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Lesson 1

INTRODUCTION TO BIOCHEMISTRY SAFETY IN BIOCHEMICAL LABORATORY

Questions to study.
1. Biochemistry and its relation to other courses.
2. Parts of biochemistry.
3. Role of biochemistry in the training of a physician.
4. Main stages in the development of biochemistry.
5. Safety code in biochemical laboratory.

SAFETY IN BIOCHEMICAL LABORATORY

All of the following rules will be followed at all times when you are in the laboratory.

1.1. Lab practicals will be only held at scheduled times. No additional lab time will be allowed, so plan to use all of your available time effectively. If you do, you will have plenty of time to finish all of the required experiments. If you do not come to the lab well prepared or if you do not use your time effectively, you will have less time and therefore, will be less likely to make a good grade. This is as it should be, for planning and self-discipline are critical parts of being a good employee.

1.2. The equipment, glassware and supplies you will be using in these courses are very expensive. Be careful with them. You are responsible for any breakage of glassware.

1.3. It is essential that you keep all common work areas (such as fume hoods, balance areas, etc.) and your work area clean. All spills should be cleaned up and reported to your teacher immediately. You should assume that all horizontal surfaces are contaminated with a corrosive and will destroy your clothing if you sit or rest your arms on them. Allow time to clean up at the end of each lab period.

1.4. You should know all of the chemical reactions and why each reagent is being used. You should continuously observe each experiment for

reasonable properties and consult your teacher if anything looks strange or is unexpected.

GENERAL

2.1. No smoking, eating (including chewing gum) or drinking is permitted in the laboratory.
2.2. Students are not permitted to apply makeup while in the laboratory.
2.3. Outdoor clothing must be left in a cloakroom. Bags must be placed on side podia provided for the purpose.
2.4. Suitable laboratory coats must be worn in the laboratory and removed when leaving. You will not be permitted to work in the lab wearing shorts with protection your legs and feet unprotected.
2.5. Safety spectacles must be worn when carrying out any procedure with a slightest risk of eye injury; gloves of the appropriate type must be worn when necessary.
2.6. Exposed hair or clothing (loose sleeves, ties, jewelry) that might come into contact with a flame or become entangled in mechanical equipment will not be permitted. Long hair must be restrained e.g. by means of a cap, ribbon or headband.
2.7. Students are NOT permitted to do any experimental work unless a supervisor (demonstrator or a member of staff) is present.

SUBSTANCES AND PROCEDURES HAZARDOUS TO HEALTH

3.1. Where a potential hazard exists in a particular practical, it will be discussed in the talk before the practical, and details of safe working methods will be highlighted in the practical notes.
3.2. Do not perform any unassigned experiments.
3.3. Students must NOT use unfamiliar equipment or procedures without having been given instruction. All safety instructions given in the preliminary talk and practical notes must be adhered to.
3.4. Label all containers containing chemicals with the name or formula of the material, the date and your initials. Read all chemical labels before use. Be sure you know what you are using.
3.5. Never use toxic substances without taking proper precautions and making arrangements for safe work. When working with volatile solvents, use a fume cupboard.
3.6. All hazardous materials are deposited in a fume cupboard.

3.7. Keep all concentrated acids and bases in the fume hoods.
3.8. Transport and dispose of all chemicals properly. If you are not sure how to do so, ask your teacher.
3.9. Mouth pipetting is forbidden for acids, bases and hazardous fluids.
3.10. Do not use chipped or broken glassware. Broken glassware will not be accepted at the end of the course and should be replaced during check-in or as soon as it is broken.
3.11. Never heat a closed system without first checking with the teacher.
3.12. Never heat flammable materials with an open flame or near an ignition source.
3.13. Do not heat or mix anything near your face (or anyone's face).
3.14. Never attempt to identify an unknown chemical by smelling or tasting it as recommended in some textbooks.
3.15. Do not operate electrical equipment with wet hands.

WASTE

4.1. Bench should be left waste-free and tidy at the end of each practical — this reduces potential for accidents and spillages and is of considerable help to the laboratory staff.
4.2. Laboratory equipment must be left clean after the practical.
4.3. Do not block aisles or fire exits.

ACCIDENTS AND FIRST AID

5.1. Be sure you know where the safety equipment is located so that you can find and use each item in an emergency.
5.2. Be sure that, in an emergency, you know how to turn off all of the utilities (gas, water, electricity) you have been using.
5.3. All accidents and spillages including any personal injuries and damage caused to equipment must be reported as soon as possible to the supervisor, chief technician or other technicians.
5.4. Concentrated acid or alkali on the skin:
   a) flush the splashed surface thoroughly with water and continue until you are satisfied that no chemical remains in contact with the skin. Soap will help to remove chemicals which are insoluble in water;
   b) remove all contaminated clothing and take care not to contaminate yourself in the process.

5.5.Splashes in the eye. Eye protection should be worn for any work where there is a potential hazard, but if accident occurs:
a) flush the eye thoroughly but gently with water;
b) seek medical advice for all eye injuries from chemicals.
5.6. Burns and scalds. Cool the affected area by immersing in cold water. Continue for at least 5 minutes or until pain is relieved.
5.7. Spillages must be cleared up immediately and the area decontaminated.
5.8. A first aid box is located in the preparatory room.
5.9. The EMERGENCY telephone number is 112

LABORATORY NOTEBOOKS

You are required to use a bound, quadrille-lined notebook in the lab to record all primary data and observations. You should prepare your notebook each week before coming to the lab by writing the title of the experiment on a new numbered page, summarizing relevant equations from the lab manual, and starting calculations involving molar masses, etc. Take note of theoretical ideas and special instructions given by your instructor at the beginning of each experiment. Your notebook should be a complete record of your work in the lab.

GUIDELINES TO BE FOLLOWED

1. Always bring your notebook with you to the laboratory test. You will be graded on the completeness of your previous note taking and your preparation for the current experiment.
2. If you make a mistake in your notebook, simply draw a line through the error and write the correction nearby.
3. Write down all observations such as color and phase changes — don't rely on your memory.

The description of each practical should be organized in the following order.

1. Title of the laboratory test.
2. Principles of the laboratory test.
3. Procedure of the laboratory test.
4. Data on measurements and calculations. Graphs are often an essential part of data analysis. They are best plotted as you go along wherever possible. Remember to label axes clearly.

5. Interpretation and conclusions. Conclusions have to correspond with the objectives of the laboratory test.
6. Significance of the laboratory test in diagnosis of diseases.

Before coming to lab this notebook should have in it:

- lab title;
- principle of the laboratory test;
- a short procedure for the laboratory test;

After lab is finished, the notebook should contain:

- raw data from experiment;
- set ups for calculations;
- any and all observations made during the lab — observations can be: color, lack of color, temperature, physical state (solid, liquid, gas);
- lastly, you need to write up a short conclusion for the laboratory test — your conclusion should address the objectives of the laboratory test.

Lesson 2

PROTEINS, THEIR STRUCTURE, SIGNIFICANCE, AND PROPERTIES. SIMPLE PROTEINS

Questions to study.

1. Proteins, their definition, biological role and classification.
2. Amino acids as monomers of proteins, their classifications and chemical properties. Structure of standard amino acids.
3. Primary structure of protein. Structure of peptide bond. Hereditary defects of primary protein structure. Relationship between structure and function of protein.
   Secondary, tertiary and quaternary structures of protein: definition, types of bonds stabilizing these structures. Their specific properties and significance.
4. Simple proteins (albumins, globulins, histones): structure, classification, representatives, biological role.
5. Physical and chemical protein properties: acidic and basic properties, denaturation, solubility, salting, osmotic pressure, dialysis.

Assignment for self-instruction

#	Task	Guidelines for performing the task
1	**2**	**3**
1	What is medical significance of the structure, functions and biological role of proteins?	1. Describe the chemical composition of living systems. 2. Describe functional diversity of proteins — enzymes, receptors, transporting proteins, antibodies. Give examples of protein hormones, structural and contractile proteins

Continued of the table

1	2	3
2	Study the levels of protein folding	1. Give definition of the term *primary structure of protein*. 2. Write the structural formula of Met-Pro-Ileu tripeptide. 3. What does *secondary structure of protein* mean and what types of secondary structure do you know? Characterize the bond stabilizing the secondary structure. 4. Draw the schemes of α-helix и β-pleated sheet. 5. What does *tertiary structure of protein* mean? Characterize the bonds stabilizing the tertiary structure. 6. What does *quaternary structure of protein* mean? Oligomeric proteins. Give examples of oligomeric proteins
3	Study the methods used for researching the protein primary structure	1. What types of protein hydrolysis do you know? 2. What is the principle of chromatography? Describe the classification of chromatographic methods. 3. Describe the sequence of determining protein primary structure. 4. What is the practical significance of knowing amino acid composition of protein and its primary structure?
4	Study modern views on protein structure	1. What does *folding of protein* mean? The role of chaperones and heat shock proteins in supporting protein native structure. 2. What does *prion diseases* mean?
5	Study the methods of researching protein secondary, tertiary and quaternary structure	1. List and characterize the methods of researching protein secondary, tertiary and quaternary structure
6	Study biological significance of proteins	1. Name biological functions of proteins in human body
7	Study the process of protein solubilisation	1. Describe the features of protein solutions. 2. What are two main factors that stabilize proteins in solutions? 3. Give the definition of the term *salting-out*. Describe the mechanism of salting-out. 4. Give examples of practical uses for salting-out. 5. Give the definition of *denaturation of proteins*. List the types of denaturation. 6. Explain the mechanism of denaturing effect of the temperature, heavy metal salts, alcohols, and phenols. 7. Give examples of practical use of protein denaturation in medicine

Ending of the table

1	2	3
8	Study electrical properties of proteins	1. Explain amphoteric properties of proteins. 2. Write down the reaction of dissociation of carboxyl groups and amino groups in the protein molecule. 3. Describe two main factors which predetermine the electrical charge of the protein molecule. 4. What does *isoelectric point of the proteins* mean? What do you know about protein properties at the isoelectric point?

Library-research paper

1. Prions and prion diseases.

LABORATORY WORK

1. Dialysis of proteins

Dialysis is the process of separating molecules in solution by the difference in their rates of diffusion through a semipermeable membrane like cellophane. Dialysis allows removal of small molecules (salts, reducing agents, dyes, etc) from high molecular weight substances such as proteins.

Procedure

- Take a tube. Pour 1 drop of saturated ammonium sulfate solution and 5 ml of 3% egg protein solution. Stir well.
- Pour 10 drops of this solution into two clean tubes.
- Make biuret test with continuation of the first tube.
- For this purpose add 10 drops of 10% NaOH solution and 1 drop of 1% copper sulfate solution. Mix well. Characteristic violet-blue coloration appears.
- Make a sulfate-ion analysis in the second one. For this purpose add 2–3 drops of 5% barium chloride solution to the second tube.
- Take the remaining mixture of protein solution with ammonium sulfate and pour it into cellophane sac (fig. 1).

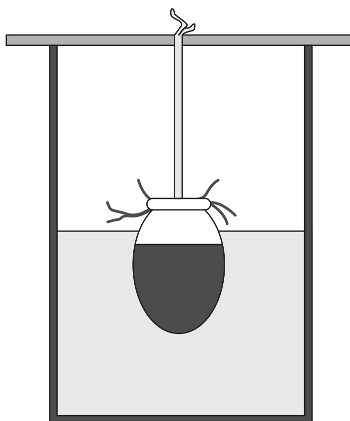


Fig. 1. Schematic drawing of dialysis device

- Fix the sac edges with special sticks. The sticks should be tightened to each other. Put it into a glass bowel with distilled water. The sac has to be fixed on the glass rims. The level of the fluid in the sac should not exceed the level of water in the glass bowel.
- In 1 hour take 10 drops of the outer liquid (from glass bowel) and pour it into two tubes. Make biuret reaction in the first tube and sulfate-ion analysis in the second one. At the same time, make these reactions with liquid from the sac.

Write down the results in the table:

Test	Before dialysis		After dialysis	
	Outer liquid	Liquid from sac	Outer liquid	Liquid from sac
Biuret reaction				
Sulfate ion analysis				

The method of dialysis is used for separation of proteins and inorganic ions, etc. The artificial kidney device based on dialysis is used for treatment of patients with renal failure.

2. Denaturation of proteins

Loss of native conformation of polypeptide chain which is accompanied by loss of function of protein is called denaturation. Denaturation of protein is not accompanied by destruction of its primary structure.

Various factors may cause denaturation. Those factors destroy weak bonds which take part in stabilization of secondary, tertiary, and quaternary structures of polypeptide chain. These are heat, extreme value of pH, organic solvents (ethyl alcohol, acetone, etc.), urea, guanidine hydrochloride, detergents among them.

2.1. Denaturation of proteins by concentrated nitric acids

Procedure

- Pour 10 drops of concentrated nitric acid into a tube.
- Tilt the tube at the angle of about 45° and add 5 drops of 3% protein solution to it.
- Watch the changes emerging at the border between two layers (a ring of denatured protein).

2.2. Denaturation of proteins by trichloroacetic acid and perchloric acid

Procedure

- Pour 10 drops of 3% protein solution into each of two tubes.
- Add 2 drops of 10% trichloroacetic acid into the first tube and 2 drops of 4 N perchloric acid into the second one. Watch the emerging changes.

2.3. Denaturation of proteins by heavy metal ions

Procedure

1. Pour 10 drops of 3% protein solution into each of two tubes.
2. Add 1–2 drops of 10% copper sulfate into the first tube, and 1–2 drops of 5% lead acetate into the second one. Watch formation of protein precipitate.
3. Add also 1–2 drops of heavy metal salts to the corresponding tube.

Watch dissolving of the sediment. Write down the result and explain the process of protein denaturation.

Protein denaturation (sedimentation, *deproteinization*) is used for:

- further determination of low-molecular substrates in biological objects (blood, fluid, tissue homogenates, etc.);
- revealing proteins in unknown solutions and biological objects;
- prevention and treatment of patients with intoxication by heavy metal salts.

3. Sedimentation of proteins

Protein undergoes sedimentation in water solution only when both factors of stabilization (electric charge and hydrophylic shell) are absent or negligible.

Procedure

Take 4 tubes and make mixtures according to data in the table:

Tube №	3% protein solution (drops)	10% trichloracetic acid (drops)	10% NaOH (drops)	$(NH_4)_2SO_4$ saturated solution (drops)	Precipitation (yes/no)	Hydrophylic shell (yes/no)	Protein charge (+, – or 0)
1	5	–	–	–			
2	5	1	–	–			
4	5	–	–	2			
5	5	–	2	–			

Write down the obtained results in the table and make a conclusion.

Lesson 3

COMPLEX (CONJUGATED) PROTEINS: GLYCOPROTEINS, LIPOPROTEINS, AND PHOSPHOPROTEINS. QUANTIFICATION OF PROTEINS

Questions to study.

1. Complex proteins: their definition and classification.
2. Characteristics of glycoproteins and proteoglycans: their structure, representatives and significance.
3. Characteristics of phosphoproteins: their structure, representatives and significance.
4. Characteristics of lipoproteins: their structure, representatives and significance. Blood lipoproteins.
5. Determination of proteins in the blood serum and its significance.
6. Fractionation of proteins by methods of electrophoresis and ion-exchange chromatography, and their significance in medicine.

Assignment for self-instruction

#	Task	Guidelines for performing the task
1	**2**	**3**
1	Study the classification, structure and chemical properties of carbohydrates	1. Write down the formula of α-*D*-glucose, α-*D*-fructose, N-acetyl galactosamine, neuraminic acid

Continued of the table

1	2	3
2	Study the structure, properties and biological significance of various representatives of glycoproteins	1. Identify O- and N-glycosidic bonds between protein and carbohydrate components in molecules of glycoproteins. 2. Give examples of glycoproteins that perform the function of enzymes, hormones, blood clotting factors, transporters. 3. Characterize the structure and biological role of mucosal glycoproteins — salivary mucin, gastromucoprotein, uroglycoproteins
3	Study the classification, structure and biological role of proteoglycans	1. Write down the classification of glycosaminoglycans. Write down the structure of disaccharide units for hyaluronic acid, chondroitin sulfate, keratin sulfate, dermatan sulfate, heparan sulfate. 2. Make a schematic drawing of proteoglycan complexes of extracellular fluid. Describe the role of hyaluronic acid in the extracellular matrix. 3. List the physico-chemical features of proteoglycan complex
4	Recall the structure of lipids	1. Describe the classification of lipids. 2. Write down the general formula of triacylglycerols (TAG), give examples of simple (triolein) and mixed (stearooleipalmitin) triacylglycerols. How does different fatty acid composition affect physico-chemical properties of TAG? 3. Remember the structure of phosphoglycerols — posphatidic acid, posphatidyl choline, posphatidyl ethanolamine, phosphatidyl serine. 4. What does *plasmalogens* mean? 5. Remember the structure of sphyngolipids — sphingomyelins, cerebrosides, gangliosides. 6. Remember the structure of cholesterol and cholesterol esters
5	Study the structure, chemical composition, methods of separation and biological role of blood serum lipoproteins	1. Make a schematic drawing of blood serum lipoprotein structure. Which methods are used to separate plasma lipoproteins? 2. What is the function of blood serum lipoproteins? 3. Fill the table to characterize the difference between chemical composition of different types of blood serum lipoproteins (see below)
6	Study the structure and function of biological membranes	1. Characterize the chemical composition of biological membranes. Make a schematic drawing of the structure of biological membranes. 2. Characterize liquid-crystal structure of biological membranes. 3. Describe the types of transmembrane transport of substances. 4. What does *liposome* mean? How can they be used in medicine?
7	Study the structure of phosphoproteins	1. Characterize the bond between apoprotein and phosphoric acid residue. 2. Give examples of most common phosphoproteins and describe their significance

Ending of the table

1	2	3
8	Study the methods of quantitative analysis of proteins in biological fluids	1. Formulate the principle of quantitative analysis of protein by biuret method. 2. Point out the reference level of protein in the blood and give examples of diseases associated with hypo- and hyperproteinemia
9	Study the dependence between light absorption and intensity of solution color	1. Draw the graph of dependence of light absorption on intensity color of the solution. 2. Make a schematic drawing of photocolorimeter and briefly describe the principle of protein quantification in solution
10	Study the principle of protein fractionation by electrophoresis	1. What does *electrophoresis* mean? Explain why various proteins move in electric field at a different rate. Formulate the principle of electrophoresis in protein fractionation. 2. Describe the main protein fractions of the blood. 3. Describe the significance of determining protein spectrum of the blood in medicine
11	Study the principle of protein fractionation by ion-exchange chromatography	1. What does *ion-exchange chromatography* mean? 2. Formulate the principle of ion-exchange chromatogramphy in protein fractionation

Fill in the table:

Type of lipoproteins	Reference level in the blood, g/l	Apoprotein, %	TAGs, %	Cholesterol, %	Phospholipids, %
Chylomicrons					
VLDL (pre-β-lipoproteins)					
LDL (β-lipoproteins)					
HDL (α-lipoproteins)					

Library-research paper

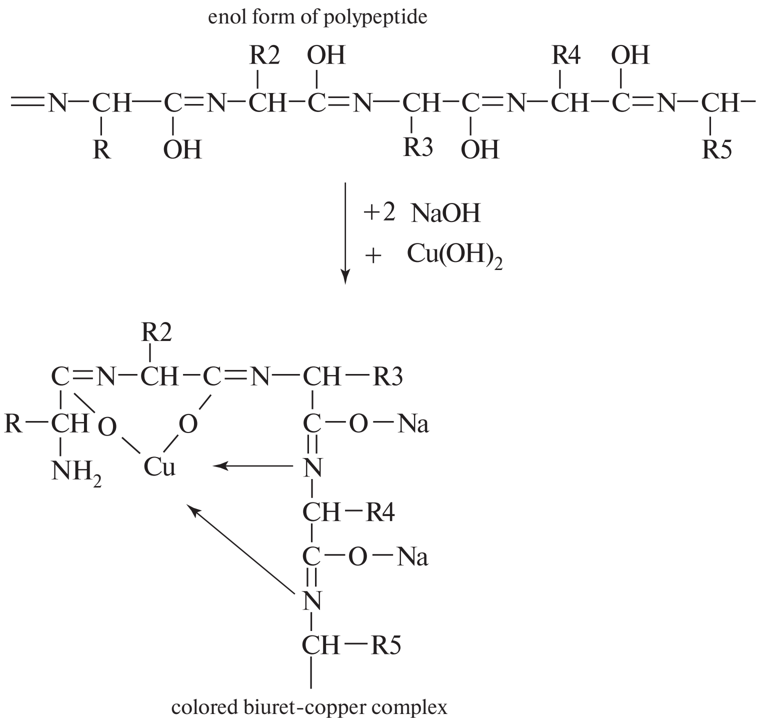
1. Casein and its role in nutrition of newborns.
2. Methods of blood serum lipoprotein fractionation.

LABORATORY WORK

1. Determination of protein in blood serum by biuret method

When protein reacts with biuret reagent, a colored complex is formed. The coloration intensity is proportional to protein concentration in the sample.

Substances containing two and more peptide bonds enter the biuret reaction. The color intensity is directly proportional to the number of peptide bonds in solution.



colored biuret-copper complex

Procedure

1. Take two glass tubes. Pour 0.1 ml serum in tube 1 (experimental) and 0.1 ml solution of 0.9% NaCl in tube 2 (control).
2. Add 5 ml of biuret reagent to both tubes and stir them.
3. Leave both tubes for 30 min at room temperature.

4. Measure light absorbance by *photocolorimetry* at 540–560 nm against the control sample.
5. Calculate the concentration of protein using calibration line.

Plot of calibration line

In order to plot the calibration line, the following components should be brought to the test tubes in accordance with data in the table:

	Reference standard solutions				
	1	2	3	4	5
Protein content (mg)	20	40	60	80	100
Protein concentration (g/l)	20	40	60	80	100
Analytical standard solution of protein (ml)	0.2	0.4	0.6	0.8	1.0
Water (ml)	0.8	0.6	0.4	0.2	0

When reference standard solutions are prepared, 0.1 ml sample is taken from each of six test tubes and mixed with 5 ml of biuret reagent. The samples are then processed in the same way as the experimental and control samples. The graph of absorbance against protein concentration in standard sample is plotted. Concentration of protein in the experimental sample may be found according to the graph.

The reference level of protein in blood serum in adults is in the range 65–85 g/l, and in children — 56–85 g/l.

Increased level of protein (*hyperproteinemia*) is observed in patients with rheumatism and plasmocytoma. Transient *hyperproteinemia* is observed in case of dehydration because of significant loss of liquid (increased sweating, vomiting, diarrhea, diabetes insipidus, cholera, severe burns). Decreased content of proteins (*hypoproteinemia*) is found in case of nephritis, tumors, cirrhosis, etc.

Preparation of reagents

1. **Biuret reagent**: 4.5 g of Rochelle salt is dissolved in 40 ml of 0.2 N sodium hydroxide. After that 1.5 g of copper sulfate and 0.5 g of potassium iodide are added. Then 0.2 N sodium hydroxide is added to 100 ml. The solution should be kept in a dark place. In order to prepare an operating solution, 20 ml of biuret reagent is mixed with 80 ml of 0.5% potassium iodide in 0.2 N sodium hydroxide solution.
2. **Standard solution of protein**: 1 g of albumin is dissolved in 100 ml of water.

Light absorbance

Determination of light absorbance (optical density) of a solution requires a special device called photocolorimeter (fig. 2).

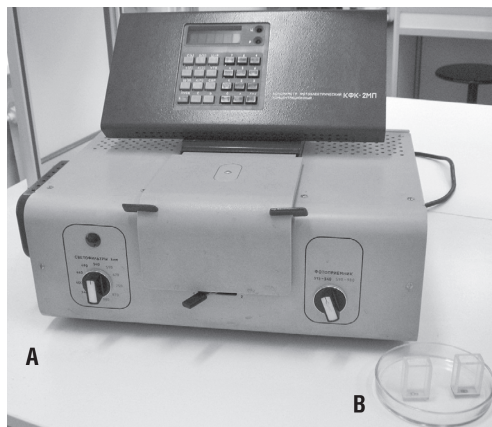


Fig. 2. КФК 2ПМ photocolorimeter (A) and cuvette (B)

Schematic representation of КФК 2ПМ photocolorimeter and cuvette is shown in fig. 3.

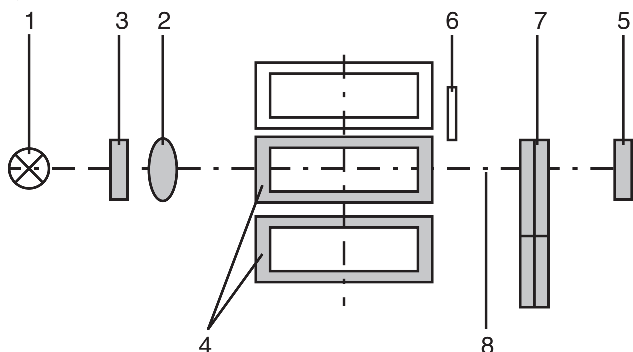


Fig. 3. Schematic representation of photocolorimeter optics (explained in the text). Light from source 1 (electric bulb) passes through the condenser (3) and then — through the light filter (2) for separating a light beam with a wavelength range necessary for the study. Next it passes through the glass cuvette (4), which is filled with solution to be studied. After exiting from the cuvette, the light beam reaches photodetector 5 (photocell). Before photocell on the way of light flux (8) mounted shutter (6), which allows overlapping of the light flux at the time when the measurement is performed, and a special unit, a photometric wedge (7), makes it possible to vary the width of the light flux

The instrument also defines two cells, one of which is a control sample and the other is the test sample. A special device allows you to set it against the light flux control or the test sample alternately.

Operating procedure for КФК 2ПМ photocolorimeter

1. Open the lid of the cuvette compartment.
2. Plug in the device (electrical network toggle switch is on the back panel).
3. Set up the length of the panel (the handle on the front panel is on the left).
4. Put the cuvette with the control (remote position) and experimental (near position) samples in the cuvette compartment.
5. Press the start button. A comma appears on the display.
6. Turn the lever to the cuvette compartment, changing the position of the cuvettes.
7. Press the τ (2) button and then press D (5) button. Write down the value of optical density (extinction).
8. Take out the cuvettes.
9. Turn off the device.

2. Separation of blood serum proteins by means of electrophoresis

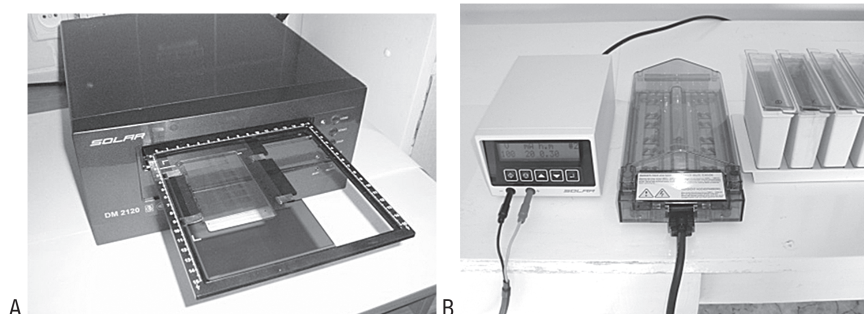
Amino acid residues with polar back side chains included in polypeptide chains under some conditions predetermine the appearance of electric charge on the protein molecule. The charge is determined by the sum of charges of all the ionized functional groups.

Proteins differ from each other in their amino acid composition, and therefore in the value of isoelectric point. For this reason, under the same conditions they show different electric charges of molecules. In this regard, electrophoresis method is one of the most widely used methods of protein mixture separation.

Electrophoresis is a process of motion of charged particles (molecules) in the electric field. The speed of movement of particles depends on their weight and charge value. Because proteins have different molecular weight and charge, in the process of electrophoresis they move to the corresponding electrodes at different rates. This enables separation of multicomponent mixtures of proteins.

Separated proteins are then fixed with the mixture acid/alcohol, and are colored with amidoblack dye solution. The excess of dye is eliminated by acidic solution. After discoloration and drying the gel can be used for densitometry.

Fig. 4 shows a device for electrophoresis on agarose supporters.



A B

Fig. 4. Device for electrophoresis on agarose gel. A — chamber for electrophoresis, source of electric current and reservoirs for plates processing; B — densito-meter with inserted plate carrying separated protein fractions

Procedure

Blood serum is diluted with buffer operating solution as follows: 1 portion of serum + 6 portions of buffer.

Electrophoresis.

- 150 ml of diluted buffer is poured into each part of the electrophoretic chamber.
- The gel is carefully removed from the packing without touching the surface and is put on the paper. In order to avoid over drying of the gel, it is recommended to open the packing right before application, when the device is ready for work and all investigated samples are appropriately diluted.
- The place for samples is drained by a quick touch with a paper strip. The wet paper is removed immediately.
- On the drained place the foil for samples is put (two of its outside slots are connected to corresponding sites on the plate) by pressing the foil lightly to the basis. The foil must fit the gel very tightly!
- 5 μl of diluted serum is placed in each cutout in the foil and is left for 5 min at room temperature after the last sample is put. The excess of serum is removed with a strip of paper.
- The foil is taken off carefully.
- The plate with agarose is bent and placed gel down in the electrophoresis chamber. The serum should be directed to the cathode. The chamber is lidded.
- Electrophoresis is carried out for 20 minutes at 100 v.

- When electrophoresis is over, the plate is removed and placed vertically in the fixer for 15 minutes.
- After removing, the plate is dried with a dryer at a temperature of up to 80 °C.
- The plate is immersed in the dye for 10 minutes and discolored in 2–3 bathes of solution.
- The discolored plate is rinsed out with distilled water and is dried with a dryer at a temperature of about 80 °C.

Results of processing.

- Densitometry of plates is carried out, and the ratio of protein fractions of blood is then calculated using computer software.
- Schematic representation of location of blood protein fractions on the plate with agarose is shown in fig. 5.

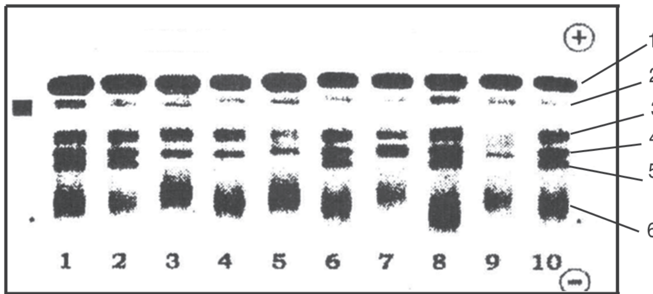


Fig. 5. Photo of agarose plate after electrophoretic separation of blood plasma proteins: 1 — albumins; 2 — α_1-globulins; 3 — α_2-globulins; 4 — β_1-globulins; 5 — β_2-globulins; 6 — γ-globulins

Typical densitogram from the plate is shown in fig. 6.

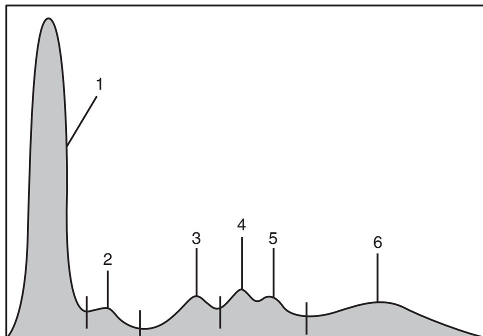


Fig. 6. Densitogram of human blood plasma with separated proteins fractions: 1 — albumins; 2 — α_1-globulins; 3 — α_2-globulins; 4 — β_1-globulins; 5 — β_2-globulins; 6 — γ-globulins

Reference values of protein content in the blood of healthy adult individuals are as follows:

Protein fraction	Relative amount (%)
albumins	52–62
α_1-globulins	4–6
α_2-globulins	7–11
β-globulins	11–15
γ-globulins	14–19

Preparation of reagents

1. **Buffer**: Contents of one bottle of concentrated buffer (TRIS-BARBITAL buffer) is diluted with distilled water to 1000 ml. Diluted buffer is stable until expiry date if stored at room temperature.
2. **Dye**: Contents of one bottle of concentrated dye (AMIDOBLACK staining solution)) is diluted with distilled water to 300 ml. Diluted dye is stable until expiry date if stored at room temperature. Ten plates can be dyed in 300 ml of the dye.
3. **Solution for discoloration**: Contents of one bottle of concentrated discoloring agent (Distaining solution) is diluted with distilled water to 10 l (or 10 ml to 1000 ml, correspondingly). Stability: 1 month at room temperature.
4. Fixing agent: 135 ml of ethanol, 30 ml of glacial acetic acid and 135 ml of distilled water are mixed. Fixing agent requires preparation for at least 15 minutes before use.

3. Quantification of protein in urine

This method is based on Heller's test — denaturation of proteins by concentrated nitric acid added to the protein solution. A white ring of denaturated protein appears between two layers . At normal protein concentration in urine (0.033 g/l) the white ring appears within 2 – 3 minutes after adding concentrated nitric acid.

Procedure

Pour 1 ml of urine to the tube and add 1 ml of concentrated nitric acid. If a white ring appears in less than 2–3 min, it is necessary to dilute the urine

(1/10, 1/20 and so on) and repeat the procedure of adding the acid until a white ring with diluted urine is formed within 2 – 3 minutes.

Calculation

For example, the white ring appeared within 2–3 minutes with urine, which was diluted 1/20. Thus, the protein concentration in urine is $0.033 \times 20 = 0.66$ g/l.