BIOCHEMISTRY

MANUAL FOR INTERNATIONAL STUDENTS
OF DENTAL FACULTY

Minsk BSMU 2025

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА БИОЛОГИЧЕСКОЙ ХИМИИ

БИОХИМИЯ

BIOCHEMISTRY

Практикум для иностранных учащихся
стоматологического факультета

4-е издание, исправленное

Минск БГМУ 2025

УДК 577(076.5)(075.8)-054.6
ББК 28.072я73
Б63

Рекомендовано Научно-методическим советом университета в качестве практикума 20.11.2024 г., протокол № 3

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Биохимия = Biochemistry : практикум для иностранных учащихся стоматологического факультета / А. Д. Таганович, И. Л. Котович, А. В. Колб [и др.]. – 4-е изд., испр. – Минск : БГМУ, 2025. – 52 с.

Б63

ISBN 978-985-21-1709-8.

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 и экзамену. Первое издание вышло в 2022 году.

Предназначен для иностранных студентов, обучающихся на английском языке по специальности «Стоматология».

УДК 577(076.5)(075.8)-054.6
ББК 28.072я73

Учебное издание

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БИОХИМИЯ

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Переводчики А. Д. Таганович, И. Л. Котович, А. В. Колб,
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Компьютерная вёрстка Н. М. Федорцовой

Подписано в печать 23.12.24. Формат 60×84/8. Бумага писчая «Снегурочка».
Ризография. Гарнитура «Times». Усл. печ. л. 6,04. Уч.-изд. л. 3,9. Тираж 55 экз. Заказ 9.

Издатель и полиграфическое исполнение: учреждение образования
«Белорусский государственный медицинский университет».
Свидетельство о государственной регистрации издателя, изготовителя,
распространителя печатных изданий № 1/187 от 24.11.2023.
Ул. Ленинградская, 6, 220006, Минск.

ISBN 978-985-21-1709-8

CONTENTS

SAFETY RULES FOR WORKING IN BIOCHEMICAL LABORATORY

Working 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 are strictly forbidden. Bringing food and beverages to the laboratory is not allowed either.
3. Students must use adequate footwear in 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. Use single-use latex gloves when working with biological material and some hazardous substances. 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 photometer:

1. Turn on the device.
2. Put two cuvettes (with the control and test solutions) into the compartments so that the light beam passing through the cuvette 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 cuvette with the test solution into a light beam by moving the handle on the front panel, and click on «Д(5)» button.
6. Take the digital data from the screen.

Operation on clinical laboratory centrifuge:

1. Turn on the device.
2. Open the cover.
3. Put the test tubes into slots opposite each other — even number of test tubes, equal volumes of solution in them (!).
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 test tubes after the centrifuge has completely stopped.
9. Close the cover.

Rules for working with the pipette pump:

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

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. 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 contains another protein — egg-albumin. Add the fine powder of ammonia sulfate to the filtrate till complete saturation, i. e. till a new portion of the powder stays unsolved. Then filter out the albumin deposit. Expose the filtrate to biuret reaction: add 2 drops of 1 % solution of $CuSO_4$ + 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 undergoes 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 at 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. 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. The temperature optimum for the majority of enzymes 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 salivary α-amylase occurs till the formation of dextrins. The presence of starch can be tested using iodine solution. When starch is present, the iodine changes from brown to blue-black or purple. Dextrins, depending on their size, can give various staining with iodine: amylodextrins — violet, erythrodextrins — red-brown, maltose — yellow. End products of starch hydrolysis, maltose and glucose, have 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 saliva with water 1 : 10. Apply 2–3 ml of diluted saliva into a clean test-tube and boil it for 5 minutes, then cool it. Take three test-tubes. Add 10 drops of pre-diluted native saliva (1 : 10) to the first test tube; 10 drops of boiled saliva into the second test-tube; 10 drops of water into the third test tube (control). Add 10 drops of 1 % starch solution into all 3 test tubes. Shake all the tubes gently and place them in a thermostat at 38 °C for 10 minutes. After incubation, perform the qualitative reactions to reveal starch or products of its degradation in each test-tube.

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

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

Fill in the table with the results of the experiment.

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

Conclusion:

2. Effect of pH on the enzyme activity.

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

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

Fill in the table with the results of the experiment:

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

Conclusion:

3. Activators and inhibitors of the saliva amylase activity.

Procedure. Add 1 ml of pre-diluted saliva (1 : 10) 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 make the conclusion where an activator and where an inhibitor is active.

Fill in the table with the results of the experiment:

Test-tube №	1 (H_2O)	2 (NaCl)	3 ($CuSO_4$)
Reaction with compound iodine (color)			

Conclusion:

Work 2. Specificity of enzymes

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

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

Add 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 perform Feling's reaction to reveal glucose.

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

Fill in the table with the results of the experiment:

Test tube №	Enzyme	Substrate	Feling's reaction
1			
2			

Conclusion:

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. Isoenzymes, 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. Lecture material.

PRACTICAL PART

Determination of saliva α-amylase activity

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

Procedure. Apply per 1 ml of water into 6 test-tubes and add 1ml of diluted 1 : 10 saliva into the first one. Stir the content of this tube by pipetting several times. Take into the pipette 1 ml of the mixture and put it into the 2nd test-tube. Stir the content of this tube and put 1 ml of it into the 3rd tube and so on to the 6th test tube. Take 1 ml of mixture from the 6th test-tube and dispose it. Add 2 ml of 0.1 % of starch solution in each tube 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, brown and violet color. Mark the last tube with yellow staining where the hydrolysis has been completed and make calculations according to the example below. 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**
	Test tubes					
	1st	**2nd**	**3rd**	**4th**	**5th**	**6th**
Staining with iodine						
Conclusions						

Example of calculation. Having marked the test-tube with a complete starch hydrolysis (solution of a yellow color) with the least amount of enzyme, calculate the saliva amylase activity with recount to the amount of undiluted saliva in this test-tube. For example, yellow staining appeared in the 4th tube where the saliva was diluted 1 : 160. If 1/160 of saliva digests 2 ml of 0.1 % starch solution; 1 ml of undiluted saliva digests ***x*** ml of 0.1 % starch solution:

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

Hence, amylase activity is 320.

Calculation: ***x*** =

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 decarboxylation of pyruvate and citric acid cycle as central metabolic pathways, the significance of a hydrogen-donor function of tricarboxylic acids cycle (TCA cycle) for further oxidation-reduction (redox) reactions in the chain of tissue respiration, to understand a catabolic and anabolic functions of the citric acid cycle.

Problems for discussion:

1. Metabolism, linear and cyclic metabolic pathways, regulatory (key) enzymes.
2. Catabolism and anabolism, their distinctions and interrelations.
3. Reactions of dehydrogenation as a basic way of oxidizing substances in the organism. Pyridine-dependent and flavin-dependent dehydrogenases. The role of vitamins PP and B_2 in redox reactions. Block-structures of coenzymes NAD^+, $NADP^+$, FAD, FMN.
4. Adenylate system of the cell, its participation in energy exchange. The central role of ATP (adenosine triphosphate) in processes coupled with energy consumption. The concept of high-energy compounds. Ways of ATP synthesis: substrate-level, oxidative and photosynthetic phosphorylation.
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. 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 with oxaloacetate catalyzed by citrate synthase. The formed citric acid is exposed to conversions in the tricarboxylic acids cycle, and the released CoA-SH can be determined by Folin 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 the work a pre-prepared homogenate of the liver is used.

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 (color):			

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 bubbles.

Experiment scheme:

№	Content of test-tubes	Control (ml)	Experiment (ml)
1	Phosphate buffer pH = 7.4	2.0	2.0
2	Acetyl-CoA solution	0.5	0.5
3	Oxaloacetate solution	0.5	0.5
4	Malonic acid solution	1.0	–
5	Incubation solution	–	1.0
6	$Ca(OH)_2$ solution	1.0	1.0
7	Homogenate of the liver	0.5	0.5
10 min incubation at room temperature			
8	0.1N solution of sulfuric acid	1.0	1.0
Observed changes (gas bubbles):			

Conclusion:

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

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

Experiment scheme:

№	Content of test-tubes	Control (ml)	Experiment (ml)
1	Phosphate buffer pH = 7.4	2.0	2.0
2	Acetyl-CoA solution	–	0.5
3	Oxaloacetate solution	–	0.5
4	Distilled water	1.0	–
5	Homogenate of the liver	1.0	1.0
6	0.001N DCPI solution	1.0	1.0
Incubation at room temperature			
Observed changes (color):			

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 energetics. To learn how to apply this knowledge in further studying of cellular metabolism. To consolidate knowledge of mechanisms of reactive 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, the mechanism of an electro-chemical gradient formation.
4. Mechanisms of mitochondrial synthesis of ATP. H^+-ATP-synthase. Coupling of respiration and phosphorylation, "coupling sites". The chemiosmotic Mitchell's theory. 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. 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 and mark the observed changes:

№	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	Trichloroacetic 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 incubation at room temperature		
5	Trichloroacetic acid (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 enzyme activity regulation: reversible and irreversible regulation, isosteric and allosteric regulation, covalent modification of the enzyme structure.
7. Isoenzymes, 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. Adenylate 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, coupling sites, 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, 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. 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 glucose oxidation. 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, its purpose, LDH isoenzymes), Cori cycle. Utilization 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), its regulation.
5. Citric acid cycle (subcellular localization, reactions, energetic balance, enzymes, regulation, biological role).
6. Aerobic oxidation of glucose to CO_2 and H_2O (stages, association 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. Lecture material.

PRACTICAL PART

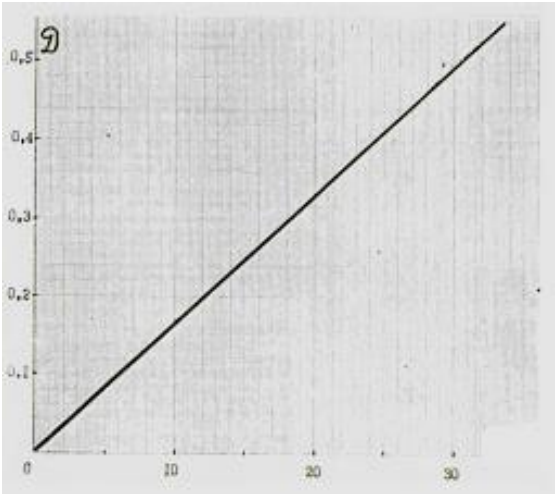
Determination of pyruvate in the urine

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. Add 1 ml of H_2O into a control tube and 1 ml of urine into a test (experimental) tube. Then add into both test-tubes 0.5 ml of 2,4-dinitro-phenylhydrosine 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 (D) of the test sample versus the control sample using 10 mm cuvettes with a green light filter (540 nm).



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.

Results:

D = Pyruvate concentration in the urine sample (μg per 1 ml) =

Pyruvate content in the 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. Uronic acid pathway (tissue and subcellular localization, biological role).
3. Regulation of blood glucose content. Mechanisms of hormonal regulation (insulin, epinephrine, glucagon, glucocorticoids etc.).
4. 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. 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 taken before applying hormones, the second one — after injecting insulin, the third one — after injecting epinephrine.

1. Determine glucose concentration in each sample.
2. According to the received data, make a conclusion which of the samples corresponds to the above 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 is of pink color. The staining 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:

Reagents	**Tested samples, ml**			**Standard sample, ml**
	№ 1	**№ 2**	**№ 3**	
Add into centrifuge test-tubes:				
Blood serum № 1	0.1	–	–	–
Blood serum № 2	–	0.1	–	–
Blood serum № 3	–	–	0.1	–
Standard glucose solution	–			0.1
Deproteinizing solution (3 % TCA)	1.0	1.0	1.0	1.0
Stir and centrifuge at 3000 rotations per minute for 15 minutes. Then add into dry test-tubes:				
Supernatant (overprecipitate fluid)	0.2	0.2	0.2	0.2
Working solution of enzymes	2.0	2.0	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	**Extinction (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, regulation).
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. EMULSIFICATION OF TRIACYLGYCEROLS BY BILE ACIDS. 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). Characteristics 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, glycerophospholipids and cholesterol esters 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. Lecture material.

PRACTICAL PART

Work 1. Emulsification of triacylglycerols by bile acids

Principle of the method. A bile acid is amphipathic, and therefore it is surface-active. Due to the surface activity, bile acids when mixed with triacylglycerols (vegetable oil) form a stable water-oil emulsion.

Procedure. Put 0.5 ml of bile into one test-tube and put 0.5 ml of water into another (control). Then add 2 drops of vegetable oil into each test-tube. Mix the contents well. Leave the test-tubes for settling. After that observe the contents of both test-tubes and make a conclusion.

Results:

Conclusion:

Work 2. Kinetics of pancreatic lipase

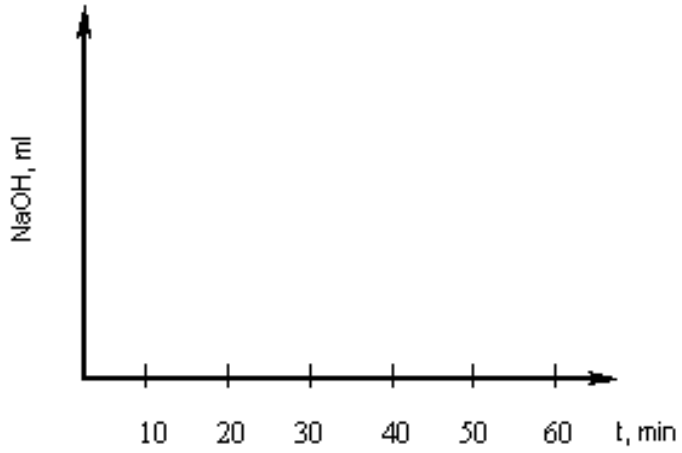
Principle of the method. The rate of lipase action in separate milk portions is evaluated by the amount of fatty acids formed in hydrolysis of milk fat for a definite interval. The amount of fatty acids is determined by alkaline titration.

Procedure. Prepare two test-tubes, each containing 5 ml of milk. Add 1 ml of water into the 1st test-tube (control) and 1 ml of bile into the 2nd one (tested). Then add per 1 ml of 5 % pancreatine (pancreatic juice) into both test-tubes and stir quickly. Take per 1 ml of the mixture from control and tested tubes into flasks for titration. 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. The obtained value of titration corresponds to the "reaction time 0". Place both test-tubes into the thermostat at 38 °C. Every 10 minutes take out per 1 ml of the mixture from control

and tested tubes into flasks and titrate by 0.05N solution of NaOH in the presence of phenolphthalein to a light-rose color. Perform 5 such determinations and fill in the table below with obtained data. Construct the graphs, that reflect the process of fat hydrolysis by lipase vs time depending on bile presence or absence.

Results:

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



Conclusion:

Work 3. Action of the pancreatic phospholipases

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

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

Result:

Conclusion:

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

Objective

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

Problems for discussion:

1. Synthesis of TAG and glycerophospholipids in the liver and adipose tissue (reactions, common steps and distinctions, sources of glycerol-3-phosphate and fatty acids, role of lipotropic factors).
2. Lipid transport forms in blood (lipoproteins) — general structure, classification. Formation and metabolism of VLDL (very low density lipoproteins), IDL (intermediate density lipoproteins), LDL (low density lipoproteins), HDL (high density lipoproteins).
3. Cholesterol, the biological role, food sources. Elimination of cholesterol from the organism, bile acids as a major end product of cholesterol metabolism, cholelithiasis. Cholesterol biosynthesis (tissue and subcellular localization, substrate, phases, reactions of the 1^{st} phase, regulation).
4. Regulatory mechanisms for maintaining cholesterol balance in cells. Delivering cholesterol into extrahepatic cells, the role of $apoB_{100}$. Unloading of the cells from excessive cholesterol, the role of HDL and LCAT.
5. Biochemistry of atherosclerosis, hypercholesterolemia as a risk factor, other risk factors. Basic principles of prevention and diagnosis of atherosclerosis (atherogenic index).
6. The mobilization of lipids from adipose tissue (scheme, cAMP-dependent mechanism of activation of hormone-sensitive lipase, hormone regulation).

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.]. 26^{th} ed. 2003. P. 205–230.
4. 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 weight, density, 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 the same mobility as α-globulins, β-lipoproteins (LDL) have the 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. Add 2 ml of 0.025M solution of $CaCl_2$ and 0.2 ml of blood plasma into a test-tube and stir. Determine the 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 determine the solution optical density (E_2) exactly in 4 minutes 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. Normal values for β-lipoproteins content — 3–4.5 g/l. The content of β-lipoproteins varies depending on the age and gender.

Results:

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 pathways of fatty acid oxidation and synthesis, sources and role of polyunsaturated fatty acids and eicosanoids. To form a notion of ketone bodies, their synthesis and catabolism in tissues, role in physiological and pathological states. To learn the role of hormones in lipid metabolism regulation. 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. Metabolism of arachidonic acid. Biosynthesis of eicosanoids (prostaglandins, leukotrienes, thromboxanes) and their biological role.
4. Ketogenesis: tissue and subcellular localization, substrates, chemistry. Synthesis of ketone bodies. The causes of ketonemia in diabetes, insufficient carbohydrate diet, starvation. Utilization of ketone bodies (interconversion, activation, including in metabolism, energy oxidation).
5. Acetyl-CoA as a central metabolite — the pathways of its formation and utilization in the cells. Interrelations between carbohydrate and lipid metabolism. Metabolism of glycerol, energy balance of its oxidation.

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. Lecture material.

PRACTICAL PART

Determination of serum cholesterol concentration by enzymatic method

Principle of the method. Cholesterol concentration in serum is determined after enzymatic hydrolysis of its esters and oxidation in the following reactions:

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

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

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

The resulting product (quinoneimine) is of pink color. The intensity of staining is directly proportional to the concentration of cholesterol and measured photometrically.

Procedure. Add the reagents 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 versus 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:

$$\mathbf{C_t\ (mmol\ /\ L) = 5.17 \times (E_t\ /\ E_{st}),}$$

where C_t — cholesterol concentration in the tested sample, 5.17 — cholesterol concentration in the standard sample, E_t — optical density of the tested sample, E_{st} — optical density of the standard sample.

Results:

$E_{t.}$ = $E_{st.}$ =

Calculation: $\mathbf{C_t}$ =

Clinical and diagnostic value. The **normal values** of cholesterol content in serum are **less than 5.2 mmol/L**. 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, the central nervous system disorders, feverish states.

Conclusion:

Qualitative reactions for acetone and acetoacetic acid

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.

Procedure: Add 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 is faster when boiling.

Results:

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

Conclusion:

14. COLLOQUIUM "LIPID METABOLISM"

Questions for preparation:

1. Lipids, general characteristics, classification. Characteristics and biological role of lipid groups (chemical structures and terminology of acylglyceroles and glycerophospholipids; block-structures of waxes, sphingophospholipids, glycolipids, sulfolipids).

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 triacylglycerols, glycerophospholipids and cholesterol esters in enterocytes. Chylomicrons — structure and metabolism.

4. Synthesis of TAG and glycerophospholipids in the liver and adipose tissue, sources of glycerol-3-phosphate and fatty acids, 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. Metabolism of arachidonic acid. Biosynthesis of eicosanoids (prostaglandins, leukotrienes, thromboxanes) and their biological role.

11. 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).

12. Acetyl-CoA as a central metabolite — the pathways of its formation and utilization in the cells. Interrelations between carbohydrate and lipid metabolism. Metabolism of glycerol, energy balance of its oxidation.

13. Hormonal regulation of lipid metabolism.

15. 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. The notion of protein putrefaction — products and mechanisms of their detoxification.
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. 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, content of free hydrochloric acid and bound hydrochloric acid is done in 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_1 = X (mmol/l) =	A_1 = X (mmol/l) =	A_1 = X (mmol/l) =
Bound HCl	A_2 = $A_2 - A_1$ = X (mmol/l) =	A_2 = $A_2 - A_1$ = X (mmol/l) =	A_2 = $A_2 - A_1$ = X (mmol/l) =
Total acidity	A_3 = X (mmol/l) =	A_3 = X (mmol/l) =	A_3 = 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:

16. 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. Lecture material.

PRACTICAL PART

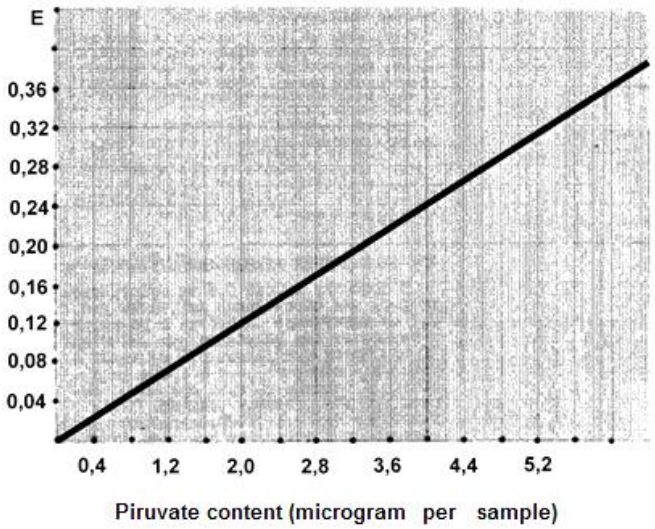
Determination of alanine aminotransferase (ALT) activity in serum

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 0.12 to 0.38 μM/h·ml for AST and 0.1–0.4 μM /h·ml for ALT.

Procedure. Prepare two test-tubes. Add per 0.5 ml of substrate solution into each test-tube, then add 0.1 ml of distilled water into the 1st one (control) and 0.1 ml of studied serum into the 2nd one (tested). Incubate both test-tubes in the thermostat at 37 °C for 30 minutes. Then add per 0.5 ml of dinitrophenylhydrasine solution and leave the samples for 20 minutes at room temperature. Then add per 5 ml of 0.4N NaOH, stir carefully and leave to stay for 10 minutes at room temperature for staining development. Measure optical density (E) of a tested sample by photometer under a green light filter (530 nm) in a 10 mm wide cuvette versus a control sample.



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

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

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

Results:

E = a =

ALT (μM/h·ml) =

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

Conclusion:

17. 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). Ammonia transport forms in blood.
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. 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 in 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-tubes must be dry. Prepare three test-tubes; add 0.2 ml of water into the 1st one (control sample), 0.2 ml of urine into the 2nd one (tested sample), and 0.2 ml of 25 mg/L urea solution into the 3rd one (standard sample). Add per 1.2 ml of 2 % solution of paradimethylaminobenzaldehyde into each of them and stir carefully. In 15 minutes perform photometry of the tested and standard samples in dry 3 mm wide cuvettes under a blue light filter (400 nm) versus a control sample.

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

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

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

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

Results:

E_t = **E_{st} =** **C_t =**

Urea content in daily urine =

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

Conclusion:

Work 2. Determination of nonprotein blood nitrogen

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

The main part of nonprotein blood nitrogen is urea nitrogen — 50 %, then nitrogen of amino acids — 25 % and nitrogen of other nitrogen-containing components. Normal values for blood nitrogen are 14.3–25.0 mmol/l; 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 (400 nm) in 5 mm thick cuvettes.

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

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

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

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

Results:

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

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

Conclusion:

18. 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. Nucleoprotein metabolism. Digestion of nucleoproteins in the gastrointestinal tract (significance, steps, enzymes).
2. Degradation of purine nucleotides (reactions, uric acid as an end-product of catabolism). Disorders of purine metabolism (hyperuricemia and gout, urolithiasis).
3. 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.
4. Degradation of pyrimidine nucleotides (end products and their fate).
5. Biosynthesis of pyrimidine nucleotides *de novo* (substrates, scheme of the process, key enzyme, regulation, role of vitamins).
6. 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. 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. Add 1.5 ml of urine into the glass for titration, then add 1 ml of 20 % solution of Sodium carbonate and 1 ml of phosphorous-tungsten Folin reagent 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.

Result:

a = **Uric acid (mg/24 h) =** **Uric acid (mmol/24 h) =**

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:

19. MATRIX BIOSYNTHESES (SYNTHESIS OF DNA, RNA, PROTEINS). MODERN TECHNIQUES OF MOLECULAR BIOLOGY

Objective

To learn molecular mechanisms of replication, DNA repair, 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. Recognition and translation as steps of genetic information realization in cells. Substrate specificity of aminoacyl-tRNA synthetases. tRNA and its role in protein biosynthesis.
4. Modern concept of protein biosynthesis. Types and role of posttranslational protein modifications. Regulation of protein biosynthesis in cells at a genetic level.
5. Modern techniques of molecular biology: polymerase chain reaction and its application in dentistry.

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. Lecture material.

20. COLLOQUIUM: “METABOLISM OF SIMPLE PROTEINS”, “NUCLEOPROTEINS METABOLISM”, “SYNTHESIS OF DNA, RNA AND PROTEINS”

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. Protein putrefaction.
4. Amino acid pool of the cell — sources of amino acids and pathways of 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. Nucleoprotein metabolism. Digestion of nucleoproteins in the digestive tract (significance, steps, enzymes).
13. Degradation of purine nucleotides (reactions, uric acid as an end-product of catabolism). Disorders of purine metabolism (hyperuricemia and gout, urolithiasis).
14. Biosynthesis of purine nucleotides. The initial substrates, scheme of reactions, enzymes. Regulation of synthesis.
15. Biosynthesis of pyrimidine nucleotides. The initial substrates of synthesis, scheme of reactions, enzymes. Regulation of synthesis.
16. Synthesis of deoxyribonucleotides.
17. The mechanism of DNA synthesis in eukaryotes. Enzymes and substrates of synthesis. Draw a scheme of replication fork, describe formation of the Okazaki fragments.
18. RNA synthesis in eukaryotes: enzymes and substrates. Maturation of pre-mRNA.
19. 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.
20. Modern concept of protein biosynthesis. Regulation of protein biosynthesis in the cell at a genetic level.
21. Posttranslational modification of protein molecules, kinds, biological role.
22. Modern techniques of molecular biology: PCR and its application in dentistry.

21. HORMONES, GENERAL CHARACTERISTICS 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 (7-TMS receptors, 1-TMS receptors, ion channels).
4. Mechanisms of steroid, amino acid-derived, protein-peptide hormones action. The role of G-proteins, second messengers (cyclic nucleotides, IP_3, Ca^{2+}, diacylglycerol), protein kinases. Peculiarities of signal transduction from intracellular, 7-TMS- 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. Lecture material.

22. BIOCHEMISTRY OF HORMONES. PITUITARY HORMONES. HORMONES OF ADRENAL CORTEX. THYROID 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, thyroglobulin, type of receptor in target-tissue, realization of thyroid 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".

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. 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. When thyroiodine is broken down, potassium iodide is formed, from which iodine is easily forced out by the potassium iodate. The released iodine is revealed by starch (blue staining) in acidic medium.

Procedure. Add 24 drops of thyroiodine hydrolyzate into the test-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 % solution of sulfuric acid until decolorization and appearance of blue staining.

Work 2. Color reactions for insulin

Growth hormone gives characteristic color reactions for protein and its constituent amino acids. There are two types of color reactions: 1) universal — biuret (reveals peptide bonds in proteins and peptides), ninhydrine (reveals α-amino groups in proteins and amino acids); 2) specific — for certain amino acids — xanthoprotein (reveals aromatic amino acids), Millon's (reveals phenol ring of tyrosine), Foll's (reveals loosely bound sulfur of cysteine).

Biuret reaction

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

Ninhydrine reaction

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

Xanthoprotein reaction

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

Reaction for tyrosine (Millon's)

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

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

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

Conclusions:

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

Objective

Consolidate the knowledge about the chemical structure and mechanisms of action of individual hormones with a special focus on the endocrine pathology of the pancreas. Learn how to construct and interpret the various types of glycemic curves.

Problems for discussion:

1. Insulin: chemical structure, synthesis, types of receptors, realization of effect at a cellular level.

2. Glucagon: chemical structure, synthesis, types of receptors, realization of effect at a cellular level.

3. Hormones of the adrenal medulla (epinephrine): chemical structure, precursor, synthesis, type of receptors in target-tissues, realization of effect at a cellular level.

4. Diabetes mellitus. Metabolic disorders. Diagnostic value of glycemic 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. Lecture material.

PRACTICAL PART

Studying of carbohydrate metabolism by glucose loading

To diagnose diabetes mellitus and some other pathologic states (endocrine diseases of adrenals, hypophysis, thyroid glands, neoplasms of the brain, kidney diseases) it is important to realize the state of carbohydrate metabolism in patients. The blood glucose level is one of most significant parameters. Normal glucose concentration in plasma/serum 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 glycemic 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 fill in the table and construct a curve. Analyze the glycemic curve, put down your conclusions.

Results:

$E_{standard}$ =

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

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

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

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

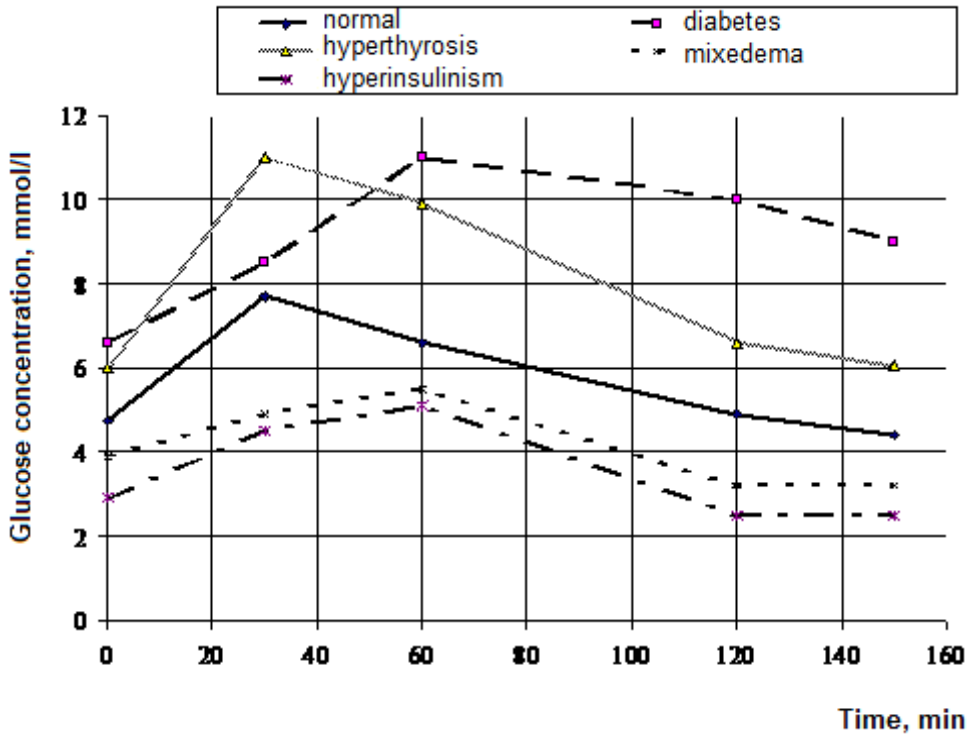
Thyroid gland diseases associated with its hyperfunction are characterized by glycemic curves with a steeper rise, which may be caused by more intensive metabolism and excitation of a sympathetic nervous system.

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

WHO criteria for diagnosing "diabetes mellitus" and "impaired glucose tolerance"

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

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



Conclusion:

24. BIOCHEMISTRY OF NUTRITION. ROLE OF PROTEINS, FATS, CARBOHYDRATES, AND VITAMINS. DETERMINATION OF VITAMIN C CONTENT IN URINE

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. 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. Tocopherol. The chemical nature, role in the metabolism, signs of deficiency, daily requirements, the main sources of vitamin E.
5. Retinol. The chemical nature, role in the metabolism, symptoms of hypo- and hypervitaminosis, daily requirements, the main sources of vitamin A.
6. Vitamin D. The chemical nature, absorption, biological role, the phenomenon of hypo- and hypervitaminosis, daily requirements, the main sources.
7. Vitamin K. The chemical nature, biological role, the phenomenon of hypovitaminosis, daily requirements, the main sources. Synthetic derivatives. Antivitamins.

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. 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 deficiency of this vitamin in tissues and organs.

The loading test is of higher diagnostic value. In healthy people taking 100 mg of vitamin C *per os* quickly results in its elevation in blood and urine. In C hypovitaminosis the tissues, suffering from its deficiency, catch the given vitamin C and its concentration in urine does not increase.

The urine of a healthy person contains 20–30 mg/24 h of vitamin C or 113.55–170.33 μM/24 h. In children the level of this vitamin decreases in scurvy as well as in acute and chronic infectious diseases.

Procedure. Add 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.

Result:

A (ml) = **X =**

Conclusion:

25. COLLOQUIUM: "HORMONES", "BIOCHEMISTRY OF NUTRITION"

Questions for preparation:

1. Hormones, classification by the chemical structure, site of synthesis. Peculiarities of hormonal action.
2. Hormone receptors, classification. Structure of intracellular receptors (nuclear and cytosolic), receptors of a plasma membrane (7-TMS receptors, 1-TMS receptors, ion channels).
3. Mechanisms of signal transduction for steroid, amino acid-derived, protein-peptide hormones. Role of G-proteins, second messengers (cAMP, cGMP, IP_3, Ca^{2+}, DAG), protein kinases. Signal transduction from intracellular, 7-TMS- and 1-TMS-receptors.
4. 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.
5. 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.
6. Thyroxine and triiodothyronine: chemical structure, precursor, thyroglobulin, type of receptor in target-tissue, realization of thyroidal hormones effects at a cellular level. The role of peroxidase and deiodase in hormones metabolism. Manifestations of hypo- and hyperthyroidism.
7. 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".
8. Hormones of the adrenal medulla: chemical structure, precursor, type of receptor in target-tissue, realization of epinephrine effect at a cellular level.
9. 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 glycemic curves.
10. 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.
11. 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.
12. Fat soluble vitamins: A (retinol), E (tocopherol), D (calcipherol), K. Structure, role in metabolic processes, diet sources, signs of hypovitaminoses. Hypervitaminoses A, D.

26. BLOOD PLASMA PROTEINS. RESPIRATORY FUNCTION OF BLOOD. HEMOGLOBINOSES. SEPARATION OF SERUM PROTEINS BY ELECTROPHORESIS ON ACETYLCELLULOSE

Objective

To consolidate knowledge of the origin of proteins and enzymes, the structure and functioning of hemoglobin, gas transport in the blood, the diagnostic value of the most important biochemical components of blood.

Problems for discussion:

1. Blood plasma proteins. Main protein fractions: albumins, globulins, fibrinogen (content, functions); albumin-globulin ratio and its diagnostic value. Biological role and diagnostic value of haptoglobin, transferrin, ceruloplasmin, trypsin inhibitors, C-reactive protein, interferon, cryoglobulins.
2. Blood plasma enzymes (secretory, indicator, excretory). Diagnostic value of plasma enzymes activity determination.
3. Features of metabolism in red blood cells. The structure of hemoglobin, heme, globin; varieties (normal and abnormal) and derivatives of hemoglobin. Synthesis of hemoglobin (scheme). Hemoglobinoses.
4. Respiratory function of the blood. Erythrocytes as a main participant of gas transport by the blood (the role of hemoglobin and carbonic anhydrase). Reversible binding of oxygen and carbon dioxide as a means of transport (mechanisms of CO_2 and O_2 binding with hemoglobin, co-operative interaction of hemoglobin subunits). Regulation of hemoglobin oxygen saturation and oxyhemoglobin dissociation (pH, 2,3-diphosphoglycerate, temperature).

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. Separation of serum proteins by electrophoresis on acetylcellulose

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

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

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

1. Electrophoretic separation of blood serum proteins.

The cuvettes of the electrophoresis apparatus are filled with a buffer solution. Strips of acetylcellulose are wetted with a buffer and tightly stretched between the cuvette compartments.

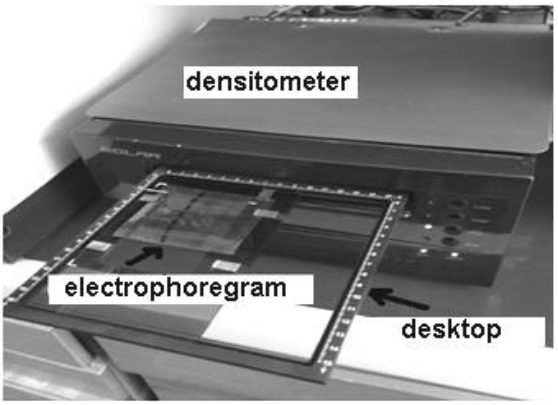
An electric current is passed through the strip for 5 minutes. Then, apparatus is turned off, and a sample of blood serum is applied on the start line on surface of the acetylcellulose film (closer to the cathode). The instrument is switched on again and electrophoretic separation of the proteins is carried out.

2. Staining of electrophoregrams.

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

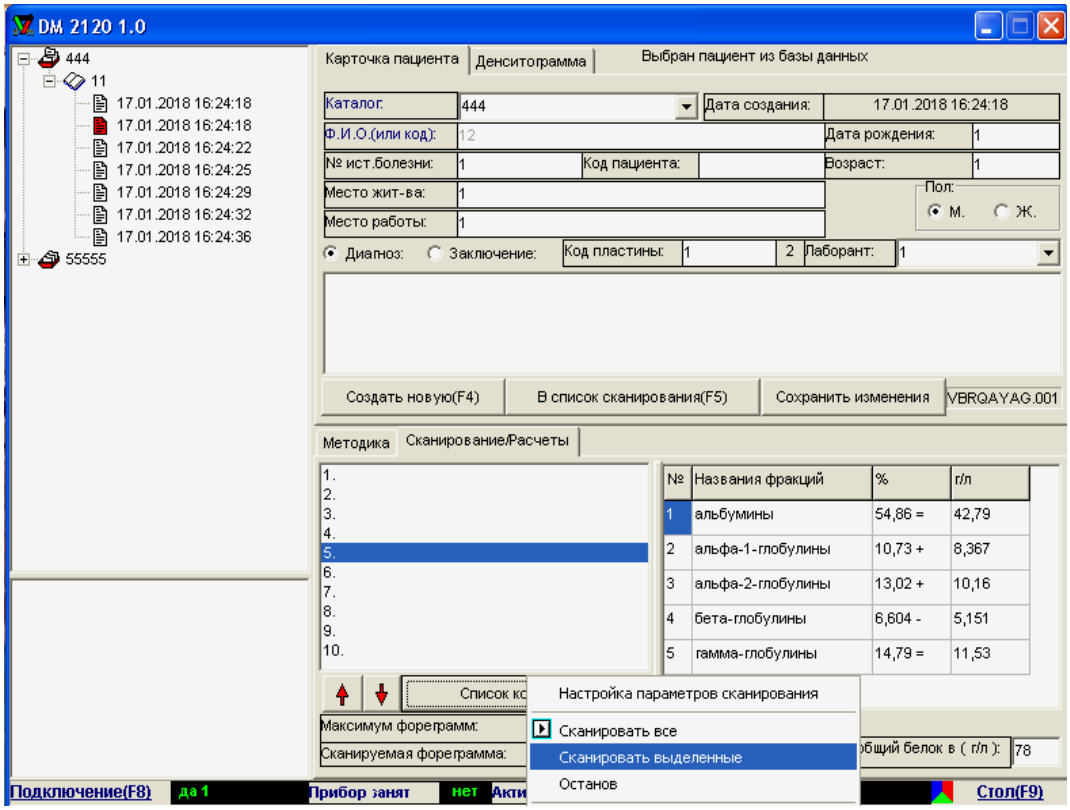
3. Quantitative evaluation of electrophoregrams is carried out by densitometry or photometry of the corresponding eluates.

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



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

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



Fill in the table with the obtained results:

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

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

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

Calculation: A / G =

Conclusion:

3.2. Photometry of the eluates of protein fractions.

Procedure.

Apply per 5 ml of 0.1 N NaOH into 5 test-tubes. Cut each fraction of proteins from the paper electrophoregram with scissors, cut the sections into small pieces and place into numbered test-tubes with alkaline. Carefully stir up the tubes and leave them for 20–30 minutes for complete extraction of the dye-stuff from the paper. Then determine the optical density of every solution versus water (control) by the photometer (a 10-mm cuvette, a red light filter with λ = 670 nm). Knowing optical densities of the solutions corresponding to every fraction, calculate the sum of all optical densities (∑) and the proportional protein content in every fraction, determine the albumin-globulin ratio.

Example.

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

Results.

$E_{alb.}$ =	$E_{\alpha1\text{-glob}}$ =	$E_{\alpha2\text{-glob}}$ =	$E_{\beta\text{-glob}}$ =	$E_{\gamma\text{-glob}}$ =	∑ =
% =	% =	% =	% =	% =	

Calculation: A / G =

Conclusion:

27. HEMOSTASIS. DETERMINATION OF CHLORIDES AND CALCIUM IN BLOOD PLASMA

Objective

To get the notion of hemostasis mechanisms. To study functioning of the blood clotting system, anticoagulant and fibrinolytic systems.

Problems for discussion:

1. Hemostasis (definition, structural-functional units and their biological role). Primary and secondary (coagulation) hemostasis. The notion of blood coagulation system functioning impairments.
2. Coagulating system (components and their origin), hemocoagulation (definition, phases and their duration, sources of phospholipid surfaces). Intrinsic and extrinsic pathways of blood coagulation.
3. Vitamin K (chemical origin, varieties, natural sources, role in coagulation).

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

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

Recommended literature

1. *Biological* chemistry. Lecture notes / A. D. Tahanovich [et al.]. Minsk : BSMU, 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. Lecture material.

PRACTICAL PART

Work 1. 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.

Results:

V_t (ml) = V_c (ml) =

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

Conclusion:

Work 2. Determination of calcium in plasma

Calcium plays an important role in realization of vital processes. It affects 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 **2.1–2.6 mmol/l**.

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

Procedure. Add 25 ml of H_2O and 1 ml of ammonia buffer solution into a flask. 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 (Vt):

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

Result:

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

Conclusion:

28. THE ROLE OF MINERALS IN THE FORMATION OF BONE TISSUE AND TEETH. THE ROLE OF CALCIUM, PHOSPHORUS, MAGNESIUM, FLUORINE, IRON, ZINC, COPPER. REGULATION OF CALCIUM AND PHOSPHORUS METABOLISM

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. Calcium and phosphate ions of the oral fluid, their role in formation of bone tissue and teeth. The role of saliva in the processes of enamel remineralization.
2. The mineral component of bone and teeth. The chemical composition. Hydroxyapatite crystals.
3. Theories of mineralization of bone tissue and hard tooth tissues. 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.
4. Ca^{2+}-binding proteins and their role in the body.
5. Hormones regulating calcium and phosphorus metabolism. Chemical nature, mechanism of signal transduction in target-cells, biological action.
6. The role of iron in the organism (absorption, transport, intracellular metabolism). Iron deficiency states and iron-deficient anemias.
7. The role of magnesium, zinc, copper in bone tissue and teeth metabolism.
8. Fluorine and its role in life processes. Fluorine sources and requirements.

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. Lecture material.

PRACTICAL PART

Work 1. 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 sulfates. In an acidic medium, 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 to boiling 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 precipitates in the presence of ammonium oxalate.

Procedure. To 20 drops of mineralizate add 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.

Results:

Work 2. 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. Add 25 mL H_2O and 1 mL of ammonia buffer 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.

Calculation. The calcium concentration in saliva is calculated from the volume of Trilon B, which was spent for titration (Vt), by the formula

$$C\ (mmol/L) = Vt \cdot 1.9992$$

Result:

Vt = C (mmol/L) =

The normal concentration of calcium in the saliva — 1.1–1.3 mmol/L.

Conclusion:

29. CONNECTIVE TISSUE PROTEINS. BIOCHEMISTRY OF TEETH AND ORAL CAVITY FLUIDS

Objective

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

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. Collagen. The amino acid composition, the spatial structure. Isocollagens. Features of the collagen synthesis. Intra- and extracellular stages of the collagen "maturation".
2. Elastin. Role in the body. Features of the primary structure. The importance of extracellular stages in the formation of mature elastic tissue.
3. Protein-carbohydrate complexes. Principles of classification, biological role, mechanisms of synthesis and degradation.
4. Fibrillar adhesive proteins of extracellular matrix and their functions.
5. Features of the protein composition of the cartilage and bone tissues. Non-collagenous proteins, the biological role.
6. The chemical composition of the tooth (enamel, dentin, cement, pulp) and bone tissue. Collagen and non-collagenous proteins, protein-carbohydrate complexes.
7. Surface formations on the enamel. Origin and chemical composition.
8. Proteins and enzymes of the oral fluid and their role.
9. Oral fluid. The chemical composition. Functions. Saliva from the ducts of the salivary glands. Unstimulated and stimulated saliva.
10. "Gingival fluid" and dental liquor, the chemical composition. The value for the oral tissues.
11. 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. 132–141.
2. *Gubsky, Yu*. Biological chemistry / Yu. Gubsky. Vinnytsia, 2017. P. 415–429, 446–465.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 535–555.
4. 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.

Result:

Work 2. Detection of proteins in bone tissue

Procedure. Put a small piece of bone into the test-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 staining indicates the presence of protein.

Result:

Work 3. 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 two test-tubes. Add 5 ml of 0.45 % $ZnSO_4$ and 1 ml of 0.1 N NaOH into each of them. Then, add 0.1 ml of saliva to the 1st test-tube (tested

sample), and 0.1 ml of distilled water to the 2nd one (control sample). Heat the test-tubes for 3 minutes (boiling). Then filter the content of each test-tube into the flask through the cotton wool (use separate flasks for tested and control samples). 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 in each flask 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 (Vc, ml), from the volume of $AgNO_3$, spent for titration of test solution (Vt, ml); multiply the difference by 100,11.

Results:

Vc (ml) = Vt (ml) =

C (mmol/L) = (Vt – Vc)·100,11 ; C (mmol/L) =

The normal concentration of chloride ions in saliva is 20–40 mmol/L.

Conclusion:

30. BIOCHEMISTRY OF 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 xenobiotics transformations 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. Role of the liver in detoxification, mechanisms: protective synthesis, acylation, microsomal oxidation, conjugation.
6. The role of the liver in pigment metabolism: degradation of hemoglobin (scheme). 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. *Gubsky, Yu.* Biological chemistry / Yu. Gubsky, Vinnytsa, 2017. P. 245–254, 398–414.
3. *Harper's* biochemistry / R. K. Murray [et al.]. 26th ed. 2003. P. 270–285.
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, stir the content carefully 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 the optical density of the tested sample (E_t) versus water in a 5 mm wide cuvette under a green light filter (500–560 nm). Determine the optical density of azobilirubin standard solution (E_{st}) corresponding to bilirubin concentration 6.84 μM/l versus water.

Calculate by the formula:

$$C_t\ (\mu M/l) = E_t \cdot C_s / E_{st},$$

where C_t — concentration of bilirubin in the tested sample of serum; C_s — concentration of bilirubin in the azobilirubin standard solution (6,84 μM/l); E_t — optical density of the tested sample, E_{st} — optical density of the standard sample.

Results:

E_t = **E_{st} =** **C_t =**

Clinical-diagnostic significance of studying pigment metabolism. Normal total bilirubin concentration in plasma (serum) is 5.1–20.5 μM/l; indirect bilirubin forms 75 % of its amount.

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 of those born premature, 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-glucuronyltransferase, which is necessary for the formation of direct bilirubin.

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

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

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

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

Conclusion:

31. COLLOQUIUM: “BLOOD BIOCHEMISTRY”, “CONNECTIVE TISSUE PROTEINS”, “BIOCHEMISTRY OF THE ORAL CAVITY ORGANS AND FLUIDS”, “MINERALS”

Questions for preparation:

1. Plasma proteins and enzymes. Their function and diagnostic value.
2. Features of metabolism in red blood cells. Mechanisms of transport of carbon dioxide and oxygen in blood. Hemoglobin structure and function, mechanism of oxygen binding. Regulation of hemoglobin affinity to oxygen. Synthesis of hemoglobin (scheme). Hemoglobinoses.
3. 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.
4. Phases of blood coagulation. Write down the schematic reactions of the 1^{st} phase, describe the factors involved in the formation of prothrombinase during extrinsic and intrinsic pathways, mechanisms of their activation.
5. The role of calcium and vitamin K and its antagonists (anti-vitamins) in blood clotting process.
6. Fibrinolysis. The biological role of fibrinolysis. Plasmin system.
7. Anticoagulant system. Classification of anticoagulants, mechanism of their action.
8. Macroelements: calcium, phosphorus. Role in metabolism. Role of saliva in enamel remineralization processes.
9. The mineral component of bone and teeth. The chemical composition. Hydroxyapatite crystals.
10. Theories of mineralization of bone and hard tooth tissues; factors influencing on mineralization. The role of organic molecules in these mechanisms. Stages of isomorphic substitution of elements of the crystal lattice and its role in the formation of apatite crystals.
11. Ca^{2+}-binding proteins and their role in the body.
12. Hormones regulating calcium and phosphorus metabolism. Chemical nature, mechanism of signal transduction in target-cells, biological action.
13. The role of iron in the organism (absorption, transport, intracellular metabolism). Iron deficiency states and iron-deficient anemias.
14. The role of copper, magnesium, zinc in tissue metabolism.
15. Fluorine and its role in life processes. Fluorine sources and requirements
16. Collagen. The amino acid composition, the spatial structure. Isocollagens. Features of the collagen synthesis. Intra- and extracellular stages of the collagen “maturation”.
17. Elastin. Role in the body. Features of the primary structure. The importance of extracellular stages in the mechanisms of formation of mature elastic tissue.
18. Protein-carbohydrate complexes. Principles of classification, biological role (call not less than 5 functions), mechanisms of synthesis and degradation.
19. Fibrillar adhesive proteins of extracellular matrix and their functions.
20. Features of the protein composition of the cartilage and bone tissues. Non-collagenous proteins, the biological role.
21. The chemical composition of the tooth (enamel, dentin, cement) and bone tissue. Collagen and non-collagenous proteins, protein-carbohydrate complexes.
22. Oral fluid. The chemical composition. Functions. Saliva from the ducts of the salivary glands. Unstimulated and stimulated saliva.
23. Proteins and enzymes of oral liquid and their role.
24. “Gingival fluid” and dental liquor, the chemical composition, their value.
25. Surface formations on the enamel. Origin and chemical composition.
26. The effect of nutrition on dental health. The role of carbohydrates, proteins, vitamins and trace elements. Caries. Causes, development mechanism, prevention.

CONTROL OVER PRACTICAL SKILLS OF BIOCHEMICAL ANALYSIS. DETERMINATION OF GLUCOSE CONCENTRATION IN BLOOD SERUM BY ENZYMATIC METHOD (1st SEMESTER)

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 is of pink color. The staining 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:

Reagents	Tested samples, ml	Standard sample, ml
Add 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. Then add 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	Extinction (E)	Glucose concentration (mmol/l)
№		
Standard		

Conclusion:

CONTROL OVER PRACTICAL SKILLS OF BIOCHEMICAL ANALYSIS. ANALYSIS OF GASTRIC JUICE. QUANTITATIVE DETERMINATION OF CALCIUM IN SALIVA (2nd SEMESTER)

1. Analysis of gastric juice

Objective. To check:

1) the skills of the students in performing a qualitative and quantitative analysis of biological fluids;

2) their ability to interpret the findings of the analysis and give them a correct assessment;

3) understanding of the origin and diagnostic significance of pathologic components of analyzed biological fluids.

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

1. Determination of gastric juice acidity.

Results:

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

Conclusion:

2. 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. Add 25 mL H_2O and 1 mL of ammonia buffer 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.

Calculation. The calcium concentration in saliva is calculated from the volume of Trilon B, which was spent for titration (Vt), by the formula

$$C\ (mmol/L) = Vt \cdot 1.9992$$

Result:

Vt = C (mmol/L) =

The normal concentration of calcium in the saliva — 1.1–1.3 mmol/L.

Conclusion:

EXAMINATION QUESTIONS

Examination questions and materials for the preparation to the exam are available on:

ISBN 978-985-21-1709-8