehydrogenase, describe its biological significance. The biological significance and mechanism of indirect deamination.

7. Usage of carbon skeletons of amino acids. Ketogenic and glycogenic amino acids.

8. The ways of ammonia detoxification. Formation of glutamine, its role in the transport of ammonia. Characteristics of the local pathways of ammonia detoxification available in non-hepatic tissues.

9. Formation of urea. The role of the liver in urea formation. Significance of the determination of blood urea in clinical practice.

10. Nonprotein blood nitrogen – components, their origin and relative content, principle of determination, clinical-diagnostic value.

11. Decarboxylation of amino acids. Enzymes, co-enzyme. The formation of histamine, serotonin, GABA, their role in the body. The formation of catecholamines (dopamine, norepinephrine, epinephrine) and their role in the body.

12. Plasma proteins and their function.

13. Regulation of acid-base status in blood. Characteristics of the buffer systems of blood.

14. Mechanisms of transport of carbon dioxide and oxygen in blood. Hemoglobin structure and function, mechanism of oxygen binding. Regulation of hemoglobin affinity to oxygen. Hypoxia.

15. Blood clotting. Phases of blood coagulation. Extrinsic and intrinsic pathways of coagulation — scheme, causes, involved factors and mechanisms of their activation. The general characteristics of blood clotting factors, mechanism of zymogens activation.

16. Phases of blood coagulation. Write down the schematic reactions of the 1st phase, describe the factors involved in the formation of prothrombinase during extrinsic and intrinsic pathways, mechanisms of their activation.

17. The role of calcium and vitamin K and its antagonists (anti-vitamins) in blood clotting process.

18. Fibrinolysis. The biological role of fibrinolysis. Plasmin system.

19. Anticoagulant system. Classification of anticoagulants, mechanism of their action.

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

Objective

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

Problems for discussion:

1. Mononucleotides, structure, terminology, biological role.

2. Primary, secondary and tertiary structures of nucleic acids (peculiarities of the structure, varieties, types of stabilizing bonds).

3. Nucleoprotein metabolism. Digestion of nucleoproteins in the gastrointestinal tract (significance, steps, enzymes).

4. Degradation of purine nucleotides (reactions, uric acid as an end-product of catabolism). Disorders of purine metabolism (hyperuricemia and gout, urolithiasis).

5. Biosynthesis of purine nucleotides *de novo* (sources of nitrogen and carbon of a purine ring, participation of folic acid, main intermediate products, key enzyme, regulation). The notion of nucleotide synthesis from free nitrogenous bases and nucleosides.

6. Degradation of pyrimidine nucleotides (end products and their fate).

7. Biosynthesis of pyrimidine nucleotides (substrates, process pattern, key enzyme, regulation, role of vitamins).

8. Synthesis of deoxyribonucleotides.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. 286–313.

4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 175–180, 185.

5. 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-tungsten Folin reagent to 1.5 ml of urine and titrate it by 0.01N 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, ml; 1.5 — the sample volume, ml.

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

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

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

Conclusion:

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

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.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. 314–414.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 180–184, 186–202.
5. Lecture material.

23. HORMONES, GENERAL CHARACTERISTIC AND PECULIARITIES OF BIOLOGICAL ACTION

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, 2015. 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. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 203–208.
5. Lecture material.

24. BIOCHEMISTRY OF HORMONES. HYPOTALAMIC HORMONES. PITUITARY HORMONES. HORMONES OF ADRENAL CORTEX. THYROID HORMONES. SEX HORMONES

Objective

Consolidate the knowledge about the chemical structure, mechanisms of action of hormones of the hypothalamus, pituitary, adrenal cortex, thyroid and sex hormones. Study their biological role in health and endocrine disorders.

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. Adenohypophysis 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. Neurohypophysis hormones: chemical structure, type of receptor in target-tissue and mechanism of a hormonal signal transduction, realization of 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. 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.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 209–248, 269–276.
5. Lecture material.

PRACTICAL PART

Work 1. Thyroid hormones

Thyroid gland synthesizes and secretes iodinated thyroid hormones thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) and hormone (polypeptide) calcitonin, the function of which is associated with regulation of calcium and phosphorus in the blood.

Qualitative reaction on thyroxine.

The principle of the method. Potassium iodide is formed in the destruction thyroidin from which iodine is easily displaced by the potassium iodate. The liberated iodine is detected by starch (blue staining) under acidic conditions.

Procedure. Poured hydrolyzate thyroidin 24 drops into the tube, add 3 drops of 1 % starch solution, 1 drop of phenolphthalein, and then 4 drops of potassium iodate and 10–15 drops of 10 % sulfuric acid solution to discoloration and appearance of blue staining.

Work 2. Hormones of sexual glands

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

Female sex hormones — estrogens. The main natural estrogen is estradiol, estrone and progesterone.

Male sex hormones — androgens. For male sex hormones include testosterone and androsterone.

Qualitative reaction to folliculin.

The principle of the method. Qualitative reaction folliculin (estrone) is carried out with concentrated sulfuric acid, leads to the formation of essential compounds straw-yellow color with green fluorescence.

Procedure. To a solution of 2 drops of oil poured estrone 30 drops of concentrated sulfuric acid. Gradually develop a straw-yellow color.

Conclusions:

25. BIOCHEMISTRY OF HORMONES. HORMONES REGULATED GLUCOSE BLOOD LEVEL. DIABETES MELLITUS. GLUCOSE TOLERANCE TEST

Objective

Consolidate the knowledge about the chemical structure and mechanisms of action of individual hormones. To pay the particular attention to the endocrine pancreas pathology. Learn how to build and interpret the results of the construction of various types of sugar curves.

Problems for discussion:

1. Insulin: chemical structure, insulin synthesis, types of receptors, realization of pancreatic hormone effect at a cellular level.
2. Glucagon: chemical structure, insulin synthesis, types of receptors, realization of pancreatic hormone effect at a cellular level.
3. Hormones of the adrenal medulla: chemical structure, precursor, type of receptor in target-tissue, realization of epinephrine and norepinephrine effect at a cellular level.
4. Diabetes mellitus. Metabolic disorders. Diagnostic value of sugar curves.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 98–99.
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. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 123–125, 249–268.
5. 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 blood glucose concentration in adults is **3.9–6.1 mmol/l**.

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

***Indications for performing a glucose tolerance test*:**

– Unambiguous results of a single blood analysis on an empty stomach.

– Glucosuria: pancreatic and non-pancreatic (the first one is associated with insufficient secretion or insufficiency of insulin itself; extrapancreatic glucosuria develops, when other organs of internal secretion are impaired, in emotional stress, kidney and liver diseases, excess of carbohydrates in the diet, in pregnancy).

– Clinical signs of diabetes mellitus and its complications in normal blood glucose concentration on an empty stomach (latent forms of diabetes).

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

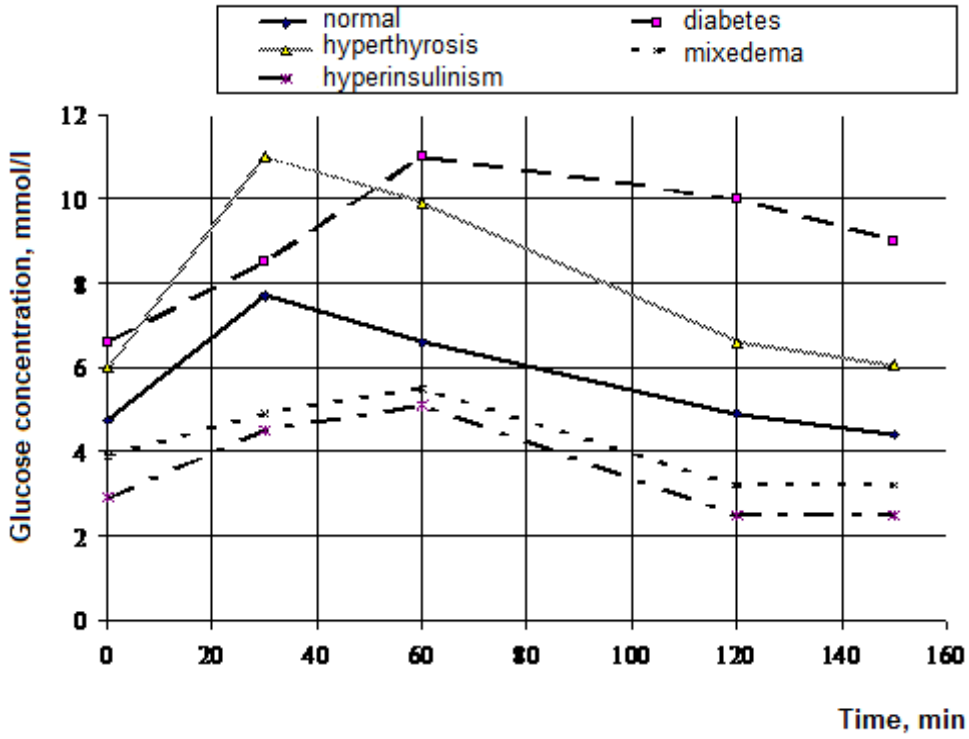
Procedure. Evaluate glucose content in analysis samples № 1–6 (See Instruction for practical class № 9 "Determination of glucose concentration in serum by an enzymatic method". Test-tube № 1 contains the serum taken before, and test-tubes № 2–6 — taken every 30 minutes after glucose loading. On the basis of received data construct a curve. Analyze the glycemic curve, put down your conclusions.

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.

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



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

Conclusion:

26. COLLOQUIUM: "NUCLEOPROTEINS METABOLISM", "SYNTHESIS OF DNA, RNA AND PROTEINS", "HORMONES"

Questions for preparation:

1. Mononucleotides, structure, terminology, biological role.
2. Modern concept of DNA structure (primary, secondary, tertiary structure). Cell localization, functions.
3. Nucleoprotein metabolism. Digestion of nucleoproteins in the digestive 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. The initial substrates, scheme of reactions, enzymes. Regulation of synthesis.
6. Biosynthesis of pyrimidine nucleotides. The initial substrates of synthesis, scheme of reactions, enzymes. Regulation of synthesis.
7. Synthesis of deoxyribonucleotides.
8. The mechanism of DNA synthesis in eukaryotes. Enzymes and substrates of synthesis. Draw a scheme of replication fork, describe formation of the Okazaki fragments.
9. RNA synthesis in eukaryotes. Enzymes and substrates of synthesis. Maturation of pre-mRNA.
10. Genetic code and its properties.
11. Recognition and translation as steps of genetic information realization in the cell. Role of t-RNA in protein synthesis, its adapter function. ARSases — role, specificity, reactions catalyzed by these enzymes.
12. Modern understanding of protein biosynthesis. Regulation of protein biosynthesis in the cell at a genetic level.
13. Posttranslational modification of protein molecules, kinds, biological role.
14. Hormones, classification by the chemical structure, site of synthesis. Peculiarities of hormonal action.
15. Hormone receptors, classification. Structure of intracellular receptors (nuclear and cytosolic), receptors of a plasma membrane (canal-forming receptors, 1-TMS and 7-TMS receptors).
16. Mechanisms of signal transduction for steroid, amino acid-derived, protein-peptide hormones. Role of G-proteins, secondary messengers (cAMP, cGMP, IP_3, Ca^{2+}, DAG), proteinkinases. Signal transduction from intracellular and 1-TMS-receptors.
17. Hypothalamus hormones: chemical structure, type of receptor in target-cells and mechanism of a hormone signal transduction, response of hypophysis cells to the liberins and statins action.
18. Adenohypophysis hormones: chemical structure, types of receptors in target-tissues and mechanism of a hormone signal transduction, realization of hormone effect at a target-tissue level. The role of excessive and insufficient secretion of hormones.
19. Neurohypophysis hormones: chemical structure, type of receptor in target-tissue and mechanism of a hormone 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.
20. Thyroxine and triiodothyronine: chemical structure, preursor, 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.
21. 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".

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

23. 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.

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

27. BIOCHEMISTRY OF NUTRITION. ROLE OF PROTEINS, FATS, CARBOHYDRATES, WATER-SOLUBLE VITAMINS. VITAMIN-LIKE SUBSTANCES

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 get acquainted 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 an organism. Essential nutritive factors.

2. 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.

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

4. 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, 2015. P. 110–114, 118–120.

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. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 285–299.

5. Lecture material.

PRACTICAL PART

Determination of vitamin C

Principle of the method. The method is based on the ability of vitamin C to reduce 2,6-dichlorphenolindophenol, which in acidic medium has red staining and decolorizes after reduction; in alkaline medium it has blue staining. To preserve vitamin C from destruction the studied solution is titrated in acidic medium by alkaline solution of 2,6-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 the content of ascorbic acid in urine is not always diminished in C hypovitaminosis. Often it is normal despite considerable insufficiency of this vitamin in tissues and organs.

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

Procedure. Measure 10 ml of urine and 10 ml of distilled water into a flask, stir, acidize with 20 drops of 10 % solution of hydrochloric acid and titrate with 0.001N solution of 2,6-dichlorphenolindophenol till a rose staining appears. Use the formula to calculate the content of ascorbic acid in urine:

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

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 0.001N 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.

Conclusions:

28. BIOCHEMISTRY OF NUTRITION. FAT-SOLUBLE VITAMINS

Objective

To consolidate knowledge of biological role of fat-soluble vitamins in cells for application in medical practice.

Problems for discussion:

1. Tocopherol. The chemical nature, involved in the metabolism, signs of vitamin deficiencies, daily demand, the main sources of vitamin E.
2. Retinol. The chemical nature, involved in the metabolism, symptoms of hypo- and hypervitaminosis, daily demand, the main sources of vitamin A.
3. Vitamin D. The chemical nature, absorption, biological role, the phenomenon of hypo- and hypervitaminosis, daily demand, the main sources.
4. Vitamin K. The chemical nature, biological role, the phenomenon of hypovitaminosis, daily demand, the main sources. Synthetic derivatives. Antivitamins.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 114–118.
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. 481–488.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 277–284.
5. Lecture material.

PRACTICAL PART

Qualitative reaction to vitamins allow to detect their presence in food, medicines and medicinal herbs.

Work 1. Qualitative tests to vitamin A (retinol)

Vitamin A has β-ionone ring and a side chain consisting of two isoprene residues and primary alcohol group. Provitamin A is the yellow pigment in plants — carotene. Carotene is converted to vitamin A in the organism.

1.1. *Drummond's test*

Principle. The method is based on the ability of concentrated H_2SO_4 to take away the water from the retinol with the formation of colored products.

Procedure. Apply 1 drop of retinol acetate and 5 drops of chloroform into a dry test tube. Stir it and add 1 drop of concentrated H_2SO_4. Blue or red-violet staining is appeared which is turn into brown-red.

1.2. ***The reaction with ferric chloride***

Procedure. Aply 1 drop of retinol acetate and 5 drops of chloroform into a dry test tube. Stir and add 3 drops of ferric chloride. Staining appears.

Conclusion:

Work 2. Qualitative tests for vitamin E (α-tocopherol)

2.1. ***The reaction with the nitric acid***

Principle. Ethanolic solution of vitamin E in the presence of concentrated nitric acid is oxidized in the quinoid compound coloring in red.

Procedure. Apply 6 drops of 0.1 % ethanolic solution of vitamin E into a dry test tube. Add several grains of sucrose. Carefully add 10 drops of concentrated HNO_3 on the wall of test tube. Gently stir. After 1–2 minutes observe the red or yellowish-red staining.

2.2. ***The reaction with ferric chloride***

Principle. Ethanolic solution of α-tocopherol is oxidized $FeCl_3$ in tokoferilquinon with appearing of a red staining.

Procedure. Apply 4–5 drops of a 0.1 % ethanolic solution of α-tocopherol into a dry test tube Add 0.5 ml of a 1 % solution of $FeCl_3$, thoroughly stir. Observe red color.

Conclusion:

Work 3. Qualitative reaction to vitamin K (naphthoquinone)

There are two natural vitamin K_1 and K_2, which are derivatives of the naphthoquinone. Artificially synthesized water-soluble analog of vitamin K1 — menadione has a biological activity of the vitamin.

Qualitative reaction to menadione

Principle. In the presence of cysteine in a basic medium menadione is colored into a lemon yellow color.

Procedure. Apply 5 drops of vikasol solution on a dry glass plate. Add 5 drops of a cysteine solution and 1 drop of 10 % NaOH. Observe lemon-yellow staining.

Conclusion:

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

Objective

To consolidate knowledge of electrolyte composition of fluids of the organism, role of micro- and macroelements in cells and extracellular fluid for application in medical practice.

Problems for discussion:

1. Sodium, potassium, calcium, phosphorus. 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, selenium, iodine in tissue metabolism.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. 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. 496–497.
4. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 277–284.
5. Lecture material.

PRACTICAL PART

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 (test 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 tested sample (Et) and standard solution (Es) versus the control sample. Use cuvettes with a working width of 5 mm, wave length of 590 nm. Stir up the samples before photometry.

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

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

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:

30. CONNECTIVE TISSUE PROTEINS

Objective

To form an idea of the variety of forms of connective tissues and the specific molecular composition of each form for understanding the mechanisms of occurrence and development of diseases (including dental), the development of methods of prevention and treatment.

Problems for discussion:

2. Collagen. The amino acid composition, the spatial structure. Isocollagens. Features of the synthesis of collagen. Intra- and extracellular stages of the formation of the "mature" collagen.

3. Elastin. Role in the body. Features of the primary structure. The importance of extracellular stages in the mechanisms of formation of mature elastic tissue.

4. Protein-carbohydrate complexes. Principles of classification, biological role, mechanisms of synthesis and decay.

5. Fibrillar adhesive proteins of extracellular matrix and their functions.

6. Features of the protein composition of the cartilage and bone tissues. Non-collagenous proteins, the biological role.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 132–136.
2. *Gubsky, Yu*. Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 446-465.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 535–555.
4. Lecture material.

31. BIOCHEMISTRY OF TEETH AND ORAL CAVITY FLUIDS

Objective

To get notion of the chemical composition of bone and tooth tissues, and to consolidate knowledge of the oral fluid composition for understanding the molecular mechanisms of dental diseases development.

Problems for discussion:

1. The chemical composition of the tooth (enamel, dentin, cement, pulp) and bone tissue. Collagen and non-collagenous proteins, protein-carbohydrate complexes. The mineral component of bone and teeth. The chemical composition. Hydroxyapatite crystals.

2. Theories of mineralization of bone tissue and hard tooth tissues. The role of organic molecules in the mechanisms of mineralization. Stages of isomorphic substitution of elements of the crystal lattice and its role in the formation of apatite crystals. Factors influencing on the processes of mineralization.

3. Ca^{2+}-binding proteins and their role in the body.

4. Oral fluid. The chemical composition. Functions. Saliva from the ducts of the salivary glands. Unstimulated and stimulated saliva. Calcium and phosphate ions of the oral fluid. The role of saliva in the processes of enamel remineralization.

5. Proteins and enzymes of the oral fluid and their role.

6. "Gingival fluid" and dental liquor, the chemical composition. The value for the oral tissues.

7. Surface formations on the enamel. Origin and chemical composition.

8. Fluorine and its role in life processes. Fluorine sources and requirements.

9. The effect of nutrition on dental health. The role of carbohydrates, proteins, vitamins and trace elements. Caries. Causes, development mechanism, prevention.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 137–141.
2. *Gubsky, Yu*. Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 415-429.
3. Lecture material.

PRACTICAL PART

Work 1. Determination of pH of the saliva

The normal pH of saliva — 6.4–7.3.

Procedure. Apply a drop of saliva on the litmus paper and determine its response:

– litmus paper turns red, the acid reaction;

– paper turns blue, alkaline reaction.

Work 2. Detection of mineral (inorganic) components in bone

Bones and teeth are related to dense connective tissue. They have a high content of mineral substances, mostly calcium phosphate. The following research methods are used for qualitative analysis of inorganic substances of bone or tooth mineralizate:

1. Determination of sulphates. In an acidic environment, sulfates of bone (tooth) mineralizate form a white precipitate ($BaSO_4$) with barium chloride.

Procedure. Take 20 drops of mineralizate and add slowly, dropwise 5 drops of HCl (10 % solution). Then add a solution of $BaCl_2$ to form a precipitate. Filter off the precipitate. Heat the filtrate over a spirit lamp flame and boil for 2–3 minutes.

The turbidity appears again due to the release of sulfuric acid.

2. Determination of phosphates. Phosphates of the bone (tooth) mineralizate form a yellow crystalline precipitate (ammonium phosphomolybdate) in the reaction with a molybdenum reagent.

Procedure. Pour 20–30 drops of a molybdenum reagent solution into the test tube and heat the solution over the flame of a spirit lamp to a simmer (do not boil!). Then add a few drops of mineralizate. A yellow precipitate is formed.

3. Determination of calcium. Calcium of bone (tooth) mineralizate is precipitated by addition of ammonium oxalate.

Procedure. To 20 drops of mineralizate pour 1–2 drops of 10 % solution of acetic acid and 2–3 drops of 5 % aqueous solution of ammonium oxalate. Crystalline precipitate of calcium oxalate appears.

Work 3. Detection of proteins in bone tissue

Procedure. A small piece of bone introduce into the tube containing 10 drops of 10 % solution of NaOH. Bring to the boil. Proteins thus dissolve in alkali. After cooling add a drop of 1 % solution of $CuSO_4$ (biuret reaction). Appearance of purple coloration indicates the presence of protein.

Work 4. Quantitative determination of chlorides in the saliva by Levinson

The principle of the method. Argentometric sedimentation method is based on the ability of silver ions to form insoluble salts with chloride ions. The amount of deposited material ($AgNO_3$) is equivalent to the content of chloride ions.

The titration of chloride ions with silver nitrate is carried out in the presence of an indicator K_2CrO_4. Upon reaching the titration equivalent point the excess of silver ions form a brick-red compound with the indicator (Ag_2CrO_4).

Procedure.

1. Sedimentation of blood proteins. Prepare a mixture solutions in two tubes: 5 ml 0,45 % $ZnSO_4$ + 1 ml 0.1 N NaOH. Then, add 0.1 ml of saliva to the 1st test tube, and 0.1 ml of H_2O dist. to the 2nd (control). Boil the tubes for 3 minutes over the flame of a spirit lamp. Then filter the content of the tubes through the cotton wool into flasks. Cotton filter should be washed twice with water (per 3 mL).

2. Precipitation of chlorine ions in the presence K_2CrO_4. Add 2 drops of 2 % K_2CrO_4 solution to the filtrate and titrate with $AgNO_3$ to change the yellow color of the solution to the brick-red.

Calculation. Subtract the volume of $AgNO_3$, gone to titrate the control solution (Vref, ml), from the volume of $AgNO_3$, spent for titration of test solution (Vtest, ml); multiply the difference by 100,11.

Vref (ml) = Vtest (ml) =

C (mmol/l) = (Vtest – Vref)·100,11 ; C (mmol/l) =

The normal content of chlorine (chloride ion) in saliva is 20–40 mmol/L.

Work 5. **Quantitative determination of calcium in saliva**

The principle of the method. The indicator chromogen black ET-00 forms a purple compound with calcium. During the titration with Trilon B (disubstituted sodium salt of EDTA forming stable complexes with calcium ions) the staining of the solution will change to the blue color in the equivalent point corresponding to binding of all calcium ions in the solution by Trilon B.

Procedure. 25 mL H_2O and 1 mL of ammonia buffer are poured into the flask. Then, 1 ml of saliva and 2 drops of indicator chromogen black are added. The solution turns purple. The solution is then titrated with 0.002 M sodium Trilon B to a blue color.

The calcium concentration in saliva is calculated from the volume of Trilon B, which was spent for titration.

Calculation:

C (mmol/l) = $V_T \cdot 1.9992$ =

The normal content of calcium in the saliva — 1.1–1.3 mmol/l.

Conclusions (compare with the content of chloride ions and calcium in the blood plasma):

32. COLLOQUIUM: "BIOCHEMISTRY OF NUTRITION", "CONNECTIVE TISSUE PROTEINS", "BIOCHEMISTRY OF THE ORAL CAVITY ORGANS AND FLUIDS"

Questions for preparation:

1. The nutritive value of proteins, fats, carbohydrates. General notion of fuel metabolism, carbohydrates and lipids storage and utilization pathways. Role of fibrous polysaccharides. Essential nutritive factors.
2. Water soluble vitamins: thiamine (B_1), riboflavin (B_2), pantothenic acid, niacin, pyridoxine (B_6), folic acid (B_9), cobalamine (B_{12}), biotin (vitamin H), ascorbic acid (vitamin C). Chemical nature, co-enzyme forms, molecular mechanisms of action, diet sources, signs of hypovitaminoses.
3. Fat soluble vitamins: A (retinol), E (tocopherol), D (calcipherol), K. Structure, role in metabolic processes, diet sources, signs of hypovitaminoses. Hypervitaminoses A, D.
4. Vitamin-like substances: bioflavonoids (vit. P), choline, lipoic acid, inositol, paraaminobenzoic acid, vit. U. Biological role.
5. Macroelements: sodium, potassium, chlorine, calcium, phosphorus, magnesium, sulfur. Role in metabolism.
6. Hormonal regulation of salt and water balance. Renin-angiotensin system, the role of aldosterone, vasopressin, atrial natriuretic factor.
7. Hormones regulating calcium and phosphorus metabolism. Chemical nature, mechanism of signal transduction in target-cells, biological action.
8. The role of iron in the organism (absorption, transport, intracellular metabolism). Iron deficiency states and iron-deficient anemias.
9. Microelements: the role of copper, selenium, iodine in tissue metabolism.
10. Vitamin-like substances: bioflavonoids (vit. P), choline, lipoic acid, inositol, paraaminobenzoic acid, vit. U, etc. Biological role.
11. Collagen. The amino acid composition, the spatial structure. Isocollagens. Features of the synthesis of collagen. Intra- and extracellular stages of the formation of the "mature" collagen.
12. Elastin. Role in the body. Features of the primary structure. The importance of extracellular stages in the mechanisms of formation of mature elastic tissue.

13. Protein-carbohydrate complexes. Principles of classification, biological role (call not less than 5 functions), mechanisms of synthesis and decay.

14. Fibrillar adhesive proteins of extracellular matrix and their functions.

15. Features of the protein composition of the cartilage and bone tissues. Non-collagenous proteins, the biological role.

16. The chemical composition of the tooth (enamel, dentin, cement) and bone tissue. Collagen and non-collagenous proteins, protein-carbohydrate complexes. The mineral component of bone and teeth. The chemical composition. Hydroxyapatite crystals.

17. Theories of mineralization of bone and hard tooth tissues. The role of organic molecules in the mechanisms of mineralization. Stages of isomorphic substitution of elements of the crystal lattice and its role in the formation of apatite crystals. Factors influencing on the processes of mineralization.

18. Ca^{2+}-binding proteins and their role in the body.

19. Oral fluid. The chemical composition. Functions. Saliva from the ducts of the salivary glands. Unstimulated and stimulated saliva. Calcium and phosphate ions of saliva. Role of saliva in enamel remineralization processes.

20. Proteins and enzymes of oral liquid and their role.

21. "Gingival fluid" and dental liquor, the chemical composition. The value for the oral tissues.

22. Surface formations on the enamel. Origin and chemical composition.

23. Fluorine and its role in life processes. Fluorine sources and requirements.

24. The effect of nutrition on dental health. The role of carbohydrates, proteins, vitamins and trace elements. Caries. Causes, development mechanism, prevention.

33. BIOCHEMISTRY OF THE LIVER

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.

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.

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 2015. P. 101–104.
2. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 270–285.
3. *Konevalova, N. Yu.* Clinical biochemistry. Materials for the state examination in biochemistry / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 19–31.
4. Lecture material.

PRACTICAL PART

Determination of total bilirubin in serum

Principle of the method. Diazoreagent 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 diazoreagent. Unconjugated (indirect) bilirubin can be soluble, if one adds 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 diazoreagent. Red-rose staining appears. In 10 minutes determine its intensity measuring the sample optical density vs water in a 5 mm wide cuvette under a green light filter (500–560 nm). The colorimetry of azobilirubin standard solution corresponding to bilirubin concentration 6.84 μM/l, is done simultaneously.

Calculate by the formula:

$$C_t\ (\mu M/l) = E_t \cdot C_s / E_s$$

Normal total bilirubin concentration in plasma (serum) is 8.55–20.52 μM/l; indirect bilirubin forms 75 % of its amount.

Conclusion:

Clinical-diagnostic significance 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-glucuroniltransferase, which is necessary for the formation of direct bilirubin.

Hemolytical jaundice (suprahepatic) is *the enhancement of erythrocyte hemolysis* that results in enhanced formation of inconjugated bilirubin because the liver has no time for its binding.

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

Mechanic jaundice (obturative, obstructive) — *the bile flow-off troubles.* It occurs in overflow of bile ducts due to obstruction, 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.

Appendix

EXAMINATION QUESTIONS (THE DENTAL FACULTY, 2018)

Enzymes

1. The role of enzymes. Principles of nomenclature and classification of enzymes.
2. The chemical nature of enzymes. General properties of enzymes.
3. The structure of the enzymes. The concept of the active site of the enzyme.
4. Coenzymes. Classification and role.
5. The mechanism of action of enzymes. Enzyme kinetics.
6. Multiple forms of enzymes and their classification.
7. General principles for the regulation of enzymatic processes.
8. The mechanism of isosteric regulation of enzymes activity.
9. The mechanism of allosteric regulation of enzyme activity.
10. Covalent modification of the enzyme structure. Types, importance.
11. Enzyme inhibitors, classification, characteristics.
12. The use of enzymes in the medical practice.

Bioenergetics

13. Metabolism and energy as an important features of life. The general concept of metabolism. Relationship between catabolism and anabolism.
14. Adenylic system and its biological significance. The mechanisms of synthesis and ways of ATP utilization.
15. The modern concept of tissue respiration. NAD- and FAD-dependent dehydrogenases, ubiquinone (coenzyme Q), cytochrome system, their chemical structure and their role in oxidative processes.
16. Oxidative phosphorylation. Chemiosmotic theory of oxidative phosphorylation.
17. Causes of the cell hypoenergetic states. Inhibitors and uncouplers of oxidative phosphorylation, the mechanism of their action.

Carbohydrate metabolism

18. Carbohydrates. Classification, the biological role.
19. Nutritional value of carbohydrates. Digestion and absorption of carbohydrates. The role of dietary fibers in digestion.
20. The synthesis and breakdown of glycogen in the liver and muscles.
21. Aerobic glucose oxidation stages, the final products. Energy yield and mechanisms of ATP synthesis.
22. Glycolysis. Energy yield, the mechanism of ATP synthesis under anaerobic conditions.
23. The fate of the final products of glycolysis — pyruvic acid and lactic acid. Gluconeogenesis. Enzymes involved in gluconeogenesis.
24. Oxidative decarboxylation of pyruvic acid and other α-keto acids, enzymes, coenzymes, biological significance.
25. Citric acid cycle. Intermediate stages, enzymes. The biological significance of the cycle. Connection with the process of oxidative phosphorylation.
26. Pentose phosphate pathway of glucose oxidation and its biological role.
27. Uronic acid pathway of glucose metabolism. The biological role.
28. Features of carbohydrate metabolism involving oral microorganisms.

Chemistry and metabolism of lipids

29. Lipids. The biological role. Classification of lipids. Their basic properties.
30. Classification of fatty acids. Polyunsaturated fatty acids. Derivatives of arachidonic acid (prostaglandins, prostacyclins, thromboxanes, leukotrienes) and their biological role.
31. Glycerophospholipids. The chemical structure, properties and biological role.
32. Cholesterol biosynthesis and biological role. Disorders of cholesterol metabolism.

33. Digestion of fats and phospholipids in the digestive tract: emulsification, enzymes, hydrolysis products, micellar dissolution. Role of bile acids in the lipid digestion.
34. Re-synthesis of triacylglycerols and phospholipids in enterocytes. Formation of chylomicrons, their composition and structure.
35. Serum lipoproteins. Classification, composition, place of formation, interconversion. The role of lipoprotein lipase, lecithin: cholesterol acyltransferase (LCAT).
36. Synthesis and mobilization of fat in adipose tissue. Hormone-sensitive lipase of adipose tissue.
37. The central role of acetyl-CoA in metabolism.
38. β-Oxidation of fatty acids. Localization in the cell. Role CoA-SH and ATP. Relationship with oxidative phosphorylation. Energy yield of β-oxidation.
39. Ketone bodies, formation of ketone bodies. Ketosis and diabetes. Significance of determination of ketone bodies in the urine.
40. The synthesis of fatty acids. Connection with glycolysis, the pentose phosphate pathway of glucose conversion, Krebs cycle. The value of CO_2, ATP, $NADPH.H^+$, biotin. Multienzyme complex for fatty acid synthesis. Activators and inhibitors of fatty acid synthesis.
41. Hormonal regulation of lipid metabolism (insulin, glucagon, thyroxine, epinephrine, norepinephrine, glucocorticoids).

Metabolism of proteins and amino acids

42. Nitrogen balance. Dietary protein requirement. The biological value of proteins.
43. Characteristics of proteases. The role of selective proteolysis.
44. Proteins digestion in the gastrointestinal tract. Role of hydrochloric acid.
45. Amino acid pool in the cells. Its utilization and sources of renewal.
46. Transamination. Enzymes. Coenzymes. The role of this process for the cell. The diagnostic value of the determination of transaminase activity in serum.
47. Ways of amino acid deamination. Enzymes and coenzymes of oxidative deamination. The biological significance of glutamate dehydrogenase reaction.
48. Use of nitrogen-free residues of amino acids. Ketogenic and glycogenic amino acids.
49. The ways of ammonia detoxification. Formation of glutamine and asparagine and their role in the transport of ammonia.
50. Formation of urea. The role of the liver in urea formation. Significance of the determination of blood urea and nonprotein nitrogen in clinical practice.
51. Decarboxylation of amino acids. The formation of biogenic amines and their role in the body.

Chemistry and metabolism of nucleic acids

52. Primary and secondary structure of DNA and RNA. Interaction of nucleic acids with proteins. The structure of the nucleoproteins.
53. The final products of pyrimidine and purine nucleotide breakdown. The value of uric acid determination in the blood and urine in clinical practice.
54. Biosynthesis of purine nucleotides. The initial substrates of synthesis. Regulation of synthesis.
55. Biosynthesis of pyrimidine nucleotides. The initial substrates of synthesis. Regulation of synthesis.
56. Matrix mechanism of DNA synthesis. Enzymes and substrates of synthesis. Features of DNA synthesis in eukaryotes.
57. RNA synthesis. Enzymes and substrates of synthesis. Features of RNA synthesis in eukaryotes.
58. The genetic code and its properties.
59. Role of t-RNA in protein synthesis. Specificity of ARSase. An adapter function of tRNA.
60. Modern concept of protein biosynthesis.

Hormones

61. Hormones. The chemical structure. Classification.
62. The mechanisms of hormone action. Role of G-proteins, second messengers, protein kinases.

63. Adenohypophysis. Role in regulating the function of the peripheral endocrine glands. Tropic hormones. Connection with the hypothalamus.
64. Hormones of the posterior pituitary: oxytocin, vasopressin. Their chemical structure, the mechanism of signal transduction in the target cells. The influence of vasopressin on metabolism.
65. Thyroid hormones. Their structure, mechanism of action, effects on metabolism. Hypo- and hyperthyroidism.
66. Hormones that regulate the metabolism of calcium and phosphorus. The chemical structure, the mechanism of signal transduction in target cells, the influence on metabolism.
67. Insulin. Chemical structure, receptors, the mechanism of signal transduction in target cells, effects on metabolism. Diabetes mellitus.
68. Glucagon. Chemical structure, receptors, the mechanism of signal transduction in target cells, effects on metabolism.
69. Glucocorticoids. Chemical structure, receptors, the mechanism of signal transduction in target cells, effects on metabolism.
70. Mineralocorticoids. Chemical structure, receptors, the mechanism of signal transduction in target cells, effects on metabolism.
71. The hormones of the adrenal medulla. Catecholamines: dopamine, epinephrine, norepinephrine. Structure, receptors, signal transduction mechanism in target cells, the influence on metabolism.
72. Male and female sex hormones, the chemical structure, receptors, signal transduction mechanism in target cells.

Water-soluble vitamins

73. General characteristics and classification of vitamins. Antivitamines.
74. Biotin. Coenzyme form. The biological role. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
75. Vitamin B_1. Participation in the construction of co-enzymes. Role in metabolism. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
76. Vitamin B_2. Structure, participation in the formation of flavin coenzymes. The biological role. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
77. Vitamin B_6. Participation in the formation of coenzymes. Role in metabolism. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
78. Vitamin B_{12}. Kobamide coenzymes. Participation in metabolism. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
79. Vitamin C. The biological significance. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
80. Pantothenic acid. Coenzymes containing pantothenic acid. The biological role. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
81. Vitamin PP. Structure. Participation in the formation of nicotinamide coenzymes. The biological significance. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
82. Folic acid. The structure, participation in the formation of coenzymes. Role in metabolism. Symptoms of vitamin deficiency. Dietary sources. The daily requirement.
83. Vitamin-like substances: bioflavonoids (vitamin P), para-aminobenzoic acid, inositol, pangamic acid, lipoic acid, choline, vitamin U and others. Biological role.

Fat-soluble vitamins

84. Vitamin A. The biological role. The phenomena of hypo-and hypervitaminosis. Dietary sources. The daily requirement.
85. Vitamin E. The biological role. The phenomena of hypovitaminosis. Dietary sources. The daily requirement.
86. Vitamin D. The structure, biological role. The phenomena of hypo- and hypervitaminosis. Dietary sources. The daily requirement.
87. Vitamin K. The biological role. Vitamin deficiency. Dietary sources. The daily requirement.

Water and mineral metabolism

88. Water. Importance of water. The biological role of sodium and potassium. Mechanisms of regulation of water and mineral balance.
89. Trace elements. Their value. The role of iron, copper, iodine, selenium.

Biochemistry of muscle

90. Chemical composition of the muscle tissue. The structure and role of contractile proteins.
91. Molecular mechanisms of muscle contraction and relaxation. Energy sources for muscle contraction.

Biochemistry of the liver

92. The role of the liver in the metabolic processes in the body. Antitoxic function of the liver.
93. Synthesis and breakdown of blood pigments. The role of the liver in the formation of bile pigments. The metabolism of bile pigments.

Blood biochemistry

94. The regulation of acid-base balance in blood. Blood buffer systems and their significance.
95. The transportation of carbon dioxide and oxygen in blood. Mechanisms of the hypoxia development.
96. Plasma proteins and their function.
97. Blood clotting. Phases of blood coagulation. The factors and mechanisms of blood coagulation.
98. The role of calcium and vitamin K in blood clotting.
99. Anticoagulant system.
100. Fibrinolysis. The biological role of fibrinolysis. Plasmin system.

Biochemistry of connective tissue, bone tissue, teeth. Biochemistry of oral fluids

101. Collagen, features of amino acid composition and spatial structure. The role of collagen in the body.
102. Elastin. Role in the body. Features of the amino acid composition and spatial structure.
103. Features of the collagen and elastin biosynthesis. The role of extracellular stages in the mechanisms of formation of extracellular matrix of connective tissue.
104. Protein-carbohydrate complexes. Classification. Role in the body. Features of synthesis and degradation.
105. Calcium and phosphorus. The metabolism in the body. Role in the processes of life. Ca^{2+}-binding proteins and their role in the body.
106. Non-collagenous proteins of the extracellular matrix. Role in the body. The mineral component of bones and teeth. The chemical composition. Hydroxyapatite crystals.
107. Chemical composition of hard tooth tissues (enamel, dentin, cement). Non-collagenous proteins of the tooth tissues and their role in mineralization.
108. The chemical composition of bone tissue. Bone proteins and their role in mineralization processes. Theories of mineralization of bone and hard tooth tissues. Factors influencing on the processes of mineralization. Stages of isomorphic substitution of elements of the crystal lattice and its role in the formation of apatite crystals.
109. The effect of nutrition on dental health. The role of carbohydrates, proteins and micronutrients. Fluorine and its role in life processes. Fluorine sources and requirement.
110. Oral fluid. The chemical composition. Functions. The role of saliva in enamel re-mineralization processes.
111. Proteins and enzymes of the oral fluid, their role in the oral cavity. The concepts of "gingival fluid" and "dental liquor", the chemical composition. The value for the oral tissues.
112. Surface formations on the enamel. Their origin and chemical composition. Caries. Causes, mechanisms of development, prevention.