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БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА БИОЛОГИЧЕСКОЙ ХИМИИ

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

Предназначено для иностранных студентов 2-го курса, обучающихся на английском языке.

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Contents

1. Topic: Introduction to practical work. Introduction to biochemistry. The structure of amino acids and peptides. Determination of the protein content in biological fluids

Objective

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

Problems for discussion

1. The subject, objectives and tasks of Biochemistry. Objects and research methods in Biochemistry.
2. Amino acids, their classification. General properties. Formulae for proteinogenic amino acids, terminology.
3. Peptides, their structure. Classification and biological role of peptides.
4. Proteins as a class of organic substances, their biological functions. Classification of proteins. Artificial synthesis of proteins and peptides.
5. Determination of total protein by a biuret method.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 4–5.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

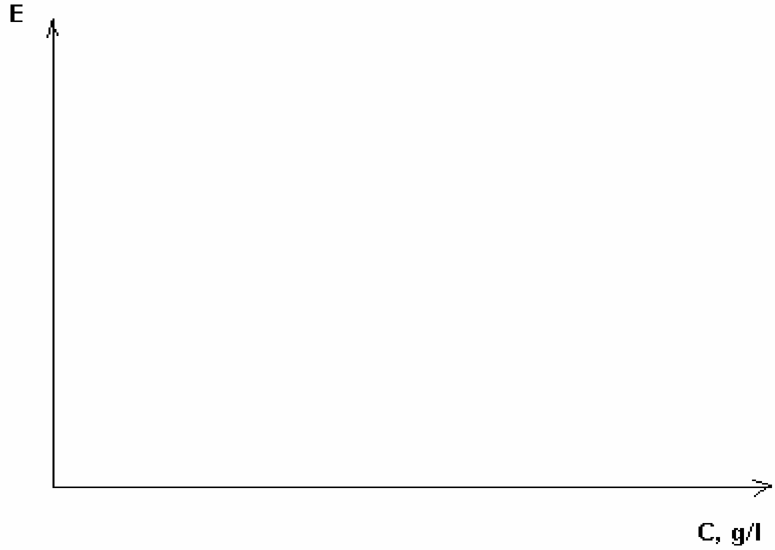
Labwork. ***Determination of total protein by a biuret method***

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

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

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

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



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

Conclusion:

2. Topic: The structure and functions of proteins. Protein hydrolysis

Objective

To form the notion of the primary structure of a protein molecule. To acquaint with the role of protein hydrolysis for vital processes and medical practice.

Problems for discussion

1. Peptide bond, its properties (X-ray structure analysis, postulates of Pauling-Corey).
2. The rules for peptides (proteins) writing, denomination and charge definition.
3. The role of protein hydrolysis for the organism and practical implementation of protein hydrolyzates in medical practice.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 5–6.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical Part

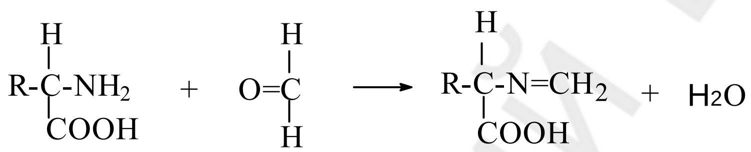
Acidic hydrolysis of the protein and formolic titration according to Söhrensen

Hydrolysis conducted in laboratory is an important step on the way to studying the primary structure of proteins. In the process of acidic hydrolysis proteins dissociate into high-molecular peptides, low-molecular peptides, dipeptides and amino acids. Some amino acids (tryptophan) are completely destroyed being exposed to such hydrolysis, while serine, treonine, cysteine, tyrosine, phenylalanine are destroyed only partially. However, the destruction percentage of these amino acids is not high. In alkaline protein hydrolysis a much more significant destruction of amino acids is observed. That is why pure 6N HCl is used in analyzing the amino acid content, it is added to a protein specimen, and then the sample, in solded ampoules, is hydrolyzed at 120^{o}C for 24, 48 and 72 hours; it is necessary for evaluating possible losses of amino acids during hydrolysis.

Protein hydrolyzates, (enzyme or acidic with tryptophan addition) are used as drugs for parenteral feeding.

Formolic titration serves for determination of the carboxylic groups number that increases when peptide bonds break down in the process of hydrolysis. As amino acids form intramolecular salts in water solutions, it is impossible to titrate carboxylic groups of amino acids by alkaline without pre-blocking of amino groups by formaldehyde.

Principle of the method. In the process of the reaction the formaldehyde blocks an α-amino group:



The formed methylene compound (methylene-amino acid) is titrated with alkaline.

Evaluating the number of carboxylic groups by titration, one can simultaneously judge by the content of amino groups, because the number of titrated carboxylic groups is equivalent to the number of amino groups bound by formaldehyde.

The method of formolic titration allows to follow the process of protein hydrolysis and to study the activity of proteolythic enzymes. In complete protein hydrolysis the number of amino and carboxylic groups in the hydrolyzate stops increasing, and the biuret reaction becomes negative (blue staining).

Accomplishment

1. Titration of carboxylic groups in the protein solution prior to hydrolysis.

Measure 1 ml of egg-white into the flask, add 5 drops of 20% neutral solution of formalin and 3 drops of 0.5% solution of phenolphthalein. Titrate from a microburette with 0.005N of NaOH to get stable light-rose colour.

2. Titration of carboxylic groups after protein hydrolysis (in *hydrolyzate*).

Measure 1.25 ml of hydrolyzate into the flask, add 3 drops of phenolphthalein solution and neutralize it adding 10% NaOH with a pipette to get stable light-rose staining (this alkaline is used to neutralize hydrochloric acid, that is why its amount is not accounted for).

If during neutralization staining becomes bright-red (a lot of NaOH), then add 1% acetic acid in drops for decolorization, its excess being neutralized by 1% solution of NaOH until staining becomes of a light-rose color. Then 5 drops of 20% neutral solution of formalin are added. Due to blocking of amino groups by formaldehyde and releasing carboxylic groups the solution gets acid reaction and phenolphthalein decolorizes. The decolorized solution is titrated by 0.005N solution of NaOH to a light-rose color and the amount of alkaline spent for titration is marked. The results of titration are put down into the table. Nitrogen of amino acids is estimated according to the amount of alkaline spent for titration proceeding from its normality. 1N alkaline solution corresponds to 14 g of Nitrogen in 1l or 14 mg in 1 ml; 1 ml of 0.005N NaOH solution corresponds to 0.07 mg of Nitrogen.

	NaOH 0.005N (ml)	**Nitrogen of α-amino groups (mg)**
Protein prior to hydrolysis		

Hydrolyzate		

Conclusion:

3. Topic: The three-dimensional structure of proteins. Physical and chemical properties of proteins. Mechanisms of proteins sedimentation

Objective

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

Problems for discussion

1. Secondary, supersecondary, tertiary, quaternary structures of a protein molecule (concept, varieties and bonds stabilizing the structure).
2. Conformational changes in functioning of proteins. Interaction of proteins with ligands.
3. Denaturation. Reversibility of denaturation. Mechanisms of denaturing factors action.
4. General physical and chemical properties of proteins (viscosity of solutions, light diffusion, optical activity, mobility in the electric field, absorption of UV rays, solubility in water).
5. Stability of protein solutions (the role of a protein charge, hydrate shell, molecular weight, molecule shape). Isoelectric state.
6. Sedimentation of proteins (reversible – "salting-out", irreversible).

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 6–9.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *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

Accomplishment. Add 20 drops of a saturated solution of $(NH_4)_2SO_4$ to 20 drops of egg-white and carefully stir. Watch the egg-globulin precipitation. Leave for 5 minutes, then filter out the deposit using a paper filter. The filtrate still have another protein — egg-albumin. Add the fine powder of ammonia sulfate to the filtrate till complete saturation, i. e. till a new portion of the powder stays unsolved. Then filter out the albumin deposit. Expose the filtrate to biuret reaction: add 2 drops of 1% solution of $CuSO_4$ + 5 drops of 10% solution of NaOH to the filtrate. A negative biuret reaction (blue staining) indicates to the absence of protein in the tested solution.

Conclusion:

Laboratory work 2. ***Irreversible sedimentation of proteins***

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

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

Accomplishment

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.

Accomplishment

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:

4. Topic: Methods of separation, isolation and purification of proteins. Compound proteins

Objective

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

Problems for discussion

1. Separation methods for proteins:
– "Salting-out";
– Chromatography (affinity, gel chromatography);
– Electrophoresis (on paper, in polyacrilamide gel using sodium dodecyl sulfate).
2. Methods of proteins purification from impurities (dialysis, gel chromatography).
3. Western-blot (purpose, steps, molecular probes).
4. The structure and functions of compound (conjugated) proteins.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 10–17.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

Column gel filtration

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

Accomplishment

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

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

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

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

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

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

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

Conclusion:

5. Topic: Enzymes. Classification, structure, properties

Objective

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

Problems for discussion

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

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 18–21, 22–26, 69–76.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

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

1. Evaluation of saliva amilase activity and its thermolability

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

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

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

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

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

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

Fill in the table with the results of the experiment.

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

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:

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

Fill in the table with the results of the experiment:

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

3. Activators and inhibitors of the saliva amylase activity

Accomplishment. Add 1 ml of saliva diluted 1:40 into 3 test-tubes. Add 2 drops of water into the 1st tube, 2 drops of 1% NaCl into the 2nd tube and 2 drops of 1% $CuSO_4$ into the 3rd one. Then add 5 drops of 1% starch solution into every tube and leave them for 2 minutes at room temperature. Then add 1 drop of compound iodine to every tube, stir, observe staining and say where an activator and where an inhibitor is active.

Fill in the table with the results of the experiment:

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

Work 2. **Specificity of enzymes**

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

Accomplishment. 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 1^{st} test-tube, 1 ml of 1% sucrose into the 2^{nd} tube. Place both test-tubes to the thermostat at $38^{o}C$ for 10 minutes, then conduct Feling's reaction to reveal glucose.

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

Fill in the table with the results of the experiment:

Test tube №	Enzyme	Substrate	Feling's reaction
1			
2			

Conclusions:

6. Topic: Regulation of enzyme activity. Determination of enzyme activity

Objective

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

Problems for discussion

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

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 21–22, 27–37.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

Work 1. ***Determination of saliva α-amilase activity***

The method is based on evaluation of the least amount of amylase (at maximum saliva dilution) that completely digests the whole added starch. Amilase activity of the saliva is expressed by the amount of 0.1% of starch solution (in ml) that is digested by 1 ml of undiluted saliva at 38 ºC 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.

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

Starch hydrolysis in the presence of saliva enzymes in various dilutions

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

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

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

Hence, amylase activity is 320.

Conclusion:

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

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

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

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

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

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

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

Conclusion:

7. Colloquium: “The chemistry of proteins, enzymes”

Questions for preparation:

1. Amino acids, their classification. General properties. Formulae for proteinogenic amino acids, terminology.
2. Peptides, their structure. Classification and biological role of peptides. Spelling rules for peptides (proteins), their names and their charge definitions.
3. Proteins as a class of organic substances, their biological functions. Classification of proteins. Artificial synthesis of proteins and peptides.
4. Qualitative determination of total protein in biological fluids.
5. Peptide bond, its properties (X-ray structure analysis, postulates of Pauling-Corey).
6. Protein hydrolysis. Role for the organism and practical implementation of protein hydrolyzates in medical practice.
7. Secondary, supersecondary, tertiary, quaternary structures of a protein molecule (concept, varieties and stabilizing bonds).
8. Conformational changes in functioning of proteins. Interaction of proteins with ligands.
9. Denaturation. Reversibility of denaturation. Mechanisms of denaturing factors action.
10. General physical and chemical properties of proteins (viscosity of solutions, light diffusion, optical activity, mobility in the electric field, absorption of UV rays, solubility in water).
11. Stability factors of protein solutions (charge of the protein, hydrate shell, molecular weight, molecule shape). Isoelectric state.
12. Separation and purification methods for proteins: “salting-out”, chromatography (affinity, gel chromatography), electrophoresis (paper, PAGE), dialysis, Western-blot. Principles.
13. Compound proteins, their classification, structure and functions.
14. Enzymes as protein catalysts.
15. Modern classification of enzymes and terminology of enzymes (systemic and working names). Enzyme code. General characteristic of classes.

16. The structure of enzymes. Coenzymes, their classification and role in catalysis. Block-structures of the NAD^+, $NADP^+$, FAD and FMN.

17. The mechanism of enzyme action. Enzyme kinetics. The effect of substrate concentration, pH, temperature on enzyme reaction velocity (molecular mechanism, graphical relationship). Michaelis constant (K_m), usage of K_m for predicting the course of biochemical reactions.

18. Specificity of enzyme action. Types of specificity.

19. Units of enzyme activity.

20. An active site of the enzyme, its organization. The theory explaining the work of the active site.

21. Structure peculiarities of allosteric enzymes, allosteric center. The concept of "key enzymes".

22. Regulation mechanisms of the enzyme-catalyzed processes rate: regulation of the enzymes amount (synthesis, break-down), enzyme activity, modification of the substrate amount, the presence of isoenzymes, joining enzymes into multienzyme complexes, compartmentation of processes.

23. Regulation of enzyme activity: covalent modification, activators and inhibitors (examples). Kinds of inhibition (irreversible and reversible, isosteric and allosteric), characteristic, examples.

24. Multiple forms of enzymes (isoenzymes and true multiple forms), examples, their biological role.

25. Medical aspects of enzymology. Examples of enzymes and inhibitors usage in diagnosis and treatment.

8. Topic: Introduction to metabolism. Central metabolic pathways (oxidative decarboxilation of pyruvate. Citric acid cycle)

Objective

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

Problems for discussion

1. Metabolism, linear and cyclic metabolic pathways, regulatory (key) enzymes.

2. Catabolism and anabolism, their distinctions and interrelations.

3. Reactions of dehydrogenation as a basic way of oxidizing substances in the organism. Pyridine-dependent and flavin-dependent dehydrogenases. The role of vitamins PP and B_2 in redox reactions. Block-structures of coenzymes NAD^+, $NADP^+$, FAD, FMN.

4. Adenilate system of the cell, its participation in energy exchange. The central role of ATP (adenosine triphosphate) in processes coupled with energy consumption. Ways of ATP synthesis: substrate-level, oxidative and photosynthetic phosphorylation. The concept of high-energy compounds.

5. Oxidative decarboxylation of pyruvate. Pyruvate dehydrogenase complex (enzymes, co-enzymes, scheme of reactions).

6. Tricarboxylic acid cycle as a central metabolic pathway. Cellular localization, reactions, enzymes, co-enzymes.

7. Dehydrogenase reactions of TCA cycle as a source of hydrogen for the system of tissue respiration. Decarboxylation in the citric acid cycle as a cellular CO_2 formation mechanism that is an end product of carbonic compounds catabolism.

8. The functions of TCA cycle: integrative, catabolic, anabolic, energetic, hydrogen-donor. Regulation. Anaplerotic reactions.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 49–68, 86–92.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

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

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

Experiment scheme:

№	Content of test-tubes	Control (ml)	Experiment (ml)
1	Phosphate buffer	2.0	2.0
2	Acetyl-CoA solution	0.5	0.5
3	Oxaloacetate solution	0.5	0.5
4	Malonic acid solution	1.0	–
5	Saline solution	–	1.0
6	Homogenate of the liver	0.5	0.5
	10 min incubation at room temperature		
7	Folin's reagent A	0.5	0.5
8	Folin's reagent B	0.5	0.5

Observed changes:

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, when sulfuric acid is added, by gas vesicles.

Experiment scheme

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

Observed changes:

Conclusion:

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

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

Experiment scheme:

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

Observed changes:

Conclusion:

9. Topic: Biological oxidation. Pathways of oxygen utilization by cells. Oxidative phosphorYlation

Object

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

Problems for discussion

1. Tissue respiration as the process of substrates' hydrogen oxidation in the respiratory chain with formation of endogenous water in cells. Distinctions of water formation in the process of tissue respiration from a similar process in vitro.
2. The structure of the respiratory chain components, enzyme complexes, co-enzymes, functioning mechanism.
3. The diagram of the respiratory chain, phosphorylation points, the mechanism of an electro-chemical gradient formation.
4. Mechanisms of mitochondrial synthesis of ATP. H^+-ATP-synthase. Coupling of respiration and phosphorylation. The chemiosmotic theory of Mitchell. Phosphorylation ratio (P/O) for various substrates supplying hydrogen to the respiratory chain.
5. Regulation of the respiratory chain and H^+-ATP-synthase.
6. Causes for the hypoenergetic states development. Uncoupling of oxidative phosphorylation (mechanism, uncoupling agents). Inhibitors of electron transport and oxidative phosphorylation.
7. Microsomal oxidation, its role for the cell.
8. Reactive oxygen species. Antioxidant defence.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 69–85.

2. *Harper's* biochemistry / R. K. Murray [et al.].

3. *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”.

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

№	Content of test-tubes	Control	Test		
		1 (ml)	2(ml)	3(ml)	4(ml)
1	Incubation mixture	1.0	1.0	1.0	1.0
2	Saline solution	0.5	–	–	–
3	Malate solution	–	0.5	–	–
4	Succinate solution	–	–	0.5	–
5	Ascorbate solution + Cytochrom c	–	–	–	0.5
6	Mitochondria suspension	0.5	0.5	0.5	0.5
	10 min incubation at room temperature, then add:				
7	Trichloracetic acid (TCA)	1.0	1.0	1.0	1.0
8	Ammonia molybdate solution	0.5	0.5	0.5	0.5
9	Reducing solution of Fiske and Subarrow				
10	Dilute the content of all test-tubes 1:8, 10 min incubation				

Observed changes (staining intensity by four-point scale):

P/O ratio:

Conclusion:

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

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

№	Content of test-tubes	Control (ml)	Experiment (ml)
1	Malate solution	0.5	0.5
2	2,4-DNP solution	–	0.5
3	Saline solution	0.5	–
4	Mitochondrium suspension	0.5	0.5
	10 min imcubation at room temperature		
5	TCA solution	1.0	1.0
6	Ammonia molibdate solution	0.5	0.5
7	Reducing solution	1.0	1.0

Observed changes (color):

Conclusion:

10. Topic: Digestion of carbohydrates. Glycogenesis and glycogenolysis. Glycolysis

Objective

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

Problems for discussion

1. Carbohydrates digestion, end products. Digestion impairments, their molecular mechanisms, symptoms. The role of cellulose and pectin in the human diet.
2. Absorption of carbohydrates digestion products, molecular mechanisms. The fate of absorbed monosaccharides. Glucose transport to cells.
3. Glycogen synthesis, purpose, sequence of reactions, expenditure of energy and regulation. Aglycogenosis.
4. Degradation of glycogen in the liver and muscles, sequence of reactions, regulation. Glycogenoses.
5. Glycolysis, its biological role, subcellular localization, phases (preparatory or unoxidative, oxidative), reactions, energy yield and mechanism of ATP formation. Glycolysis regulation, key enzymes.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 93–98, 112–119.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

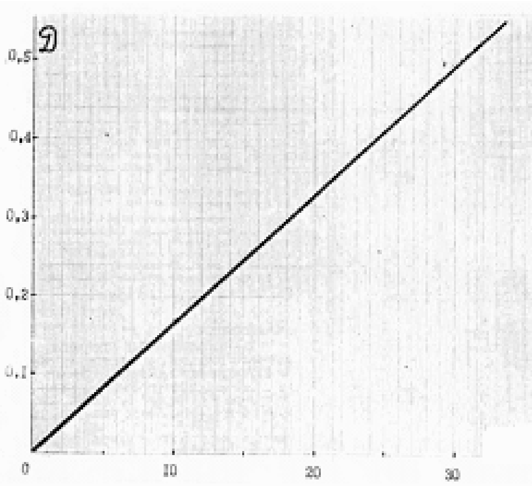
Determination of pyruvate in the urine

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.

Accomplishment. Take 2 test-tubes: apply 1 ml of H_2O into a control one and 1 ml of urine into a test tube. Then add into both test-tubes 0.5 ml of 2,4-dinitrophenylhydrosine solution and leave them for 20 minutes at room temperature. Then add 5 ml of 0.4N NaOH into each tube and in 10 minutes determine the optical density of the test sample versus the control sample using 10 mm cuvettes with a green light filter.



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

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

Clinical and diagnostic value. In avitaminosis and hypovitaminosis of B_1 in the blood and other tissues, especially in the brain, a great amount of pyruvate is accumulated and its excretion with urine increases. The content of this acid in the blood increases in diabetes mellitus, cardiac insufficiency, hyperfunction of the hypophysis-adrenal system. The amount of pyruvate increases after injection of some medicines — camphor, strychnine, epinephrine. In anesthesia the content of this acid in the blood decreases.

Conclusion:

11. Topic: Metabolic pathways of pyruvate. Gluconeogenesis. Aerobic oxidation of glucose to end-products (CO2 AND H2O). Determination of blood glucose

Objective

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

Problems for discussion

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

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 93–108.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

Determination of glucose concentration in serum by enzymatic method

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

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

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

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

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

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

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

where C_t — glucose concentration in serum (mg per 100 ml); C_s — glucose concentration in standard solution (100 mg per 100 ml); E_t — extinction of the tested sample; E_s — extinction of the standard sample. Conversion factor to SI units (mmol/l) — 0.0555.

Normal values of glucose concentration in plasma and serum — 70–110 mg per 100 ml (3.9–6.1 mmol/l), in cerebrospinal fluid — about 50 mg per 100 ml (2.78–3.89 mmol/l).

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

Decrease of blood glucose level (hypoglycemia) occurs in affection of the liver parenchyma, impairment of enzyme activity in glycogen break-down; hypofunction of the thyroid gland, adrenal glands, 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:

12. Topic: Secondary pathways of glucose metabolism. Effect of hormones on the blood glucose level

Objective

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

Problems for discussion

1. Pentose phosphate pathway (subcellular localization, steps, key enzymes, metabolites, biological role).

2. Glucuronic pathway (tissue and subcellular localization, biological role).

3. Metabolism of fructose, galactose.

4. Regulation of blood glucose content. Mechanisms of hormonal regulation (insulin, epinephrine, glucagon, glucocorticoids etc.).

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 108–111, 119–126.

2. *Harper's* biochemistry / R. K. Murray [et al.].

3. *Lecture* material.

Practical part

Effect of hormones on blood glucose content

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

1. Evaluate glucose content in every sample.

2. On the basis of received data make a conclusion which of the samples corresponds to the above states.

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

Accomplishment: see the preceding classes. A control sample can be prepared one for the whole group.

Calculate glucose concentration according to the formula:

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

Conversion factor to SI units (mmol/l) = 0.0555.

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

Conclusion:

13. Colloquium: "Introduction to metabolism, central metabolic pathways, biological oxidation, oxidative phosphorylation, carbohydrate metabolism"

Questions for preparation:

1. Metabolism, catabolism and anabolism, their distinctions and interrelations. Linear and cyclic metabolic pathways, regulatory (key) enzymes.

2. Pyridine-dependent and flavin-dependent dehydrogenases. Block-structures of co-enzymes NAD^+, $NADP^+$, FAD, FMN.

3. Adenilate system of the cell, its participation in energy exchange. Ways of ATP synthesis: substrate-level, oxidative and photosynthetic phosphorylation.

4. Oxidative decarboxylation of pyruvate as a central metabolic pathway. Pyruvate dehydrogenase complex (enzymes, co-enzymes, scheme of reactions).

5. Tricarboxylic acid cycle as a central metabolic pathway. Cellular localization of TCA cycle, reactions, enzymes, co-enzymes. Functions of TCA cycle. Anaplerotic reactions

6. Tissue respiration. The structure of the respiratory chain components, enzyme complexes, co-enzymes, functioning mechanism. The diagram of the respiratory chain, phosphorylation points, the mechanism of an electro-chemical gradient formation. H^+-ATP-synthase. The chemiosmotic Mitchell theory. Phosphorylation ratio (P/O) for various substrates supplying hydrogen to the respiratory chain. Regulation of the respiratory chain and H^+-ATP-synthase.

7. Causes for the hypoenergetic states development. Uncoupling of oxidative phosphorylation (mechanism, uncoupling agents). Inhibitors of electron transport and oxidative phosphorylation.

8. Microsomal oxidation, its role for the cell.

9. Active oxygen forms. Antioxidant defence.

10. Carbohydrates — classification, main representatives.

11. Carbohydrates digestion, end products, digestion impairments. The role of cellulose and pectines in the human diet.

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

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

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

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

16. Pyruvate as a central metabolite. Pathays of pyruvate conversion depending on the energetic status and peculiarities of oxidative cellular metabolism.

17. Gluconeogenesis (purpose, substrates, key reactions and enzymes, regulation, expenditure of energy).

18. Aerobic oxidation of glucose to CO_2 and H_2O (stages, energy yield, mechanisms of ATP formation).

19. Pentose phosphate pathway (subcellular localization, steps, key enzymes, metabolites, biological role, regulation).

20. Glucuronic pathway (tissue and subcellular localization, biological role).

21. Peculiarities of fructose and galactose metabolism.

22. Regulation of blood glucose content. Mechanisms of hormonal regulation (insulin, epinephrine, glucagon, glucorticoids etc.). Determination of glucose concentration in blood (the principle of glucose oxidase method).

14. Topic: Lipid metabolism. Digestion and re-synthesis. Transport of exogenous lipids. Evaluation of lipase activity

Objective

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

Problems for discussion

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

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

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

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

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 127–129, 134–135.

2. *Harper's* biochemistry / R. K. Murray [et al.].

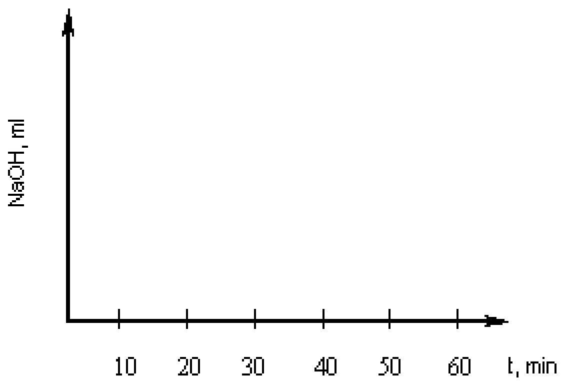
3. *Lecture* material.

Practical part

Work 1. ***Kinetics of pancreatic lipase***

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

Accomplishment. Prepare two test-tubes containing 5 ml of milk and 1 ml of 5% pancreatine (pancreas juice). Add 1 ml of water into one test-tube and 1 ml of bile — into the other. Quickly stir the fluid in the test-tubes. Take 1 ml of the mixture from every tube and apply into flasks, add 1–2 drops of 0.5% phenolphthalein solution and titrate by 0.05N solution of NaOH to a light-rose color, which doesn't disappear for 30 seconds. Place both test-tubes with remaining mixture into the thermostat at 38 ºC. Every 10 minutes take out 1 ml of the mixture and titrate by 0.05N solution of NaOH in the presence of phenolphthalein to a light-rose color. Perform 5 such determinations and on the basis of received data construct 2 curves, they will reflect the process of fat hydrolysis by lipase vs time and dependence on the presence or absence of bile.



Conclusion:

Work 2. ***Action of the pancreatic phospholipases***

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

Accomplishment. 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 ºC for 30 minutes. After incubation add 5 drops of molybdenum reagent into both tubes, heat them over the burner and cool under running water.

Conclusion:

15. Topic: Lipid transport in blood. Cholesterol metabolism. Storage and mobilization of lipids. Determination of plasma β-lipoproteins

Objective

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

Problems for discussion

1. Synthesis of TAG and glycerophospholipids in the liver and adipose tissue (reactions, general steps of synthesis, distinctions, the role of lipotropic factors). Transport forms of lipids in blood. The structure and metabolism of VLDL (very low density lipoproteins), IDL (intermediate density lipoproteins), LDL (low density lipoproteins), HDL (high density lipoproteins).
2. Cholesterol, the biological role, food sources. Elimination of cholesterol from the organism, bile acids as a major end product of cholesterol metabolism, cholelithiasis. Cholesterol biosynthesis (tissue and subcellular localization, substrates, phases, reactions of the 1st phase, regulation).
3. Mechanisms of maintaining cholesterol balance in cells.
4. Biochemistry of atherosclerosis, hypercholesterolemia as a risk factor, other risk factors. Basic principles of prevention and diagnosis of atherosclerosis (atherogenity index).
5. Mobilization of lipids from the adipose tissue. Hormone-sensitive lipase.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 135–136, 142–153.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

Determination of plasma β-lipoproteins (low density lipoproteins)

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

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

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

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

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

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

Conclusion:

16. Topic: Intracellular metabolism of fatty ACIDS. Ketone bodies. Determination of cholesterol in serum

Objective

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

Problems for discussion

1. β-oxidation as a central pathway of fatty acids catabolism. Subcellular localization of the process, activation of fatty acids, transport to mitochondria. Oxidation reactions, participation of vitamins. Association with oxidative phosphorylation, energetic yield. β-oxidation of fatty acids with an odd number of carbons, unsaturated fatty acids. Peculiarities of β-oxidation in peroxisomes.
2. α- and ω-oxidation of fatty acids as secondary metabolic pathways. Subcellular localization, products, biological role.
3. Biosynthesis of fatty acids. Subcellular localization, substrates, reactions, regulation. Peculiarities of fatty acid synthase structure. The role of malic-enzyme.
4. Polyunsaturated fatty acids as essential nutritive factors: representatives, biological role.
5. Metabolism of arachidonic acid. Biosynthesis of eicosanoids (prostaglandins, prostacyclins, leukotriens, thromboxans) and their biological role.
6. 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).
7. Acetyl-CoA as a central metabolite.
8. Hormonal regulation of lipid metabolism.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 129–133, 137–142.
2. *Harper's* biochemistry / R. K. Murray [et al.].

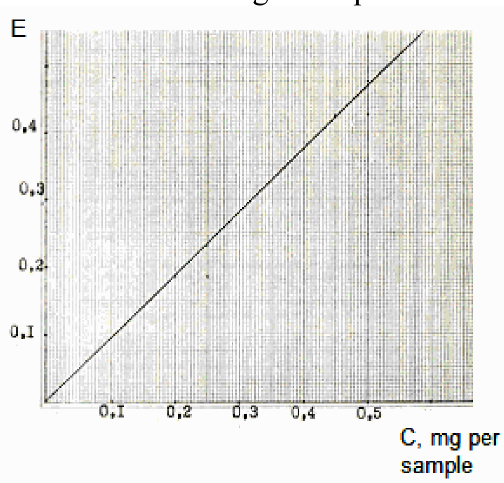
3. *Lecture* material.

Practical part

Work 1. ***Determination of total cholesterol concentration in serum***

Principle of the method. The method is based on the ability of cholesterol to give green staining in chloroform in association with acetic anhydride and concentrated sulfuric acid.

Accomplishment. Apply 0.1 ml of blood serum to the bottom of a dry test-tube. Measure 2.1 ml of reagent №1 (a mixture of glacial acetic acid, acetic anhydride and concentrated sulphuric acid) with a cylinder. **Work with reagent №1 very carefully, don't take it with a pipette and don't spill, as it is a mixture of concentrated acids!** Pour reagent №1 into the test-tube with serum, stir up and place into the thermostat at 37°C for 20 minutes (or at room temperature). Green staining appears. The intensity of staining is measured photometrically in a 5 mm thick cuvette vs water under a red light filter. Use a readymade calibration graph to determine the content of cholesterol in serum sample. Multiply the found cholesterol concentration by 1000, because 0.1 ml of serum was taken during the experiment. Conversion factor to SI units (mmol/l) is 0.0258.



Calibration graph for determination of cholesterol concentration

E=

C (mg per 100 ml) =

C (mmol/l) =

The normal values of cholesterol content in serum are 3.9–6.2 mmol/l (150–240 mg per 100 ml).

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

Conclusion:

Work 2. ***Qualitative reactions for acetone and acetoacetic acid***

Accomplishment.

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

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

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

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

Conclusion:

17. Colloquium: "Lipid metabolism"

Questions for preparation:

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

18. Topic: Control over practical skills of biochemical analysis

Objective

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

Work 1. ***Color reactions for proteins and amino acids***

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

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

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

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

Reaction	Principle of the reaction	Summary of accomplishment	Notes
Biuret reaction	This reaction reveals peptide bonds in protein. It is due to the formation of a biuret complex in alkaline medium as a result of binding copper with a protein peptide group. The solution becomes of a violet-blue color	5 drops of studied solution + 5 drops of 10% NaOH + 2 drops of 1% copper sulfate ($CuSO_4$). Stir the content of the test-tube	Biuret reaction is produced by substances containing not less than two peptide bonds
Ninhydrine reaction	Amino acids, polypeptides and proteins while boiling with water solution of ninhydrine give blue or blue-violet staining. As a result of interaction of α-amino acid with ninhydrine a shiff base is formed that is re-grouped, decarboxylated and split into aldehyde and aminodiketohydrinden	5 drops of studied solution + 5 drops of 0.5% water solution of ninhydrine. Boil the mixture for 1–2 minutes. The appearance of staining is observed	It is characteristic for amino groups in α-position included into protein, polypeptides and free amino acids
Xanthoprotein reaction	While processing the protein solution with concentrated nitric	5 drops of studied solution + 3 drops of	Positive reaction proves the presence

	acid a yellow staining appears. Aromatic amino acids in interacting with concentrated HNO_3 form nitrocompounds stained in yellow color	concentrated nitric acid (HNO_3). Carefully boil the mixture	of aromatic amino acids in the solution (trp, phe, tyr)
Reaction for tyrosine (Millon's)	Tyrosine interacting with Millon's reagent and while boiled forms bloody-red deposit of mercury salt of dinitrotyrosine due to the presence of a phenol ring in tyrosine	5 drops of studied solution + 3 drops of Millon's solution. Carefully heat the mixture	Compounds comprising a phenol ring also give positive Millon's reaction
Foll's reaction (for cysteine)	Thiol groups (-SH) in protein or in peptide are exposed to alkaline hydrolysis resulting in splitting-off sulfur in the form of lead sulfide that together with plumbite gives black or brown insoluble deposit of lead sulfide (PbS)	5 drops of studied solution + 5 drops of Foll's solution. Boil and leave it to stay for 1–2 minutes	The reaction is positive only with amino acids that contain loosely bound sulfur (cystein)

Conclusion:

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

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

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

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

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

Conclusion:

19. Topic: 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. Amino acid pool of the cell — its sources and utilization.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 154–156.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

Determination of gastric juice acidity

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

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

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

Accomplishment. 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 staining appears and record the result (the 1st mark).Without adding alkaline into the burette continue titration till lemon-yellow color appears and record the result (the 2nd mark from 0). Continue titration till rosy staining appears (the 3rd mark from 0).

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

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

where A — the amount of 0.1N 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 0.1N solution, mmol; 1000 — re-count to 1 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 (achilia) are noted.

Conclusion:

20. Topic: 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. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 156–161.

2. *Harper's* biochemistry / R. K. Murray [et al.].

3. *Lecture* material.

Practical part

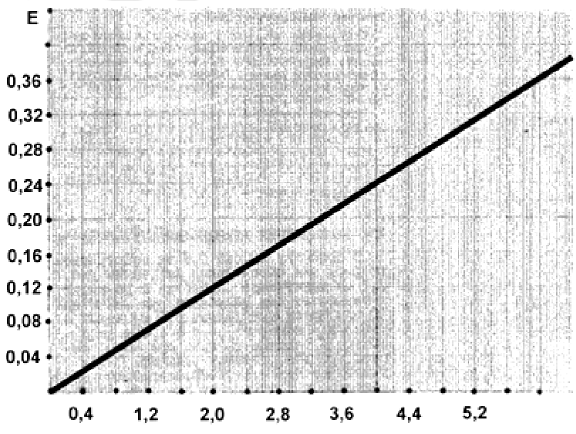
Determination of alanine aminotransferase (ALT) activity

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

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

Aminotransferase activity is expressed in micromoles of pyruvate produced in 1 incubation hour at 37°C by 1 ml of blood serum. Normal aminotransferase activity in the blood is not high and is from 0.1 to 0.45 μM/h·ml for AST and 0.1–0.68 μM /h·ml for ALT.

Accomplishment. Apply 0.5 ml of substrate solution into a test-tube, then add 0.1 ml of studied serum and incubate it in the thermostat at 37 ºC for 30 minutes. Then add 0.5 ml of dinitrophenylhydrasine solution and leave the samples for 20 minutes at room temperature. Then add 5 ml of 0.4N NaOH, carefully stir and leave to stay for



Pyruvate content (microgram per sample)

10 minutes at room temperature for staining development. Measure optical density by photoelectrocolorimeter under a green light filter (530 nm) in a 10 mm wide cuvette versus a control sample for reagents. The control sample contains all ingredients of the tested sample excluding serum, it being substituted by 0.1 ml of distilled water.

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

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

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

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

Conclusion:

21. Topic: Detoxification of ammonia. Determination of nonprotein nitrogen in blood and urea in urine

Objective

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

Problems for discussion

1. Ways of ammonia binding in cells (reductive amination of α-ketoglutarate, synthesis of glutamine and asparagine, formation of carbamoyl phosphate). Transport forms of ammonia.

2. Ammonia salts formation in kidneys (source of ammonia, the role of glutaminase and glutamate dehydrogenase, the significance of renal glutaminase activation in acidosis).

3. The role of hepatic cells in detoxification of ammonia. Ornithine cycle of urea formation (cycle pattern, substrates, enzymes, energetic supply, relation to the citric acid cycle, regulation). Fate of urea.

4. Nonprotein blood nitrogen (main components and their relative content). Principle of determination and clinical-diagnostic significance.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 162–169.

2. *Harper's* biochemistry / R. K. Murray [et al.].

3. *Lecture* material.

Practical work

Work 1. ***Determination of urea in urine***

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

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

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

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

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

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

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

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

Conclusion:

Work 2. ***Determination of nonprotein blood nitrogen***

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

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

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

Accomplishment. 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 into the second tube (standard sample) and 10 ml of water into the third one (control). Then apply per 0.5 ml of Nessler's reagent into all tubes. Perform photometry of the tested and the standard sample versus the control under a blue light filter in 5 mm thick cuvettes.

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

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

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

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

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:

22. Topic: Nucleoproteins chemistry and metabolism. Determination of uric acid in urine

Objective

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

Problems for discussion

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

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 175–180, 185.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *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.

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

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

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

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

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

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

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

Conclusion:

23. Topic (seminar): Matrix biosyntheses (synthesis of dna, rna, proteins)

Objective

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

Problems for discussion

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

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 180–184, 186–202.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

24. Colloquium: "Metabolism of simple proteins and nucleoproteins. Synthesis of dna, rna and proteins"

Questions for preparation:

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

25. Topic: Hormones, general characteristic and peculiarities of biological action. Qualitative reactions for hormones

Objective

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

Problems for discussion

1. Terminology and classification of hormones by the site of synthesis, chemical structure.
2. Peculiarities of hormones biological action.
3. Concept "hormone receptor". Classification and structure of receptors: intracellular receptors (nuclear and cytosolic), receptors of a plasma membrane (canal-forming receptors, 1-TMS and 7-TMS receptors).
4. Mechanisms of steroid, amino acid-derived, protein-peptide hormones action. The role of G-proteins, secondary messengers (cyclic nucleotides, IP_3, Ca^{2+}, diacylglycerol), proteinkinases. Peculiarities of signal transduction from intracellular and 1-TMS-receptors.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 203–208.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

Qualitative reactions for hormones

Hormones of the thyroid gland

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

Work 1. ***Qualitative reaction for thyroxine***

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

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

Hormones of the pancreas

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

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

Work 2. ***Color reactions for insulin***

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

Biuret reaction

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

Ninhydrine reaction

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

Xanthoprotein reaction

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

Reaction for thyrosine (Millon's)

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

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

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

Hormones of the adrenal medulla

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

Work 3. ***Qualitative reactions for epinephrine***

Reaction with chlorous iron

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

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

Diazoreaction

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

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

Work 4. ***Fluorescence of epinephrine oxidation products***

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

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

Hormones of sex glands

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

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

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

Work 5. ***Qualitative reactions for folliculin***

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

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

Conclusions:

26. Topic: Biochemistry of hormones. Glucose tolerance test

Objective

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

Problems for discussion

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

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

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

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

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

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

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

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

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 123–125, 209–276.

2. *Harper's* biochemistry / R. K. Murray [et al.].

3. *Lecture* material.

Practical part

Studying of carbohydrate metabolism by glucose loading

To diagnose diabetes mellitus and some pathologic states (insufficiency of the liver and kidneys function, some endocrine diseases, neoplasms of the brain, pancreas and adrenal glands, B_1 hypovitaminosis, some hereditary enzymopathies) it is important to realize the state of carbohydrate metabolism in patients. The blood glucose level is one of most significant parameters. Normal blood glucose concentration in adults is **3.9–6.1 mmol/l**.

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

Indications for performing a glucose tolerance test

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

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

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

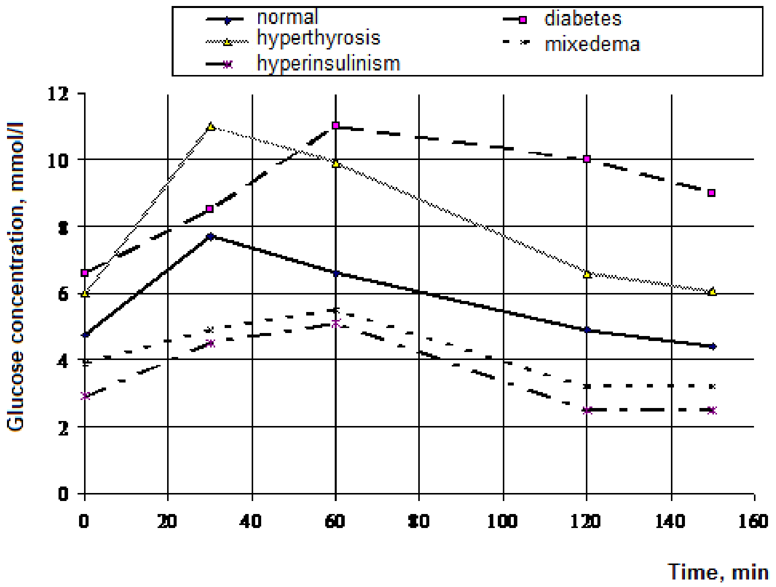
Loading. Blood is taken in the morning on an empty stomach from the patient's finger to determine the glucose content, then he is given 200 ml of glucose to drink (calculated as 1 g of glucose per 1 kg of body weight) during 5 minutes. Then the patient is taken blood from his finger every 30 minutes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

Accomplishment. Evaluate glucose content in analysis samples № 1–6 (See Instruction for practical class № 11 "Determination of glucose concentration in serum by an enzymatic method". Test-tube № 1 contains the serum taken before, and test-tubes № 2–6 — taken every 30 minutes after glucose loading. On the basis of received data construct a curve. Analyze the glycemic curve, put down your conclusions.

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

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

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



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

Conclusion:

27. Topic: Physical and chemical properties of the blood. Hemoglobinoses

Objective

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

Problems for discussion

1. Chemical composition of plasma (physiological concentrations of the most important plasma components and their origin).

2. The most important blood buffer systems: bicarbonate, hemoglobin, phosphate, protein (components and their proportion, mechanism of action, capacity). The notion of acid-base disturbances (acidosis, alkalosis).

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

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

Recommended literature

1. *Harper's* biochemistry / R. K. Murray [et al.].

Practical part

Work 1. ***Buffer properties of serum***

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

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

Conclusion:

Work 2. ***Determination of chlorides in blood according to Levinson***

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

Principle of the method. The argentometric method is based on the ability of silver ions to form insoluble salts with ions of chlorine. The amount of depositing substance ($AgNO_3$) is equivalent to the content of chloride-ions.

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

Accomplishment.

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 1^{st} tube, 0.1 ml of $H_2O_{dist.}$ into the 2^{nd} 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 0.355, if the result is expressed in mg of chlorine per 0.1 ml of blood. To get a percentage value, multiply the received value by 1000. The conversion factor to SI units (mmol/l) = 0.282.

V_t (ml) =

V_c (ml) =

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

Conclusion:

28. Topic: Blood plasma proteins. Blood clotting system

Objective

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

Problems for discussion

1. Blood plasma proteins. Main protein fractions: albumins, globulins, fibrinogen (content, functions); albumin-globulin ratio and its diagnostic value.
2. Blood plasma enzymes (secretory, indicator, excretory). Diagnostic value of plasma enzymes activity determination.
3. Understanding of hemostasis (definition, structural-functional units and their biological role). Vascular-thrombocytic and coagulation hemostasis. The notion of blood coagulation system functioning impairments.
4. Coagulating system (components and their origin), hemocoagulation (definition, phases and their duration, sources of phospholipid surfaces). Intrinsic and extrinsic pathways of blood coagulation.
5. Vitamin K (chemical origin, varieties, natural sources, role in coagulation).
6. Anticoagulant system, classification of physiological anticoagulants: primary and secondary (representatives, mechanism of action). Artificial anticoagulants of direct and indirect action.
7. Fibrinolytic system, mechanisms of fibrinolysis. Plasmin system (components and their origin, mechanism of action).

Recommended literature

1. *Konevalova, N. Yu.* Clinical biochemistry. Materials for the state examination in biochemistry / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 11–18.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *Lecture* material.

Practical part

Work 1. ***Separation of serum proteins by paper electrophoresis***

The most common methods of studying serum (plasma) protein specter in clinical laboratories are methods of electrophoresis.

Principle of the method. The method is based on the fact, that under constant electric field, electrically charged serum proteins move on the paper moistured by buffer solution with the speed depending on the amount of electric charge and molecular mass 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 which is evaluated photometrically. The relative content of protein fractions in the blood serum of a healthy adult person is expressed by the following figures: albumins — 52–65%; globulins — 29–54%; α_1-globulins — 2–5%, α_2-globulins — 7–13%, β -globulins — 8–14%, γ -globulins — 12–22%.

Accomplishment.

1. Electrophoretic separation of blood serum proteins

Soak a strip of chromatographic paper with buffer solution, dip the ends of the strip into cuvettes with this solution, place the electrodes there too — a cathode and an anode. Place the studied serum in the middle of the paper strip, on a start line and switch on electric power. At a definite time switch off the power and dry the paper at 90 ºC (the proteins being denaturized and fixed on the paper). Then dye the protein fractions.

2. Coloring of electrophoregrams

Place electrophoregrams into a cuvette with the dye-stuff (amidoblack 10 B) and leave there for 15–20 minutes. To remove the excess of the dye-stuff transfer the paper strips into a cuvette with 2% solution of acetic acid. Pour out the acid in 5–10 minutes and overflow the strips with a pure solution of acetic acid. After such processing blue spots corresponding to various fractions of proteins will be clearly seen on white background. Dry the received electrophoregrams by air.

3. Evaluation of electrophoregrams

Apply per 5 ml of 0.1N NaOH into 5 test-tubes. Cut with scissors the sections of the electrophoregram appropriate for each fraction, cut them into small pieces and place into numbered test-tubes with alkaline. Place an undyed section of a paper strip 3–10 mm wide into the 6^{th} test-tube as a control. Carefully stir up the tubes and leave them for 20–30 minutes for complete extraction of the dye-stuff from the paper. Then determine optical density of every solution versus the control by the photoelectrocolorimeter (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.

Example.

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

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

Conclusion:

Work 2. ***Determination of calcium in plasma***

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

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

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

Accomplishment. Apply 25 ml of H_2O into a flask and add 1 ml of ammonia buffer solution. Then add 1 ml of studied blood plasma and 2 drops of indicator chromogen black. The solution becomes rose-violet. Then titrate the solution with 0.002M solution of EDTA to a blue-rose color. Calculate the content of calcium in blood plasma by the volume of EDTA spent for titration:

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

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

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

Vt (ml) =

X (mg per 100 ml) =

C(mmol/l) =

Conclusion:

29. Topic: Liver biochemistry

Objective

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

Problems for discussion

1. Basic functions and chemical composition of the liver.
2. The role of the liver in carbohydrate metabolism.
3. The role of the liver in lipid metabolism.
4. The role of the liver in protein metabolism.
5. Detoxificating function of the liver, mechanisms: (protective syntheses, acylation, microsomal oxidation, conjugation).
6. The role of the liver in pigment exchange. Synthesis and degradation of hemoglobin (schemes). Normal bilirubin metabolism and its disorders.
7. Biochemical methods of diagnosing liver disturbances.

Recommended literature

1. *Konevalova, N. Yu.* Clinical biochemistry. Materials for the state examination in biochemistry / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 19–31.
2. *Harper's* biochemistry / R. K. Murray [et al.].
3. *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 cab be made soluble, if one adds ethyl alcohol to blood serum.

Accomplishment. Measure 1 ml of blood serum to a centrifuge test-tube, 2 ml of ethyl alcohol, carefully stir the content with a glass stick and centrifuge for 15 minutes at the speed of 3000 turns/min. Then pour off the supernatant into another test-tube and add 0.25 ml of diazoreagent. Red-rose staining appears. In 10 minutes determine its intensity measuring the sample optical density vs water in a 5 mm wide cuvette under a green light filter (500–560 nm). The colorimetry of azobilirubin standard solution corresponding to bilirubin concentration 0.4 mg per 100 ml (C_s), is done simultaneously.

Calculate by the formula:

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

Normal total bilirubin concentration in plasma (serum) is 0.5–1.2 mg per 100 ml (8.55–20.52 μM/l); indirect bilirubin forms 75% of its amount.

Conclusion:

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

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

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

Mechanic jaundice (obturative, obstructive) — *the bile flow-off troubles.* It occurs in overflow of bile ducts due to obstruction, their rupture and consequent entrance of bile into the blood.

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

30. Colloquium: "Hormones, biochemistry of the liver, biochemistry of the blood"

Questions for preparation:

1. Hormones, classification by the chemical structure, site of synthesis. Peculiarities of hormones biological action.
2. Hormones receptors, classification. Structure of intracellular receptors (nuclear and cytosolic), receptors of a plasma membrane (canal-forming receptors, 1-TMS and 7-TMS receptors).
3. Mechanisms of signal transduction for steroid, amino acid-derived, protein-peptide hormones. Role of G-proteins, secondary messengers (cAMP, cGMP, IP_3, Ca^{2+}, DAG), proteinkinases. Signal transduction from intracellular and 1-TMS-receptors.
4. Hypothalamus hormones: chemical structure, type of receptor in target-cells and mechanism of a hormone signal transduction, response of hypophysis cells to the liberins and statins action.
5. 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.
6. 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.

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

8. 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".

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

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

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

12. Chemical composition of the blood plasma (physiological concentrations of the most important components of the blood plasma and their origin).

13. Blood buffer systems: bicarbonate, hemoglobin, phosphate, protein (components and their proportion, mechanism of action, capacity). The notion of acid-base disturbances (acidosis, alkalosis).

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

15. Respiratory function of the blood. Transport forms of O_2 and CO_2 in blood. Reversible binding of oxygen and carbon dioxide to hemoglobin as a means of transport, co-operative interaction of hemoglobin subunits. Hypoxia, forms, mechanisms of development.

16. Blood plasma proteins. Main protein fractions: albumins, globulins, fibrinogen (content, functions); albumin-globulin ratio and its diagnostic value.

17. Blood plasma enzymes (secretory, indicator, excretory). Diagnostic value of plasma enzymes activity determination.

18. Hemostasis (definition, structural-functional units and their biological role). Vascular-thrombocytic and coagulation hemostasis. The notion of blood coagulation system functioning impairments.

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

20. Vitamin K (chemical origin, varieties, natural sources, role in coagulation).

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

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

23. Functions of the liver. Role in carbohydrate, lipid, protein metabolism.

24. Detoxificating function of the liver, mechanisms: (protective syntheses, acylation, microsomal oxidation, conjugation, etc).

25. Role of the liver in pigment exchange. Synthesis and degradation of hemoglobin (schemes). Normal bilirubin metabolism and its disorders.

26. Biochemical methods of diagnosing liver disturbances.

31. Topic: Biochemistry of the urine. Physiological and pathological components of the urine

Objective

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

Problems for discussion

1. Normal characteristics of urine volume, its density, color, transparency, pH.
2. Inorganic and organic components of the urine.
3. Diagnostic significance of the urine pathologic components determination:
   a) Renal and extrarenal proteinuria;
   b) Glucosuria in diabetes mellitus and renal glucosuria;
   c) Renal and extrarenal hematuria;
   d) Ketonuria in fasting, diabetes.

Recommended literature

1. *Harper's* biochemistry / R. K. Murray [et al.].

Practical part

Work 1. ***Determination of urine specific gravity (density)***

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

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

Work 2. ***Determination of urine acidity***

Normal urine reaction is mildly acidic.

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

1) The blue litmus paper becomes red, the red one doesn't change the color — acidic reaction;

2) The red paper becomes blue, the blue paper doesn't change the color — alkaline reaction;

3) Both papers don't change the color — neutral reaction.

One can use other indicator papers.

Work 3. ***Qualitative determination of inorganic urine components***

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

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

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

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

Accomplishment. Add 5 drops of 10% solution of HCl to 20 drops of urine. Then slowly, drop by drop, add the solution of $BaCl_2$ till the sediment forms. Filter out the formed sediment. You will reveal salts of ether-sulfuric acids while heating the filtrate in boiling water bath (5–10 minutes). Again turbidity will appear as sulfuric acid is released from ether-sulphuric acids.

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

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

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

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

Work 4. ***Qualitative determination of organic urine components***

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Work 5. ***Quantitative determination of organic urine components***

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

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

Glucose + H_2O + O_2 → Gluconolacton + H_2O
Glucose oxidase

H_2O_2 + 4-aminoantipyrin + phenol → Chinonimine + $4H_2O$
Peroxidase

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

Accomplishment

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

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

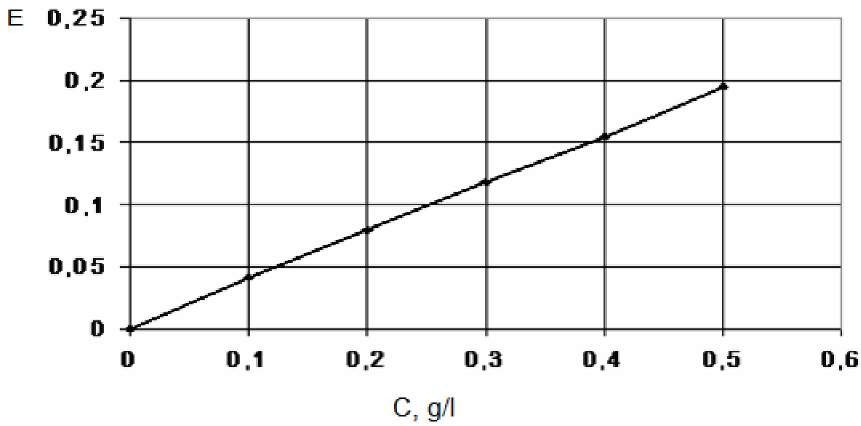
The control sample contains 0.2 ml of water and 2.0 ml of working enzyme solution. The control sample can be prepared one for the group.

Calculate by the formula:

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

where C_t — glucose concentration in urine (mg per 100 ml); C_s — glucose concentration in the standard solution (100 mg per 100 ml); E_t — extinction of the tested sample; E_s — extinction of the standard sample. Conversion factor to SI units (mmol/l) is 0.0555.

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



Calibration graph for determination total protein content in urine

5.2. Determination of protein concentration in urine

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

Accomplishment. Add 3 ml of 3% solution of sulphosalycilic acid to 1 ml of tranparent urine, stir up the mixture and in 5 minutes measure the optical density of the test-tube content by PEC under a red light filter (wave length 630–650 nm) in a 10 mm wide cuvette vs the control sample (control sample: add 3 ml of NaCl isotonic solution to 1 ml of urine). Make the calculation by a calibration graph. Take into consideration the diuresis (1200–1500 ml) while making calculations of daily loss of protein.

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

Glucose content in urine (mmol/24 h)	
Protein content in urine (g/24 h)	

Conclusion:

32. Topic: Biochemistry of nutrition. Role of proteins, fats, carbohydrates, vitamins

Objective

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

Problems for discussion

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

2. Syndrome of malnutrition (forms, characteristic).

3. Vitamins of B group: a) thiamine (B_1); b) riboflavin (B_2); c) pantothenic acid; d) niacin; e) pyridoxine (B_6); f) folic acid (B_9); g) cobalamine (B_{12}). Chemical nature, co-enzyme forms, molecular mechanisms of action.

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

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

Recommended literature

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

2. *Harper's* biochemistry / R. K. Murray.

3. *Lecture* material.

Practical part

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

Work 1. ***Qualitative reactions for vitamin B_1***

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

1. Oxidation reaction

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

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

2. Diazoreaction

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

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

Work 2. ***Qualitative reaction for vitamin B_2***

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

Accomplishment. Apply 10 drops of vitamin B_2 into a test-tube, add 5 drops of concentrated hydrochloric acid and dip a granule of metal zinc. The release of hydrogen vesicles starts, the fluid gradually becomes rosy, then decolorizes. Compare both forms of vitamin B_2 by fluorescence.

Work 3. ***Qualitative reaction to vitamin PP***

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

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

Work 4. ***Qualitative reaction for vitamin B_6***

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

Accomplishment. To 5 drops of vitamin B_6 add the equal amount of 1% solution of chlorous iron and stir. Red staining develops.

Work 5. ***Qualitative reaction to vitamin B_{12}***

B_{12} is the only vitamin containing a metal (cobalt) in its structure.

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

Accomplishment. Apply 2–3 drops of thiourea solution on a paper filter and dry over the burner. Then apply 1–2 drops of mineralizate (B_{12}) on the filter and heat again over the burner. Green staining appears, more often along the edge, it evidencing to the presence of cobalt.

Fill in the table with the results:

№	Experiment	Observed changes	Conclusions

Work 6. ***Determination of Vitamin C***

Principle of the method. The method is based on the ability of vitamin C to reduce 2,6-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 in C hypovitaminosis the content of ascorbic acid in urine is not always diminished. Often it is normal despite considerable insufficiency of this vitamin in tissues and organs.

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

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

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

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

Conclusions:

33. Topic: Biochemistry of nutrition. Mineral substances. regulation of water-electrolyte balance. Integration of metabolism

Objective

To understand principles and mechanisms of interaction of various metabolic pathways for maintaining vital activity and adaptation. To consolidate knowledge of electrolyte composition of fluids of the organism, role of micro- and macroelements in cells and extracellular fluid for application in medical practice.

Problems for discussion

1. Sodium, potassium, chlorine, calcium, phosphorus, magnesium, sulfur. The role in metabolism.
2. Hormonal regulation of salt and water balance. Renin-angiotensin system, the role of aldosterone, vasopressin, atrial natriuretic factor.
3. Hormones regulating calcium and phosphorus metabolism. Chemical nature, mechanism of signal transduction in target-cells, biological action.
4. The role of iron in the organism (absorption, transport, intracellular metabolism). Iron deficiency states and iron-deficient anemias.
5. The role of copper, zinc, manganese, cobalt, selenium, iodine and fluoride in tissue metabolism.
6. The necessity of metabolism integration, its principal components. Mechanisms of metabolic regulation.
7. Peculiarities of metabolism in the liver in fed state and between meals.
8. Inter-organ metabolism in fasting.

Recommended literature

1. *Konevalova, N. Yu.* Biochemistry lecture course / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 277–284.
2. *Konevalova, N. Yu.* Clinical biochemistry. Materials for the state examination in biochemistry / N. Yu. Konevalova, S. V. Buyanova. Vitebsk, 2005. P. 4–10.
3. *Harper's* biochemistry / R. K. Murray.
4. *Lecture* material.

Practical part

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

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

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

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

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

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

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

Conclusion:

Clinical-diagnostic significance. Normal content of Na^+ in serum is 135–150 mmol/l. Decrease of sodium concentration in blood serum results in a clinical symptom complex characterized by the appearance of apathy, loss of appetite, nausea, vomiting, impairment of reflexes, tachycardia, falling down of blood pressure, psychoses.

Hypernatremia is associated with a severe general condition of patients, elevation of body temperature, tachicardia.

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

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

Accomplishment. Take 2 test-tubes. Apply 2.0 ml of reagent into the 1st tube and add 0.04 ml of blood serum (tested sample), introduce 2.0 ml of reagent + 0.04 ml of standard solution into the 2nd tube (standard sample) and add only reagent (2.0 ml) into the 3rd tube (control sample). **It is necessary to strictly follow the sequence of introducing reagents into test-tubes (serum should be added to reagent)!** Stir and incubate for 2 minutes. Again stir carefully and incubate exactly 10 minutes at room temperature. Measure optical density of the tested sample (Et) and standard solution (Es) vs the control sample. Use cuvettes with a working width of 5 mm (wave length 590 nm). Stir up the samples before performing photometry.

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

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

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

Conclusion:

Clinical-diagnostic significance. Normal content of K^+ in serum is 3.4–5.6 mmol/l.

Decrease of potassium concentration in blood serum results in severe impairments — up to the incidence of peripheral flaccid paralysis. Psychic and mental activity becomes worse, intestinal peristalsis is depressed, meteorism develops, the stomach and urine bladder become dilated.

Hyperkalemia is associated with a sensation of tingles, disappearance of tendon reflexes, cardiac impairment. In 2-fold excess of potassium in the blood the heart stops in the phase of diastole.

34. Colloquium: biochemistry of nutrition, biochemistry of urine. Integration of metabolism

Questions for preparation:

1. The nutritive value of proteins, fats, carbohydrates. General notion of fuel metabolism, carbohydrates and lipids storage and utilization pathways. Role of fibrous polysaccharides. Essential nutritive factors.

2. Syndrome of malnutrition (forms, characteristic).

3. 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.

4. Fat soluble vitamins: A (retinol), E (tocopherol), D (calcipherol), K. Structure, role in metabolic processes, diet sources, signs of hypovitaminoses. Hypervitaminoses A, D.

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

6. Macroelements: sodium, potassium, chlorine, calcium, phosphorus, magnesium, sulfur. Role in metabolism.

7. Hormonal regulation of salt and water balance. Renin-angiotensin system, the role of aldosterone, vasopressin, atrial natriuretic factor.

8. Hormones regulating calcium and phosphorus metabolism. Chemical nature, mechanism of signal transduction in target-cells, biological action.

9. The role of iron in the organism (absorption, transport, intracellular metabolism). Iron deficiency states and iron-deficient anemias.

10. Microelements: the role of copper, zinc, manganese, cobalt, selenium, iodine and fluoride in tissue metabolism.

11. Metabolism integration, its principal components; levels and mechanisms of metabolism regulation.

12. Peculiarities of inter-organ metabolism in fed state and between meals. Inter-organ metabolism in fasting.

13. Normal characteristics of urine volume, density, color, transparency, pH. Inorganic and organic components of the urine.

14. Pathologic urine components, diagnostic value and determination: renal and extrarenal proteinuria; glucosuria in diabetes mellitus and renal glucosuria; renal and extrarenal hematuria; ketonuria (mechanism of development in fasting, diabetes).

35. Topic: Control over practical skills of biochemical analys is: analysis of gastric juice and urine

Objective

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

Having received individual control samples of gastric juice and urine the students start their analysis using methodic recommendations of class №19 (Analysis of gastric juice) and № 31 (Physiological and pathological components of urine).

Conclusions:

1. Determination of gastric juice acidity.

2. Evaluation of urine content.

EXAMINATION QUESTIONS FOR INTERNATIONAL MEDICAL STUDENTS (2008/2009)

1. The subject and objectives of biological chemistry. The role of biochemistry in theoretical and practical medicine.

CHEMISTRY OF PROTEINS

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

ENZYMES

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

BIOENERGETICS

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

29. Causes of cell hypoenergetic states. Inhibitors and uncouplers of the tissue respiration and oxidative phosphorylation, mechanisms of their action.

CARBOHYDRATE METABOLISM

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

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

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

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

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

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79. Mechanisms of hormonal action on cells. Role of G-proteins, second messengers, protein kinases.
80. Hormones of adenohypophysis. Their role in regulation of peripheral gland functions.
81. Hormones of neurohypophysis: oxytocin and vasopressin. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
82. Hormones of thyroid gland. Their structure, the mechanism of action, the influence on metabolism. Hypo- and hyperthyroidism.
83. Hormones which regulate calcium and phosphorus metabolism. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
84. Insulin. Chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism. Diabetes mellitus.
85. Glucagon. Chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
86. Glucocorticosteroids. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
87. Mineralocorticosteroids. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.

88. Hormones of adrenal medulla. Catecholamines: dopamine, epinephrine, norepinephrine. Their structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
89. Male sex hormones. Their chemical structure, receptors. The mechanism of signal transmission in target cells, the influence on metabolism.
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94. Vitamin B_2. Structure, participation in flavin coenzymes arrangement. Biological role. Specific symptoms of deficiency. Food resources, daily requirement.
95. Vitamin B_6. Its participation in coenzyme arrangement. The role in metabolism. Specific symptoms of deficiency. Food resources, daily requirement.
96. Vitamin B_{12}. Cobalamins. The role in metabolism. Specific symptoms of deficiency. Food resources, daily requirement.
97. Vitamin C. Biological importance. Specific symptoms of deficiency. Food resources, daily requirement.
98. Pantothenic acid. Coenzymes which contain pantothenic acid. Biological importance. Specific symptoms of deficiency. Food resources, daily requirement.
99. Vitamin PP. Structure, participation in nicotinamide coenzymes arrangement. Biological role. Specific symptoms of deficiency. Food resources, daily requirement.
100. Folic acid. Structure, participation in coenzymes arrangement. Biological role. Specific symptoms of deficiency. Food resources, daily requirement.
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103. Vitamin E. Biological role. Vitamin E deficiency. Food resources, daily requirement.
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105. Vitamin K. Biological role. Vitamin K deficiency. Food resources, daily requirement.

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106. Water, its biological significance. The biological role of sodium, potassium, chlorine. Regulation of the water and salt balance, mechanisms.
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108. Microelements, their significance. The biological role of manganese, copper, zinc, selenium, iodine, cobalt, fluoride.
109. The biological role of sulfur. The role of thiol and disulfide groups in the formation of specific structure and properties of proteins and hormones. Glutathione, sulfolipids, thiamine, biotin, participation in detoxification.
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112. Interorgan metabolism and fuel supply between meals and in fasting.
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