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Theoretical and methodical fundamentals of the study of metabolic processes in human and animals using blood indicators

(Manual)

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Теоретичні та методичні основи дослідження метаболічних процесів у людини та тварин за показниками крові (Посібник): К.:, ФОП Ямчинський О.В., 2019.- 296 с.

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The manual combines the theoretical and methodological principles of studying metabolic processes in human and animals based on blood parameters, which make it possible to evaluate virtually the work of all organs and a whole organism and to obtain valuable advanced information about the state of health and reflect changes that occur in the norm or pathology.

For students of higher educational institutions.

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CONTENT 3

PREFACE

Training experts in veterinary medicine, biological and medical professions require a deep fundamental understanding of the morphological, physiological and biochemical basis of a functioning organism, individual organs, tissues, and cells. As it is known, a special role belongs to blood in all vital functions of an organism. Along with the published literature devoted to studying some questions of biochemistry, physiology, and morphology of the blood of animals and human, there are needs in providing additional educational materials, which can illuminate questions of theory and practice in a complex manner, to students, scientific and pedagogical staffs.

The proposed educational textbook successfully combines theoretical and methodical principles of the study of metabolic processes in animals and human for blood indices which allow evaluating the work of almost all organs and whole organism and getting expanded information about health status and changes occurring under normal or pathological conditions as well as the basics of mathematical modeling and correlation analysis for the interpretation and presentation of experimental data.

The first part of the educational textbook presents extensive information field concerning the blood, its constituents, and functions in animals and human. The functioning of the kinin-kallikrein system and metabolic features of blood cells are highlighted along with such traditional questions of studying the blood, as buffer systems, the role of hemoglobin, proteins, and enzymes in the plasma and serum.

The second part of the educational textbook is devoted to methodological resources for studying metabolic processes in animals and human for blood indices; it presents a number of different specific biochemical analyzers. Methods of determination of indices of metabolism and molecular diagnostics using the reaction of an interaction of antigen with antibody and conducting polymerase chain reaction are presented along with the methodological approaches of obtaining and studying blood cells.

The third part of the educational textbook presents basics of mathematical modeling and correlation analysis for the interpretation and presentation of biochemical investigation data.

The presented schemes, tables and informative material of additions promote better understanding of the textbook material.

Thus, the educational textbook can be useful for students, professionals of various areas of medico-biological directions and for anyone interested in these topics to solve a lot of educational and scientific-practical questions, which arise every day in practical and fundamental biochemistry.

LIST OF ABBREVIATIONS

2,3-DPG – 2,3-diphosphoglycerinic acid

ATP – adenosine triphosphate

ADP – adenosine diphosphate

AMP – adenosine monophosphate

ALT – alanine aminotransferase

AST – aspartate aminotransferase

GTP – guanosine triphosphate

EDTA – ethylenediaminetetraacetic acid

ABS – acid-base status

LDG – lactate dehydrogenase

MDG – malate dehydrogenase

NAD - nicotinamide adenine dinucleotide

NAD+- nicotinamide adenine dinucleotide (oxidized)

NADH – nicotinamide adenine dinucleotide (reduced)

NADP– nicotinamide adenine dinucleotide phosphate

NADP+- nicotinamide adenine dinucleotide phosphate (oxidized)

NADPH– nicotinamide adenine dinucleotide phosphate (reduced)

Pi – inorganic phosphate

CRP – C-reactive protein

Hb – hemoglobin

HbO2 – oxyhemoglobin

HbCO2 – carbhemoglobin or carbaminohemoglobin

HHb – deoxyhemoglobin

HbCO – carboxyhemoglobin

HbOH (MetHb) – methemoglobin

Na_2 EDTA – disodium salt of ethylenedyamintetraacetic acid

LHD – lipoproteins of high density

LLD – lipoproteins of low density

LVLD – lipoproteins of very low density

GIT – gastrointestinal tract

CK – creatine kinase

ALP – alkaline phosphatase

ACP – acid phosphatase

KKS - kinin-kallikrein system

ROS – reactive oxygen species

PART 1

THEORETICAL FUNDAMENTALS OF STUDYING METABOLIC PROCESSES IN HUMAN AND ANIMALS USING BLOOD INDICATORS

CHAPTER 1.1

BLOOD AS BIOFLUID: STRUCTURE AND FUNCTIONS

1.1.1. Blood: concept, composition, and function

Blood is a liquid connective tissue, which provides a continuous relationship between organs and systems of the whole organism. Blood contains plasma (pale-yellow liquid substance) and blood cells (erythrocytes, leukocytes, and thrombocytes) (fig. 1.1, 1.2, 1.3).

Percentage of blood in a human organism varies according to sex, body weight, metabolic rate, physical fitness and it is in an average of 7% of body weight of an adult (15% of body weight of the newborn infant). So, about 5 liters of blood are in the human body with the weight of 70 kg.

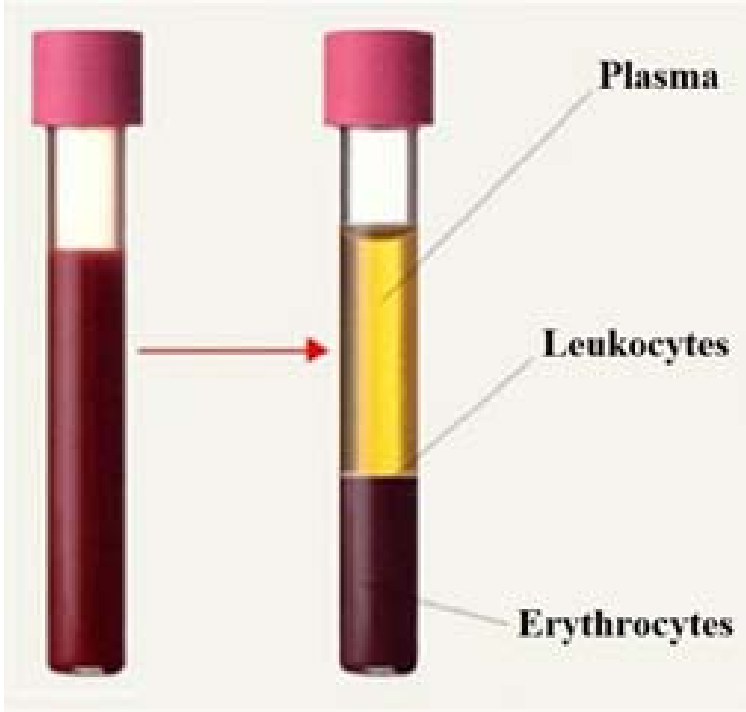


Fig. 1.1. Blood plasma and cells in mammals.

The amount of blood (that circulates through the vessels with a rate per kilogram of body weight) for men is 52-86 ml/kg, and for women – 50-75 ml/kg. In human organism, blood circulates not only in the vessels. It can also be in so-called depots, such as the liver – 20%, the skin – 10%, spleen – 1.5-2% of its total amount. Depot of blood contains more form elements than in vascular channel and circulates in 10-20 times slower. [4].

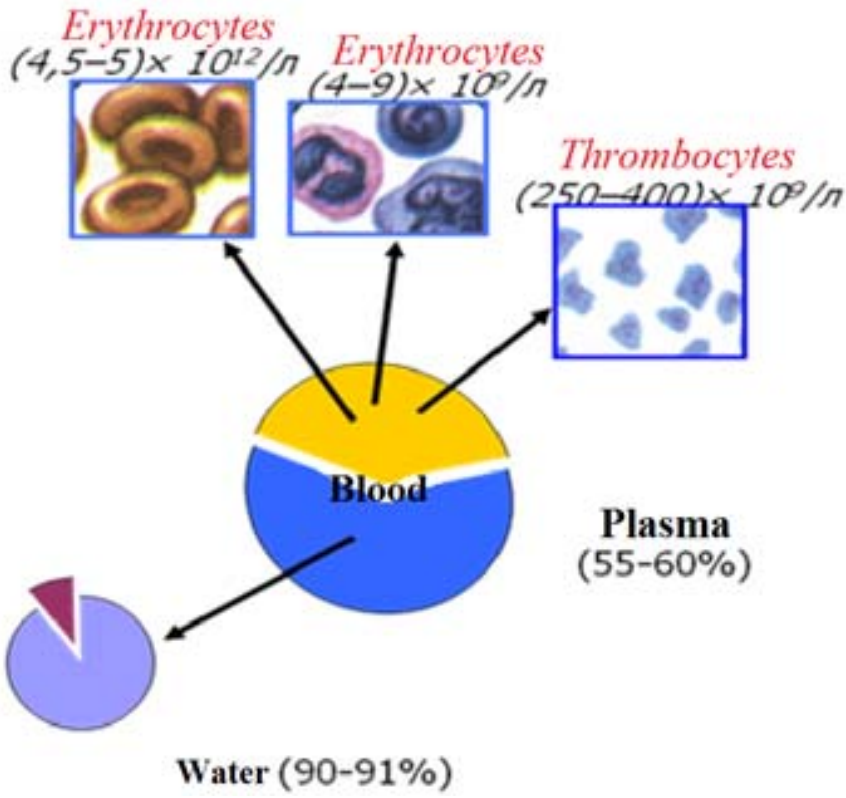


Fig. 1.2. Blood composition of mammals [14].

A system of blood contains not only blood but also organs involved in forming blood cells and their elimination (the bone marrow, thymus, lymphatic nodes, the liver, and the spleen).

The bone marrow is the main place for forming blood cells. The degradation of erythrocytes, hemoglobin synthesis, and accumulation of reserve lipids are also found in it.

The formation of T-lymphocytes takes place in the thymus gland. These cells are involved in cellular immunity. In addition to the thymus gland for immunity, the spleens and lymph nodes are also responsible for immunity. The spleen is involved in

the process of lymphocyte formation, the synthesis of immunoglobulins, the destruction of leukocytes, red blood cells, and platelets, and forming depot blood. Lymph nodes produce and deposit lymphocytes [10].

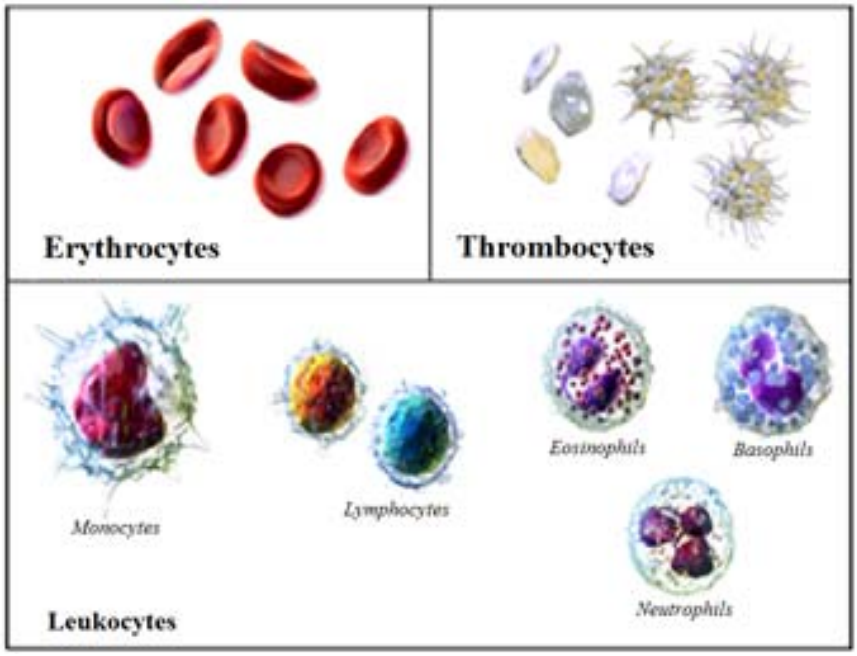


Fig. 1.3. The types of blood cells [14].

Blood enters all parts of the body and performs the following important functions as

1) transport - transferring various substances between organs and tissues (oxygen, carbon dioxide, nutrients, transmitters, enzymes, electrolytes, end products of metabolism, hormones, etc.). These substances are transported in a free state or in complex with proteins;

2) nutritional (trophic) function – blood provides transport of nutrients (carbohydrates, lipids, amino acids, etc.) from the digestive tract to the cells of the organism;

3) excretory – the essence of this function is the removal of unnecessary and even harmful to the organism final products of metabolism, excess water, mineral, and organic substances that come from food or formed in the organism during the process of metabolism (urea, uric acid, ammonia, etc.);

4) respiratory - this function of the blood ensures the binding and transferring oxygen from the lung to the tissues and carbon dioxide from the tissues to the lungs (in the reverse direction). In lungs and tissues, the exchange of gases occurs due to diffusion of O2, CO2 which are contained mainly in the bound state and in the dissolved state when they are presented only in small quantities;

5) regulatory - biologically active substances are contained in the blood, and blood carries the connection between different organs. Because of this, the organism functions as a single system that provides adaptation to the environmental conditions, ie humoral unity and adaptive reactions;

6) protective - the blood contains components (leukocytes, immunoglobulins, complement system) that protect the organism from alien agents; coagulation system protects the organism from blood loss;

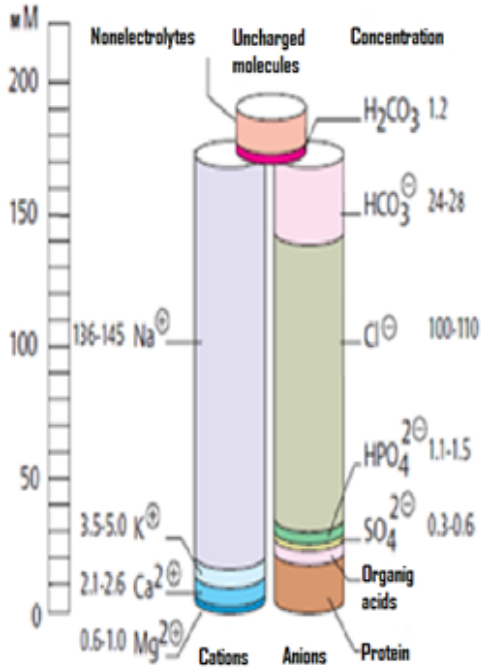
7) thermoregulatory - blood participates in the maintaining and redistribution of heat throughout the organism. The circulating blood combines the organs, in which the heat is produced, with the organs that dissipate it. For example, during intense muscular work, the formation of heat increases in muscles. Blood absorbs heat that spreads through the body, causing excitation of the hypothalamic centers of regulation of temperature. This leads to a change in the product-to-heat ratio. As a result, this is maintaining the temperature at a constant level;

8) homeostatic – blood participates in maintaining the constancy of the internal environment of the organism: constant pH, water-electrolyte balance, level of glucose in the blood, etc.;

9) the function – that consists of the transferring plasma and the blood cells macromolecules, which carry out information communications in the organism. Due to this regulated intracellular processes of protein synthesis, cell differentiation, preservation of cellular structure constancy can be performed [10, 11, 20, 31].

1.1.2. Composition of blood plasma (Plasma electrolyte composition. Non-Protein nitrogen-containing blood components. Nitrogen-free organic components of blood plasma. Plasma protein composition)

Blood plasma is a liquid part of the blood. Its percentage content in the blood is 52-61%. Macroscopically, it is a homogeneous turbid (sometimes almost transparent) yellowish liquid that is collected in the upper part of the vessel with blood after the precipitation of the blood cells. It consists of water (91%), in which there are dissolved proteins (7-8% of the mass of the plasma) and other organic and mineral compounds (fig. 1.4).



Metabolite	Concentration (mM)
Glucose	3.6 - 6.1
Lactate	0.4 - 1.8
Piruvate	0.07 - 0.11
Urea	3.5 - 9.0
Uric acid	0.18 - 0.54
Creatinine	0.06 - 0.13
Amino acids	2.3 - 4.0
Ammonium	0.02 - 0.06
Lipids (total)	5.5 - 6.0 г·л$^{-1}$
Triacylglycerols	1.0 - 1.3 г·л$^{-1}$
Cholesterol	1.7 - 2.1 г·л$^{-1}$

Fig. 1.4. The composition of blood plasma [14].

Albumins are the major plasma proteins. They make 55-65% of the total protein content. In addition, there are α1-globulins (2-4%), α2-globulins (6-12%), β-globulins (8-12%), γ-globulins (2-4%), and fibrinogen (0.2-0.4%). In the blood plasma, there are also dissolved nutrients (in particular, glucose and lipids), hormones, vitamins, enzymes, intermediate and final metabolic products, as well as inorganic substances [11, 38, 39]. On average, 1 liter of human plasma contains 900-

950 g of water, 65-85 g of protein and 20 g of low molecular weight compounds. The plasma density ranges from 1.025 to 1.029, pH is 7.34-7.43 (Fig. 1.4).

Plasma electrolyte composition

Inorganic substances make 0.9 - 1% of blood. They include: cations Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, anions Cl^-, PO_4^{3-}, HCO_3^-, I- and water (tab. 1.1, 1.2). The presence of ions is important condition for maintaining osmotic pressure, the blood cells and vascular mesh functions, acid-alkaline state, enzymes activity, processes of blood coagulation and fibrinolysis.

Table 1.1

Electrolyte content (mmol/L) in blood plasma and human erythrocytes [27]

Ions	Blood plasma	Erythrocytes
Cations		
Na^+	135-150	10-25
K^+	4.0-5.5	95-110
Ca^{2+}	2.2-2,5	0.12-0.30
Mg^{2+}	0.5-0.9	1.7-2.3
Anions		
Cl^-	90-110	40-60
HCO_3^-	23-33	13-18
HPO_4^{2-}	0.8-1.2	38-48
SO_4^{2-}	0.4-0.6	6-8

Because blood plasma constantly exchanges electrolytes with cells microenvironment, the electrolyte content in it largely determines the fundamental properties of cellular elements – excitability and contractility, secretory activity and permeability of membranes, bioenergetic processes [7, 41].

The content of the main electrolytes in blood plasma and human erythrocytes is shown in the table 1.1, and in blood serum in animals - in the table 1.2.

From the table shown above, it is evident that content of sodium and potassium in blood plasma and erythrocytes is different and it is due to differences in permeability of membranes and the activity of the Na+/K+-ATPases (sodium-potassium pump of cells work). Part of blood plasma cations is combined with organic acids and albumins anions, which plays a role in maintaining the acid-alkaline state and it is necessary for albumins functions [5, 45].

Sodium is the major cation of the extracellular fluid. Its content in the blood plasma is 135-150 mmol/L. Sodium ions participate in maintaining osmotic pressure of extracellular fluid. Hypernatremia is observed due to hyperfunction of the adrenal cortex, with administration of hypertonic solution of sodium chloride parenterally. Hyponatremia can be due to salt-free diet, adrenal insufficiency, and diabetic acidosis.

Potassium is the major intracellular cation. Like sodium, potassium maintains osmotic and acid-base homeostasis in a cell. Potassium ions concentration in blood plasma is 5 mmol/L. Hyperkalemia is observed due to increased cellular destruction (hemolytic anemia, prolonged crushing syndrome), disturbance of removal of potassium by the kidneys, organism dehydration. Hypokalemia is observed due to hyperfunction of the adrenal cortex, with diabetic acidosis [5, 45].

Calcium in blood plasma is contained in the following forms: bound with albumins (it is 0.9 mmol/L), ionized (1.25 mmol/L) and nonionized (0.35 mmol/L), but biologically active is only ionized calcium. The regulation of the level of ionized calcium in the blood plasma is carried out by a special humoral system, which includes a number of calcium-regulating hormones: parathyroid glands (parathyrin), thyroid gland (calcitonin and its analogs), and calcitriol (an active form of vitamin D). Hypercalcemia is observed due to hyperparathyroidism, hypervitaminosis D, Cushing's syndrome, destructive processes in the bone tissue. Hypocalcemia occurs due to rickets, hypoparathyroidism, and kidney diseases [5, 45].

Chlorides participate in maintaining osmotic pressure, acid-base state of the extracellular fluid. Hyperchloremia is observed due to heart failure, arterial hypertension, hypochloremia due to vomiting, kidney diseases [5, 45].

Phosphates in blood plasma are components of the buffer system. Hyperphosphatemia is observed due to kidneys diseases, hypoparathyroidism, hypervitaminosis D. Hypophosphatemia is noted due to hypoparathyroidism, myxedema, rickets.

Blood plasma also contains a large number of various microelements, the contents of which is insignificant [5, 45].

Table 1.2

The content of some electrolytes (mmol /L) in blood plasma of animals [5]

Ion	Animal species			
	Cattle	Sheep	Pigs	Horses
Ca^{2+}	2.5-2.55	2.8-2.85	2.5-3.0	2.75-3.25
K^{+}	4.35-4.86	4.66-4.86	4.86-5.12	4.86-5.63
Na^{+}	143.0-147.4	140.8-149.6	145.2-149.6	136.4-145.2
Mg^{2+}	0.82-0.98	0.82-1.03	1.03-1.44	0.82-0.92

At least 15 microelements, which are contained in blood plasma, such as: copper, cobalt, manganese, zinc, chromium, strontium etc., play important role in cellular metabolic processes and providing their functions, because they are the part of enzymes, catalyze their action, participate in the formation of blood cells and hemoglobin (haematopoiesis) [5, 45].

Non-Protein nitrogen-containing blood components

This group of substances includes: urea, uric acid, amino acids, creatine, creatinine, ammonia, indican, bilirubin and other compounds (fig. 1.5).

Urea (3.33-8.32 mmol/L) is formed in the liver as a final product of ammonia neutralization. The urea itself is low-toxic, however, when its concentration increases, there is swelling of the parenchymal organs, myocardium and the tissues of the central nervous system. In the clinic, it is determined to diagnose kidney diseases. In addition, for the differential diagnosis of kidney diseases and degenerative lesions

of liver parenchyma, a special Urea ratio coefficient is used [27]. Urea ratio = (nitrogen of urea/residual nitrogen) × 100%.

For a healthy human, this indicator must be 46-60 %. This indicator increases due to chronic nephritis (almost to 90 %), peritonitis, pernicious anemia, burns, and in severe forms of hepatitis, it significantly decreases.

Amino acids are entering the blood during absorption from the gastrointestinal tract or are the products of degradation of tissue proteins. In the blood of healthy people, alanine and glutamine are dominated among the amino acids, which along with participation in the biosynthesis of proteins are the transport forms of ammonia [5, 27].

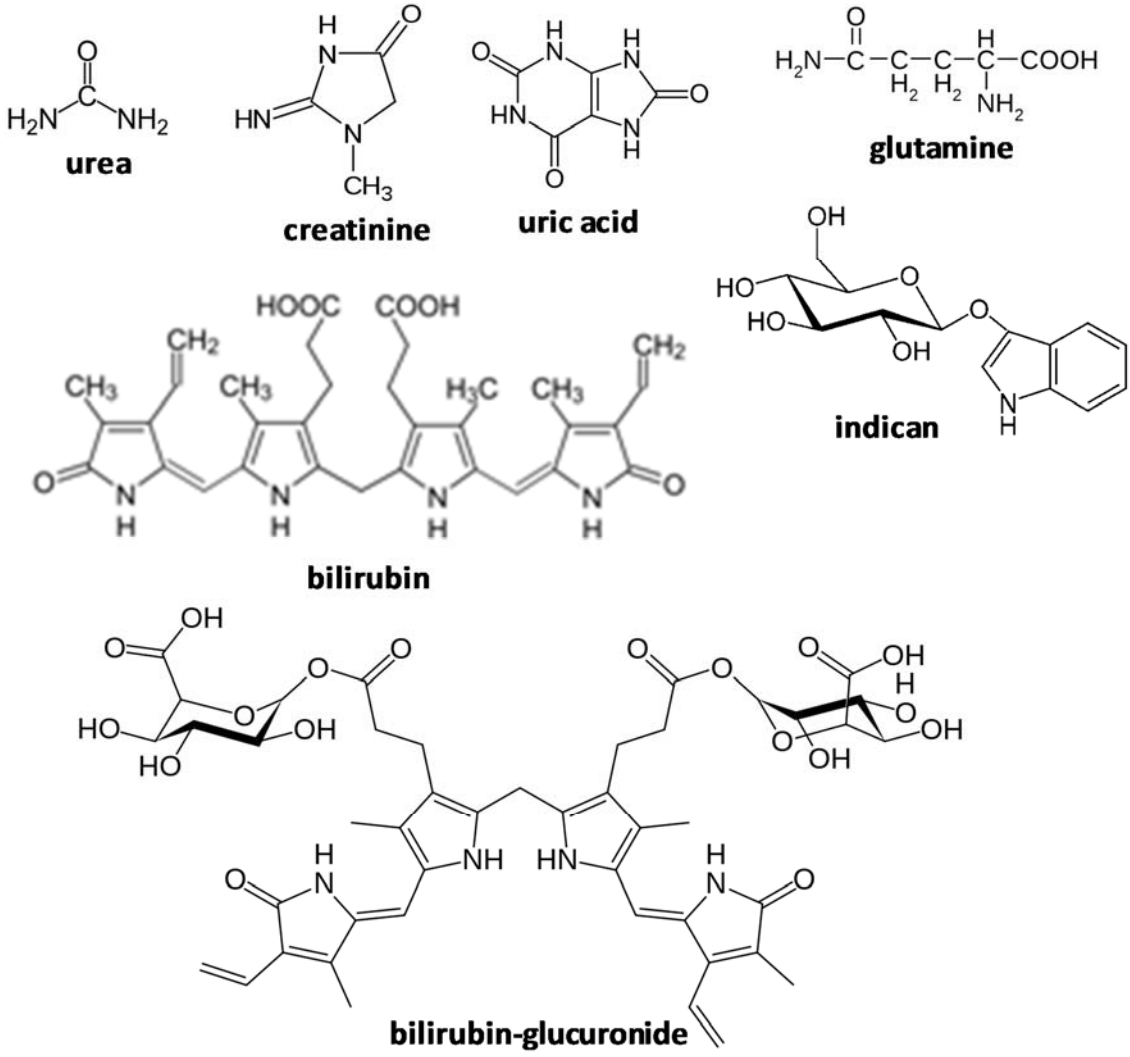


Fig. 1.5. Non-protein nitrogen-containing blood plasma components of human and animals [20].

Uric acid (for men – 0.12-0.38 mmol/L, for women – 0.12-0.46 mmol/L) – are the final product of decaying purine nucleotides metabolism. Uric acid is a weak acidic substance, so after secretion in blood it forms salts with alkaline cations (98% with sodium) – urates that bind to α-globulins and are excreted by the kidneys (2/3) and through the small intestine (1/3). Concentration uric acid in blood increases (hyperuricemia) due to gout, Lesch-Nyhan syndrome, states that are accompanied by an increase in the collapse of cells, disruption of uric acid excretion, changes in the endocrine regulation of the exchange of purines. In addition, food rich in purines (liver, kidneys, red wine), long starvation, alcohol also cause increasing its content in the blood. Reduced concentration of uric acid in the blood (hypouricemia) is observed due to Wilson's disease, Hodgkin's lymphoma, after taking piperazine, salicylates, corticotropin [5, 27].

Creatine is the final product of nitrogen metabolism, it is formed as a result of dephosphorylation of creatine phosphate in the muscles, is removed from the body by the kidneys. Normally, plasma concentrations of creatinine are 53-106 μmol / L. The content of creatinine in the blood decreases due to diseases of the muscular system but increases due to kidneys insufficiency [5, 27].

Indican (potassium or sodium salt of indoxyl sulfuric acid) is formed in the liver as a result of neutralizing one of the products of decay of proteins in the intestine – indole. Hyperindicanemia is observed in patients with an initial stage of renal failure development, with the strengthening of the processes of protein breakdown in the intestine (strangulated hernia, constipation, intestinal obstruction), tumors, abscesses, tuberculous lung disease, biliary duct obstruction, etc. [5, 27].

Bilirubin (direct and indirect) is a catabolism product of hemoglobin. The content of bilirubin in the blood increases due to jaundices: hemolytic (due to indirect bilirubin), obstructive (due to direct bilirubin), parenchymatous (due to both fractions) [5, 27].

The content of the remaining nitrogen in the blood plasma of healthy people is 15-25 mmol/L. Increasing the content of the remaining nitrogen in the blood is called

azotemia. Depending on the cause, azotemia is divided into retention and production [5, 27].

The retentional azotemia occurs due to the infraction of removal of nitrogen exchange products (foremost urea) with urea and typical for kidney function insufficiency. In this case, up to 90% of non-protein nitrogen in the blood falls on the nitrogen of urea (50% in norm).

The production azotemia develops due to excessive intake of nitrogen-containing substances in the blood as a result of the increased breakdown of tissue proteins (prolonged starvation, diabetes mellitus, serious injuries and burns, infectious diseases) [5, 27].

Determination of the remaining nitrogen is conducted in a protein-free filtrate of blood serum. As a result of mineralization of non-protein filtrate due to heating with concentrated H_2SO_4, the nitrogen of all non-protein compounds is converted into the form $(NH4)_2SO_4$. NH_4^+ ions are determined using Nessler's reagent.

Ammonia (17-18 μmol/L) is a final product of protein metabolism, that also is a component of remaining nitrogen. Its sources are processes of deamination, that occurs in tissues, and catabolism of nitrogen-containing compounds in the intestine under the influence of rotting bacteria. Increasing the concentration of ammonia in the blood is observed due to hepatic coma, the effects of hepatotropic poisons (carbon tetrachloride, methionine and others), partial removal of the liver, acute hepatic insufficiency, thermal shock, protein starvation, hereditary enzymopathy of the ornithine cycle, fatty liver infiltration, acute renal failure, intestinal dysbiosis etc. [5, 27].

Nitrogen-free organic components of blood plasma

This group of substances contains nutrients (carbohydrates, lipids) and their products of metabolism (organic acids). The greatest importance for the clinic is determination of the content of blood glucose, cholesterol, free fatty acids, ketone bodies and lactate. Formulas of these substances are presented in fig. 1.6.

Carbohydrates of blood plasma. Blood plasma contains mainly monosaccharides, mostly hexoses (glucose, fructose, galactose), trioses (lactic acid, pyruvic acid) and some pentoses (ribose, deoxyribose).

Glucose is the main energetic substrate of an organism. The content of glucose in the arterial blood is higher than in venous blood because it is continuously used by tissue cells. In a healthy person venous blood contains 3.6-6.9 mmol/L (65-119 mg%) glucose, moreover, the fluctuations of its level are mainly related to the time of food intake and absorption from the gastrointestinal tract. In general, the level of glucose in the blood depends on the ratio of such factors: absorption from the gastrointestinal tract, mobilizing it from the depot (liver glycogen), tumors from amino acids and fatty acids (gluconeogenesis), tissue utilization and depositing in the form of glycogen. Increasing the content of glucose in the blood (hyperglycemia) is observed after the meal, due to emotional stress, in patients with diabetes mellitus, hyperthyroidism, Cushing's syndrome. Decreasing the content of glucose (hypoglycemia) is observed due to starvation, intensive physical activity, acute alcohol poisoning, an overdose of insulin [23, 24, 26].

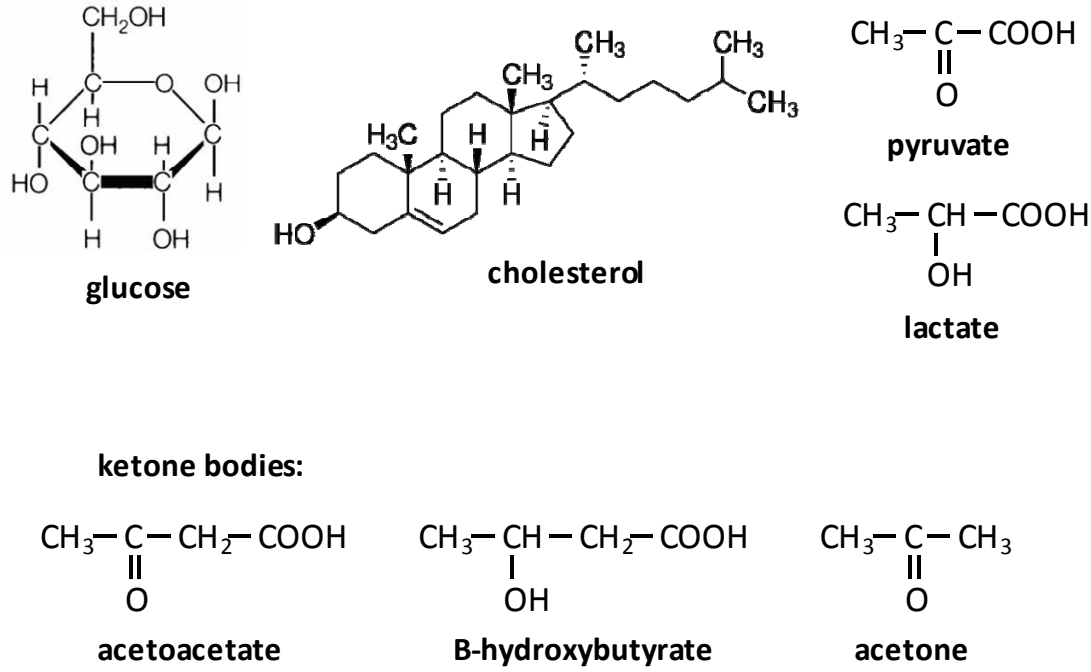


Fig. 1.6. Nitrogen-free organic components of blood plasma [20].

Lactic acid (lactate) is the final product of anaerobic oxidation of carbohydrates. Its content in the blood (normally 8-17 mg%) increases with hypoxia (physical activity, diseases of the lungs, heart, blood) [23, 24, 26].

Pyruvic acid (pyruvate) is an intermediate product of catabolism of carbohydrates and some amino acids. The most rapid increase in the content of pyruvic acid in the blood (in the normal range of 0.4-2.5 mg%) is observed in muscle work and vitamin B1 deficiency [23, 24, 26].

Lipids of blood plasma. The total concentration of lipids in human`s blood plasma varies depending on the diet mode and quality of food and the constitutional features of the body (age, sex), averaging 5-7 g/L. Under physiological conditions, the total amount of lipids in the blood can increase to 10-15 g/L after eating a meal, rich in fats (alimentary hyperlipaemia).

The largest number of blood plasma lipids are compounds of the following classes:

- Triacylglycerols – 0.5-1.9 g/L;
- Phospholipids – 1.1-2.75 g/L;
- Total Cholesterol – 1.5-2.6 g/L;
- Cholesterol – 1,0-2,1 g/L;
- Fatty acids (not esterified) – 0.08-0.2 g/L.

Lipids, as hydrophobic compounds, are not capable of being in a free (soluble) state in blood plasma, which from the physicochemical point of view is a water-saline solution.

Stabilizers of plasma lipids are special proteins (apoproteins or apolipoproteins) that promote the formation of lipoprotein micelles, in which various types of lipids can be transported by blood. There are five classes of apoproteins (A, B, C, D, E), which in certain quantitative ratios, are the part of various lipoproteins.

Distinguish the following transport forms of plasma lipoproteins:

- Hylomicrons (1-2,5 g/L) – the major transport form of triacylglycerols;

- Lipoproteins of very low destiny (LVLD) or pre-β-lipoproteins (1,3-2,0 g/L) - contain a significant amount of triacylglycerols, an also phospholipids and cholesterol;
- Lipoproteins of low destiny (LLD) or β-lipoproteins (2,1-4,0 g/L)- the major transport form of cholesterol; increasing concentration of LLD in blood promotes the penetration of cholesterol in the endothelium and the formation of an atherosclerotic plaque, which is a risk factor for the development of atherosclerosis;
- Lipoproteins of high destiny (LHD) or α-lipoproteins (0.2-0.25 g/L) - contain a significant amount of phospholipids, an also cholesterol and triacylglycerols. LHD are considered as "antiatherogenic" lipoproteins that contribute to the release of cholesterol from the vascular wall.

Increasing concentration in blood plasma of various classes of lipoproteins (hyperlipoproteinemia) indicates significant changes in lipid metabolism, most often associated with certain genetic disorders. There are five main types of hyperlipoproteinemia, which depend on defects in the enzyme systems of cleavage or conversion of certain lipoproteins (types I, II, III, IV, V) and may be manifested by the early development of atherosclerosis, obesity, xanthomatosis, liver and kidney disease, and decreased glucose tolerance.

Cholesterol is an obligatory lipid component of biological membranes, a precursor of steroid hormones, vitamin D3, bile acids. Cholesterol content in blood plasma of healthy people is 3.9-6.5 mmol/L. The increasing content of cholesterol in the blood (hypercholesterolemia) is observed due to atherosclerosis, diabetes mellitus, myxedema, gallstone disease. The decreasing level of cholesterol in the blood (hypocholesterolemia) is manifested in hyperthyroidism, liver cirrhosis, intestinal diseases, starvation, under the administration of choleretic drugs.

Free fatty acids (FFA) are used by tissues and organs as energy materials. The content of FFA in blood increases due to starvation, diabetes mellitus, after the introduction of adrenaline and glucocorticoids; decreases due to hypothyroidism, after the insulin injection.

Ketone bodies include acetoacetate, β-hydroxybutyrate, acetone – products of partial oxidation of fatty acids. The content of ketone bodies in blood increases (hyperketonemia) due to starvation, fever, diabetes mellitus [23, 24, 26].

Plasma protein composition

In this chapter, information is provided about medium molecular wheight peptides and hemolymph proteins.

Medium molecular weight peptides have molecular weights from 300 to over 5000 Daltons. They are not precipitated by trichloroacetic acid (TCA). The peptides are part of the fraction of the residual blood nitrogen that includes hormones, neuropeptides, mediators of immune reaction, some vitamins, nucleotides, products of disintegration of fibrin etc. Composition of medium molecular weight peptides can be different. It depends on kind of pathology and character of complications. An increase of the concentration of medium molecules in the blood is observed in all pathological states accompanied by the intoxication (festering peritonitis, intracranial injury, inflammatory condition of the lungs, liver diseases, acute and chronic kidney diseases). For control of intoxication, hemo- and enterosorptions are carried out, which reduce the concentration of peptides and non-polar amino acids in blood plasma [9, 17, 34].

Proteins (MV over 300) in the blood plasma are in the dynamic equilibrium with proteins of tissues. Their quantitative and qualitative composition displays state of protein metabolism in the organism.

Their main functions are to:

- support the colloid-osmotic (oncotic) blood pressure. Osmotic pressure of the blood plasma is 7.3-7.6 atm (5600 mmHg or 745 kPa);
- define blood viscosity;
- take part in functioning the coagulation and anticoagulation systems of blood;
- form part buffer system (supporting constant pH);
- be involved in transporting different substances (hormones, lipids, pigments, mineral substances, fat-soluble vitamins);

- use as a plastic material for the synthesis of proteins of tissues;
- be as the transmitters of innate and acquired immunity.

Most of the proteins in the blood plasma are synthesized in the liver. There are an albumin (10-16 g/day; a half-decline period of albumins is 10-15 days), α-globulins, some of the β-globulins, fibrinogen, components of the system of coagulation (II, V, VII, IX, X, XI factors). The typical number of proteins in plasma is 65-85 g/L but the daily variations of the number are possible (10-20 g/L). In the new-born infants, a number of the total protein in the blood is 50-60 g/L.

Decrease (hypoproteinemia) or increase (hyperproteinemia) of total protein in the blood plasma and some protein fractions can be caused by many reasons. Hyperproteinemia can be caused by water loss from the organism as a result of polyuria, diarrhea, vomiting or conditioned by an increase of content γ-globulins and some other proteins during acute inflammatory processes, injuries, and myeloma. They are called proteins of acute phase (for instance, C-reactive protein, haptoglobin, and fibrinogen). Mainly, hypoproteinemia is the consequence of a disturbance of synthesis or loss of albumin from an organism that's called a hypoalbuminemia. It’s observed under conditions of nephritis, hepatitis, cirrhosis, burns, prolonged starvation.

In the norm, the ratio of albumins to globulins (A/G) is 1.2-2.0. A decrease of a value of the ratio is possible in a case of decreasing albumins concentration and increasing quantity of globulins in the blood. So, for an example, it can be a result of inhibition of the synthesis of albumins in the liver, or loss of proteins with urine, or in a case of increased synthesis of γ-globulins as a responce to an infection [27].

CHAPTER 1.2
HEMOGLOBIN AND PROTEINS OF BLOOD PLASMA

1.2.1. Hemoglobin: Structure, properties, derivatives

Hemoglobin (Hb; Haima (gr.) is a blood, and Globus (lat.) is a globule) is the conjugated Ferrum-containing protein of erythrocytes of animals and human. It is able to bind reversibly with oxygen providing its transfer to tissues. Hemoglobin is the conjugated protein of a class of chromo proteins which contain a prosthetic group heme (an iron-containing compound of the porphyrin class that forms the nonprotein part of hemoglobin and some other biological molecules).

In the blood of new-born infants, the concentration of Hb is 192-232 g/L, in adult women - 120-150 g/L, men - 130-160 g/L. Molecular mass of hemoglobin of human is about 66.8 kDa [23, 24, 26].

There are three main physiological functions of hemoglobin:

1) respiratory (transferring oxygen and carbon dioxide);
2) providing constant pH (the hemoglobin buffer system is the most powerful system which supports constant pH in the blood);
3) transport of the respiratory gases (oxygen, nitric oxide, and carbon dioxide)

Hemoglobin is a tetramer that consists of four subunits (fig. 1.7). Each subunit contains a heme which binds to protein part of hemoglobin molecule by a histidine residue. Two different kinds of polypeptide chains form hemoglobin molecule. In the adult human, there are α1, α2, β1 and β2 polypeptide chains in the molecule of hemoglobin. Subunits are bound to each other on the principle of isologic tetrahedron. Subunits of hemoglobin mainly bind each other by hydrophobic interactions.

Hemoglobin is synthesized in the predecessors of red blood cells, which have a nucleus. The synthesis of heme requires Ferrum and vitamins: B12 (cyanocobalamin) and folic acid. The synthesis of globin requires amino acids, the sources of which are full protein foods [23, 24, 39].

Anemias develop due to the lack of these factors and due to the congenital deficiencies some enzymes of exchange of glucose (glucose-6-phosphate dehydrogenase, pyruvate kinase etc.). Accerelated destruction of red blood cells (hemolysis) is the cause of another group of anemias (hemolytic).

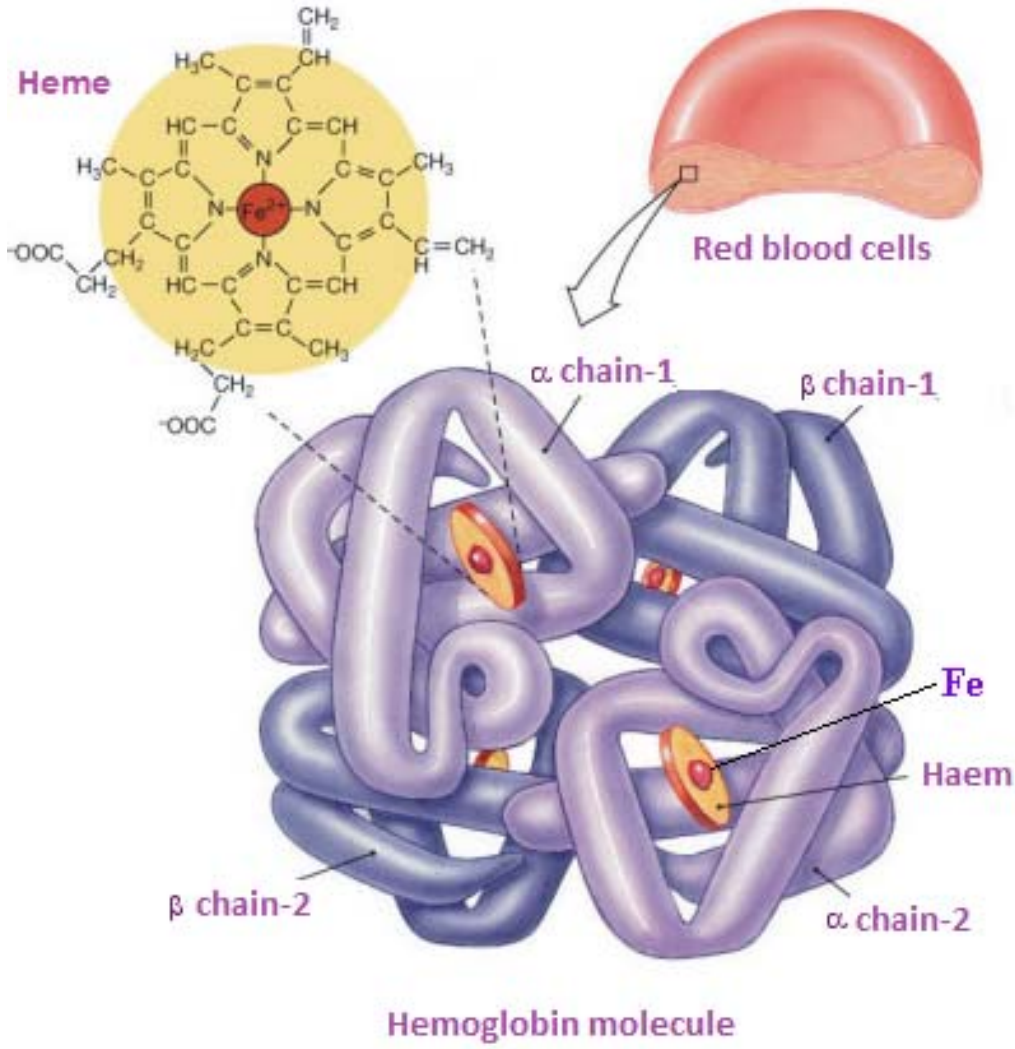


Fig. 1.7. Structure of hemoglobin [14].

There are fetal hemoglobin (hemoglobin of fetus orfoetus, HbF) and adult hemoglobin (HbA). In adult humans, the most common form of hemoglobin is HbA1 (96-99% of all hemoglobin). It consists of two α- and two β-chains (α2β2). Some of the HbA1 is glycated. Glycosylated hemoglobin (HbA1c) is the form of hemoglobin to which glucose is bound without the help of an enzyme. The normal concentration of HbA1c is 4-7% [23, 24, 39].

There are, next to the most common form (HbA1), minor quantities (2-3%) of another form of hemoglobin with a higher affinity with O_2 (the β chains are gradually replaced by δ chains (HbA2, α2δ2)) in the blood. Two other forms of Hb present only during embryogenesis. Fetal hemoglobin of composition ξ2ε2 and α2γ2 are formed in the first three months. Then the fetal hemoglobin (HbF, α2γ2) prevails up till the birth. It is replaced gradually by HbA during the first month of life (fig. 1.8).

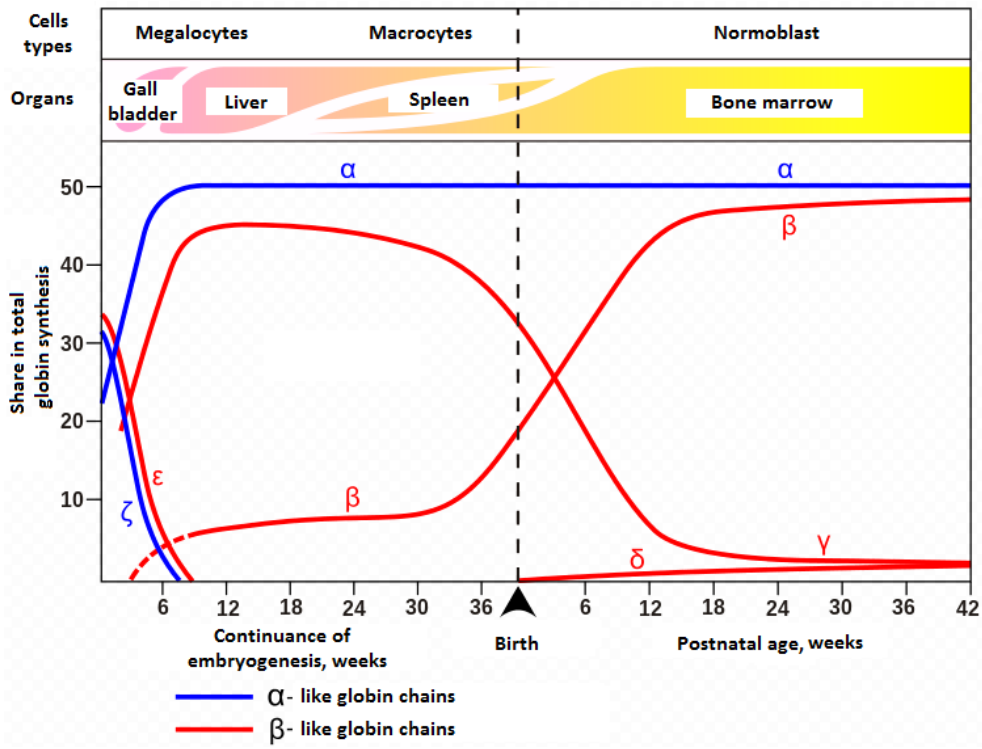


Fig. 1.8. Gene expression of hemoglobin before and after birth. It is also specified types of cells and organs in which the gene expression occurs [57].

Fetal hemoglobin binds oxygen with higher affinity than adult hemoglobin. It takes oxygen from the maternal circulatory system. HbF is close to the myoglobin by his oxygen-binding capacity. The oxygen binding curve of fetal hemoglobin is hyperbolical. It is unable to fast dissociation by releasing O2 at the decline of partial pressures of oxygen, like HbA. The HbF concentration in the blood of adult human can increase in 10-20 times as a result of parental multidrug resistance. A heavy hypoxia appears as a result of the inability of such hemoglobin to mediate normal oxygen transport between arterial and venous blood [23, 24, 39].

A heme is the complex of protoporphyrin IX, which belongs to the class porphyrin compounds, with the atom of ferrum (II). This cofactor contacts with hydrophobic depression of molecules of hemoglobin and myoglobin by non-covalent interaction. Ferrum (II) has octahedral coordination. It binds with six ligands. Four of them are made up of the atoms of nitrogen of porphyrin rings which lies in one plane. Two other co-ordination positions lie on the axis which is perpendicular to the porphyrin plane. One of them is occupied by Nitrogen of histidine residue in 93 positions of the polypeptide chain (F area). Oxygen molecule, which is bound by hemoglobin, is coordinated to Ferrum from the reverse side and it appears placed between atoms of Ferrum and Nitrogen of another histidine residue. It is located in the chain (E area, the 64-th position). Total hemoglobin has four sites of oxygen binding (one gem per subunit), that is, four molecules of oxygen can be linked simultaneously to the hemoglobin complex [39, 54].

Distinguish the following hemoglobin compounds: HbO2 – oxyhemoglobin, has bright red color.

The formation of oxyhemoglobin occurs in the pulmonary capillaries. It depends on partial pressure (tension) of oxygen. The valence of Ferrum does not change, it remains bivalent.

The oxygen binding curve of hemoglobin and, accordingly, dissociations of oxyhemoglobin, has S-shaped form. It tis consistent with the co-operative character of this process (fig. 1.9).

The binding of O_2 molecule to the first subunit of hemoglobin (as a result of conformation change) increased the ability of hemoprotein to combined with the other three molecules of oxygen. Thus, the affinity of hemoglobin to the fourth molecule of oxygen is almost in 300 times higher than to the first one.

Oxyhemoglobin is stronger acid as compared with deoxyhemoglobin. It gives protons easier. In the red blood cells cations of potassium force out protons of oxyhemoglobin with formation of potassium salt (K^+HbO_2):

$$H^+Hb^- + O_2 + K^+ \rightarrow K^+HbO_2^- + H^+$$

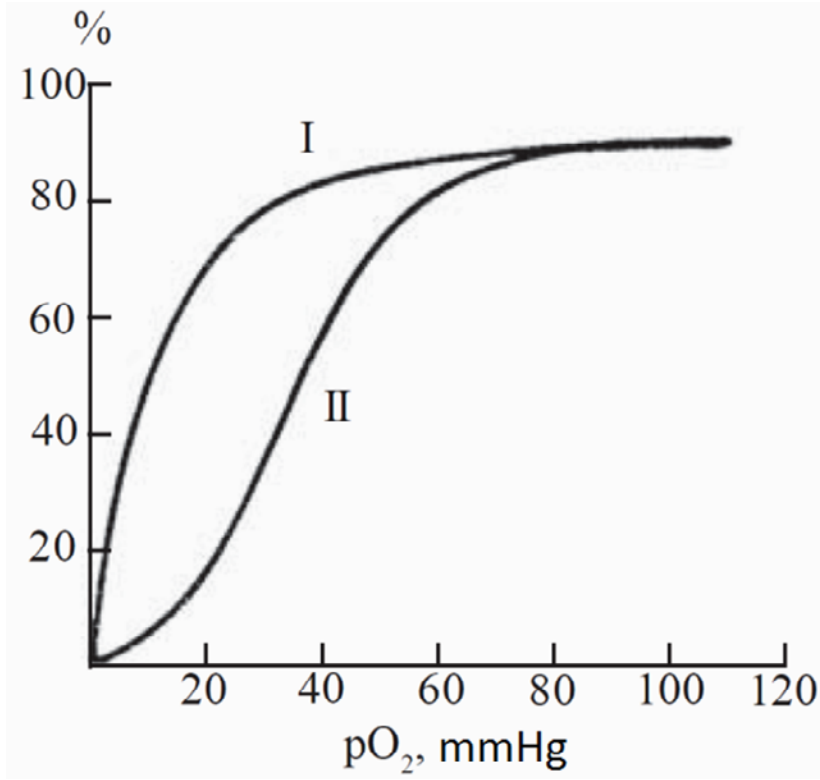


Fig. 1.9. The dependence of the degree of oxygenation (% from maximal) from the partial pressure of O_2 for hemoglobin (II) and myoglobin (I) which is an oxygen-binding protein of muscles that does not have co-operative properties [95].

Hemoglobin binds to O_2 (a process of oxygenation) in the pulmonary capillaries (pressure of O_2 is about 100 mmHg.) and becomes oxygenized hemoglobin - oxyhemoglobin. The dissociation of oxyhemoglobin to the hemoglobin and O_2 happens in the capillaries of tissues, where the tension of O_2 is considerably lower (about 40 mmHg). O_2 comes to the cells of organs and tissues, where the partial pressure of O_2 is lower (5-20 mmHg.). It decreases to zero in the inside of the cell. The difference of partial pressure of O_2 between alveolar air and intercellular liquid, where the oxygen gets from blood, equals 65 mmHg. This considerable difference provides transport of oxygen from alveolar into the blood and farther - to the intercellular liquid. Besides, the functioning of cytochrome oxidase of the respiratory chain conduces to the continuously using of oxygen and decrease of partial pressure of oxygen in mitochondria to 4-5 mmHg. Thus, an "oxygenic

vacuum" is practically created in mitochondria, which directs the flux of oxygen into the cells.

A considerable part of the oxygen is transported as HbO_2. Around 3.2 ml of O_2 is dissolved in 1 liter of blood plasma. Hemoglobin (which is in the red blood cells) is able to bind 220 ml O_2/L.

The intensity of formation of HbO_2 depends on the partial pressure of gases in the blood, the value of pH, the concentration of CO_2 and content of 2,3-biphosphoglycerate (2,3-BPG) [26, 39, 54].

HHb is deoxyhemoglobin or the reduced hemoglobin (that gave oxygen) has a dark-red color, which determines the color of venous blood. It appears in the capillaries of tissues where is less oxygen. Its partial pressure is lower. Oxyhemoglobin breakes down into hemoglobin and oxygen there.

$HbCO_2$ is carboxyhemoglobin (CO_2 combines with N-eventual groups of hemoglobin) has a cherry color and appears by the passing of the blood through tissues. 20% of CO_2 is transported in the form of this derivative.

Negative influence, decrease of pH and increase of the concentration of carbon dioxide (CO_2), to the formation of oxyhemoglobin is called “the Bohr effect”.

The molecular mechanisms of the Bohr effect are related to the conformation changes in the molecule of hemoprotein. It happens by its co-operating with the above mentioned ligands.

The processes, that are the basis of the ability of hemoglobin to take a part in transporting CO_2, are described by the following reaction equations :

A. In the pulmonary capillaries

Oxygenation of hemoglobin which increase its acid properties (degree of dissociation of the acid groups of its protein portion):

$HHb\ O_2 \rightarrow HHbO_2$

Interaction of the acid form of hemoglobin with the potassium bicarbonate, entering into the erythrocytes from blood plasma):

$HHbO_2 + KHCO3 \rightarrow KHbO2 + H2CO3$

The cleavage of carbonic acid that formed, under the action of the enzyme to the carbonic anhydrase:

$H_2CO_3 \rightarrow H_2O + CO_2$

B. In the capillaries of peripheral tissues

The cleavage of the oxygen from potassium salt of oxyhemoglobin:

$KHbO_2 \rightarrow KHb + O_2$

The formation of carbonic acid inside the red blood cells from carbon dioxide (which is generated due to the processes of decarboxylation):

$CO_2 + H_2O \rightarrow H_2CO_3$

The formation of the bicarbonate in the red blood cells by combining of carbonic acid with potassium salt of hemoglobin:

$KHb + H_2CO_3 \rightarrow HHb + KHCO_3$

Bicarbonate (HCO_3^-), which formed in this reaction, comes from erythrocyte in blood plasma (due to anionic exchange with the anion of Cl^-) and is transported to the lungs.

HbCO is a carboxyhemoglobin, which has a bright red color. It is formed in the presence of CO in the surrounding air. Affinity of Hb for CO is in 200 times higher than for oxygen that is why formation of carboxyhemoglobin blocks the formation of the oxyhemoglobin and transport of oxygen (fig. 1.10).

Therefore, even negligible quantities of carbon monoxide in the air are dangerous for life. Level of carboxyhemoglobin in the person that does not smoke is 0.5 -1.5% Hb; that smokes - 8-9% (especially its number is increasing after deep puffs).

HbOH (MetHb) is methemoglobin that has fulvous color. It is formed under some pathological conditions, for example, in poisoning by powerful oxidants (potassium manganite, potassium chlorate, sulfonamide etc.). The iron in the heme group is in the ferric state. Methemoglobin content is 0-37 μmol/L. This form of hemoglobin connot binds oxygen. Normally there are to 2% methemoglobin in the red blood cells. It is produced as a result of self-oxidation.

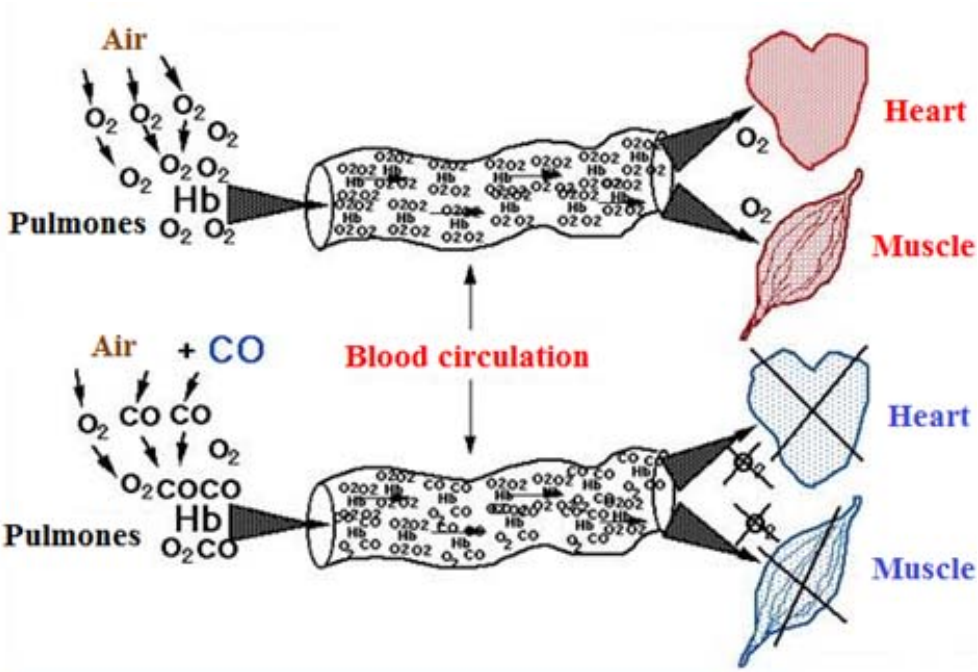


Fig. 1.10. Blocking oxyhemoglobin production and oxygen transport by the carbon monoxide (schematic image) [14].

Such very small amount does not supress gas exchange. Functioning of methemoglobin reductase enzyme (which renews MetHb) prevents accumulation of methemoglobin. Methemoglobinemia (increase in MetHb concentration) could have hereditary character (deficit of methemoglobin reductase) and developed as a result ointake of a great amount of oxidants – nitrites, aniline, nitrobenzene etc. (acute toxic methemoglobinemia developed) [37].

The binding of hemoglobin with different ligands, such as H^+ (at decreased pH) and CO_2, results in conformation changes in the hemoglobin molecule and changes the affinity of Hb to oxygen. CO_2 forces out O_2 from hemoglobin in the tissues. But in the lungs, O_2 forces out CO_2 from the blood to the alveolar air. It is known as the Bohr Effect. This effect is involved in the processes of pH blood regulation. In the tissues capillaries, proton binds to hemoglobin and, thus, it prevents acidification. Furthermore, increased quantities of H^+ in tissues (under formation of carbonic acid with CO_2) reduce affinity of Hb for oxygen. Conversely, in the pulmonary capillaries, when proton released, O_2 binds to Hb [39, 54].

2,3-biphosphoglycerate is a metabolite. It is formed from 1,3BPG (metabolic intermediate of glycolysis). It reduces affinity of hemoglobin for oxygen, which assists release O_2 in the tissues.

Hemoglobinopathies and thalassemias arise under hemoglobin synthesis disorders. These are "molecular diseases" which have hereditary character.

Hemoglobinopathies are the result of the changes of quantitative and qualitative amino acid composition of polypeptide chains of the hemoglobin. It belongs to the qualitative hemoglobinopathies.

Thalassemia (Thalassa- (gr.) is a sea) is the most common hereditary human disease. It is classified into groups, depending on which hemoglobin chains is pathologically synthesed. There are two main types: α- and β-thalassemias. Alpha-thalassemia is caused by disorders of synthesis of α-chains of Hb (as a result of deletion or inactivation of one of four genes encoding alpha-chains of globin). Beta-thalassemia is the result of disorders of synthesis of β-chains.

β4-tetramer is hemoglobin H or HbH that is formed in the organism of an adult human under conditions of alpha-thalassemia. These tetramers are unstable. There is no the S-shaped curve of oxyhemoglobin dissociation. In patients, hypochromic microcytic anemia is observed. Hemolytic symptoms of the disease are caused by the presence of such tetramers.

There is excess of α-chains of the hemoglobin under conditions of beta-thalassemia. Alpha-Chains do not form tetramers. α-Chains of Hb bind to membranes of the red blood cells and damage them. Homo- and heterozygote has clinical symptoms, which have various severities. There are varieties of symptoms: from heavy anemia with clinical semiotics, when human doesn`t live over 20 years (Cooley anemia) to mild microcytic anemia.

Erythropathy syndrome is typical for hemoglobinopathies and thalassemias. It accompanies with: reduction of the lifespan of the red blood cells, increased hemolysis, erythrocyte function abnormalities. There are about 300 abnormal hemoglobins. But some pathology has not clinical sympthoms. The first abnormal hemoglobins were named with Latin letters. There are many abnormal thalassemias

forms that's why to the names of the hemoglobins started to add the names of the place of its discovery (Boston) or the names of the hospitals.

S, C. D, E, H hemoglobins are often observed under hemoglobinopathies.

HbS is a mutant hemoglobin. In the β-chains, glutamic acid is replaced by valine (6Glu→6Val) at position 6. Valine has nonpolar radical. Consequently, that change caused a decrease of solubility of hemoglobin. The structure of the red blood cells changes as a result of the synthesis of HbS. Crystallization of the hemoglobin accompanies with an extension of the membrane and it becomes sickle-shaped. That is why this pathology is named sickle-cell anemia. As a result, there is an increase in blood viscosity, a decrement of the speed of blood flow, a decrease in the mechanical resistance of red blood cells; consequently, they lose the ability to pass through small capillaries. Such erythrocytes can be stuck in the capillaries, destroyed and they form a blood clot, resulting in chronic capillaropathy [37, 39, 45, 54].

HbC is abnormal hemoglobin in which a glutamic acid residue is substituted with a lysine (6Glu→6Lys) at the 6th position of the β-chain. This hemoglobin is crystallized in the red blood cells which are hemolyzed. As result, anemia is developed. The high content of HbC causes the development a mild form of hemolytic anemia.

HbD is a form of hemoglobin in which a glutamic acid (Glu) residue is substituted with a glutamine (Gln) at the 121st position of the β-chain. A mild form of hemolytic anemia is developed under a high content of this abnormal hemoglobin.

HbE is a form of hemoglobin in which glutamic acid (Glu) residue is substituted with a lysine (Lys) at the 26th position of the β-chain. This hemoglobinopathy is similar to β-thalassemia according to semiotics as a result of the deficit of normal β-chains. It is accompanied by the development of the hypochromic microcytic anemia and presence of the target red blood cells. There are also more severe forms of this pathology, which is accompanied by expressed splenomegaly.

HbM is a hemoglobin M group. In the polypeptide chains of HbM, a residue of histidine (which takes part in the binding to Ferrum-containing heme) is substituted with the other amino acid residue. For example, there is a change in HbMBoston

hemoglobin (α58His→58Tyr), in HbMSaskatoon (β 63His→63Tyr). The ferric Fe^{3+} form of iron cannot be reduced by methemoglobin reductase to Fe^{2+} in the hemoglobin which contains such molecular defect. Since methemoglobin (which contains the ferric (Fe3+) form of iron) is accumulated in the red blood cells. It is not able to perform the normal oxygen transport. This methemoglobinemia is the most evident in the homozygous state, in consequences; patients die under heavy hypoxia [27, 37, 45].

1.2.2. The main protein fractions of blood plasma (albumins, globulins, lipoproteins, fibrinogen, and indicator-proteins of blood)

Albumin (serum albumin) is a highly dispersed fraction of plasma proteins characterized by high electrophoretic mobility and low solubility in water and saline solutions [20].

Albumin molecule contains 600 amino acid residues. Its molecular weight is 67 kDa. Albumins are synthesized in the liver, as the most of the serum proteins. About 40% of albumins are in the blood plasma, the rest – in the interstitial liquid and lymph. It is characterised by its high hydrophilicity and high concentration in the blood plasma.

Proportion of albumins is 40-50 g/L (52-65% of the general protein).

Their main functions are to:

1) support the oncotic pressure of the blood plasma. Therefore, decrease of the quantity of albumins in the plasma, causes the oncotic pressure fall. The liquid flows out of the bloodstream into the tissue. A “hunger” edema is thus developing. Albumins provide about 80% of the total oncotic pressure of plasma. Albumins are easily lost easily with urine upon kidney disease. Therefore, they play important role in reduction of oncotic pressure by diseases which cause “kidney” edema;

2) transport carbohydrates, lipids, hormones, pigments, mineral substances. They bind to free fatty acids and transport them. This binding promotes the decrease concentration of physiologically active free fatty acids in 10000 times. Fatty hepatosis can be caused by decrease of the quantity of albumins. It is known, a half of

all calcium ions is bound with albumins in the blood. Albumin retains cations (mostly Na^+, Ca^{2+}, Zn^{2+}) due to high concentration of dicarboxylic amino acids in its structure. The dynamic balance between ionic and bounded Ca depends on albumins concentration. Such drugs as penicillin, sulfonamide, and aspirin make complexes with albumins. It ensures more continuance these compounds in the blood, thus, extend its action.

Albumins have also different specific binding sites with hormones (thyroid, steroid, insulin). It is known that saturation of albumins with one hormone does not suppress its binding with others. The quantity of albumins controls the amount of free hormones, which are active. Thus, it regulates the activity degree of some hormones. Decrease of the albumin amount leads to serious metabolic and physiological disorder which is related to with hormonal activity increase;

3) has buffer properties which depend on free amino and carboxyl groups in the protein structure.

4) carry out reserve and plastic functions. Albumins are a reserve of free amino acids in an organism. It is produced by proteolytic cleavage of proteins.

Considerable decrease in the concentration of albumins (to 5 g/L) is possible under kidney disease with expressed nephrotic syndrome [20]

Globulins are a heterogeneous mixture of protein molecules in which there are α-, β- and γ-globulins (α1 – 1-4 g/L, α2 – 4-8 g/L, β – 6-12 g/L, γ – 8-16 g/L). Each of these fractions contains specific proteins that perform certain biochemical functions. The normal concentration of globulins in serum is 20-30g/L. Unlike albumins, the globulins are not soluble in water but soluble in weak saline solutions [20].

α-Globulins have two fractions: α1- (4,5±0,2 g/L) and α2- globulins (5,6±0,2 g/L), which are identified by electrophoresis.

α1-Globulins functions are to:

1) transport lipids, while forming complexes - lipoproteins. Among the proteins of this fraction, there is a special protein - thyroxine-binding protein that intended for transport of the thyroid hormone thyroxine;

2) participate in the functioning of the blood coagulation system and the complement system - as part of this fraction, there are also some factors of blood clotting and components of the complement system;

3) regulate, for instance, some proteins of α1- globulins fraction are endogenous inhibitors of proteases;

α1-globulins have high hydrophilicity and low molecular weight. Therefore, in the pathology of the kidneys, they easily lost with urine. However, their loss does not significantly affect oncotic blood pressure because their content in the blood plasma is small. The fraction of α1-globulins includes proteins, the prosthetic group of which is carbohydrates, preferably hexoses and hexosimines. These include α1-antitrypsin, α1-acid glycoprotein, α-fetoprotein and others. There is the highest plasma concentration of α1-antitrypsin [17, 20, 29].

α1-Antitrypsin (2-2.5 g/L) is a glycoprotein (a natural proteinase inhibitor) with a molecular weight of 58-59 kDa. Its main function is the inhibition of elastase - an enzyme that hydrolyzes elastin (one of the major proteins of the connective tissue). α1-antitrypsin is also an inhibitor of proteases: thrombin, plasmin, trypsin, chymotrypsin, and some enzymes in the blood coagulation system. The amount of this protein increases with inflammatory diseases, with the processes of cellular decay, decreases in severe liver diseases. This decrease is the result of a disturbance of the synthesis of α1-antitrypsin, and it is associated with the excessive splitting of elastin. Hereditary disturbance of α1-antitrypsin synthesis lead to the development of lung emphysema in people aged 20-40 years and neonatal hepatitis, which may lead to cirrhosis of the liver. The cause of emphysema is the absence of mechanisms of inhibition of elastase (also proteinases) that is involved in destructive processes in the lungs.

The α1-acid glycoprotein (orosomucoid, AGP-α1-acid-glycoprotein) is a macromolecular protein whose physiological role has not yet been established, but there is a point of view on its immunomodulatory properties-AGP can bind to endogenous and exogenous inflammatory mediators. Orosomucoid can protect the body in conditions of increased production of inflammatory cytokines (for example,

at endotoxic shock). During the development of an inflammatory reaction, the concentration of this protein increases by 2-4 times, so AGP also belongs to the proteins of the acute phase [27, 29].

An increase in the concentration of α1-acid-glycoprotein is observed in inflammations, malignant tumors, and chronic pain syndrome. Its decrease is observed in early childhood, when taking contraceptives, genetic defects.

a-fetoprotein (AFP, α-Fetoprotein) is a glycoprotein of blood serum, which begins to be produced at the 5th week of fetal development. The structure of AFP is similar to albumin and this protein performs a similar function in the body of the fetus. In obstetrics and gynecology, this protein is one of the indicators of the state of fetal development and the presence of hereditary pathology. Determination of the concentration of AFP in the mother's blood, along with the level of β-chorionic gonadotropin (hCG) and free estradiol, is a "triple test" for diagnosing the risk of abnormalities in fetal development. In oncology, AFP is used as one of the embryonic immunological markers of malignant tumors (oncoprotein antigen). In adults, this protein is determined in blood at high concentrations in the case of benign or malignant proliferative processes in cells in which it is produced in the embryonic period. Thus, in patients, with hepatocellular carcinoma, the concentration of this protein can increase in 100 times (80-90% of patients), with chronic hepatitis - 10-25 times (30% of patients). In addition, the determination of AFP concentration is intended to detect liver metastasis, evaluation of the treatment of malignant tumors, screening of risk groups (patients with cirrhosis of the liver, deficiency of α1-antitrypsin) [27, 29].

α1-antichymotrypsin. It suppresses chymotrypsin and some proteinases of the formed blood elements.

α2-Globulins are high-molecular proteins, namely: haptoglobin, α2-macroglobulin, ceruloplasmin, C-reactive protein, thyroxine-binding protein, transcobalamin (vitamin B12-binding globulin), bilirubin binding globulin, transcortin (cortisol-binding globulin).

Haptoglobins (Hp) are glycoproteins, the biological function of which is the binding of free hemoglobin released by intravascular hemolysis. Thus, Hp prevents hemoglobin from being lost by the body because the Hp-Hb complex cannot pass through the renal glomeruli. In addition, it has been found that the Hp-Hb complex has high peroxidase activity and is involved in the functioning of the antioxidant system of the organism. The Hp-Hb complexes are destroyed by cells of the reticuloendothelial system (cells of the mononuclear phagocyte system), after which the globin splits into amino acids, the gem breaks down to bilirubin and excretes in bile, and the Ferrum remains in the body and can be re-mobilized.

The content of these proteins is approximately 1/4 of all α2 globulins. This protein is synthesized in the liver and belongs to the proteins of the acute phase. Its quantity increases and is the result of stimulation of synthesis by interleukins in all exudative-inflammatory processes. Hp content indicates the state of connective tissue - the amount of this protein increases with destructive changes in the tissue and decreases with glucocorticoid therapy. An increase in the concentration of haptoglobin is possible with nephrotic syndrome, tumors, cholestasis, collagenoses, lymphogranulomatosis, and the like. Reducing the concentration of this protein is observed in acute and chronic liver diseases, with all types of hemolysis, deficiency of glucose-6-phosphate dehydrogenase [27, 29].

α2-Macroglobulin (AMG) is a α2-globulin fraction protein, a universal serum proteinase inhibitor whose blood levels are highest in comparison with other proteinase inhibitors, averaging 2.5 g / L. α2-macroglobulin is a glycoprotein with a molecular weight of 725 kD. Concentration in the blood plasma is 1.5-3 g/L.

This is an acute phase protein, which is synthesized in the pancreas, is an inhibitor of proteases (like antitrypsin, but with a wider spectrum of activity). It inactivates plasmin, as well as reduces the activity of thrombin. It is an endogenous proteinase inhibitor of all classes, has antioxidant properties, and also binds insulin hormone. The half-life of AMG is very small - 5 minutes. It is a universal "purifier" of blood, complexes "AMG-enzyme" are able to absorb immune peptides, for example, interleukins, growth factors, tumor necrosis factor, and remove them from

the bloodstream. In children, the content of AMG increases with nephrotic syndrome. [27, 29].

The determination of this protein in the blood does not have an independent significance. In combination with other indicators (C-reactive protein, sialic acids, etc.), it reflects the presence of inflammation, especially in rheumatism and rheumatoid arthritis. The decrease of AMG concentration is observed in lesions of the pancreas and myocardial infarction.

Ceruloplasmin is the main Copper-containing metalloprotein of blood (contains 95% of the blood Copper and 3% of Copper of the organism). This protein has 8 binding regions for Copper. It performs several functions. First, ceruloplasmin binds to and provides transport of Copper in the blood. Secondly, it has enzymatic properties - it is oxidase of ascorbic acid, epinephrine, norepinephrine, DOPA, serotonin, inactivates ROS (an antioxidant property). It can also catalyze the oxidation of Fe^{2+} to Fe^{3+}, that is why it is also called ferroxidase. Ceruloplasmin belongs to acute phase proteins. An elevated concentration of this protein in the blood is observed in acute and chronic infectious diseases, cirrhosis, hepatitis, myocardial infarction, lymphogranulomatosis, some malignant tumors, and in patients with schizophrenia. The inadequacy of ceruloplasmin is the result of inhibition of its synthesis in the liver and is observed in the disease of Wilson-Konovalov or severe liver damage. The deficiency of this protein may also be due to its loss by the body (nephrotic syndrome).

C-reactive protein (CRP) is a glycoprotein, which is called so due to its ability to react with a pneumococcal C-polysaccharide (this is an important mechanism for early protection of organism from infection). CRP belongs to the proteins of the acute phase; it is a sensitive indicator of tissue damage in inflammation, necrosis, trauma. By its chemical nature, it is a glycoprotein.

CRP is synthesized predominantly in the liver. In serum of healthy human blood, it is contained at very low concentrations, so it is not determined by usual methods. The rapid increase in the concentration of C-reactive protein (hundreds of times) is observed in inflammatory processes of various etiologies and localizations,

infectious diseases, injuries, tumors that are accompanied by necrosis and inflammation. After myocardial infarction, the increase in the amount of this protein is present already on the 2nd day of the disease (in patients with angina pectoris, increasing the content of CRP is not observed). C-reactive protein is also found in the blood of the rheumatism phase, with collagenoses, cancer, bacterial and viral infections. During transition of the disease to the chronic phase, the concentration of C-reactive protein in the blood again decreases practically to 0 and increases with the aggravation of the process [27, 29].

Relatively new possibility of using the definition of CRP in the blood is an assessment of the risk of developing atherosclerosis and its complications. To perform this, very sensitive methods are used to evaluate the change in protein concentration in the range <10 mg / L.

The decrease in the concentration of α-globulins is observed in severe degenerative processes in the liver, cirrhosis, and myeloma. An increase in the content of α1- and α2-globulins in the blood is observed in all acute inflammatory processes. An increase in the α2-globulin fraction is a signal of exacerbation of the chronic disease, observed during the first few hours after myocardial infarction [27, 29].

β-Globulins. The β-globulins include important protein carriers of lipids and polysaccharides. The importance of lipoproteins is that they keep in solution insoluble in water fats and lipids and thus ensure their blood transfer. β-globulins are involved in the transport of phospholipids, cholesterol, steroid hormones, metal cations. About 75% of all fats and plasma lipids are part of lipoproteins. A small amount of lipoproteins is included in the α1-fraction of globulins. This fraction consists of transferrin, hemopexin, β2-microglobulin, low density lipoproteins, components of the complement system (C3) [27, 29].

Transferin (Tf) is a glycoprotein of plasma, which is synthesized in the liver and is the main carrier of ferrous ions (each transferrin molecule contains two atoms of trivalent ferrum). The synthesis of Tf in the liver depends on the functional state of the body, the needs and reserves of the ferrum in the body. Normally, about 30% of

this protein is bound to a ferrum; each protein molecule contains 2 metal atoms. 1 g of transferrin binds 1.25 mg of the ferrum. Transferin has high affinity for the ferrum, therefore, theoretically, 1 liter of blood contains only 1 free ferrous atom. The Tf content for women is 10% higher than for men. Its concentration is reduced in elderly people. With a lack of ferrum in the case of estrogen administration, the concentration of transferrin in the blood also increases. This protein belongs to acute phase proteins, but the response to acute inflammation is accompanied by a decrease in its content. For example, in a fever, the synthesis of Tf inhibits interleukins. The decrease in the content of transferrin is accompanied by diseases such as chronic inflammatory processes, cirrhosis, malignant neoplasms, androgens and corticosteroids, hemochromatosis, myeloma, etc. [27, 29].

Hemopexin (P-globulin) is a protein that binds free hem of hemoproteins (hemoglobin, myoglobin, catalase) and transports it into PEC liver cells for re-utilization. This prevents the removal of heme by the kidneys and the loss of Ferrum with the urine. In clinical and laboratory diagnosis, hemopexin is considered to be better an indicator of hemolysis than haptoglobin, because this protein does not belong to acute phase proteins. The concentration of hemopexin decreases in hemolysis, liver and kidney diseases; it increases with inflammation [27, 29].

β2-Microglobulin (β2-MG) is a low molecular weight protein of surface antigens of cellular nuclei. This is a component of the first class proteins of the main complex of histocompatibility. β2-MG has antioxidant properties. In the blood, it has a concentration of 2.4 mg/L, it is removed with urine (about 130 μg/liter). The increase in the content of this protein is observed in malignant diseases, rheumatoid arthritis, myocardial infarction, burns, autoimmune diseases, cellular immunity disorders (eg AIDS), organ transplantation, etc. In urine, the concentration of β2-MH increases with diabetic nephropathy, intoxication with heavy metals (eg, cadmium) [27, 29].

γ-Globulins. This fraction contains the bulk of antibodies (immunoglobulins) that provide the humoral protective response of the organism, as well as blood

agglutinins (α-and β-) and cryoglobulins. Globulins are formed in the liver, bone marrow, spleen, lymph nodes.

Immunoglobulins (antibodies) comprise a group of proteins that are produced in response to the entry of 'alien structures' (antigens) into the body. They are synthesized in lymph nodes and spleen by lymphocytes B. There are 5 classes of immunoglobulins - IgA, IgG, IgM, IgD, IgE.

Immunoglobulin molecules have a structure plan (fig. 1.11). The structural unit of the immunoglobulin (monomer) forms four polypeptide chains, which are joined together by disulfide bonds: two heavy (chain H) and two light (chains L) chains (fig. 1.11). N-terminal portions of both the L- and H-chains are called variable regions (V) since their structure is characterized by significant differences in different classes of antibodies. Inside the variable domain, there are 3 hypervariable regions that are distinguished by the largest variety of amino acid sequence [27, 29].

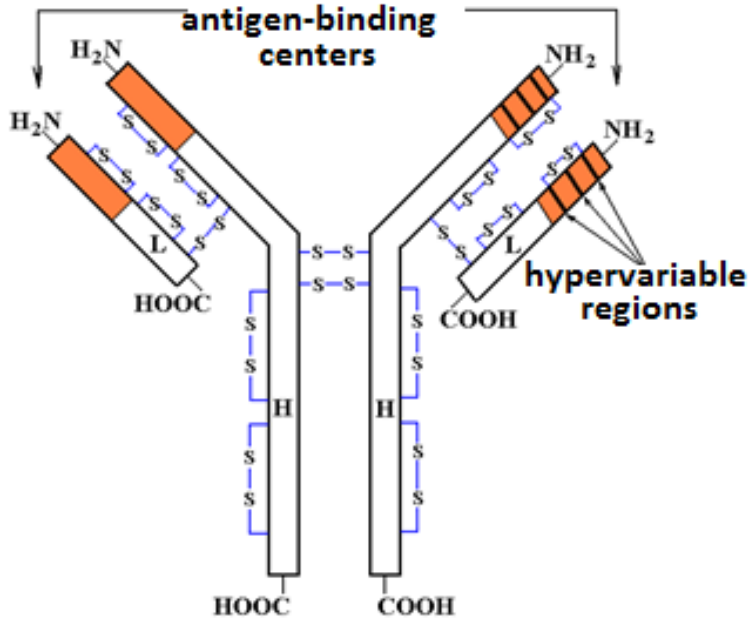


Fig. 1.11. Scheme of the structure of immunoglobulins (orange shows the variable region, white - the constant region) [14].

It is the variable portion of the antibodies which is responsible for the binding of antigens by the principle of complementarity; the primary structure of the protein chains in this region determines the specificity of the antibodies.

The C-terminal domains of the H- and L-chains have a relatively constant primary structure within each class of antibodies and hence these are called a constant region or ‘C-region’. The constant region determines the properties of different classes of immunoglobulins, their distribution in the body; they can participate in the launch of mechanisms that cause the destruction of antigens.

IgG, IgD and IgE in their structure, as a rule, are monomers. IgM molecules are composed of five monomers, IgA consist of two or more structural units or are monomers (fig. 1.12).

Immunoglobulins of different classes differ in biological properties, namely: in their ability to bind to antigen [26, 27, 29].

Functions of immunoglobulins.

IgG (59-55 μmol/L) are the major antibodies of secondary immunity. They form 80% of all blood serum immunoglobulins. So, these are the main blood serum immunoglobulins, that ensure the protection of the body from many bacteria, viruses, and their toxins. They are the only immunoglobulins that can penetrate through the placenta to the fetus body. It is known that there are four subclasses of IgG, which differ in the structure of heavy chains.

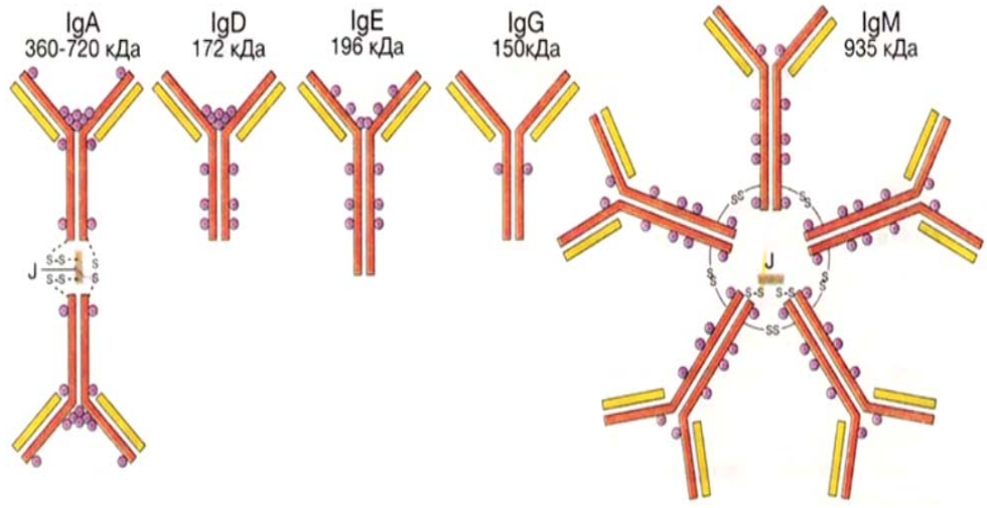


Fig. 1.12. Classes of immunoglobulins [14].

IgA (19-25 μmol/L) occurs in the serum of blood and is the major antibody in serous-mucous secretions (saliva, bronchial mucus, tears, female colostrum, mucous membrane of the intestine).

IgM (0,8-1,2 μmol/L) is the major antibody of primary immunity. It is a powerful activator of the complement system. Immunoglobulins of this class are Wasserman antibodies, rheumatoid factor, cold agglutinins, and iso-gem-agglutinins. These immunoglobulins are the first to be synthesized in the fetus body and due to immunizing adults with most antigens.

IgD (0,26 μmol/L) is presented on the surface of B-lymphocytes, it participates in the recognition of antigen.

IgE (0,3-30 nmol/L) is presented on the surface of mast cells and basophils. There is a point of view that they are involved in immune protection from helminths and in reactions of hypersensitivity of an instant type. It also involved in allergic reactions [26, 27, 29].

Due to electrophoresis, immunoglobulins move in the γ-globulin zone, but IgA and IgM are found in the β- and α2-globulin fractions.

IgG, IgA, IgD, IgE are secreted mainly by plasma cells, IgM - predominantly by lymphocytes. The major weight of immunoglobulins has IgG.

The amount of γ-globulin in blood plasma depends on the morphological maturity and functional integrity of the immunoreactive tissue. The amount of γ-globulin of the newborn is the same as that of the mother. In the first year of life of a child, γ-globulins are mainly represented by mother`s IgG immunoglobulins. After birth, their number gradually decreases and, starting from 4 months, the synthesis of own IgG is terminated [26, 27, 29].

There are diseases, in which the production of certain classes of Ig is disturbed. Almost always, alterations in the level of Ig are due to a disturbance of the rate of synthesis or secretion of these molecules [27].

Cryoglobulins are proteins that are able to precipitate due to cooling serum. In healthy people, they are not in serum. They appear in patients with rheumatic arthritis, myeloma.

Among the cryoglobulins, there is fibronectin protein. This is a high molecular weight glycoprotein (molecular weight 220 kDa). It is present in the plasma of blood and on the surface of many cells (macrophages, endothelial cells, platelets, fibroblasts).

Fibronectin functions. Fibronectin: 1) provides interaction of cells with each other; 2) promotes platelet adhesion; 3) prevents metastasis of tumors. Fibronectin in plasma is opsonin, and it enhances phagocytosis. It plays an important role in clearing blood from the products of proteins metabolism, for example, collapsed collagen. Fibronectin can bind heparin thus binding with heparin, it participates in the regulation of blood coagulation processes. Currently, this protein is widely studied and used for diagnosis, especially under conditions that are accompanied by inhibition of the macrophage system (sepsis, etc.) [27, 45].

Lipoproteins

Lipoproteins are complex compounds that carry out lipid transport in the blood. They include a hydrophobic nucleus containing triacylglycerols and cholesterol esters, and an amphiphilic membrane formed by phospholipids, free cholesterol, and proteins-apoproteins (fig 1.13).

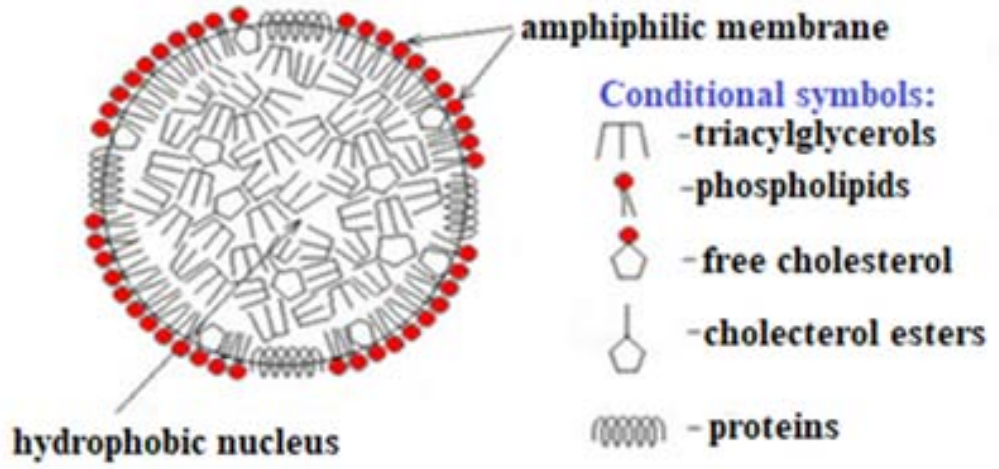


Fig. 1.13. Scheme of the structure of blood plasma lipoprotein [14].

Human blood plasma contains the following fractions of lipoproteins:

High-density lipoproteins (HDL) or α-lipoproteins, they move along with α- during electrophoresis. They contain many proteins and phospholipids, and transport cholesterol from peripheral tissues to the liver.

Lipoproteins of low density (LDL) or β-lipoproteins, as well as during electrophoresis, move along with β-globulins, are enriched by cholesterol and transport it from the liver to peripheral tissues.

Very low-density lipoproteins (VLDL) or pre-β-lipoproteins (located on the electrophoregram between α- and β-globulins) are a transport form of endogenous triacylglycerols and precursors of low-density lipoproteins.

Chylomicrons are electrophoretically immobile; in the blood, taken on an empty stomach, they are absent. They are a transport form of exogenous (food) triacylglycerols [20].

Fibrinogen

Fibrinogen is a colorless, fibrous protein from a group of globulins dissolved in blood plasma. Its molecular weight is about 350 kDa.

Blood plasma, which does not contain fibrinogen, is called serum. Fibrinogen is formed in the liver.

The molecule has a globule shape of about 22 nm in diameter.

Synthesis of fibrinogen in the body occurs in parenchymal cells of the liver. The content of fibrinogen in the blood plasma of a healthy person is 300-500 mg. Due to fibrinogen deficiency in the body or due to the formation of molecules with an abnormal structure, bleeding may occur [20, 42].

Fibrinogen molecules consist of two outer D domains, each connected by a coiled-coil segment to its central E domain. The molecule is comprised of two sets of three polypeptide chains termed Aα, Bβ, and γ, which are joined together in the N-terminal E domain by five symmetrical disulfide bridges. Non-symmetrical disulfide bridges form a ‘disulfide ring’ in this region. Due to activating the blood coagulation system,fibrin is formed after thrombin cleavage of fibrinopeptide A from fibrinogen Aα-chains leading to initiating fibrin polymerization (fig. 1.14).

Fibrinogen is a valuable indicator of hemostasis (coagulogram). Analysis of fibrinogen is a necessary stage of preoperative examination, prenatal diagnosis, is carried out in inflammatory and cardiovascular diseases. The content of fibrinogen in the blood increases due to the occurrence of acute inflammatory diseases that can lead to cell death. and death of tissues.

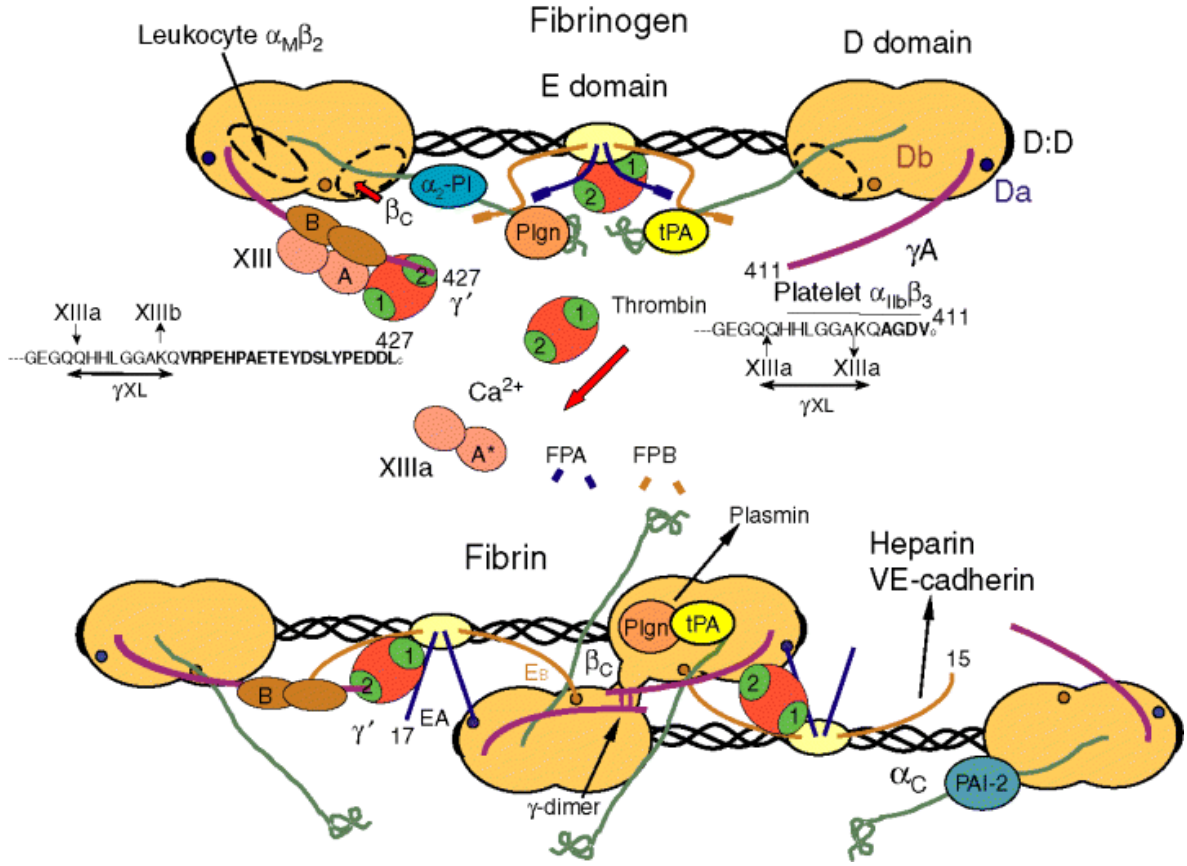


Fig.1.14. Schematic diagram of fibrinogen structure, its conversion to fibrin, and the thrombin-mediated conversion of native factor XIII to XIIIa. Binding sites for proteins, enzymes, receptors, and other molecules that participate in fibrin(ogen) functions [66, 109]

Fibrinogen also affects the rate of erythrocyte sedimentation (RES). Normal fibrinogen level for adults is in the range 2-4 g/L. Normal fibrinogen level for a newborn is in the range 1.25-3 g/L. The norm for fibrinogen in pregnancy is slightly higher. During this period, there is a gradual increase in fibrinogen and in the third

trimester of pregnancy the level of fibrinogen reaches 6 g/L. In other cases, elevated fibrinogen in the human blood is a symptom of the following diseases: acute inflammatory and infectious diseases (influenza, tuberculosis), stroke, myocardial infarction, hypothyroidomyloidosis, pneumonia, malignant tumors (cancer of the lungs). The increase in the level of fibrinogen accompanies burns, surgical intervention, and the administration of certain hormonal drugs [29, 30].

The normal level of fibrinogen is reduced due to such diseases as: DVS syndrome, liver disease (hepatitis, cirrhosis), pregnancy toxicosis, vitamin C and B12 deficiency, embolism with amniotic fluid (in newborns), chronic myeloleukosis, polycythemia.

The level of fibrinogen also decreases due to poisoning with snake venom, due to receiving anabolic hormones, androgens and fish oil [29, 30].

Fibrinogen, obtained by plasma deposition of ethanol, is used to stop bleeding in operations, in obstetric and gynecological practice, in hemophilia, and in diseases associated with low levels of fibrinogen in the blood.

Fibrinogen products are available for laboratory testing. Fibrinogen, derived from human blood, is used for clinical purposes [29, 30, 57].

CHAPTER 1.3
FRACTIONATION OF BLOOD SERUM PROTEINS. HYPO-, HYPER-, DYSPROTEINEMIA. PARAPROTEINEMIA

1.3.1. Blood serum protein fractionation

Electrophoresis is used for determination of serum proteins and detection of paraproteins. The method was developed by A. Tiselius, who won the Nobel Prize in Chemistry in 1948 for this important discovery in biomedical practice.

Based on international standards, estimation of electrophoresis results is conducted by comparison of electropherograms investigation blood plasma proteins with molecular-weight size markers..

Electrophoretic principle of separation serum proteins (and other biological liquid) consists in the difference of molecules moving in the constant electric field depending on their charge value and molecular weight mass. In clinic practice, electrophoresis is most often used on chromatographic paper, cellulose acetate membranes, different gels, compound carrier. Electrophoresis on paper is the simplest method of protein fractions separation but it has some essential drawbacks deficiences [57, 89].

Different devices are used for electrophoresis, which are equipped with computers, electronic color scanners, mini-cameras to increase research accuracy..

The movement direction of protein molecules under electrophoresis depends on pH value of the buffer solution and its ionic strength. All serum proteins have negative charge and move from cathode to anode under pH=8.6 or 8.9 and ionic strength of 0.08-0.15 mol/L.

At the end of electrophoresis, electropherograms are dyed by Coomassie Blue R-250, paper or film slips are dried and then to dye (for example, by bromophenol blue).

Thus, there are slips which are called electrophoregrams or proteinograms. The color intensity corresponds to the concentration of some fractions and is determined by photometer or densitometer.

The results are expressed in relative units (%) and or absolute concentrations values (g/L). Concentration expressed in g/L is more accurate, thus avoiding of the errors relative measurements. For this percent content of some fractions is multiplied by the value of concentration of the total protein in serum.

As a rule, the serum is used for blood protein fractionation because plasma`s fibrinogen makes strips in the β2-globulin zone. It complicates identification of some blood proteins and it could bring an error in determination of paraproteins [7, 31, 57].

In serum of a healthy patient, 5 fractions are detected, such as albumins, globulins (α1, α2, β, and γ) (fig. 1.15) by using the simplest method of electrophoresis on paper.

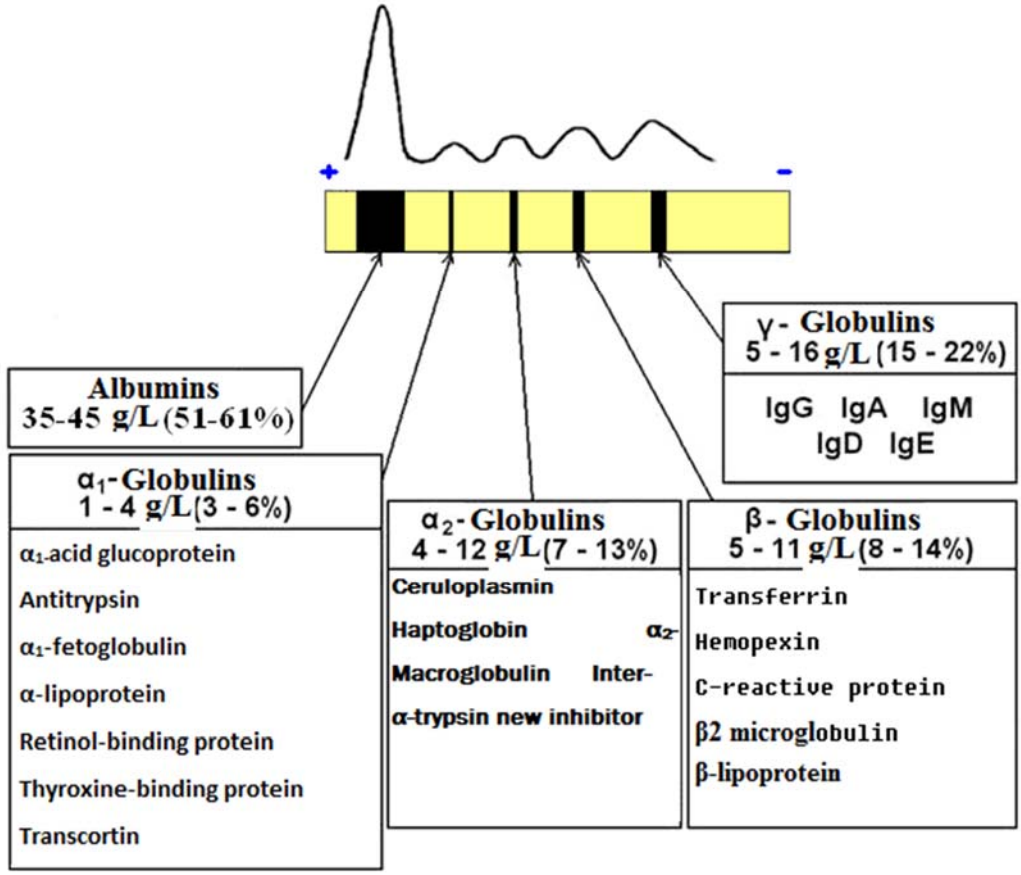


Fig. 1.15. Densitogram and proteinogram of the blood plasma proteins in the norm [57].

Table 1.3 shows the electrophoregrams (proteinograms) of blood serum proteins of a healthy person and under some pathological states that are obtained by electrophoresis on paper (5 fractions).

Table 1.3

Typical proteinograms obtained by electrophoresis on paper [57]

Type	Diseases	Albumin	Globulin			
			α_1	α_2	β	γ
1	Acute inflammation processes	↓	↑	↑	→	→
2	Chronic inflammation processes	↓m	↑m	↑	→	↑
3	Nephrotic syndrome	↓	→	↑	↑	↓m
4	Malignant neoplasm	↓	↑	↑	↑	↑
5	Hepatitis	↓m	→	→	↑m	↑
6	Liver cirrhosis	↓	→	↓	↑	↑
7	Jaundice	↓m	→	↑m	↑m	↑m
8	β-globulins plasmocytoms	↓	↓	↓	↑	↓
9	γ-globulins plasmocytoms	↓	↓	↓	↓	↑
10	α_2-globulins plasmocytoms	↓	↓	↑	↓	↓
↓ - decrease; ↓m - moderate decrease; ↑ - increase; ↑m – moderate increase; → - without changes						

The 1st type of proteinogram is typical for acute inflammation processes (the sepsis, the first study of pneumonia, myocardial infarction, exudative tuberculosis); an increase of synthesis of the acute phase proteins is observed. That is why the concentration of α1- and α2- globulins increases. Thus, albumin synthesis decreases but concentration of main protein in the blood does not change.

The 2nd type of proteinogram is typical feature for chronic inflammation (cholecystitis, cystitis, final stage of pneumonia, chronic form of tuberculosis, collagenosis). These diseases are accompanied by a decrease of ratio albumins to (α2- + γ-globulins) which is lower than 2.2. It shows that α2- and γ-globulins synthesis is increased.

The 3rd type of proteinogram characterises alterations in renal function, such as decreased glomerular filtration rate, gestational toxicosis, and cachexia, neurological symptoms, which are is depended on lipidosis or kidney amyloidosis and some other

diseases. An appreciable decrease of albumin concentration is observed (protein is caused by the elevated glomerular permeability,), increased concentration of α2-globulins and β-globulins (owing to increased concentration of α2-microglobulin or pre-β-lipoproteins).

The 4th type of proteinogram is typical at metastatic neoplasm with different localization primary tumor. It is characterized by decreased albumin synthesis in the liver and enhanced protein formation of all globulin fractions.

The 5th type of proteinogram is typical for hepatitis, liver toxic damage, leukemia, tumors of lymphatic system, dermatosis, some forms of polyarthritis etc. There observed albumin concentration decreasing (due to hepatic protein synthesis inhibition) and increased γ-globulin level (in lower grade β-globulin).

The 6th type of proteinogram is observed at cirrhosis, heavy forms of pulmonary tuberculosis, collagenosis, and chronic polyarthritis. It is characterized by substantial reduction of albumin concentration and α2-globulins (because of dystrophic liver changes, which results in the inhibition of protein synthesis) and a slight increase of γ-globulin fraction (compensatory mechanism).

The 7th type of proteinogram is incidental for jaundice, pancreatic head tumor, which support cholestasis syndrome. There observed decreased concentration of albumins and increased number of α2-, β- and γ- globulins.

The 8th type of proteinuria is characteristic of beta-globulin plasmacyde (for example, Waldenstrom macroglobulinemia); it is accompanied by a decrease in the content of albumins and globulins (with the exception of β-globulins).

The 9th type of proteinogram is observed in γ-globulin plasmacytomas; it is accompanied by a decrease in the concentration of albumins and all globulin fractions, with the exception of γ-globulins, the content of which increases in the blood.

The 10th type of proteinuria is inherent to α2 globulin plasmacytoma; an increase in the concentration of only α2-globulins is observed with a decrease in the protein content of all other fractions [7, 31, 57].

When using an agarose gel, 6 fractions are observed, namely:

Albumin: albumins;

Alpha-1-zone: alpha-1-antitrypsin, orosomucoid, alpha-1-antichymotrypsin;

Alpha-2-zone: haptoglobin, ceruloplasmin, alpha-2-macroglobulin, alpha-lipoproteins;

Beta-1-zone: hemopexin-transfers;

Beta-2-zone: betalipoprotein, C3 component of complement;

Gamma Zone: IgG, IGA, IgM, IgD, IgE.

17 protein fractions are observed during electrophoretic separation on a polyacrylamide gel. An even greater amount can be obtained by immuno-electrophoresis, which is the most informative to detect the minimum concentrations of abnormal immunoglobulins (paraproteins), for example, in myeloma or Waldenstrom's disease.

The mobility of some proteins may vary (especially albumins), when they are bound to drugs or other ligands (for example, bilirubin). This leads to the formation of additional bands on the electrophoregram [7, 31, 57].

Proteinograms are considered as an additional diagnostic test because their significance for diagnosis and control of treatment is quite controversial.

1.3.2. Hypo-, hyper-, dysproteinemia. Paraproteinemia

In clinical practice, there are conditions characterized by changes in both the concentration of total proteins of the plasma and the percentage of individual protein fractions.

The change in protein concentration may be relative or absolute and depends on the volume of circulating blood, may be accompanied by a decrease in the concentration of total proteins (hypoproteinemia) or an increase (hyperproteinemia). Hypoproteinemia is almost always associated with a decrease in the concentration of blood albumins (hypoalbuminemia), while hyperproteinemia is the result of increased concentrations of globulins (hyperglobulinemia) [29, 57].

Hypoproteinemia is the most common type of changes in the concentration of proteins in the blood and is observed in most diseases of the internal organs.

Absolute hypoproteinemia develops as a result of:

1) insufficiency of intake of proteins in the body with food (fasting, esophageal tumors, stomach ulcers and others - conditions that are accompanied by deterioration of digestion and absorption of proteins in the gastrointestinal tract);

2) disturbance of the synthesis of proteins in the liver (parenchymatous hepatitis, cirrhosis, malignant neoplasms, thyrotoxicosis, the action of certain chemical compounds);

3) increased breakdown of proteins in the body, which is observed with a shortage of energy and plastic resources (thermal burns, malignant neoplasms, hyperthermia, alterations in the hormonal status of the body);

4) proteins loss by an organism under chronic kidney diseases with a nephrotic component (the protein content can decrease to 25-30 g / L) or with blood when bleeding;

5) defectoproteinemia, which arises as a result of an inherited disturbance of the synthesis of blood proteins (albuminemia, agammaglobulinemia, absence or deficiency of ceruloplasmin in the disease of Wilson-Konovalov, etc.).

Relative hypoproteinemia is the result of an increase in the volume of the liquid part of the blood (with anuria, disturbance of the regulation of water-salt metabolism in the hypersecretion of the antidiuretic hormone or aldosterone, metabolic acidosis, etc.) [29, 57].

Hyperproteinemia is an increase in the total number of plasma proteins; it can also be absolute or relative. Most often, relative hyperproteinemia develops, as observed in patients with surgical diseases and is considered as a sign of dehydration. This type of hyperproteinemia occurs when burns, generalized peritonitis, cholera, diabetes mellitus, chronic nephritis in the stage of polyuria, respiratory acidosis and the like.

Absolute hyperproteinemia develops rarely and is associated with an increase in γ-globulins as a result of a toxic or infectious process. An increase in total proteins up to 200 g/L is possible due to "pathological" proteins - paraproteins. Such proteins are: C-reactive protein, the concentration of which increases in the blood in

rheumatism, myocardial infarction; Bens-Jones protein that appears in the blood with myeloma (plasmacytoma); cryoglobulins.

Dysproteinemia is developed at a change in the ratio of individual protein fractions to normal total protein content. The most pronounced dysproteinemia results due to changes in organs and systems, in which the synthesis of plasma proteins is carried out. Below are indicated the possible causes of dysproteinemia occurrence [29, 57].

Hypoalbuminemia can be primary and acquired (secondary). The main causes of hypoalbuminemia are:

1) decrease in the synthesis of albumins (malnutrition, defects in digestion and absorption of proteins in the digestive tract (GIT), destructive changes in the liver);

2) abnormal albumin losses (through the kidneys, bleeding, anaphylactic shock, the formation of exudates, transudates, etc.).

Secondary hypoalbuminemia is observed in fasting, long-term obstructive jaundice, portal cirrhosis, viral hepatitis. A significant decrease in albumin concentration in serum is observed in chronic kidney damage - nephrotic syndrome (up to 5 g/L, with a decrease in the total protein content to 25-30 g/L). In addition, the albumin concentration decreases due to burns, infectious diseases, tuberculosis, malignant tumors (cancerous cachexia), leukemia, anemia, myocardial infarction, pregnancy, hyperhydration, and the like. In some diseases and pathological conditions (elevated cortisol and thyroid hormones secretion, after significant surgical interventions, etc.) catabolism of albumins is increased, which also leads to hypoalbuminemia.

Alterations in the concentration of albumins in the blood may be primary. They include albuminemia and double albuminemia. These hereditary abnormalities are rare. With albuminemia, the concentration of albumins in the blood can decrease significantly - up to 10 g/L. Double albuminemia is characterized by the presence of two identical homogeneous lines in the region of albumins on the blood electrophoregram [27, 29, 57].

Hyperalbuminemia is observed rarely, it has a relative nature as a consequence of hypo- and dehydration.

A decrease in the concentration of α-globulin fraction is observed in lymphocytic leukemia, myeloma, diabetes mellitus, acute liver dystrophy, hereditary deficiency of α-1-antitrypsin.

The increase of α-globulins is characteristic for acute and subacute inflammatory processes (diphtheria, colposic pneumonia, pyelocystis, cholecystitis, etc.), allergic, stress states, non-fibrotic processes. The increase of protein of this fraction is observed in mechanical jaundice, malignant tumors (α2-globulins), pregnancy, α-2-plasmacytomas.

The β-globulin fraction contains the largest number of lipids. It contains ¾ of all lipids in the blood. That is why increasing the protein content of this fraction is always observed in primary and secondary hyperlipoproteinemia. Also, an increase in the content of β-globulins occurs in chronic infections, liver cirrhosis, toxic liver damage, obstructive jaundice, diabetes mellitus, malignant tumors, cachexia, starvation, pregnancy, and others. [27, 29, 57].

The decrease of β-globulins is accompanied by hypobetalipoproteinemia.

The development of hypogammaglobulinemia is associated with hereditary lesions of humoral immunity and plasmacytic tissue (Bruton's disease, Swiss agammaglobulinemia) or secondary inhibition of immunoreactive tissue with toxin and toxic-infectious effects, with loss of Ferrum (kidney disease, burns, eczema, etc.), treatment with corticosteroids, immunosuppressants, chemotherapy, radiation therapy, etc.

Hypergammaglobulinemia is due to a total increase in the content of virtually all Ig classes (due to septic infections, pneumonia, scarlet fever, collagenoses, tumors, toxic liver damage, obstructive jaundice, autoimmune diseases, liver cirrhosis, cystitis, starvation, etc.) [27, 29].

Paraproteinemia is caused by the presence of pathological immunoglobulins (Pig), which are secreted by tumor cells of the system of B-lymphocytes and are not detected at physiological conditions. These proteins appear in blood and urine in

patients with signs of their degradation, allergies, tumors, inflammation. For detection paraproteins in the blood and urine electrophoresis is used in agar or immuno-electrophoresis. Due to electrophoresis, Pig-monoclonal immunoglobulins have mobility in the region between γ- and β- or α-globulins, and on the electrophoregram give a homogeneous band of the M-gradient.

As mentioned above, examples of paraproteins can be Bens-Jones, Cryoglobulins and some others. By chemical structure, these proteins are similar to immunoglobulins but have no antibody properties. Paraproteins are detected, for example, with myeloma (plasmacytoma), Waldenstrom macroglobulinemia.

At Waldenstrom's disease, electrophoregrams show a clear peak of abnormal paraproteins with a high molecular weight of the M-fraction [27, 29].

With myeloma in urine, there is the presence of Bens-Jones protein. This protein consists of Ig light chains, has a small molecular weight, and therefore easily passes through a renal filter.

On the electrophoregram, it gives a band that is located between γ- and β-globulins. A characteristic feature of this protein is that it precipitates at urine temperature of 40-60 ° C and then dissolves again with further heating to 85-100 ° C.

Cryoglobulins, which are γ-globulins, also belong to paraproteins. These proteins were discovered in 1974, they received their name due to the general property of precipitation and heliation when cooling blood serum. It is established that if their precipitation occurs at a temperature of 15 ° C, cryoglobulinemia passes without symptoms. In the event that this threshold is higher, severe circulatory disorders (rash, gangrene, etc.) may develop. Cryoglobulins that form a gel at 37 ° C are dangerous for the development of thrombosis. Cryoglobulins can be detected in cirrhosis of the liver, leukemia, malaria, myocardial infarction, tuberculosis, myeloma, Waldenstrom macroglobulinemia, chronic kidney damage, and others. The presence of cryoglobulins in the blood is determined after the patient's blood serum is kept in the refrigerator for 12 hours at 4 ° C.

Elevation of the concentration of paraproteins in the blood significantly increases the concentration of total proteins (up to 200 g/L), the viscosity of blood, sediment samples become sharply positive [27, 29].

CHAPTER 1.4
BUFFER SYSTEMS OF BLOOD

1.4.1. General understanding of the buffer systems of organism

The normal function of the cell depends on the constancy of its volume, composition, and pH of the body's fluid. Regulatory mechanisms that control the normal volume, osmotic pressure, ionic composition and H^+ content in the body fluids are interrelated.

The system that maintains pH is a system of dynamic equilibrium: H^+ is constantly present in the intracellular medium as a metabolite and is released from it. The acidity or basicity of blood depends on the concentration of H^+. The pH of the arterial blood of a human is normally – 7.35-7.45, venous - at 0.02 units below. Their metabolism is closely interconnected, and at the same time acids are formed - carbonic, lactic and pyruvic acids, acetoacetate, β- hydroxybutyrate, sulfuric acid, hydrochloric acid, and others. Various pH changes depend on the chemical buffers of the body's fluid, the activity of the lungs, kidneys and intestines. In human blood, there are special buffer systems that help to maintain blood pH. It is believed that, due to the very existence of buffer systems, blood pH is the most stable parameter of the internal environment.

Normally, the pH value in different liquid compartments of the organism is not the same, but its fluctuations are small, the pH of the intracellular, tissue fluid and blood belongs to rigid homeostatic constants. In table 1.4, the pH values of different compartments are indicated.

As a rule, the buffer system is a mixture of weak acids with salts of these acids and strong alkalis that neutralize, respectively, alkalis and acids that can accumulate in the body during metabolism, thus counteracting the deviation of *pH* from the physiological level [26, 29, 57].

The pH of any buffer system can be calculated from the equation:

$$pH = pK_{of\ acid} + \lg \frac{[salt]}{[\ acid\ ]}$$

Releasing and absorption of protons from the environment helps to stabilize the normal values of the *pH* of the body fluids (blood and tissues) in the case of receiving of acids or alkalis.

Table 1.4

The pH value of the main biological fluids in the organism [27]

Name of liquids	pH
Arterial blood	7.36 – 7.42
Venous blood	7.26 – 7.36
Cerebrospinal fluid	7.4 – 7.5
Intercellular tissue fluid	7.26 – 7.38
Tissue fluid of muscle	6.7 – 6.9
Pancreatic juice	7.8 – 8.4
Hepatic bile	7.3 – 8.0
Gall bladder	6.0 – 7.0
Saliva	5.8 – 7.8
Gastric juice	1.4 – 1.8
Content of the small intestine	7.5 – 8.6
Content of the large intestine	8.0 – 9.0

The buffer solution contains weak acid and its salt formed by a strong base. Such a mixture has a certain and relatively constant concentration of hydrogen ions, which depends on the ratio of the acid and salt and the ionic strength of the mixture [26, 29, 57].

The following buffer systems function in the blood (in brackets the acid-base pairs of each system is indicated):

- hemoglobin (reduced Hb - potassium salt Hb: HHb-$KHbO_2$);

- hydrocarbonate or bicarbonate (carbonic acid - sodium bicarbonate: H_2CO_3 - $NaHCO_3$);
- phosphate (monobasic-sodium biphasic phosphoric acid: NaH_2PO_4 - Na_2HPO_4);
- protein.

Each of them has a certain buffer capacity and their interaction is interconnected. The most powerful buffer system of blood is hemoglobin. Its buffer capacity is 73-76%. The capacity is 17-27% for bicarbonate, 2-5% for protein, 1-2% for phosphate buffer. The main physicochemical system of plasma pH regulation is the bicarbonate buffer system. In addition, hemoglobin, protein, and phosphate buffer systems regulate blood pH precisely because of the stabilization by this buffer [26, 27, 29, 57].

1.4.2. Hemoglobin buffer system

The hemoglobin buffer system provides the largest buffer capacity of the blood. The dissociation constant in hemoglobin is lower than the *pH* of blood, so hemoglobin dissociates as an acid. Oxyhemoglobin is a stronger acid than hemoglobin itselfDue to the dissociation of oxyhemoglobin in the capillaries of tissues with the oxygen release, there is a greater amount of alkaline-reactive salts of hemoglobin, capable of binding H-ions coming from acids of a tissue fluid, for example, carbonic acid. Oxyhemoglobin is usually potassium salt. In the interaction of acids with the potassium salt of oxyhemoglobin, an appropriate potassium salt of the acid and free hemoglobin with the properties of very weak acid is formed [57, 94-96].

The hemoglobin buffer system is represented by several subsystems - $HHb/HHbO_2$, KHb/HHb, $KHbO_2/HHbO_2$.

In erythrocytes of peripheral tissues, $KHbO_2$ releases oxygen, which diffuses into tissues, and K^+ ion. After that, Hb binds H^+, which is released by the dissociation of carbonic acid and converted to HHB - a weaker acid. Potassium ions are coupled with hydrocarbon, which was formed from CO_2. The sequence of reactions looks like this:

1) $CO_2 + H_2O \leftrightarrow H_2CO_3$;

2) $H_2CO_3 \leftrightarrow H^+ + HCO_3^-$;

3) $HCO_3^- + K^+ \leftrightarrow KHCO_3$;

4) $KHb + H_2CO_3 \rightarrow KHCO_3 + HHb$.

Venous blood that goes to the lungs contains erythrocytes with reduced hemoglobin. $HHbO_2$ is formed from the HHB in the lungs. In addition, in the lungs, due to the release of a large amount of CO_2, an excess of anions HCO_3^- is observed, therefore there is a risk of shifting the *pH* to the alkaline side. This is not happening due to the fact that the anions of HCO_3^- are transported into erythrocytes and become the basis of $KHCO_3$. In the interaction of $KHCO_3$ with $HHbO_2$, $KHbO_2$ and H_2CO_3 are formed. Next, the carbonic acid under the action of carbonic anhydrase dissociates into H_2O and CO_2. Carbonic acid is excreted through the lungs [57, 94-96].

Thus, the functioning of the hemoglobin buffer system is associated with the main function of hemoglobin, namely, the transport of oxygen.

It is important that the hemoglobin buffer system interacts with the bicarbonate system, which is the main alkaline reserve of blood.

In tissue capillaries, the interaction of hemoglobin with carbonic acid leads to the preservation of hydrocarbons, that is, alkaline reserves of the blood.

In the lungs, there is oxygenation of hemoglobin, which is accompanied by the simultaneous separation of CO_2 from $KHCO_3$, because oxyhemoglobin is a stronger acid. Ions K^+ bind to HbO_2, as a result of which a potassium salt of oxyhemoglobin is formed. Hydrocarbonate (HCO_3^-) binds proton, resulting in the formation of carbonic acid. Carboanhydrase splits H_2CO_3 with the formation of CO_2, which is transported from erythrocytes to the plasma, further - in the lung alveoli and excreted from the body. Isolation of CO_2 from the body prevents lowering the pH of blood.

The binding of oxygen to Hb in the lungs, the return of oxygen in tissues, penetration into the erythrocytes of CO_2 is a rather complicated process involving chloride anions, potassium cations and other ions [57].

1.4.3. Bicarbonate buffer system

This system, in its capacity, ranks the second place in the system of regulation of blood *pH*.

Acidity in a bicarbonate system can be calculated according to the Henderson–Hasselbalch equation:

$$pH = 6.1 + \lg \frac{[HCO_3^-]}{[CO_2]}$$

The carbonic acid in plasma and intercellular fluid is present in four forms: physically dissolved carbon dioxide (CO_2), carbonic acid (H_2CO_3), the anion of carbonate (CO_3^{2-}) and the anion of bicarbonate (HCO_3^-).

There is a steady correlation between the concentration in blood plasma of carbon dioxide and sodium bicarbonate:

$$\frac{[CO_2]}{[NaHCO_3]} = 1/18 - 1/20 ,$$

that is, the bicarbonate content exceeds the content of free carbon dioxide by 18-20 times, which also causes some alkalinity of blood (fig. 1.16).

Bicarbonate is present in the form of sodium and potassium salts. Sodium bicarbonate ($NaHCO_3$) is predominantly in the plasma, and potassium bicarbonate ($KHCO_3$) in erythrocytes [5, 27, 57].

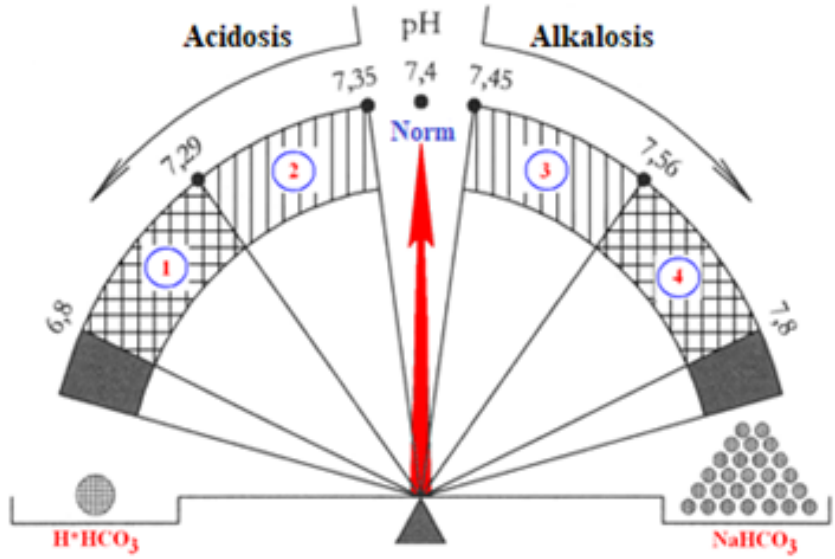


Fig. 1.16. Acid-alkaline state [14].

Scales reflecting the acid/base ratio or respiratory/non-respiratory components of the Henderson–Hasselbalch equation in the norm (1/20) and its displacements leading toward alkalosis or acidosis.

1 - decompensated acidosis;

2 – sub-compensated acidosis;

3 – sub-compensated alkalosis;

4 - decompensated alkalosis.

When acid, which is stronger than carbonic acid, is entered in the blood, salt of stronger acid and weakly carbonic acid are formed. The carbonic acid under the action of carbonic anhydrase is cleaved to H_2O and CO_2 which is released by the lungs:

1) $Na^+ + HCO_3^- + H^+ + X^- \longrightarrow NaX + H_2CO_3$

(X is an anion of acid);

2) $H_2CO_3 \rightarrow H_2O + CO_2$.

In case of excess of alkali, the reaction with carbonic acid occurs and hydrocarbonate and water are formed:

$$HCO_3 + Na^+ \rightarrow NaHCO_3.$$

Excess bicarbonate is excreted by the kidneys [5, 27, 57].

1.4.4. Phosphate buffer system

This buffer system consists of sodium dihydrogenphosphate (NaH_2PO_4) and hydrophosphate (Na_2HPO_4). The first one has the properties of weak acid and interacts with alkaline substances that enter the bloodstream. The second component has the properties of a weak alkali and reacts with stronger acids. This buffer system is able to maintain pH changes in the range 6.2-8.2, thus can provide a significant proportion of the buffer capacity of the blood.

This buffer capacity is lower because of low phosphate content in the blood. At the same time in cells and especially due to renal acid-alkaline displacement compensation the value of the phosphate buffer is larger.

During increasing the level of acids in the blood, the following reaction occurs:

$$2Na^+ + HPO_4^{2-} + H^+ + X^- \rightarrow NaX + Na^+ + H_2PO_4^-.$$

Excess sodium hydrophosphate is excreted with the urine.

Due to increasing the amount of alkalis, the process that occurs is illustrated by the following reaction:

$$Na^+ + H_2PO_4^- + Na^+ + OH^- \rightarrow H_2O + 2Na^+ + HPO_4^{2-}.$$

There is a certain connection and dynamic equilibrium between inorganic buffer systems. For example, a phosphate buffer system can increase bicarbonate reserves by restoring sodium bicarbonate:

$$2Na^+ + HPO_4^{2-} + H_2CO_3 \leftrightarrow NaHCO_3 + Na^+ + H_2PO_4^-$$

As a result, the excess H2CO3 in the plasma is eliminated and the constant correlation of the concentrations of carbonic acid and sodium bicarbonate (1/20) is restored [5, 27, 57].

1.4.5. Protein buffer system

Proteins carry out a buffer role because of their amphoterism (the presence of free-COOH and -NH_2 groups), and the nature of their dissociation depends on the nature of the protein and the pH of the internal environment. For example, globulins have more acidic dissociation, that is, disconnect more protons than hydroxyl ions, and thus play an important role in the neutralization of alkalis. Proteins that contain a lot of diamino acids, dissociate more as alkalis, and therefore to a greater extent, neutralize the acids. The buffer capacity of blood plasma proteins is lower compared to the bicarbonate system, but its role in tissues can be very significant.

1.4.6. Efficiency of buffer systems

It was noted above that the normal value of such an index as blood pH is 7.4 ± 0.04 (or 7.36-7.44). Blood plasma and erythrocyte buffer systems have different relative blood efficacy (Table 1.5).

Thus, the efficiency of the buffer systems of erythrocytes is higher (due to hemoglobin buffer) than plasma.

It is known that a decrease in the concentration of H-ions in the direction of "cell → intercellular medium → blood", suggests that the largest buffer capacity has the whole blood, and the smallest intracellular environment. The acids formed in cells during the metabolism enter the intercellular fluid the easier, the more they are formed in the cells, as the excess of H-ions increases the permeability of the cell membrane. In the buffer properties of the intercellular medium, the connective tissue plays an important role, especially collagen fibers, known as "acidophilic". At the minimum accumulation of acids, they react to swelling, absorbing very quickly the acid and releasing the H-ions into an intercellular fluid. This ability of collagen is explained by the absorption property [5, 27, 57].

Table 1.5

Relative efficiency of blood buffer systems [26]

Blood plasma	**%**	**Erythrocytes**	**%**
Bicarbonate	35	Hemoglobin	35
Proteinaceous	7	Bicarbonate	18
Phosphate	1	Phosphate	4
Total:	43%	Total:	57%

In order to characterize the regulatory physicochemical, physiological and other mechanisms that provide *pH* stability, the term "acid-base state" of the organism (ABS) is introduced. In the regulation of ABS, in addition to the buffer systems of the body, the lungs, kidneys, liver, and intestine are involved. Through the lungs, as we found out, volatile acids, primarily carbon, are removed. With urine, non-fatty acids (sour phosphates) and alkaline compounds (hydrocarbons, ammonium ions, etc.) are removed. The role of the liver in maintaining the sustainability of the organism's ABS consists in the fact that in the body there are processes of complete catabolism of low-oxidized products formed during metabolism. In the case of pathological changes in the liver parenchyma the blood substances can reduce the pH. Participation of the gastrointestinal tract in the regulation of ABS is actively

manifested in the loss of ions of hydrogen, chlorine, potassium in the course of vomiting and bicarbonate - in diarrhea [5, 27, 57].

The Astrup micro-method based on the Zigurd-Andersen principle is used to characterize the body's ABS. The basis of the method (according to the Henderson–Hasselbalch equation) is the dependence between pH and pCO_2. The method is used to determine the pH of blood plasma before and after equilibrium of a blood sample with two gas mixtures containing 4% and 8% CO_2. The data obtained during the experiment is used to determine pCO_2, SB (SB - standard bicarbonate) and BE (base excess or excess bases) on a standard nanogram. The nanogram shows a relation between pH and pCO_2, that is, a graphical representation of the buffer line of blood.

ABS assessment can be done, at least, with three basic pH, pCO_2, and BE (or SB):

pH - negative decimal log of concentration of hydrogen ions;

pCO_2 - partial pressure of CO_2 in plasma, mm Hg;

SB (standard bicarbonate) is the content of the bicarbonate ion in the blood plasma, which is completely saturated with Ferrum and is brought to standard conditions. This is a basic indicator that directly reflects the renal component of ABS regulation;

BE displays the number of unused blood buffers, mainly bicarbonate and hemoglobin. This is the difference between the actual and the required values of the sum of all anions of undiluted blood (BB - buffer base) in metabolic alkalosis.

In addition, the following indicators are used to characterize the ABS:

BB (buffer base) – the sum of all anions of undiluted blood (in fact, SB + anionic stock of protein buffer).

BD (base deficit) – is the difference between the actual and the required values of BB in metabolic acidosis.

AB (current bicarbonate of blood) is the concentration of hydrocarbons (HCO3-) under physiological conditions [5, 27, 57].

Below are the normal values of the main indicators of ABS organism:

pH – 7.36-7.42 (arterial);

7.26-7.36 (arterial);

7.35-7.44 (capillary);

pCO_2 – 35.1-45.1 mmHg (arterial);

46-58 mmHg (venous);

BB – 42-52 mmol/L;

BE and BD – ±2,5 mmol/L;

SB – 20- 26 mmol/L;

AB – 21 -26 mmol/L.

Acidosis and alkalosis are a disturbance of the body's ABS when regulatory systems (including buffers) can not maintain a constant pH at a constant level that is why a significant amount of acids or alkalis accumulated in the blood.

In acidosis in the blood, a relative or absolute excess of acids appears, with a decrease in the pH of the blood (pH <7.37).

Alkalosis is characterized by an increase in the level of alkalis in the blood and an increase in pH (pH> 7.43).

Each of these two types of ABS changes is divided into several types depending on the reasons for the disturbance of pH constancy [5, 27, 57].

Allocate respiratory (respiratory, gas) and metabolic (non-respiratory) acidosis/alkalosis.

Below is a classification of ABS disturbances and causes that induce them.

Acidosis:

1. Gas

2. Non-gas:

a) metabolic: ketoacidosis, lactic acidosis, acidosis in the accumulation of organic and inorganic acids (with inflammation, trauma);

b) excretory: acid reflux (diffuse nephritis, uremia), loss of alkalis (kidney);

c) exogenous: long-term use of sour food, drug intake, acid poisoning;

d) Combined forms.

3. Mixed (1 + 2): with asphyxia, cardiovascular insufficiency.

Alkalosis:

1. Gas:

a) hyperventilation;

b) enhanced allocation of CO2 as a consequence of other causes.

2. Non-gas:

a) excretory: delay of alkalis, loss of acids, hypochloremic (metabolic);

b) exogenous: long-term use of alkaline food, administration of drugs (bicarbonates and other alkaline compounds).

3. Mixed forms (1 + 2).

Non-gas (metabolic) acidosis is the most common form of ABS disturbance.

Conditions that lead to the appearance of non-toxic acidosis.

Increase in the entering of H+ ions (exogenous acidosis) is the reason that can form acids or some acidic salts ($CaCl_2$, NH_4Cl).

Increasing the formation of H^+ (actually "metabolic" acidosis) is the formation of a large amount of acidic metabolism products (ketone bodies, lactate, other organic acids). Ketoacidosis occurs in patients with diabetes mellitus, thyrotoxicosis, in medical starvation, and the like. Lactic acidosis is the result of tissue hypoxia with insufficient function of the cardiovascular system, airborne anesthesia, shock, complicated pneumonia, and the like. Mixed forms of metabolic acidosis arise in sepsis, burns, acute liver failure, rickets (in children), and the like.

Decrease in the allocation of H^+ ions in the urine is observed with oliguria or anuria, renal insufficiency (which is accompanied by accumulation of residual sulfuric and phosphoric acids), kidney disease with reduced glomerular filtration.

Increased loss of bicarbonate through the intestine (with diarrhea), with urine (distinct acidosis) - with tubular nephritis, under the influence of inhibitors of carboanhydrase (diarrhea diacortica, diamonds), which disturb the processes of "reabsorption" of bicarbonate.

Gas (respiratory) acidosis : this type of disturbace of ABS occurs as a result of the accumulation of carbon dioxide in the body (hypoventilation of the lungs, pulmonary edema, disruption of respiratory mechanics, etc.).

Non-gas (metabolic) alkalosis develops as a result of the administration of large doses of sodium bicarbonate (with correction of metabolic acidosis), loss of a large amount of acids and chlorides (multiple vomiting), incorrect hormonal therapy, which does not take into account potassium losses (in the case of Itsenko-Cushing's, Conna syndrome), etc.

Gas (respiratory) alkalosis is the result of hyperventilation of the lungs, occurs in hematological and cardiovascular diseases, stimulation of the respiratory center by organic lesions of the central nervous system, sepsis, a neurotic mood that is caused by stress, whiteness, severe crying in children, etc. [5, 27, 57].

CHAPTER 1.5
ENZYMES OF BLOOD PLASMA

1.5.1. Enzymatic systems functioning in blood plasma

The basis of many pathological and prepathological conditions of an organism is disturbances of the functioning of enzyme systems. The enzymes which posses activity are localized preferably inside of the cells, in blood serum or plasma their activity are low or even non-existent. That is why by analyzing the extracellular fluid (blood), activity of certain enzymes, it is possible to detect changes occurring within the cells of various organs and tissues of the body. Other enzymes are constantly contained in the blood with known quantities and have specific functions (e.g., enzymes of the blood coagulation system).

The enzyme activity in the serum reflects the balance between the rate of synthesis of enzymes within cells and release them from the cells. The increase of activity of enzymes of blood may be the result of the acceleration of the processes of synthesis, reducing the clearance rate, increase the permeability of cell membranes, action of activators of cell necrosis. The decrease of enzymatic activity is caused by increasing the rate of excretion of the enzyme inhibitors, inhibition of synthesis. Increased activity of a particular enzyme in the blood is an early diagnostic test [5, 27]. Currently, at the clinic, it is common to determine the activity of a specific set of enzymes. Therefore, the characteristics of those blood enzymes are very important for diagnosis, information about the course of the disease and therapy with a prognostic purpose.

Typically, blood serum (whose enzymes are relatively constant and of different origin) is used to determine the activity of blood enzymes.

Blood serum or plasma enzymes are divided into three groups [23, 24, 27]:

1) an indicator (cellular marker) enzymes localized in the cells of the tissues, enter the blood due to physiological aging and destruction of cells or as a result of increasing the permeability of cell membranes. The blood contains several tens of indicator enzymes. Normal cellular enzymes in the blood have low activity and do

not perform specific functions. When entering the blood they are inactivated by proteases in serum and tissues. The activity of these enzymes increases in the organ under condition of a powerful destruction of cellular membranes. Enzymes of this group are divided into nonspecific and organ-specific. Non-specific indicator enzymes catalyze universal metabolic reactions and are localized in most organs and tissues. Organ-specific enzymes are contained only in those organs and tissues where specific reactions occur, characteristic only for cells of that organ. That is why increasing the activity of these enzymes in the blood indicates the organ localization of the pathological process;

2) secretory (plasma-specific) enzymes are synthesized in the liver, released into the blood, where certain physiological functions are performed (enzymes of blood coagulation, fibrinolysis, cholinesterase, ceruloplasmin, proteins of renin-angiotensin and kallikrein systems, etc.);

3) excretory enzymes are synthesized in the liver, pancreas, and the mucous membrane of the intestine. The appearance of these enzymes in the blood is associated with the natural destruction of the cellular structures in which they are formed (alkaline phosphatase, leucine-peptidase, enterokinase, gamma-glutamyl transpeptidase, trypsin, lipase, etc.).

There are several types of changes in the activity of enzymes in the blood: hyper-, hypo- and dys-enzymemia [23, 24, 27].

Hyper-enzymemia is the increased activity of enzymes in serum. This may be due to 1) the entering of enzymes into the bloodstream from damaged organs and tissues; 2) increase of the catalytic activity of enzymes, directly in the damaged part of body, and when they enter the bloodstream.

Hypo-enzymemia is a decrease in the activity of blood enzymes, which is the result of inhibition of the synthesis of enzymes in tissues. This type of changes in the activity of enzymes is characteristic only for certain enzymes, for example, for cholinesterase.

Dys-enzymemia characterizes the appearance of some blood enzymes, the activity of which is normally absent. Such changes may be characteristic of some

organ-specific enzymes, such as sorbitol dehydrogenase, fructose monophosphate aldolase, and others [23, 24, 27].

1.5.2. Indicator-enzymes of blood plasma

The specific field in clinical trials is enzymes diagnosis. Some enzymes, proenzymes, and their substrates normally circulate in the blood. In the blood, there are also non-functional enzymes, the concentration of which in plasma is several orders of magnitude lower than in tissues and organs. The appearance of these enzymes in plasma at elevated concentrations is usually associated with pathology.

Usually, the disease of one or another organ, accompanied by the destruction of its cells, leads to the release of enzymes in the blood plasma, where the enzymes activity is increased.

Aspartate and alanine aminotransferase

Aminotransferases (transaminases) are enzymes of the group of transferases that carry out nitrogen-containing groups of atoms. In this group of enzymes there are such important enzymes for clinical laboratory diagnostics, as AST and ALT. Aspartate aminotransferase (AST; glutamate-oxaloacetate transaminase; L-aspartate: 2-oxoglutarate-glutamate aminotransferase, EC 2.6.1.1) catalyzes the transamination reaction between aspartate and α-ketoglutarate:

α-ketoglutarate + L-aspartate ↔

↔ L-glutamate + oxaloacetate

Normal activity: 0.1-0.45 mmol/(h×L), 8-40 U/ml.

In mammals, the highest activity and concentration of AST is noted in the liver, nerve tissue, skeletal muscle, and myocardium. In most tissues, there are at least two isoenzymes AST: mitochondrial isoenzymes (MAST), which moves to the cathode on all supported media, and AST isoenzymes soluble in the cytosol (CAST) that migrate to the anode. The CAST consists of two identical subunits; It is a dimer with a molecular weight of 93 kDa. MAST is also a dimer with a molecular weight of 91 kDa. The isolation of isoenzymes revealed the existence of several isoforms with

similar kinetics, but expressed immunological differences. AST isoenzymes also have a certain difference in kinetic characteristics: the optimal action of the MAST is lower than that of CAST [5, 27].

Alanine aminotransferase (ALT, glutamate pyruvate transaminase; GPT, L-alanine: 2-oxoglutarate-aminotransaminase, EC 2.6.1.2) catalyzes the transamination reaction between L-alanine and α-ketoglutarate: L-alanine + α-ketoglutarate ↔ pyruvate + L-glutamate. Normal activity: 0.1-0.68 mmol/(hour · L), 5-30 U/ml. ALT is present in many organs: the liver, kidneys, skeletal muscle, myocardium, pancreas. Low activity of ALT is noted in the serum of blood of healthy people. Like AST, ALT is present in cells in the form of two isoenzymes - cytosolic and mitochondrial, but the latter is unstable and its content in the cell is low [23, 24, 27].

Clinical significance of aminotransferase concentration determination. The increased activity of aminotransferases is noted in a numerous pathological processes in which the liver is involved. Activity of AST and ALT is increased in acute pancreatitis, cholecystitis, parasitic diseases, psoriasis, burns, and the use of propiolactone as an antiviral agent, an overload of the body with Ferrum, hepatobiliary pathology.

An increase in the activity of aminotransferases can also occurs in healthy people with a diet rich in protein or containing 25-30% sucrose [23, 24, 27].

The activity of ALT in the blood increases with tuberculosis of the lungs, septicemia, herpetic infection, viral hepatitis, tumors. ALT activity decreases in malaria and pregnancy and does not change with pulmonary artery embolism, pneumonia, abscesses of the lungs, rheumatoid arthritis. The increased ALT activity was noted in patients which consumed certain drugs, especially antibiotics, hypolipidemic agents, ketoacidosis, and azotemia.

Both aminotransferases - AST (cytoplasmic and mitochondrial) and ALT (cytoplasmic) - are in the normal state present in plasma of human blood, spinal fluid, and saliva, but not detected in the urine. Usually, blood serum is taken for the study. It should be noted that its hemolysis needs to be avoided, since the activity of AST and ALT in erythrocytes is higher than in normal serum [23, 24, 27].

In myocardial infarction, the activity of AST in serum increases from 8 to 12 hours; maximum activity is achieved in 24-36 hours, and its return to the normal level which occurs for 4-6 days (Fig. 1.17).

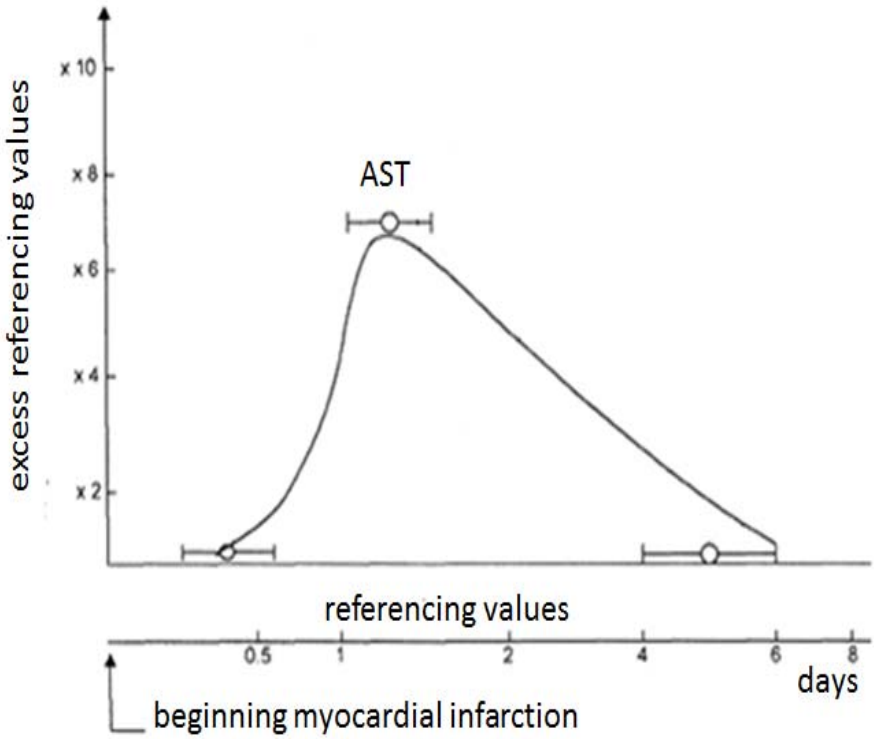


Fig. 1.17. Kinetics of release in the plasma of tissue AST during acute myocardial infarction [29].

In about 95% of patients with myocardial infarction, the serum AST activity is 2-10 times higher than the upper limit of the normal value. In myocardial infarction, there is also an increase in the activity of AST in serum, but to a much lesser extent than the increase of AST activity [23, 24, 27].

In myocardial infarction, the ratio of AST/ALT activity in most cases is 2, and the ratio, less than 1, indicates predominantly liver damage [23, 24, 27].

Amylase

α-Amylase (α- (1,4) -glucan-4-glucanohydrolase, EC 3.2.1.1) is an enzyme that hydrolyzes the internal 1,4-α-glycoside bonds of starch, glycogen and other glucose carbohydrates. In the human body, the main sources of α-amylase are pancreas and salivary glands. Numerous isoforms isolated from human biological fluids and tissues are probably heterogeneous products of post-translational modifications of two

families of isoenzymes of amylase, the synthesis of which is coded by two loci of the gene Atu-1, salivary (S) type of amylase and Atu-2, pancreatic (P) type of enzyme closely linked on chromosome 1. Isoenzymes S and P have no significant differences in the amino acids composition. Sephadex gel filtration in the S-amylase fraction has two isoforms, one of which contains carbohydrates. The molecular weight of isoforms containing carbohydrates, 57 kDa, does not contain carbohydrates - 55 kDa. The isoelectric point of the main isoforms of S-isoamylase is from 5.5 to 6.5. The P-group isoamylase has a lower molecular weight (53 kDa) and does not contain carbohydrates. The isoelectric point of these proteins is varied from 5.7 to 7.0 [41].

The convincing evidence that the P-type amylase is formed only in the pancreas is the absence of this isoenzyme in subjects with total pancreatectomy. High activity of S-amylase was recorded in the tissues of fallopian tubes and the content of ovarian cysts. A specific group of isoamylases produced by the tissues of female genital organs is not detected in the blood serum or urine of healthy people; the distribution of isoamylases is identical in men and women. Isoamylases belonging to S-group were found in female milk and wound fluid [9].

Clinical significance of amylase activity determination. The normal amylase (diastase) activity is 12-32 g/(h × L) in the blood serum; and 20-160 g/(h × L) in urine.

Determination of α-amylase activity in serum is the most common diagnostic test for acute pancreatitis. In acute pancreatitis, the activity of the enzyme in the serum increases after 3-12 hours after a pain attack and reaches a maximum in 20-30 hours and returns to normal level during four days. α-Amylase (diastase) activity in the urine increases after 6-10 hours after the rise in serum activity and returns to normal level in three days after increase [23, 24, 27].

It is now widely known that the increase in total α-amylase activity is nonspecific for pancreatitis and other pancreatic diseases. Clinical studies have shown that increased α-amylase activity occurs in a numerous diseases, which include intestinal obstruction, biliary disease, appendicitis, parotitis, ectopic pregnancy. The sensitivity and specificity of the determination of α-amylase in the

diagnosis of acute pancreatitis increase with a change in the normal values of the boundaries (the discriminatory level of norm and pathology - activity 1.5-2 times exceeds the upper limits of the norm). In this case, the determination of α-amylase activity is the most informative in the first day of the acute pancreatitis development. According to some authors, the determination of α-amylase activity in the blood serum in chronic pancreatitis has no diagnostic value [24, 27].

In humans, α-amylase freely passes through the filtration barrier of the renal corpuscle; cells of the epithelium of the renal tubules reabsorb it, as well as other low molecular weight serum proteins. An increase in serum amylase activity may be due to a disturbance of the elimination of enzymes. An example is a condition called macroamylasemia, when the enzyme is bound to immunoglobulins of the blood serum and forms a macromolecular complex. Such a complex is not filtered into urine and cannot be removed by any other mechanisms, which leads to a significant and sometimes prolonged increase in the activity of α-amylase in serum. Macroamilaseemia occurs in healthy people with a frequency of 1%, in persons with hyperamylasemia at a frequency of 2.5%. Macroamylasemia can be diagnosed by ultracentrifugation, electrophoresis, gel chromatography, and a simple test for the complexation of polyethylene glycol [4] is also proposed.

The α-amylase activity in serum is often increased under condition of renal insufficiency, but it is not entirely clear whether the cause of such an increase is the activation of the synthesis of the enzyme or the decrease of its elimination. In such cases, additional determination of the rate of excretion of α-amylase or measurement of enzyme clearance can be provided.

According to many authors, the level of P-isoenzyme α-amylase activity is the most sensitive and specific test for diagnosis of pancreatitis. The particular importance of this test is obtained, if a patient with a predicted diagnosis of pancreatitis revealed the normal total activity of amylase. Chronic pancreatitis can be diagnosed with decreased activity of P-amylase. The proportion of P-isoamylase in the overall activity of α-amylase is significantly higher in urine than in serum, possibly due to differences in the excretion of isoenzymes in the kidneys.

Nevertheless, the diagnostic value of the determination of the activity of iso-enzymes α-amylase in urine is inferior to that in the blood [23, 24, 27].

γ-glutamyltransferase

In recent years, in the clinical biochemistry, the determination of the activity of γ-glutamyltransferase (GGT; γ-glutamyltranspeptidase, EC 2.3.2.2) was taken as an appropriate test.

GGT catalyzes the transfer of γ-glutamyl to an amino acid or peptide to another substrate or water molecule. GGT is a protein composed of one polypeptide chain with a molecular weight of 90 kDa. An enzyme contains hydrophilic and hydrophobic fragments. The active center is located in the hydrophilic region of the polypeptide chain. A hydrophobic section is part of the chain by which the enzyme is attached to the membrane. If extraction of the enzyme from tissues is carried out using bile acid detergent or Triton X-100, GGT, along with the hydrophobic portion, can be transferred to a solution. If only proteases (papain or trypsin) are included in the extracted mixture, the hydrophobic fragment of GGT remains on the membrane, and the hydrophilic portion of the GGT polypeptide is detected in the solution [5].
Hydrophilic fragments of GGT, isolated from the liver or kidneys, are immunologically similar, as well as hydrophobic fragments of GGT, isolated from different organs.

GGT plays an important role in the metabolism of amino acids. A set of biochemical reactions of reabsorption of amino acids from primary urine, filtered by a filtration barrier of renal bodies, is called glutamyl cycle. This process begins with the action of GGT, which is located on the outer surface of the cell membrane and binds the amino acid of the primary urine to the epithelial cells of the tubules with the involvement of the natural substrate of glutathione, forming a triple complex: the amino acid-GGT-glutathione. Also, inhibition of reaction with high concentrations of substrates may also occur. As a result of the binding of two substrates in the active center of the enzyme, the transfer of glutamyl acid from glutathione to amino acids occurs. In this phase, the amino acid enters the cell where the following reactions

occur, in which glutamyl cyclotransferase cleaves the bond between amino and glutamyl acid using the energy of ATP. The presence of GGT on the membrane of cells of other organs (a thin section of the intestine, a choroidal plexus, etc.) suggests that in these organs, the enzyme is involved in the transport of amino acids. The role of GGT in the liver is not fully understood; Probably the enzyme binds molecules of substances that need to be excreted. The biological role of the enzyme is also related to the regulation of glutathione levels in the tissues. This is precisely the reason why high levels of glutathione in plasma and urine of patients with a genetically determined lack of GGT synthesis can be explained. By regulating the level of glutathione, GGT can affect protein synthesis, which explains the increased specific activity of the enzyme in tissues with the high metabolic rate. This may be due to an increased level of the enzyme in the tissues and blood of the newborns [19].

GGT is found mainly in the membrane of cells with high secretory or adsorption capacity: epithelial cells of the biliary tract, hepatic tubules, proximal tubular nephron, pancreatic exocrine tissue and excretory ducts. In order of decreasing the specific activity of GGT, tissues are arranged in the following sequence: kidneys, liver, pancreas, thin intestine. GGT activity was not detected in skeletal muscle and myocardium [24].

Clinical value of determination of GGT concentration in blood serum. Normal activity: men - 15-106 U/L, women - 10-66 U/L. The most common cause of GGT activity increasing in blood serum is liver disease. Weak toxic effects on the liver causing fatty infiltration, alcohol, and drugs are accompanied by a moderate increase in GGT activity. A more pronounced increase in the activity of the enzyme is due to extrahepatic and intrahepatic obstruction, the secondary involvement of the liver in the oncological processes of the body by metastasis. The highest activity of GGT in serum is noted when bile duct closure or malignant tumors, directly or indirectly affecting the liver [27].

In the absence of jaundice, the determination of GGT is a sensitive test for the detection of liver disease; Clinical sensitivity is higher than that of such enzymes as alkaline phosphatase (ALP) and 5’-nucleotidase. In cancer cases, the normal activity

of GGT in serum indicates a lack of metastasis in the liver, whereas high GGT activity (12 times or more compared to normal) serves as an indicator of liver damage by metastases. In acute viral hepatitis, multiple studies of GGT activity can monitor the course of the disease: the ever-increasing activity of GGT indicates the development of a chronic form of the disease. With the increase in ALP activity and the difficulty in determining its isoenzymes, it is expedient to determine the activity of GGT to identify the possible source of hyperenzymemia: GGT activity remains within the normal range if the increase in ALP activity is due to bone isoenzyme and is increased when the enzyme is formed in the liver. In pediatrics, the determination of GGT activity is more appropriate than the definition of ALP, since the activity of GGT does not change with age and the results obtained are easier to interpret. High activity of GGT is observed in the blood of people abusing alcohol. In 74% of alcoholics who suffer from histologically confirmed liver damage, an increase in GGT activity was constant even during periods of abstinence.

There are certain differences between the activity of GGT in the blood of an alcoholic and the person who took a significant dose of alcohol. In the first, there is an increase in the activity of GGT up to 140% of normal values with peak activity in 18 hours, while in others, even after severe intoxication, the increase in GGT activity does not exceed 15% for a further 12 hours [31].

The development of myocardial infarction is accompanied by changes in the activity of serum enzymes (CK, LDH, AST) and GGT, the activity of which after an angina attack remains elevated for a long time.

Increased activity of GGT was noted in 50% of patients with angina and in part of patients with other diseases (coronary insufficiency, circulatory failure). In myocardial infarction, obscure mechanisms of increasing GGT activity remain, as it is absent in the cardiac muscle.

The GGT activity in the urine is higher than in serum. Present in urine, GGT has a renal origin: the enzyme is excreted in the urine from the affected cells of the proximal tubule sections, which contain high concentrations of GGT [23, 24, 27].

Creatine Kinase (CK)

Creatine kinase-ATP: creatine-1,4-phosphotransferase (EC 2.7.3.2) catalyzes the transfer of phosphate residue between ATP and creatine to form ADP and creatine phosphate. The common name of the enzyme is creatine kinase (CK). CK is a cytosolic and mitochondrial enzyme that is functioning in cells of many tissues. The product of the reaction is phosphocreatine - high-energy substance, which provides energy for muscle contraction, their relaxation, and transport of metabolites to the myocyte. For the first time, the CK activity in blood was of interest to clinical biochemists, as a test to detect lesions of transversal muscle. Currently, the CK activity is a leading test in the diagnosis of myocardial infarction [27].

The highest content of CK was found in the myocardium and skeletal muscle, high content - in the muscle of the diaphragm, low - in the kidneys, lungs, liver. CK is a dimer; in the cytosol of human tissue cells, there are 2 subunits: M - muscular type and B - brain type. Subunits are proteins with a molecular weight of 41 kDa with an active center in each subunit. Accordingly, the two forms of the subunits of the dimeric form of the molecule of the CK are muscle type CK-MM, hybrid dimer CK-MB, characteristic for myocardium and isoenzyme CK-BB, localized mainly in the brain tissue (fig. 1.18).

The CK activity and its isoenzymes is organ-specific, therefore the identification and quantitative determination of isoenzymes in the blood serum is not used to establish an increase in permeability of the membranes or damage to a specific organ, but also makes it possible to establish a diagnosis, observe the clinical course and estimate the prognosis of the disease.

In the electric field, the most mobile is CK-BB, the smallest - CK-MM, the hybrid form of CK-MB occupies an intermediate position. Accordingly, in the classification of the International Union of Theoretical and Applied Chemistry, CK-BB is designated as isoenzyme I CK, CK-MB - II, CK-MM - III. To understand the nature of the activity in the blood of CC-MB, it should be noted that the fetus to the 16-week age in the muscle tissue is dominated by the embryonic form of CK, namely, CK-MB, which is later replaced by CK-MM. A certain rearrangement of the

isoenzymatic composition of the CK occurs with hypertrophy of the myocardium: the percentage of CK-MB and CK-BB increases, and the proportion of CK-MM is reduced. Myocytes in conditions of prolonged hyperfunction increase the synthesis of isomers of CK-BB [23].

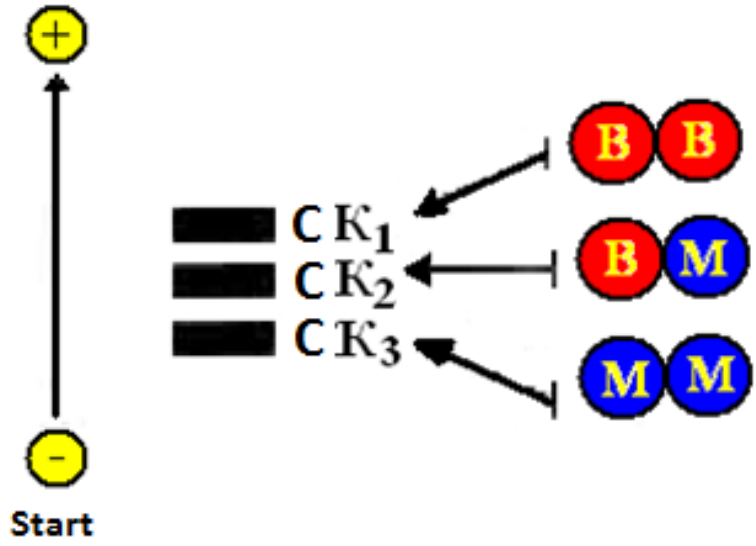


Fig. 1.18. Structure and electrophoretic mobility of various isoforms of creatine kinase (CK) [14].

Clinical significance of determination of CK activity and its isoenzymes. Normal activity: up to 100 nmol/(s × L) or up to 6 IU at 30 ° C. Increasing the activity of CK in the blood may be the result of injury, overcooling or overheating, fasting and bacterial intoxication, dehydration, electric shock. Increased activity of CK-MB was noted for muscular dystrophy of various etiologies, ischemic rhabdomyolysis with myoglobinuria, poisoning with carbon monoxide. The activity of CK-MB increases, more than an order of magnitude higher than that, in patients with acute pericarditis [27].

The experience of using isoenzyme diagnostics in the block of intensive care has shown that an increase in the activity of CK-MB in the blood can occur with severe circulatory failure, heart catheterization, pulmonary edema, arrhythmias, cardiogenic shock, alcohol intoxication, bleeding in the gastrointestinal tract, traumatic epilepsy, trauma chest. The activity of CK-MB is elevated in the blood of patients with shock developing with anemia, hypercapnia, hypoxemia, lactic acidosis,

severe hypotension. However, only in some cases, the activity of CK-MB may exceed the activity of the CK by 6%. Considering the possibility of a non-specific increase in the activity of CK-MB, one should also remember the risk of a minor myocardial infarction. In patients with hypertrophy of the myocardium, the activity of CK-MB in the period of a heart attack can reach 1/3 of the activity of the CK [27].

Increased activity of CK in blood serum may be due to an increase in the activity of isoenzyme CK-MM. The activity of CK-MM in blood serum increases with progressive muscular dystrophy, dermatomyositis, polymyositis, myocardial infarction, hypothyroidism, and some diseases of the nervous system. The activity of CK-MM also increases after physical activity, intramuscular injections, surgical operations. With muscular dystrophy of the Duchenne type (hereditary, associated with the sex of a recessive disease), the activity of CK-MM can increase up to 90 times in comparison with the norm, the activity of the isoenzyme is also increased in the carriers of the corresponding gene.

The activity of CK-BB in the blood was also noted during coronary artery bypass grafting. It is believed that the activity of CK-BB can be a test for anoxia of tissues. The activity of CK-BB in the blood may also be a consequence of hypoxic brain damage, especially in conditions of perinatal hypoxia. CK-BB activity increased in 53% of newborns with asphyxiation. The activity of CK-BB occurs in smooth muscle, but it is not determined in the blood serum of people with benign illnesses of these tissues. One possible explanation for an increase in the activity of CK-BB in blood in vascular operations is the assumption that the walls of the veins, as well as the aorta, contain only one isoform of the CK, namely, CK-BB. The activity of CK-BB isoenzyme may be increased in blood in prostate cancer, small cell lung cancer, adenocarcinoma of the stomach, leukemia, chronic renal insufficiency, overdose of muscle relaxants. Metastasis of prostate cancer is accompanied by a particularly high activity of CK-BB in the blood. Researchers believe that the activity of CK-BB can be used as a nonspecific marker of the tumor process of some organs [23, 24].

In numerous cases, with myocardial infarction, and sometimes in its absence, high activity of the CK persists indefinitely. Observed phenomena are caused by circulation in blood of unusual forms of CK and its isoenzymes. After separation at the EF of fractions of the CK on the electrophoregram, bands that do not correspond to the position of the bands of CK-MM, CK-MB and CK-BB become visible, and it is possible to note the presence of isoforms of CK moving to the cathode, which is characteristic for immunoglobulins of the blood serum. It is known that the oxidation of sulfhydryl groups in the CK changes the electrophoretic mobility of molecules, but at the same time, they still move to the anode [5].

The activity of CK-MB plasma isoenzyme begins to increase already in 4-8 hours after myocardial infarction, reaches the maximum value in 12-24 hours, which is somewhat faster than for the increase of the total activity of CK. This activity returns to the normal level for the 3rd day (fig. 1.19).

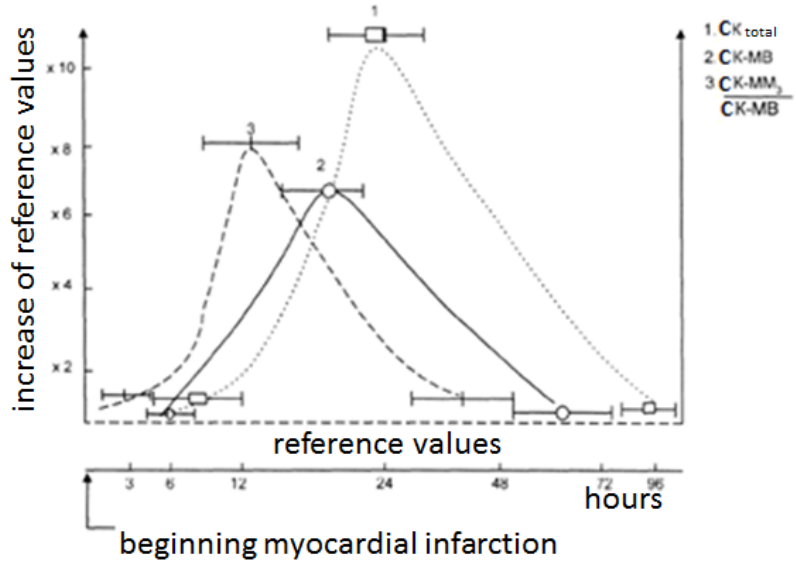


Fig. 1.19. Creatine kinase (CK), its isoenzyme of CK-MB, and the ratio of CK-MM3/CK-MB isoforms in blood plasma during myocardial infarction [27].

It is believed that the best enzymatic test for confirming or excluding the diagnosis of acute myocardial infarction is CK-MB, especially with the use of immunochemical methods for the determination of this isoenzyme. There was no

clear dependence of the diagnostic value of CK on the localization of myocardial infarction and hemodynamic state of the patient.

In many cases, a single determination of the activity of the isoenzymes CK-MM or CK-MB and their isoforms during the first 48 hours after the onset of pain can accurately determine the time of the onset of myocardial infarction. On the other hand, the absence of increased CK-MB activity for 24 hours in a patient who did not have additional pain can exclude a heart attack [42].

Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH, L-lactate-NAD-oxidoreductase, EC 1.1.1.27) is zinc-containing enzyme, catalyzes the reverse reaction of the recovery of pyruvic acid in lactic acid with the participation of NADH2. An enzyme in crystalline form was obtained from the muscle of the heart. Similarly, crystalline enzyme preparations were obtained from skeletal muscle and liver. At pH 7.0, the equilibrium of the reaction is shifted towards the formation of lactate, in the alkaline medium the reaction proceeds in the opposite direction. LDH may also use NADP as a coenzyme but much less often than NAD [23, 27].

LDH is a tetramer, two loci of genes encoding the synthesis of two oligomers - the M- and H-subunits. The M-subunit is synthesized mainly in tissues with anaerobic metabolism, while the H-subunit is present in tissues with a predominance of aerobic processes. The molecular weight of each subunit is 35 kDa, each tetramer is 140 kDa. The polypeptide chain of both subunits contains 330 amino acid residues; the differences in their sequence in subunits are detected within more than 25% of the length of the polypeptide chain. In tetrameric LDH structure, the subunits are bound by ionic and hydrogen interactions. Each subunit has a catalytic center; the tetramer dissociation of dimers or monomers leads to loss of enzyme activity.

In the cytoplasm of the cells and blood serum, LDH has 5 isoenzymes, which are indicated by their mobility to the anode in the electric field: LDH1 (HHHH), LDH2 (HHHM), LDH3, (HHMM), LDH4 (HHHM) and LDH5 (MMMM). LDH participates in the oxidation of lactate to pyruvate in tissues with an aerobic type of

metabolism (myocardium, brain, kidneys, red blood cells, platelets). LDH5 is optimized by nature to convert pyruvate to lactate in tissues with high levels of glycolysis (skeletal muscle, liver). Not all LDH isoenzymes are homogeneous: the electrophoretic division of isoenzymes of LDH blood serum and erythrocytes in polyacrylamide gel revealed the splitting of LDH3 into two lanes, indicating the synthesis of two forms of LDH3 in tissues. The presence of molecular structures of the two forms, it was confirmed by reactions with antiserum, which explain the spatial (cis- and trans-) location of H - and M -subunits in the tetramer [17, 30, 45].
In human tissues, LDH activity per gram of dry mass decreases in sequence: kidneys - skeletal muscle - pancreas - spleen - liver - placenta. Isoenzymes of LDH1 and LDH2 predominate in erythrocytes, leukocytes, myocardium, kidneys, LDH4 and LDH5 - in the liver, skeletal muscle, neoplastic tissues, the highest content of LDH3 is found in lymphoid tissue, platelets, and tumors.

Properties of LDH isoenzymes are determined by the peculiarities of subunits that are part of their composition. Isoenzymes LDH are characterized by various kinetic characteristics: pH, in which they exhibit maximum activity, affinity to the substrate and cofactors.

Clinical significance of LDH activity determination. Normal activity: up to 3200 nm/(s × L) or up to 195 IU at 25 ° C, to 5330 nm/(s × L) or to 320 IU at 30 ° C. The activity of LDH in serum is increased in many pathological conditions; therefore, for differential diagnostics of diseases, it is more appropriate to investigate changes in the spectrum of LDH isoenzymes. At present there is a large amount of data on the distribution of LDH isoenzymes in tissues and on changes in the spectrum of LDH isoenzymes in blood serum under various diseases. The isoenzyme spectrum of skeletal muscle shows the predominance of LDH5. In muscular dystrophy, an increase in more mobile isoenzymes of LDH and a decrease in LDH5 activity, which is characteristic of many neurological diseases, are also noted. The reason for changing the range of isoenzymes may be the rapid removal of slow-moving isoenzymes from circulation. The activity of LDH5 in blood serum is a sensitive indicator of hepatitis, liver hypoxia (including blood stagnation in the liver due to

heart failure), drug intoxication, cirrhosis, tumors and a numerous injuries. The activity of LDH in blood serum does not increase in chronic kidney and uremic diseases, but sometimes increases after hemodialysis or plasmapheresis, that can be explained by removal of blood from inhibitors (urea, oxalates) [23, 24, 27].

The overall activity of LDH in myocardial infarction is most significantly increased during the first 2 days after an angina attack and is slowly returning to the baseline, in the course of 14-16 days, episodic increase in LDH can be detected at a later date (fig. 1.20).

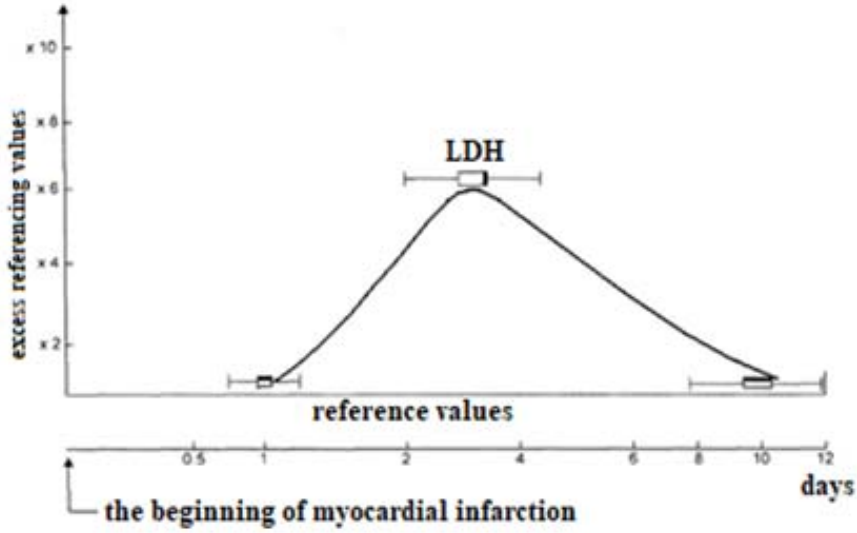


Fig. 1.20. Kinetics of tissue LDH release into blood plasma during acute myocardial infarction [27].

The activity of LDH can also be affected by the hormonal influence. High doses of thyroxine lowered the synthesis of enzymes, while more inhibited synthesis of subunit M. Noradrenalin and adrenaline cause an increase in the overall activity of LDH with the predominance of LDH1 and LDH2 activity.

The enzymatic activity in the blood increases with the action of anabolic steroids and ethanol, as well as a numerous drugs - clofibrate, caffeine, sulfanilamides, and others [23, 24, 27].

The isoenzyme spectrum of LDH may vary with neoplastic processes. In such cases, it is difficult to interpret, because the source of isoenzymes LDH is not only neoplastic tissue but also tissues that have been exposed to metastasis. However, the

isoenzyme-spectrum of transudates with tumor lesion is similar to that found in inflammatory exudates - the activity of LDH1 and LDH2 prevails. For LDH, as well as for other enzymes, the tumor process is characterized by the synthesis of isoenzymes inherent in embryonic tissues. Undifferentiated embryonic tissues have a spectrum of isoenzymes LDH, in which LDH2 and LDH3, as well as LDH4, predominate. In malignant tumors, three types of LDH isoenzymes have been identified. An increase in the content of LDH4 and LDH5 was found in prostate tumors, uterus, mammary glands, stomach, thick intestine, bladder, and some types of brain tumors. In patients with leukemia, malignant lymphoma, neuroblastoma, pheochromocytoma, as well as oral cavity tumors, bronchial cancer and certain types of brain tumors, the activity of LDH2, LDH3, LDG4 is increased. An increase in the activity of LDH3 was found in the serum of patients with certain types of brain tumors and various types of genital tumors [27].

Ability to increase the activity of LDH, in the tumor process should be taken into account in the diagnosis of myocardial infarction. Sometimes, in the cases of brain tumors, esophageal cancer, neuroblastoma, it is observed an unusual additional band during electrophoresis of blood serum and tumor tissues. Determination of the spectrum of LDH isoenzymes in the blood serum in cancerous diseases is expedient not only for diagnosis but also for controlling the effectiveness of treatment. It was found that the normalization of the LDH isoenzymes spectrum correlates with the effectiveness of the patient treatment [23].

The presence of complications in myocardial infarction and concomitant diseases can change the LDH spectrum and LDH activity. Detection of the spectrum of isoenzymes characteristic for myocardial infarction may occur with stagnant blood in the liver and kidneys due to heart failure, with ischemic lesions of some organs (fig. 1.21).

With pulmonary embolism, which in some cases has to be differentiated with myocardial infarction, an increase in the activity of LDH2 and LDH3 in the blood may be explained by the release of enzymes from platelets, liver pathology caused by venous hypertension, anemia of the cortical layer of the adrenal glands and kidneys.

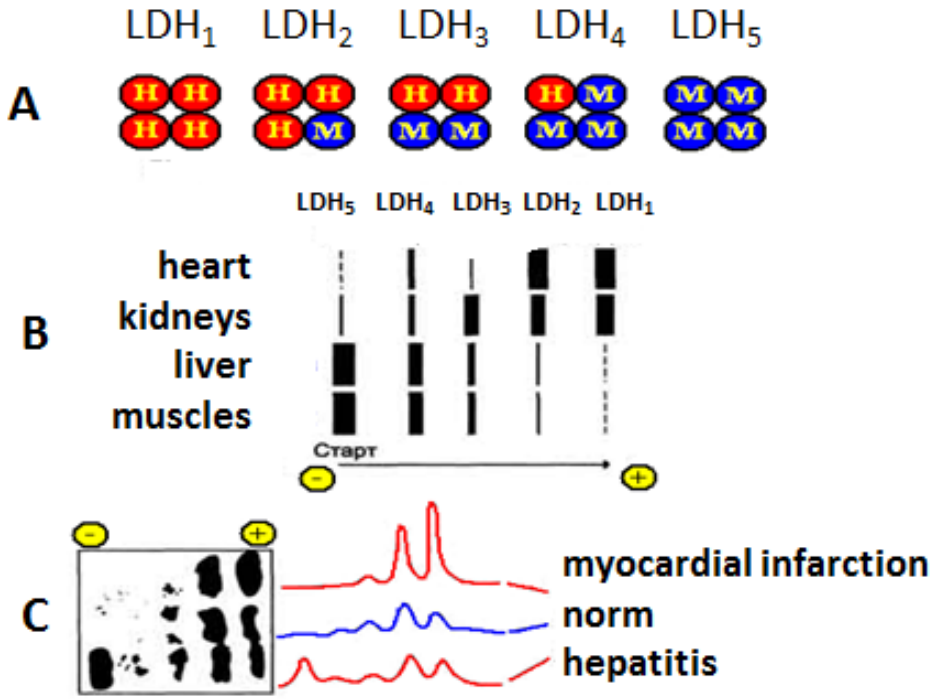


Fig. 1.21. Isoforms of lactate dehydrogenase. A - the structure of different isoforms of LDH; B - distribution on the electrophoregram and the relative number of LDH isoforms in different organs; C - the content of LDH isoforms in the plasma in normal and in pathology (electrophoregram - on the left and photometric scan - on the right) [14].

Since these disturbances are not always possible to distinguish, it is not easy to interpret the LDH spectrum of isoenzymes.

In the blood serum of patients with severe illness (usually in the terminal state) by the electrophoretic method on cellulose acetate, an additional band is found, closer to the cathode than LDH5, it is called LDH6.

In all observations in the tissues of the liver, skeletal muscle, kidneys, spleen and adrenal glands, the presence of LDH6 was found; in some cases, the appearance of this fraction was of a transient nature, LDH6 was not detected in myocardial tissues. It is believed that there is no nosological form of the disease, which is characterized by LDH6, but there are difficult clinical conditions that predispose its appearance, which include pronounced acidosis, hypotension, and sepsis [23].

Alkaline (ALP) and acid phosphatase (ACP)

Alkaline phosphatase (ALP) - phospho-hydrolase monoester of orthophosphoric acid (EC 3.1.3.1) - hydrolyzes various synthetic substrates at an optimum pH equal to 10.0; enzyme substrate *in vivo* is not exactly known. ALP - glycoprotein; the structure is a dimer with a significant variation in the molecular weight of enzyme in different tissues. ALP is a metal-containing enzyme, the active center of which includes a zinc atom. It is believed that the zinc atom increases the activity of the enzyme, providing conformational alterations and hydrolysis of the monoester of orthophosphoric acid. Each monomer ALP contains three metal-forming centers. An enzyme loses activity when it loses zinc ions but restores it after the addition of metal. The activity of the enzyme increases in the presence of magnesium ions, a certain ratio of magnesium ions to zinc is necessary for its optimal activity [4, 38].

ALP is present in all organs of the person; the highest specific activity of the enzyme was found in the epithelium of the small intestine, the epithelial cells of the renal tubules, osteoblasts, hepatocytes, and placenta. The enzyme is tightly bound to the cell membrane by the hydrophobic carboxyl residue of the polypeptide chain; the hydrophobic end of the chain is identical in all isoenzymes of ALP. ALP is attached to the plasma membrane with the phosphatidylinositol-glycan "anchor". ALP molecules on the surface of the plasma membrane are irregular [23, 24, 27].

The molecular weight of the ALP is 130-220 kDa. The association of ALP with glycosaminoglycans (neuraminic acid) can change not only the molecular weight of the enzyme but also the charge of the molecule and its mobility in the electric field. The degree of glycosylation of ALP in tissues can affect its thermolability.

In the human body, the biosynthesis of the enzyme is encoded by three genes: one - hepatic, bone and kidney isoenzymes, another - intestinal isoenzyme and the third - placental ALP. It is believed that there is also a 4th gene that codes for the synthesis of germline ALP. The latter is localized in the subtle part of the fetal gut until about 30 weeks of gestation. Embryonic and mature forms of ALP are similar in catalytic activity. Isolation of ALP from liver tissue with phosphatidylinositol-

specific phospholipase leads to the formation of forms present in blood serum. These data suggest that the forms of ALP in blood serum are produced by enzyme hydrolysis of tissue forms [24].

Clinical significance of ALP activity determination. Normal activity: male - 54-137 U/L (900-2290 nmol/s × L), female - 44-126 U/L (740-2100 nmol/s ×L). Increasing activity of alkaline phosphatase in blood serum does not always allow with sufficient degree of reliability to make an idea about organotrophic pathology [27].

In clinical biochemistry, determination of ALP activity is most often used in the diagnosis of pathology of the hepatobiliary system and bone tissue. ALP activity in blood serum is often increased with an obstructive liver disease, cholestasis, hepatitis, hepatotoxicity, Paget's disease, osteomalacia, neoplasms of the liver and bone tissue. Low activity of alkaline phosphatase was noted at the hepatic degeneration of Konovalov-Wilson, cretinism, accumulation of radioactive substances in the bones, scurvy, and congenital hypophosphatasia. The use of hypolipidemic drugs also inhibits ALP activity.

Low or even negligible ALP activity was detected in hepatic degeneration. The mechanism of this phenomenon is not finally clear, suggesting that the copper ion competes with zinc for a site in the active center of alkaline phosphatase, which leads to a sharp drop in the activity of the enzyme. The use of hypolipidemic drugs, in particular, clofibrate, also inhibits enzyme.

In preterm infants, for the purpose of early diagnosis of rickets, it is recommended to determine the activity of ALP.

Investigation of ALP activity in patients with hypophosphatasia syndrome indicates the important role of ALP in mineralization of bone tissue. Hypophosphatasia syndrome is a congenital bone disease characterized by selective ALP synthesis deficiency. In patients with a similar pathology in the tissues and blood serum, the activity of the hepatic, bone and renal isoenzymes of the ALP in the normal activity of the placental and intestinal isoenzymes is significantly reduced. Another feature of the disease is the accumulation of phosphorus-containing

complexes in the blood and urine. These include phosphoethanolamine, pyrophosphate, P-5-P. The disease is caused by point mutations in the gene encoding the enzyme synthesis [23, 24, 27].

The increased activity of ALP occurs not only in the conditions of active growth of bone tissue but also in its destruction such as osteomalacia and subsequent osteoporosis. In severe osteomalacia and osteoporosis, ALP activity in serum can be normal or slightly elevated (2-3 times). Osteomalacia, verified histologically, may occur with normal ALP activity.

The activity of ALP in the blood serum can be increased with the osteo myodystrophy, which develops as a complication of prolonged hemodialysis. When cyclosporine is administered after transplantation, the increased activity of ALP depends more on the toxic effect of the drug on hepatocytes and is less associated with the pathology of osteoblasts. In patients with hyperparathyroidism, the activity of ALP blood serum in the normal range, but with the development of osteoporosis, especially osteonecrosis, can be significantly increased [24].

Investigation of ALP activity is also advisable in the differential diagnosis of intra-and extrahepatic cholestasis. In the case of extrahepatic-biliary obstruction with stones in the bile duct and gallbladder, as well as tumors in these organs, the activity of ALP increases up to 10 times or more. With intrahepatic obstruction of the bile ducts and hepatitis, there is also an increase in ALP activity, but the degree of hyperenzymemia does not exceed 2-3-fold magnitude. Acute necrotic changes in hepatocytes may not be accompanied by an increase in ALP activity until bile tubules are involved in the pathological process and delayed biliary excretion. At the same time, far from all cases of liver parenchyma, there is a clear correlation between the activity of ALP blood serum and the content of bilirubin in it. At the beginning of the development of intra-hepatic cholestasis, an increase in ALP activity may be due to an increase in the synthesis of protein in hepatocytes; further increase in the activity of alkaline phosphatase in blood serum, especially in the form of macro-ALP, is associated with a disturbance of the integrity of the cells of the bile ducts [9].

Acid phosphatase (ACP). Normal activity: 0.025-0.12 mmol/(hour × L), 0.1-0.8 units/L. Major sources: the main source of this lysosomal enzyme is the prostate gland, although the enzyme is also present in erythrocytes, platelets, liver, spleen, and bones.

Since erythrocytes contain a significant amount of ACP, hemolyzed blood samples are not at all suitable for the diagnostic determination of prostatic enzyme.

Diagnostic changes in ACP activity are used to monitor prostate carcinoma. Increased blood serum coronary activity may be observed in other prostate diseases, for example, adenomas. A small amount of ACP is released into the blood during the study of the prostate "per rectum", 1-2 days after surgical intervention on the prostate gland, as well as in its biopsy. The activity of ACP can increase in other conditions: hemolysis, bone diseases, hyperparathyroidism, metastatic carcinoma of the mammary gland, lymphoblastic leukemia. Prostatic causes can be differentiated from nonprostatic effects of ACP inhibitors. Tartrate inhibits the prostatic enzyme, and formaldehyde inhibits ACP from other sources. The use of clofibrate in women and men and androgens in women also increases the activity of ACP [7].

Cholinesterase

Normal activity: 2666-5666 U/L, 44.4-94.4 mkatal/L. Cholinesterase is an enzyme of the class of hydrolases. In the human body, 2 types of cholinesterase are identified: "true" cholinesterase (acetylcholinesterase, EC 3.1.1.7) localized in the liver, red blood cells and the nerve tissue and pseudocholinesterase is found in the blood serum, liver, pancreas, and intestinal mucosa. Acetylcholinesterase cleaves acetylcholine, pseudocholinesterase hydrolyzes both choline and non-cholines ethers. Investigations of acetylcholinesterase activity are carried out to determine the sensitivity to suxamethonium. Suxamethonium (scoline) is a muscle relaxant used for the endotracheal intubation procedure. Suxamethonium is normally cleaved by acetylcholinesterase.

The activity of blood serum pseudocholinesterase decreases with liver disease, especially low its activity is in malignant neoplasms of the liver. Organophosphorus

insecticides inhibit both types of cholinesterases, and the determination of their activity can be used for screening poisoning [26; 29].

Lipase

Lipase is an enzyme formed in the pancreas and is released in large quantities into the duodenal gut with pancreatic juice. Normal activity: 0-28 μmol/(min × L), 14-26 U/L. Unlike other cellular lipases involved in the lipolysis process in the liver and fat cells, the pancreatic lipase belongs to the secretory enzymes that break down the triacylglycerol formed in the small intestine after the emulsification of the fats coming from the food to the mono- and diacylglycerols and free fatty acids, which are then absorbed into the bloodstream [23, 24, 27].

The causes of increased serum lipase activity may be:

1) acute pancreatitis of any origin, in which there is a particularly significant increase in the activity of the enzyme;

2) other diseases of the digestive system, in which also it is impossible to exclude the presence of acute changes in the pancreas: bilious colic, intestinal obstruction, peritonitis, intestinal infarction, perforation of the stomach or intestine. In these cases, a moderate increase in lipase activity is usually observed [5].

Aldolase

Aldolase is an enzyme involved in the processes of glycolytic glucose splitting, is a class of lyase. Normal activity: 0.09-0.57 mmol/(h × L) or 1.47-9.5 ODI/L Aldolase catalyzes the cleavage of fructose-1,6-diphosphate to triose phosphate. An enzyme is present in all tissues and organs, but the greatest activity is found in skeletal muscle, myocardium, liver, red blood cells and brain. Isolated by 3 isoenzymes: A - skeletal muscle, myocardium; B - liver, kidneys, leukocytes; C - brain.

5'-nucleotidease

This is a specific phosphomoneesterase, which is formed in the liver tissues. Normal activity: 1.3-13.3 U/L or 27-233 nmol/(s × L). This enzyme is organ-specific

for the liver. Its diagnostic value is similar to the value of alkaline phosphatase or γ-glutamyltransferase but 5'-nucleotidase is a more sensitive and specific biochemical marker of cholestasis than alkaline phosphatase. Increased blood serum 5'-nucleotidase is observed in biliary cirrhosis, carcinoma of the liver and biliary structures, choledocholithiasis. However, there are methodological difficulties to determine this enzyme as diagnostic test due to its weight [32].

Leucyl aminopeptidase (Leucine aminopeptidase)

The leucine aminopeptidase acts on the N-terminal amino acid residues of the proteins especially effectively if the N-terminal amino acid is L-leucine. The enzyme also cleaves amide groups from various amino acids.

Normal activity: up to 6 U/L.

The main sources are the liver, the small intestine, the kidneys, the pancreas.

The activity of blood serum leucine amaminopeptidase is a sensitive indicator of pathology of the liver parenchyma. In acute infectious hepatitis, its activity is significantly increased already in the pre-jaundice period. In addition, leucine aminopeptidase can be considered as one of the biochemical markers of cholestasis [23, 24, 27].

CHAPTER 1.6
KININ–KALLIKREIN SYSTEM OF BLOOD PLASMA

1.6.1. The concept, structure and value of the kinin-kallikrein system

The kinin-kallikrein system (KKS) is a group of blood proteins that play a role in regulating vascular tone, diuresis, inflammation, coagulation and pain sensation. KKS was described in 1949 at the International Congress of Physiologists in Copenhagen, where Brazilian scientists Roha and Silva reported a new high-level antihypertensive substance called bradykinin. Biologically active components of it are polypeptide hormones - bradykinin and kallidin:

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg Bradykinin

Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg Kallidin (lysyl-bradykinin)

This is one of the key cascade proteolytic systems of blood plasma, which together with the renin-angiotensin system, complement and system of blood clotting participates in the regulation of some of the basic functions of the body, such as maintaining blood pressure, antigenic compatibility and hemostasis [19, 34]. KKS consists of a group of macromolecular precursor proteins (kininogens), active polypeptides (kinins), as well as a set of activators (kallikrein, aminopeptidases, kininase I) and inhibitory enzymes (kininase II), (fig. 1.22). In functional terms, in humans two independent KKS can be identified, which include different types of kallikreins, kininogens and kinins: the blood plasma (circulating) and the organ (local) [19]. Blood plasma KKS includes the so-called high molecular weight kininogen (HMWK) and plasma pre-kallikrein which are synthesized in the liver and secreted as the rest of the plasma proteins.

Blood plasma pre-kallikrein is proteolytically activated by the XII blood coagulation factor and other proteases (amino-peptidases) and in this form activates coagulation and releases biologically active kinin-bradykinin from a high molecular complexes acting as a pro-inflammatory factor. Local KKS consists of locally

synthesized or hepatic low molecular weight kininogen and tissue kallikrein. They interact following the same scheme. Local KKS is found in many internal organs and tissues, in particular in the myocardium, renal tubules, central nervous system, pancreas, prostate, salivary glands and granulocytes [23, 24, 27].

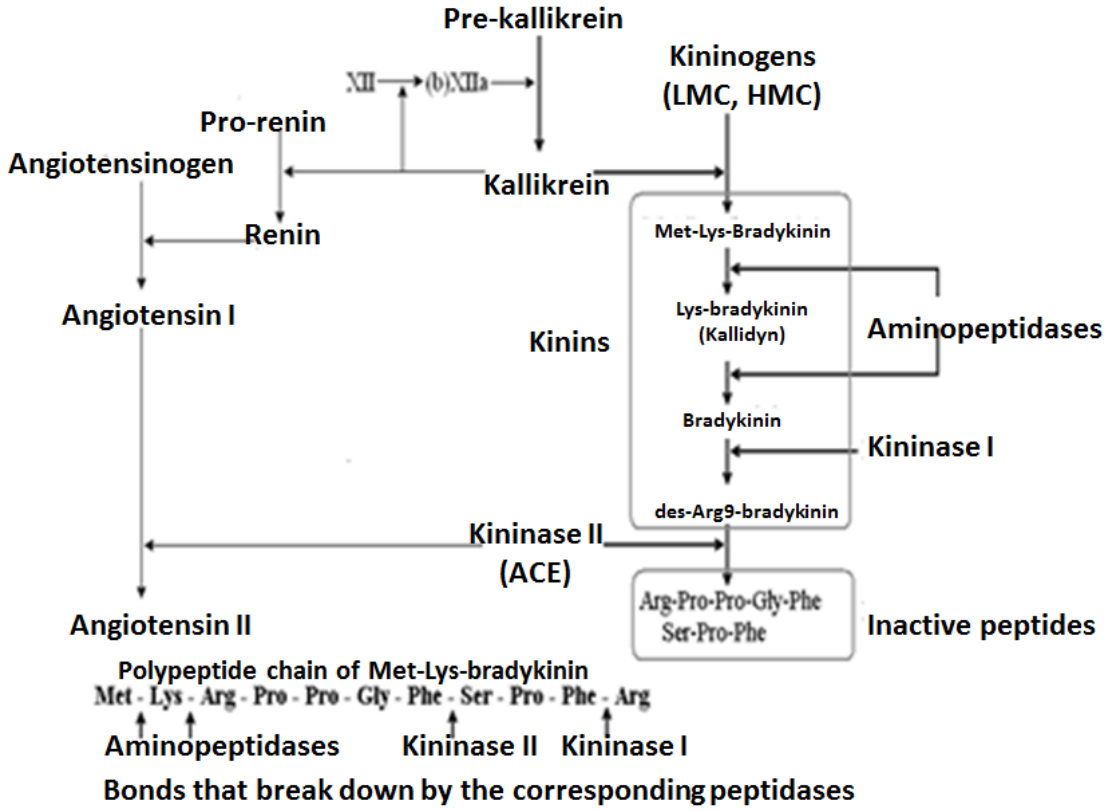


Fig. 1.22. Kinin-kallikrein system [19].

In contrast to the blood plasma KKS, organ-specific tissue systems can continuously produce kallikrein, respectively, and the kallidin is formed there continuously from the systemic or local kininogen: some local systems synthesize their own, low molecular weight kininogen. This is especially true in the kidneys, where a large amount of kallikrein and kininogen are synthesized by the epithelium of the tubules and excreted in the urine [19, 27].

The conversion of kininogens into kinins is carried out by specific enzymes - kallikreins. Kallikreins are also in inactive form - pre-kallikrein which is activated by the factor XII of the blood coagulation system. Kinins have a short-term effect - the

half-life of these molecules is 20-30 seconds. This is due to the high activity of kinases - the enzymes that break them down [19, 24].

1.6.2. Kinins biological effects and the KKS interconnection with other enzymatic systems in blood plasma

System Effects. Along with vasodilation, causing local hyperemia and lowering blood pressure, kinins stimulate pain and cause edema due to increased vascular permeability. Because of these properties, kinins are considered as classical inflammation mediators. They contribute to the development of shock in pancreatitis, sepsis and intravascular coagulation. There is also evidence that kallikrein has a strong chemotactic activity, and kinins provoke the release of cytokines from monocytes.

Local effects. In contrast to blood plasma corticosteroids, in the normal physiological state, kinins are produced continuously in different organs. This allows the KKS to be involved in the regulation of many physiological functions. Thus, the formation of kinins in the glandular organs (salivary, sweat, pancreas, prostate, kidneys) is of great importance, where kinins regulate their perfusion, secretion, and contractility of smooth muscles. The presence of KKS components in the central nervous system and myocardium was also shown. In the kidneys, kininogen and kallikrein are synthesized by the epithelium of the tubules and secreted into their lumen. Thus, they are present in large quantities in urine. It has been found that in the kidneys, kinins increase local blood circulation, as well as diuresis and natriuresis. In endothelial cells under the influence of kinins, synthesis of such products as nitrogen oxide and prostacyclin (prostaglandin I_2) is stimulated [19, 26, 27].

Connection to the system of coagulation. It is known that the activated blood coagulation factor XII not only provides a further enzymatic chain in the coagulation system but also cleaves pre-kallikrein to active kallikrein. Kallikrein, in turn, as a serine protease, along with the cleavage of kininogens to kinins, is capable to activate the factor XII. The connection of the KKS with the system of blood clotting indicates what the first can be activated in the process of contact coagulation (as occurs with

arthritis, allergic rhinitis, dialysis) as well as a result of interaction with bacterial or endogenous proteases (as with sepsis, pancreatitis or operations in the prostate gland) [19, 26].

Relationship with the renin-angiotensin system. Circulating KKS has a close relationship with the renin-angiotensin system (RAS), due to the common enzymes for them - the blood plasma kallikrein and kininase II. Kallikrein cleaves the prorenin to renin and thereby triggers a cascade of proteolytic reactions, the terminal active product of which is angiotensin II, a powerful vasoconstrictor. Kininase II, also called angiotensin-converting enzyme (ACE), provides direct conversion of inactive angiotensin I to angiotensin II. Thus, ACE, on the one hand, activates the RAS and, thus, promotes the increase of blood pressure, and on the other - degrades kinins to inactive metabolites, leveling their antihypertensive effect.

Investigation of kinin potentiating effect of snake poisons allowed discovering the first ACE inhibitors. They are widely used in the treatment of arterial hypertension. The significant role of kinins in the wide spectrum of ACE inhibitors was explained when it became known that the vasodilating effect of bradykinin is mediated by the release of endothelial mediators of nitrogen oxide (NO) and prostacyclin. Now, this is almost the most numerous group of antihypertensive drugs (about 15 varieties of ACE inhibitors). Most often they are used in combination with thiazide diuretics. The common side effect of ACE inhibitors is a dry cough due to the accumulation of non-split kinins in lung tissue [19, 26, 27].

1.6.3. The kinin-kallikrein system state in various pathological processes

It is known that KKS of blood is the central link in the complex of the humoral system that regulates homeostasis and makes adaptive-protective reactions of the organism. KKS is actively involved in the supply of organs and tissues by Ferrum in hypoxic states. One of the most important factors in its activation is tissue ischemia. In patients with Ferrum deficiency anemia (FDA), there is activation of KKS with the involvement of the inhibitory link. In this case, the type of response to KKS depends on the duration of the disease. At an insignificant term, FDA is marked activation of

KKS, characterized by the preservation of connections within the system. The long course of the disease contributes to the appearance of a pathological type of KKS response, indicating its inhibition and disruption in the proteinase system - proteinase inhibitors. Treatment performed in patients with a disease duration of fewer than 2 years, leads to the normalization of the state of KKS (with the preservation of the high activity of the α1-proteinase inhibitor in patients with a severe course of illness) [19, 20].

Changes in KKS in severely ill patients are more stable and retained after treatment.

One of the leading directions in the study of arterial blood pressure (ABP) pathology in adults and children is to study the role of pressor and depressor mechanisms for its regulation. Among the latter, the KKS of blood is quite relevant, due to the strong influence of kinins on arterial pressure, functioning of the heart, kidneys and vessels, the close connection of this system with the autonomic nervous system (ANS), prostaglandins (PG), catecholamines (CA), the system renin-angiotensin and other humoral factors in the regulation of arterial pressure.

The simultaneous participation of CA, serotonin, histamine, and kinins in the regulation of vascular tone and heart function, the presence of the relationship between the components of the kinin system and angiotensin, PG and corticosteroids allow the cause of the "balance of mediators" responsible for regulating homeostasis in the body. The influence of kinins on the vascular tone depends on the rheological properties of the blood as kinins play an important role in making corrections between the factors regulating blood composition and vascular tone.

The physiological essence of kinins consists in reducing the tone of the vessels in accordance with the rheological state of the blood. The most important physiological regulator of KKS is the level of functional activity of the ANS. In physiological conditions, hypotensive effects of kinins prevail. In the system of central hemodynamics, the physiological role of bradykinin is to limit the hypertensive effect of angiotensin and CA [19, 27].

In more than 80% of children with arterial hypotension, activation of KKS of

blood has been detected, whereas for children with arterial hypertension activation of KKS is not characteristic. The results of the studies allow for adequate and vascular therapy of vascular dystonia in children.

This information is very relevant since it allows characterizing the pathogenetic positions of a disturbance of systemic and regional blood flow in children and adolescence, providing an early implementation of primary and secondary prevention of identified pathology and conducting adequate therapy [33].

Many studies point to the obligatory reaction of KKS to viral hepatitis, with the most stable sign of this pathology - a decrease in the blood the the content of the precursor of kallikrein. Reducing the level of pre-kallikrein may be a result of the activation and functioning of the system in the formation of kallikrein, and the disturbance of its synthesis by the affected liver.

The main inhibitor of trypsin and trypsin-like proteinases, which provides 90% of the antitrypsic activity of the blood serum, is α1-antitrypsin (α1-AT or α1-proteinase inhibitor), which is synthesized by the liver in the endoplasmic reticulum of hepatocytes. Hereditary deficiency of α1-AT leads to the development of chronic liver disease [19].

In the scientific community the most probable hypothesis of the mechanism of liver damage with α1-AT deficiency is considered to be the inability of the body to prevent the cytolytic action of proteases of various origins (viral, bacterial, formed from the elements of inflammatory infiltrates). Under the action of these proteases from affected hepatocytes, new enzymes are released, which increase the liver damage, thus resulting in a chain reaction.

In viral hepatitis, a significant increase in α1-AT level in blood, an increase in the functional activity of α1-MG and C-1 inactivators, which is important in the development of adaptive reactions, has been detected. With a quantitative or functional deficiency of proteinase inhibitors, the progressive course of hepatitis, as well as the development of acute liver failure [19].

Activation of KKS is an universal response to damage, including the structural disruption of cell membranes due to the activation of lipid peroxidation (LPO). The

activation product of KKS, vasoactive peptide bradykinin, is a pathogenetic factor in the development of many diseases that can lead to the extinction of vasodilation, edema, pain, microcirculation.

The activity of KKS is regulated by proteinase inhibitors, therefore the decrease in the activity of the main inhibitors leads to uncontrolled activation of this system.

Activation of KKS may indicate an unfavorable course of the meningococcal disease and meningococcal meningitis, some bacterial and viral infections, bronchial asthma, allergic reactions, metabolic syndrome, type 1 diabetes mellitus.

Increasing the activity of KKS may also be a marker for reducing the adaptive properties of the organism [19, 26].

CHAPTER 1.7
BIOCHEMISTRY OF BLOOD COAGULATION SYSTEM

The blood coagulation system includes the enzyme and non-enzyme proteins in plasma, tissues, supramolecular complexes, calcium ions.

The process of blood coagulation and the formation of a blood clot consist of a cascade of consistent enzymatic reactions, which are catalyzed by specialized proteins - coagulation factors. In the cascade system of blood clotting, each protein factor causes activation of the next component of the cascade based on the principle of pro-enzyme (inactive) → enzyme (active), which ensures a consistent enhancement of the process and implements a rapid protective response in response to the vascular impression [62].

Nomenclature (numbering and trivial designation) of blood coagulation factors (according to the recommendations of the International nomenclature committee):

Factor I (fibrinogen).

Factor II (prothrombin).

Factor III (tissue thromboplastin).

Factor IV (calcium ions).

Factor V (proaccelerin).

Factor VII (converting).

Factor VIII (anti-hemophilic globulin A, Willebrand factor).

Factor IX (anti-hemophilic globulin B, Christmas factor).

Factor X (Stuart-Provera factor).

Factor XI (Rosenthal factor or thromboplastin plasma precursor).

Factor XII (factor Hageman).

Factor XIII (fibrin-stabilizing factor).

Along with the indicated major coagulation factors predominantly in the blood plasma, there are platelet coagulation factors involved in various stages of vascular thrombocyte, coagulation hemostasis and fibrinolysis. Thrombocyte coagulation

factors denote the Latin letter P (from English platelets - blood platelets) with the numerical indices: P1-P11 [10, 11].

Ways of activation and functioning of cascade system of coagulation of blood. The cascade blood clotting circuit can be activated by activating two alternative coagulation pathways (internal and external) that are different from initial reactions and converge into a single common path of coagulation, which begins with the activation of factor X.

The central event in implementing the blood coagulation process is the formation of an active factor X (factor Stuart-Provera).

Factor X is a Ca^{2+}-dependent glycoprotein that is synthesized in the liver with the participation of vitamin K. It consists of light and heavy polypeptide chains, connected by disulfide bridges, and is activated by limited proteolysis. The activated factor X (f.Ha) is a serine protease, which converts prothrombin (f. II) into active thrombin (f. IIa), necessary for the transformation of fibrinogen (f. I) into fibrin, the basis of a fibrin clot or thrombus [10, 11]

Conditionally three phases can be identified in the coagulation cascade: 1) the formation of prothrombinase (Va-Xa and calcium ions) of the complex on the phospholipid membrane, 2) thrombin generation, and 3) fibrin formation. Depending on how the prothrombinase has been formed, the external and internal mechanisms of blood coagulation are distinguished. The relationship between phospholipid cell membranes and the subsequent relationship between blood coagulation factors is due to protein-protein interactions that include IXa-VIIIa (intravenous tenase) or the interaction of factor VII with tissue factor (TF), resulting in an active complex of TF-VIIa (tenase of the external coagulation path). The generated thrombin converts fibrinogen to fibrin, which strengthens the thrombocyte thrombus, stabilizing the clot [56, 62, 73, 74].

The essence of the main (internal) pathway of blood coagulation [62, 66, 104] is when the endothelium is damaged, the surface of activated platelet collagen is highly effective for factor XII and high molecular kininogen, which is in combination

with pre-kallikrein and factor XI. Formation of the complex leads to the activation of these components, thus changing the molecular structure and activation of each of the proteins [99]. The factor XIIa in the presence of high molecular weight kininogen activates the factor XI, which converts the factor IX to factor IXa, which together with factor VIII, phospholipids and Ca^{2+} ions forms a complex that activates factor X [62, 66].

It is also possible to implement the external pathway of blood coagulation at the initiation of TF, which, in the presence of calcium ions, forms a complex with the factor VII which circulates in the blood. The VIIa-TF complex activates factor X and converts it into an active form [62,100,104].

The separation of the internal and external blood coagulation pathways is rather conditional and both blood coagulation cascades act together, complementing and regulating each other [62, 101]. Due to the action of these enzyme cascades, factor Xa of blood clotting in the presence of calcium ions on the phospholipid surface is associated with the factor Va. The complex of factors Ha and Va on the phospholipid surface acts as prothrombinase and converts pro-enzyme - prothrombin, into active enzyme - thrombin [15].

With a slight damage to the vascular wall of TF in combination with the factor VIIa activates the factor IX. Factor IXa forms a complex with factor VIII in the presence of calcium ions and phospholipids. This complex activates factor X. Such a process is called alternate. It enhances the activation mechanism that results in the emergence of a small amount of thrombin (insufficient for fibrin formation), but that promotes the activation of cellular hemostasis [85].

At the same time, blood clotting is under control, which determines the velocity and volume of the enzymatic processes. A key role in the initial stages of activation of the blood coagulation system is played by the amount of tissue factor and its inhibitors, which ensure the normal functioning of the hemostasis system. Disturbance of the regulation of this complex system leads to severe complications, the development of thrombophilia and hemorrhages [82].

The initial stage of blood coagulation is blocked by the tissue factor inhibitor (TFPI), which is a proteinase inhibitor that, in turn, participates in initiating blood clotting. In the bloodstream, there is a free form of the inhibitor and it bounds by lipoproteins [36]. It has been found that TFPI inhibits factor Xa by binding directly to the active center of the enzyme, thereby inhibiting the action of the factor VIIa-TF complex, affecting the process of thrombin activation [36].

Directly inactivating thrombin, there is a family of the so-called serpins (serine protease inhibitors) that include antithrombin III (AT III), α1-inhibitor of proteases (α1-antitrypsin), cofactor II of heparin and α2-macroglobulin in its own group. The main thrombin inhibitor is antithrombin III, which corresponds to 75% of the antithrombin potential of blood plasma. It blocks thrombin and other serine proteases of plasma (blood coagulation factors Xa, IXa, XIIa, and plasmin), suppressing not only the effect of thrombin but also preventing its formation [25]. The product of catalytic action of antithrombin III is heparin.

An important regulatory function of the blood coagulation system is the complex multifunctional system of protein C. The action of this system is considered as one of the early and fastest mechanisms that are included in the process of regulation of hemostasis with the threat of thrombotic formation. Active protein C acts as an anticoagulant, which fundamentally differs from the activity of specific inhibitors, coagulation factors and the action of the fibrinolysis system, while indirectly stimulating fibrinolysis [25]. At the same time, the active use of protein C is considered as one of the main indicators of the threat of thrombophilia.

Fibrinogen is practically absent until 4 months of fetal development in the embryo. It appears in very small quantities for only 5 months and in the process of fetal development, its content is gradually increasing. Prothrombin also appears only for 5 months of intrauterine development and its quantity is gradually increasing. In the period of fetal development, the concentration of blood coagulation factors is very low, and the factors that prevent coagulation are present in a large quantity. The concentration of anticoagulants is reduced only at the time of birth [23, 24].

In the blood of the fetus, the quantity of factors of coagulation and anticoagulation does not depend on their content in the mother's body. During pregnancy in the mother's blood, the concentration of fibrinogen is greatly increased.

Newborn blood coagulation is slowed down, especially on the second day of the life of the newborn. Low concentration in the blood of newborns of the main factors of blood coagulation is determined largely by the functional immaturity of the liver and insufficient synthesis of vitamin K by the microflora of the large intestine [57, 89].

During the first year of life, there is an increase in the blood concentration of individual factors of the hemostasis system, and their characteristic level for adults is reached only during puberty.

An insignificant increase in the quantity of platelets, an increase in their ability to adhesion and aggregation, an increase in the concentration of fibrinogen, soluble fibrin-monomeric complexes, and the activity of the fibrin-stabilizing factor is observed in the process of aging [89]. Therefore, such alterations in the elderly cause increased blood-coagulation.

CHAPTER 1.8
PECULIARITIES OF METABOLISM IN BLOOD CELLS

1.8.1. Peculiarities of metabolism in erythrocytes

The mature erythrocyte of an animal and a human is a simplified cell in both organization and structure. In the erythrocyte, there is no nucleus, and it is not able to synthesize the protein, nucleic acids, porphyrins, purines. But the erythrocyte is well adapted to its main function - the transport of O_2 from the lung to the tissues and CO_2 - from the tissues to the lungs. Enzyme systems support the activity of erythrocyte in the bloodstream for 120 days. A characteristic feature of erythrocytes is the high content of 2,3-di-phosphoglycerol acid (2,3-DPG), which plays (along with ATP) an important role in the regulation of the affinity of hemoglobin (Hb) to O_2 [15, 46].

The main pathway of energy exchange in erythrocytes is glycolysis. In the process of glycolysis ATP and NADH are produced. Glycolysis energy is used for the active transport of cations through the cell membrane and the maintenance of the equilibrium between potassium and sodium ions in erythrocytes and blood plasma, as well as to preserve the integrity of the membrane and the biconcave shape of the cell. The formed NADH is used to maintain the active functional state of Hb - preventing its oxidation into methemoglobin [3, 39].

In addition to anaerobic, direct redistribution of glucose (in the reactions of the pentose-phosphate pathway) is carried out in erythrocytes. The significance of this process consists in the formation of a restored NADPH, the hydrogen of which is used in erythrocytes to restore glutathione, which plays an important role in reduced state for preserving the activity of a number of enzymes containing SH and participating in the cleavage of hydrogen peroxide with the help of glutathione peroxidase [42, 88].

Mature erythrocytes circulating in the bloodstream are metabolic cells, even though there is no ability for the protein synthesis and tricarboxylic acid cycle processes. In physiological conditions, they use little O_2 [90]. Preservation of functional activity of erythrocytes is connected not only with ATP but also with

NADH, which is formed in the process of glycolysis. This compound is used to restore pyruvic acid to lactate and to restore methemoglobin with methemoglobin reductase [49].

The alteration in the surrounding erythrocyte environment (osmotic pressure, temperature, pH, the concentration of P_{in}, etc.) is reflected in the rate of glycolysis, concentration of metabolites and cofactors [48, 58, 86]. When studying the influence of various factors on erythrocyte functioning, data on the mechanism of regulation of glycolysis was accumulated:

1. Influence of temperature. The glycolysis is relatively insensitive to temperature rise and its rate remains constant at 45 °C and pH 7.3 and 48 °C and pH 8.2 within one hour. Further increase in temperature leads to a rapid decrease in the intensity of the process. Lowering the temperature leads to increased activity of enzymes, especially aldolase and hexokinase [42, 70].

2. Influence of pH. The intensity of glycolysis, depending on the pH of the medium, is closely related to the changes in the activity of enzymes. At different values of pH, the enzymes of glycolysis change their activity in different ways: with a decrease in the pH of the blood to 6.8 - the hexokinase was most sensitive, at pH 6.8-7.4 - mostly phosphofructokinase, at pH 7.6-8.3 hexokinase, phosphofructokinase, and glyceraldehyde phosphate dehydrogenase [47, 93].

3. Influence of inorganic phosphate (P_{in}). The concentration of P_{in} in a suspended medium significantly influences the rate of red blood cell glycolysis and the content of intermediate glycolysis [92].

Along with the anaerobic conversion of glucose, which leads to the formation of lactic acid in red blood cells, there is an additional way of utilizing glucose - a pentose-phosphate pathway, in which about 11% glucose is oxidized to carbon dioxide and water. This process can vary depending on pH and pO2. It was found that an increase in glucose level, which is oxidized to CO_2, does not lead to the accumulation of lactic acid [88, 97].

The release of CO_2 as a byproduct of the pentose cycle is accompanied by the use of a small amount of O_2. This process is not good breathing since it does not

occur in mitochondria and is not related to oxidative phosphorylation due to the absence of Krebs circulating enzymes in mature erythrocytes. The general result of the pentose pathway is the oxidation of one of the 6 molecules of glucose-6-phosphate with the formation of 6 molecules of CO_2 and the production of 12 NADPH molecules. The significance of NADPH is its participation in numerous reactions that are necessary to maintain the functional activity and integrity of erythrocytes. These include the restoration of methemoglobin in hemoglobin and the restoration of oxidative glutathione [15, 93].

Despite the relatively high activity of enzymes, only one-tenth of glucose is oxidized in human erythrocytes in the pentose pathway [88].

The regulation of the pentose pathway depends on the velocity of the two competing metabolic processes - glycolysis and the pentose pathway. The subject of competition in these pathways is one substrate - glucose-6-phosphate, which can take part in glycolysis, where isomerization in fructose-6-phosphate undergoes further transformations or oxidized to 6-phosphogluconate under the action of glucose-6-phosphate dehydrogenase.

The erythrocyte has all the necessary conditions for the conversion of glucose-6-phosphate in the pentose pathway. But this inhibits the cyclical inhibition of metabolites (eg, erythrose-4-phosphate) activity of glucose-6-phosphate isomerase, which contributes to an increase in the amount of glucose-6-phosphate. Therefore, the possibility of a competition of the corresponding dehydrogenase for the glucose-6-phosphate substrate with the glycolytic system decreases. On the other hand, erythrose-4-phosphate also inhibits transketolase in hemolysates, but at the same time, this compound is required to maintain active transaldolase [67].

An increase in the concentration of P_{in} (inorganic phosphate) promotes the activation of glycolisis enzymes and reduces the activity of glucose-6-phosphate dehydrogenase. In addition, P_{in} directly affects the decrease in the activity of transketolases and transaldolase in erythrocytes and hemolysates [46].

The value of the pH of the intracellular medium plays a role in the metabolism of red blood cells: the optimal pH for the pentose pathway is 7.6, for glycolysis - 8.2 [93].

A special place in the structure of the erythrocyte belongs to hemoglobin, which actually determines its function (fig. 1.23). The structure of the hemoglobin molecule consists of 4 pairwise identical polypeptide chains, the totality of which forms the protein part - the globin. Human Hb molecule is heterogeneous, due to the properties of the polypeptide chains that are part of it.

Hb A - the main component of erythrocytes (95-98% of Hb blood) consists of 2α- and 2β-chains, Hb A2 (2-2,5%) - with 2α- and 2δ-chains, Hb F (0.1-2%) - from 2α- and 2γ-chains [42, 68].

The α and β Hb A chains consist of 141 and 146 amino acid residues, which are bound in a genetically determined sequence, respectively. In general, the Hb molecule contains 574 amino acids [37].

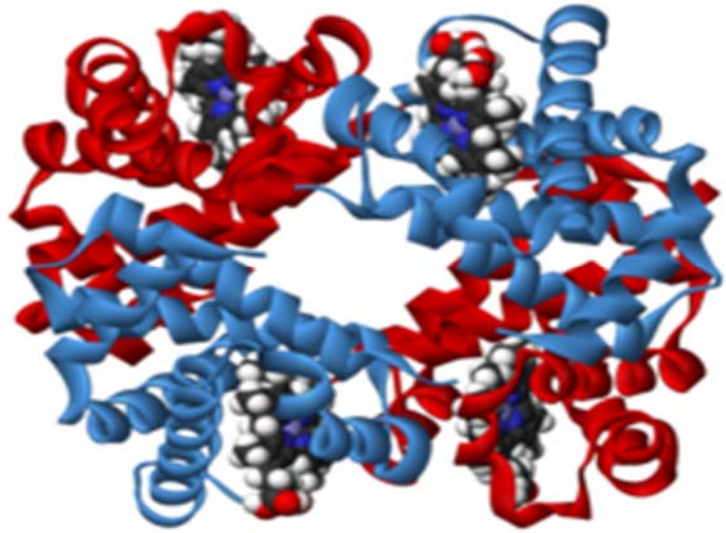


Fig. 1.23. Scheme of the quaternary structure of the hemoglobin molecule [37].

The X-ray structural method for analyzing Hb crystals allowed us to understand the peculiarities of the spatial arrangement of both individual chains and the Hb molecule as a whole. It turned out that α- and β-chains are twisted into spiral segments of different lengths that have the structure of right α-helixes: 7 helices in α-

chains and 8 in β-chains. The structure thus formed is called the secondary structure of the protein chains of globin. The degree of spiralization, that is, the relative number of amino acid units mounted in the spiral chain sections, makes up 70-75% in the Hb molecule [77].

Each polypeptide chain is coupled with heme. Gem is a complex compound of protoporphyrin IX with Ferrum (fig. 1.24). In heme, Ferrum is located in the center of the protoporphyrin nucleus and is bound to 4 nitrogen atoms of the pyrrole rings - two main and two additional bonds. The specificity of the connection between heme and globin depends on their native nature. Disturbances of their structure lead to the emergence of non-specific communication centers between them with the loss of the ability to attach O_2 [52].

The specificity of the interaction of heme and globin is due to the multiple contacts that arise between them. Inside each polypeptide chain of globin near the surface there is a hydrophobic hollow, in which heme is placed, forming three types of connection with globin.

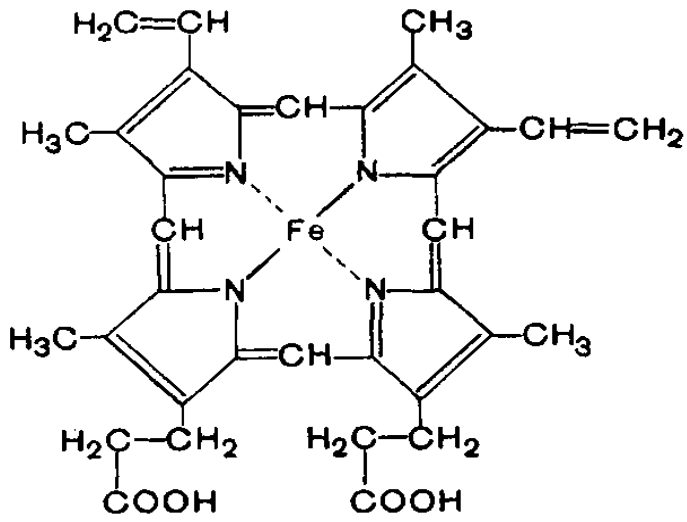


Fig. 1.24. The structure of the heme [72].

When combined with globin, the heme is oriented so that its polar substituents (vinyl and methyl groups) are directed to the inner part of the cavity and are part of

the already formed hydrophobic nucleus of the protein globulus. As a result, there are about 60 non-polar contacts of the heme with the globin chain. It is precisely nonpolar contacts that play the main role in combining heme with globin. Any disturbance of nonpolar contacts near heme leads to substantial changes in the properties of Hb [38, 81].

In its turn, the disturbance of the polar contacts of the heme with globin virtually does not affect the functional properties of the Hb molecule. Polar contacts of heme with globin are not essential for oxygenation, but they have a definite role for stabilizing the native structure of the Hb molecule [94].

The connection between the heme Ferrum and histidine F-8 globin is not very important for establishing the native structure of the Hb molecule. The absence of the influence of the Ferrum on the structure of the Hb molecule is shown in the work of numerous researchers. The binding of the heme Ferrum to globin does not significantly affect the stability of the Hb molecule. But the association of histidine F-8 with the heme Ferrum is essential for the implementation of the Hb oxidation process [76].

Regarding the origin of histidine F-8, this compound refers to invariant amino acids. Invariant amino acids surround the gem in the hydrophobic globin depression; they are the same for different species of animals and play an important role in the transport of oxygen. Among the invariant amino acids in this region of the molecule, the main role belongs to the two histidines and valine: F-8 (87) - α, F-8 (92) - β, E-7 (58) - α, E-11 (62) - α, E-7 (63) -β, E-11 (67) - β [95].

The role of histidine is as follows: as a strong σ-donor, it captures heme in globin, raises the basicity of Fe^{2+} and thereby contributes to the formation of π-bond with the π-acceptor, which is the O_2 molecule. Histidine, as an invariant amino acid, stabilizes Fe^{2+} against irreversible oxidation in $Fe3^{+}$ [90].

The advantage of histidine over other amino acids (in particular: arginine and tyrosine) is that it contains nitrogen, which can act as a σ- and π-donor [103].

In the Hb molecule, 4 subunits of 2 α- and 2 β-chains are placed in the corners of the tetrahedron around the double axis of symmetry, which passes through an

internal cavity filled with water. Therefore, Hb refers to proteins that have a quaternary structure that combines 4 globular subunits into a regular system of the α-chain in the Hb molecule located above the β-chains and are somewhat compressed between them. All four hemes are far removed from each other. The quaternary structure is stabilized by saline bonds α_1-, α_2- and β_1-, β_2-chains, which include their final residues, as well as two types of nonpolar contacts between α- and β-chains: α_1-, β_1 - the contact is equivalent to α_2-, β_2- and α_1-, β_2-contact is equivalent to α_2-, β_1- [96].

Heme, in the absence of globin, is not able to bind oxygen. Only the modification of globin changes its properties, turning the heavily soluble and chemically inert gem into a well-soluble form that can inverse O_2. In turn, hem strongly affects the chemical and physicochemical properties of globin. When a heme is combined with α-β-globin chains, changes in their conformation occur and there is a secondary and tertiary structure specific to them. This leads to the unification of polypeptide chains that contain heme, with the formation of the native quaternary structure of the Hb molecule [50, 52].

Globin is very labile to heat denaturation. The addition of heme to it greatly increases its resistance to the thermal factor. The coagulation temperature at the 15-minute exposure increases from 38 to 55 ° C. The resistance of Hb to globin and to the cleavage by proteolytic enzymes increases. Study of acid denaturation of globin and methemoglobin showed that the rate of denaturation of globin in combination with hemoglobin decreases in 2000 times [72, 95].

Inorganic and organic ions and CO_2, which are present in the erythrocyte, can join the molecule Hb, changing its affinity to O_2. In contrast to O_2 and some other ligands attached to hemic groups Hb, these compounds are inversely coupled to free amino groups of the protein part of the molecule, forming the so-called carbamine compounds. The relationship between these groups and centers that attach O_2 is allosteric. It occurs as a result of conformational changes that occur in the protein part of the molecule in the process of oxygenation Hb. When Hb interacts with O_2, as well

as when the latter has be released from HbO2, the quantity of ions and CO_2 associated with Hb can be changed [72, 81].

The influence of H^+ ions on the affinity of Hb to O_2 is called the Bohr effect and is a typical example of an allosteric link between hemes and groups of protein part of the Hb molecule. Concerning the features of the Bohr effect, according to Antonini E., Brunori M., the affinity of hemoglobin to oxygen is minimal at pH 6.2 (20 ° C) and increases on both sides of this pH value. If pH is higher than 6.2, then the effect of Bohr is called alkaline or normal, if the pH below 6.2 - acid or reverse. In the range of pH 6-9, the affinity of Hb to O_2 varies 10 times. The decrease in the affinity of Hb to O_2 with a decrease in pH is due to the addition of H^+ ions to dioxygen-hemoglobin and the stabilization of its structure. In the oxygenation of Hb, the ionization constants of the groups joining the protons increase, then the ions of H+ are secreted into the medium. At physiological values of pH (7.2-7.5), there is the largest difference in the quantity of protons that are coupled with deoxyhemoglobin and oxyhemoglobin. This feature of hemoglobin has an important physiological significance, since in the tissue capillaries the transition of CO_2 into the blood is much easier, and in the capillaries of the lungs it is allocated. At pH 6.2, a reverse pattern is observed [52, 94].

The affinity of Hb to O_2 decreases when it interacts with 2,3-DPG and ATP. These compounds, when added to dilute solutions of purified Hb, increase the release of O_2 from Hb at physiological values of pO_2 [59].

The role of the regulator to a greater extent belongs to 2,3-DPG since ATP in erythrocyte is 4-5 times less than 2,3-DPG. 2,3-DPG is able to reduce the affinity of Hb to O_2 with an increase in its concentration above the physiological level of pO_2 [54, 95].

The degree of formation of the complex of 2,3-DFG from Hb in the dioxin form largely depends on the environmental factors. The presence of various anions and cations in it, including the H^+ ions, CO_2, as well as changes in temperature and Hb concentration, affect its formation. It has a great physiological significance because with the help of 2,3-DPG changes occurring in erythrocytes under the influence of

any factors can greatly affect the transport of O_2, increasing or decreasing its impact on tissues [55].

It is established that the influence of carbon dioxide on oxygen affinity is caused by the appearance of a chemical bond of CO_2 with Hb and the formation of carboxyhemoglobin and the electrostatic interaction of Hb with the ions of HCO^-_3. However, with an increase in the concentration of HCO^-_3 ions, that is, in cases of compensated respiratory acidosis or uncompensated respiratory alkalosis, this type of connection can greatly affect the amount of CO_2 associated with Hb, as well as the overall total effect of CO_2 on affinity of Hb to O_2 [80, 96].

1.8.2. Leukocytes metabolism peculiarities

In all forms of leukocytes, there are two ways of securing the cell energy: the conversion of carbohydrates (glycolysis and pentose phosphate pathway) and the cycle of tricarboxylic acids. Compared with most other living cells, all leukocytes are characterized by intense glycolysis, which is associated with a small quantity of mitochondria in their structure [21].

Several authors point out that granulocytes undergo more intense energy exchange processes than lymphocytes (per cell). In addition, when studying the processes of respiration and glycolysis in both types of cells, it was found that the proportion of respiration in the sum of these processes in lymphocytes is higher than in granulocytes, in which glycolysis dominates [6, 32].

In granulocytes, glycolysis can occur not only in anaerobic, but also in aerobic conditions, but with less intensity. These cells are characterized by the Pasteur Effect. This effect is also clearly expressed in lymphocytes [22, 87].

It has been established that granulocytes and lymphocytes are capable to maintain a stable level of macroergic phosphates for some time in both aerobic (irrespective of the presence of glucose) and in anaerobic conditions (in the presence of glucose). This indicates the presence in these cells of mechanisms that allow the effective switching of the oxide type of energy exchange to glycolytic and, conversely, [22].

In a comparative study of energy metabolism in granulocytes in a state of rest and in actively functioning, using as a model of phagocytosis in "in vitro", various microbial bodies or inert particles, significant use of oxygen and only a moderate increase in glycolysis after the contact of cells with particles was revealed. It was suggested that the energy that is needed in the process of phagocytosis is ensured, above all, by breathing. However, experiments with metabolic inhibitors have shown that the particle capture process is completely suppressed by glycolytic, not respiratory metabolites. Thus, it was proved that the energy consumption during phagocytosis is compensated precisely at the expense of the glycolytic pathway of the transformation of carbohydrates. In other phagocytic cells - macrophages - phagocytosis, as in leukocytes, is inhibited only by glycolytic metabolites, and foam cytosis - both glycolytic and respiratory [12, 53].

Consequently, lymphocytes are able to adapt to the conditions of rapidly increasing functional activity, reinforcing one of the ways of energy metabolism in the case of a shortage of another [2].

The notion that a cycle of tricarboxylic acids takes place in leukocytes is based on the presence of mitochondria in them, as well as the presence in the cells of such dehydrogenases as a NAD-isocitrate dehydrogenase, aconitate hydratase, succinate dehydrogenase, fumarate-hydratase, malate dehydrogenase, glutamate-dehydrogenase, glyceryl-3-phosphate oxidase and aspartate aminotransferase [44].

Research conducted on whole leukocytes with the purpose of determining the intensity of oxygen absorption by them and the production of macroergic bonds when various Krebs circle metabolites (acetate, pyruvate, isocitrate, fumarate, malate, glutamate, aspartate, palmitate, α-ketoglutarate, α- glycerophosphate) showed that the absorption of oxygen by these cells was stimulated by succinate (especially intensively) and malate and α-glycerophosphate [98].

The main substrate of carbohydrate-phosphate metabolism in leukocytes is glucose. An important role belongs to glycogen, especially in granulocytes, where it is contained in relatively large amounts - from 1.4 to 5% of dry weight. In lymphocytes, it is much less (about 0.9% per dry mass). Glucose is used much more

by granulocytes than by lymphocytes. Leukocytes can use not only glucose but also other monosaccharides: hexoses (galactose, fructose) and some pentoses (ribose, xylose, and arabinose). Despite the fact that the final product of carbohydrates catabolism in leukocytes is lactate, its accumulation under aerobic conditions is only 70% of the quantity of used substrates. In anaerobic conditions, this discrepancy is even greater. The main reason for this imbalance is the use of part of these substrates for the synthesis of lipids. Proof of this may be the fact of accumulation in large quantities of α-glycerophosphate - one of the precursors of such synthesis in the conditions of anaerobiosis [98].

It has been established that in leukocytes there are nono of the four enzymes necessary for the synthesis of glucose: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, hexose diphosphatase and glucose-6-phosphatase. The absence of these enzymes makes it impossible to direct the synthesis of glucose from pyruvate, amino acids, as well as various phosphorus-containing intermediate products of glycolysis [8, 98].

1.8.3. Peculiarities of metabolism in the platelets

Blood platelets or plates were first described by Donne in 1842. Their physiological role in coagulation of blood was first discovered by Hayem (1878) and M. Lovdovsky (1883). Some morphological features of the blood platelets have been described by Bizzozero (1882), which was observed in vitro. This scientist also conducted a count of platelet quantity in the blood. The formation of blood platelets from megakaryocytes was discovered by Wright in 1906. He observed the separation of these formed cells from bone marrow cells. Subsequently, this process was studied in a tissue culture by many researchers. It was found that from one megakaryocyte 3000-4000 platelets are formed [25].

One of the characteristic features of thrombocytes is their ability to stick and spread on a wettable surface: on the glass, damaged blood vessel (adhesion). In this case, the blood platelets change their shape, becoming a split star-shaped figures with

numerous short spikes or elongated pseudopods. Dissolution is accompanied by a sharp increase in platelet size [28].

Thrombocytes are characterized by high intensity of glycolytic process, both in anaerobic and aerobic conditions (but in the absence of oxygen, this process is almost twice as intense). Glucose concentration in human platelets is low; the main source is endogenous glycogen cleavage. When glucose is added to the blood platelets, which are suspended in a saline solution, the energy cleavage of the substrate occurs, with the accumulation of lactic acid. When exhausted glucose reserves, endogenous glycogen is cleaved. Addition of glucose inhibits the cleavage of glycogen [60].

The value of the Pasteur effect in platelets depends on the substrate of anaerobic cleavage: when using endogenous glycogen of cells, inhibition of glycolysis by breathing is expressed stronger than when added to glucose [63].

The effect of Pasteur in platelets is imperfect because breathing completely does not inhibit glycolysis and, in aerobic conditions, lactic acid is also accumulated. This is due to the fact that the activity of the enzymes of the tricarboxylic acid cycle is much lower compared with the activity of enzymes glycolysis [64].

Pyruvate, which is formed in the process of anaerobic cleavage of carbohydrates, is mainly recovered in lactic acid and is partially oxidized in the citric acid cycle. The main importance in the regulation of anaerobic transformations of carbohydrates belongs to phosphofructokinase, which determines the rate of both glycogenolysis and glycolysis. Glycolytic oxidoreduction limits the rate of anaerobic digestion of carbohydrates and the possibility of partial glycolysis recovery at the stages that are catalyzed by hexokinase, phosphofructokinase and dehydrogenase phosphoglycerol aldehyde. In order to maintain a certain level of glycolysis, it is necessary, in addition to the presence of substrates of the reaction, the preservation of a certain level of ATP and NAD and its derivatives, as coenzymes. The beginning and regulation of glycogenolysis are carried out by phosphorylase of blood platelets [79].

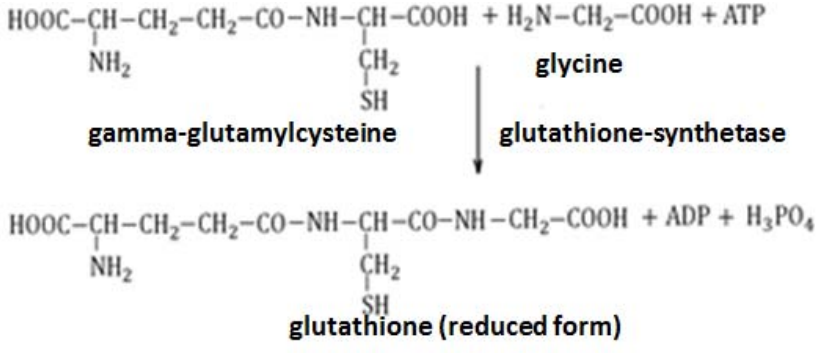
Blood platelets contain all enzymes throughout the pathway of glyconeogenesis, which carry out the synthesis of glycogen from pyruvate and citrate.

These include fructose-1,6-diphosphatase, which activates AMP and ADP and is inhibited by ATP and is a limiting factor in glycogenolysis at the physiological concentration of adenyl nucleotides [78].

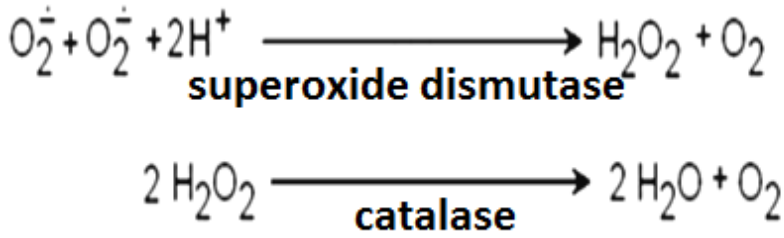
According to some authors, glycogen, and glucose, which are cleaved by blood platelets stoichiometrically, are converted into lactate, that is, the substrates of oxidation are not glycogen with glucose, and other compounds, in particular, fatty acids, which are intensively oxidized in the platelets. In the blood platelets, there is oxidative phosphorylation, which consumes a small amount of oxygen. The bulk of the breathing occurs regardless of the energy processes that occur in the blood platelets [77].

1.8.4. Mechanisms of reactive oxygen species (ROS) neutralization in erythrocytes

Molecular oxygen, under certain conditions, can be converted into active forms, which include superoxide anion O_2^-, hydrogen peroxide H_2O_2, hydroxyl radical OH · and singlet oxygen $^1O_2$. These forms of oxygen have high reactivity, can have a detrimental effect on the proteins and lipids of biological membranes, and cause cell destruction. The higher the content of O_2, the more its active forms are formed. Therefore, erythrocytes that constantly interact with the Ferrum contain effective antioxidant systems that can neutralize the reactive oxygen species. An important component of antioxidant systems is the tripeptide glutathione, which is formed in erythrocytes as a result of the interaction of γ-glutamylcysteine and glycine:



In erythrocytes are also enzymes superoxide dismutase and catalase, which carry out the following transformations:



Antioxidant systems have a special significance for erythrocytes since erythrocytes do not replenish proteins by synthesis.

CHAPTER 1.9
CHARACTERISTICS OF BLOOD PRESERVATION SYSTEM

Blood preservation is the creation of conditions for long-term maintenance of blood outside the body sterile, with the preservation of biological and functional properties of blood cells, as well as the physiological composition of the plasma.

Cannabis and its derivatives are widely used in various fields of medicine and their concentration can be determined in blood. The development and practical application of blood preservation systems are also actively taking place in veterinary medicine.

The theoretical basis for the development of methods for stabilizing donor blood is the theory of blood coagulation. The basics of this doctrine were developed by the Russian physiologist A.A. Schmidt in 1863-1864.

The process of haemo-coagulation is a complex "cascade" of reactions, which can only be stopped by removing or blocking the component in the chain of continuous enzyme reactions [4].

At one time, it was proposed to isolate five major groups of blood stabilizers, depending on which component of the haemo-coagulation system (ionized calcium, thrombokinase, prothrombin, thrombin, fibrinogen) directed its action. However, later it became clear that such a division is not entirely correct. It has been shown that a stabilizer, whose action is directed primarily to someone factor of the coagulation system, may also affect others. This property is clearly manifested in the conservation of canned blood [83].

For the general characteristics of blood preservation systems, the following classification was developed:

1 - defibrillation of blood;

2 - use of cationic components;

3 - blood mixing with an anticoagulant solution [43].

Defibrillation is carried out by mechanical methods (shaking blood with balls, mixing blades with blades), which is accompanied by traumatism of the formed

elements. Thrombocytes (with such manipulation) are completely destroyed, the quantity of erythrocytes decreases by 15%, and leukocytes - by 50%. The main reasons for the fact that there is no procurement of defibrillated blood is the cumbersome and complexity of the technological process in the processing of blood, as well as the fact that it is almost impossible to avoid traumatism of the formed elements and their significant loss when removing the fibrin clot [1].

More progressive, at one time, the method of blood stabilization was the method of adsorption of blood plasma ionized calcium by a cationic resin. External chemicals in the sorbent method of stabilization in the blood are not added. The passage of blood through a special column can remove up to 98% of ionized calcium. However, cationic blood rapidly loses its high transfusional properties and has become unusable for transfusion for up to 10 days. The disadvantages of this method of stabilization include low speed of blood filtration through resin filled columns; the regular complex procedure of regeneration of the sorbent; penetration from the resin into the blood of reactogenic impurities and fine particles of ionite, which increases up to 30% posttransfusion complications [16].

Most of the blood stabilization methods used in practice are based on mixing a preservative solution with blood [51, 69, 91].

By mechanism of action, stabilizers are conventionally divided into stabilizers-antithrombin and substances that form insoluble or complex compounds with ionized calcium. The group of antithrombin stabilizers includes heparin and synanthropic substance. The mechanism of stabilizing the action of heparin is quite complex. It is established that heparin itself is not active and is activated by a cofactor - antithrombin II, which is contained in the plasma. Under the action of heparin, the affinity of antithrombin to thrombin increases, and the inactivation of the latter. In addition to antithrombin, heparin also has anti-thromboplastin action, that is, activates the process of binding thrombin with fibrin clot. However, agglutination of platelets and mechanisms of fibrinolysis, on the contrary, are inhibited [16].

However, heparinized blood has a very significant disadvantage, which is a major obstacle to its widespread use. In a very short period of time, it loses its

qualitative properties and becomes unfit for further use and causes the condition of "artificial" hemophilia. By the mechanism of action on the system of coagulation of blood to heparin is close to a group of stabilizing substances, which are the product of hydrolysis of pre-sulfated cellulose. The compound of this class is synanthropic substance. When comparing the properties of heparin and synanthropic substance, the latter, as a stabilizer, has no advantages over heparin, respectively, all the disadvantages of heparinized blood are also characteristic of synanthropic substance blood [18].

Therefore, synanthropic substance, as a serial blood stabilizer, did not get a widespread incarnation in practice.

The group of stabilizing substances bind free calcium ions (factor IV of the blood coagulation system) and form insoluble or weakly dissociating complexes include citric acid and its salts. Numerous other drugs, including magnesium sulfate, sodium hyposulfite, sodium salts of malic, oxalic and tartaric acids, sodium phosphoric acid, are also able to inhibit blood clotting. However, they did not become practical agents in transfusiology. These substances were either less reliable stabilizers than citrates, or more toxic [33, 47].

Citric acid and its salts deserve special attention . At the beginning of the last century, researchers used sodium citrate as a blood stabilizer intended for transfusion. It later became clear that Ca^{2+} ions play an important role in almost all stages of blood coagulation: they are needed to form the active factor X (Stuart-Prawer factor) necessary for the synthesis of active thromboplastin and thrombin from prothrombin; take part in the activation of proconvertin; and permeability of platelet membranes [28].

Figure 1.25 shows the mechanism of binding of sodium with ionized calcium citrate (factor IV) and its conversion into non-ionized form.

P. Rous and J. Turner in 1916 suggested adding glucose or sucrose to citrate blood, which allowed a significant lengthening of the shelf life of such blood. Subsequently, the mechanism of action of glucose in glucose-citrate solutions was

disclosed [15, 35]. Sodium citrate and glucose are the basis of almost all modern hemocontaining solutions [40, 43].

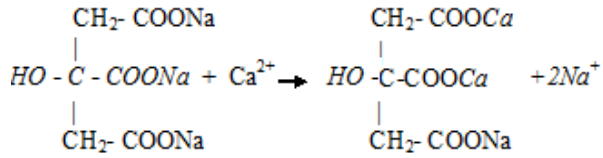


Fig. 1.25 The reaction of binding the ionized calcium with sodium citrate [27].

When mixed with a certain preservative solution and stored in a certain temperature mode, the blood can be stored for up to 21 days.

Terms of storage of canned donor blood are established on the basis of the use of red blood cells in the recipient's circulatory system.

Depending on the duration of preservation, blood is divided into:

1. Blood with a term from 1 to 5 days of storage ("blood of early terms"), is recommended in obstetrics, traumatology, surgery - 90% of life expectancy.
2. From 5 to 10 days - ("medium terms") - 80%.
3. From 10 to 21 days ("late terms") - 70-75%.

But despite the considerable practical achievements in the field of canning and blood transfusion, the universal system of hemoconstriction has not yet been developed. Analysis of literature shows that all methods of blood conservation are not devoid of some or other disadvantages. Therefore, the search of new and improving existing prescriptions for hemo-conserving solutions [65, 75, 84, 102, 103] and other biochemical investigations [105-108] is an urgent problem for scientists at present.Therefore, the search for new and improved existing formulations for haemo-conserving solutions [65, 75, 84, 102, 103] and other biochemical investigations [105-108] is an urgent task for scientists at present.

REFERENCES
(PART 1)

1. Антонова Е.В., Ротфельд Л.С. Изучение некоторых биохимических, физико-химичиских изменений крови, заготовленной без стабилизатора, в процессе ее хранения // Проблемы гематологии. 1958. – Т. 3, № 3. – С. 43-48.
2. Бабаева А.Г. Единство и противоположность цитогенетической активности лимфоцитов и их антителообразующей функции при восстановительных процессах в органах // Бюл. эксперим. биологии и медицины. – 1999. – Т.128, – №11. – С. 484-490.
3. Белоус А.М. Грищенко В.И. Криобиология – К.: Наукова думка, 1994. – 431с.
4. Березов Т.Т., Коровкин Б.Ф. Биологическая химия. М.: «Медицина» - 2004. – С. 704.
5. Ветеринарна клінічна біохімія / Левченко В.І., Влізло В.В., Кондрахін І.П. та ін. – Біла Церква., 2002. – 400с.
6. Влияние длительных сроков хранения миелоконсерванта на его физико-химические и биологические свойства / Ларичева Н.И., Когут Г.И., Волошина М.С., Харченко Н.К. // II Всесоюзный сезд гематологов и трансфузиологов: Тез.докл. – М.: Медицина, -1985. – С. 137.
7. Гарбарець Б.О., Висоцький І.Ю., Качанова А.А. Практикум з біологічної хімії. - Суми, 1997.
8. Гомоляка І.В., Тумасова К. П. Морфометрична характеристика нейтрофільних гранулоцитів крові // Вісник морфології. – 1999. – №5. – С. 20
9. Гонський Я.І. Біологічна хімія: лабораторний практикум.- Тернопіль: Укрмедкнига, 2001.
10. Гонський Я.І., Максимчук Г.П. Біохімія людини. – Тернопіль: Укрмедкнига, 2001. – С. 25-74.

11. Губський Ю.І. Біологічна хімія. – Київ; Тернопіль: Укрмедкнига, 2000. – С. 112-350.
12. Зак К.П. Большие гранулосодержащие лимфоциты (естественные клетки – киллеры) в патологии. – К.: Наук. думка, 1992. – 164с.
13. Зубаиров Д.М. Механизмы образования тромбина / Д.М. Зубаиров // Республиканский межведомственный сборник Биохимия животных и человека. Свертывание крови и фибринолиз. – 1982. – Вып. 6. – С. 3-14.
14. Кольман Я., Рем К.-Г. Наглядная биохимия. 3-е изд.: Пер. с нем. М.: Мир, 2009. – 469 с.
15. Кребс Г., Корнберг Г. Превращения энергии в живых системах. Пер. с англ. -М., Изд-во иностр. лит., 1959. – С. 3-24.
16. Кузьмин В.А. Сравнительная оценка методов и аппаратов, применяемых для переливания крови. Автореф. дисс. ... к.м.н. –Ярославль, 1969. – С. 5-10.
17. Кушманова О.Д., Ивченко Г.М. Руководство к лабораторным занятиям по биологической химии.- М.: Медицина,1983.– С. 42-90.
18. Лаврик С.С., Глухенькая Г.Т. Консервирование гемопоэтической ткани при умеренно низкой температуре. – Киев, 1983. – 16 с.
19. Левчин А.М. Калікреїн-кінінова система – універсальний компонент біохімічного гомеостазу // Перспективи медицини та біології. Т. 5, №1, 2013. – С. 14-18.
20. Ленинджер А. Основы биохимии. – М. МИР, 1965.– С. 30-362.
21. Луганова И.С., Сейц И.Ф. Дыхание, гликолиз и сопряженное фосфорилирование в лейкоцитах // Докл. АН СССР. –1957, № 6. –С. 1082.
22. Луганова И.С., Сейц И.Ф. Дыхание, гликолиз и сопряженное фосфорилирование в лимфоцитах человека // Вопр. мед.химии. – 1959. – В. 4. – С. 285.
23. Марри Р., Греннер Д., Мейс П., Родуэлл В. Биохимия человека – М.: Мир, 2009. – Т. 1. – 381 с.

24. Марри Р., Греннер Д., Мейс П., Родуэлл В. Биохимия человека – М.: Мир, 2009. – Т. 2. –414 с.
25. Маркосян А.А. Физиология тромбоцитов. – Л., 1970. – С. 4-35.
26. Мак-Мюрей У. Обмен веществ у человека. – М.: МИР, 1980.– С. 15-175.
27. Маршалл В. Дж. Клиническая биохимия. – М.: БИНОМ, 1999.– С. 35-250.
28. Мислицький В.Ф., Подоляк С.К. Патологічні зміни тромбоцитарно – судинного та коагуляційного гемостазу під впливом хлористого свинцю та їх корекція за допомогою синтетичного аналога простацикліну // Фізіол. журнал – 1999. – Т. 45, №4. – С. 99-104.
29. Мусил Я. Основы биохимии патологических процессов. – М.: Медицина, 1985.– С. 112-305.
30. Николаев А.Я. Биологическая химия. – М.: «Высшая школа», 1989.– С. 10-86.
31. Посібник до практичних занять з біологічної хімії /за редакцією Я.І. Гонського. - Тернопіль: Навчальна література для медичних вузів, 1993.– С. 75-86.
32. Проценко В.А. Тканевые базофилы и базофильные гранолуциты крови. - М.: Медицина, 1987. – 128 с.
33. Рубинштейн Д.Л. Классификация стабилизаторов крови. – В сб.: Современные проблемы гематологии и переливания крови. – М., 1946. – В. 22–23. – С. 21-32.
34. Савицкий И.В. Биологическая химия. – К.: «Вища школа», 1982.– С. 65-340.
35. Северин С.Е. Биохимические основы благоприятного действия глюкозы при консервировании крови. // Биохимия. – 1946. – В. 11, № 2. – С. 139-148.
36. Современные представления о системе гемостаза / [Волков Г.Л. [и др.]. – К.: «Наукова думка», 2005. – 295 с.

37. Стародуб Н.В., Назаренко В. И. Гетерогенная система гемоглобина: структура, свойства, синтез, биологическая роль. – К.: Наук. думка, 1987. – 200с.
38. Строев Е.А. Биологическая химия. – М.: «Высшая школа», 1986.– С.125-234.
39. Структура и функции эритроцитов в норме и патологии / Под.ред.М. И. Лосевой. – Новосибирск: НГМИ, 1988. – 102 с.
40. Типовий технологічний регламент виготовлення розчину «Глюгіцир» для консервування донорської крові. – К.: 1997. – 44с.
41. Тюкавкина Н. А., Бауков Ю. Н. Биоорганическая химия. – М.: «Медицина», 1991.– С. 260-296.
42. Федоров Н.А. Нормальное кровотворение и его регуляция. М.: «Медицина» – 1976. – С. 543.
43. Филатов А.Н. Руководство по применению крови и кровезаменителей. – Л.: «Медицина», 1978. – С. 35-42.
44. Черняк Н.Б. Окислительное и гликолитическое фосфорилирование в лейкоцитах // Докл. АН СССР. – 1958, № 5. – С. 1004.
45. Хмелевский Ю.В., Усатенко С.К. Основные биохимические константы человека в норме и при патологии. – К.: Здоров’я, 1987.– С. 91-205.
46. Черняк Н.Б. Фосфорные фракции эритроцитов и плазмы и их изменения при хранении консервированной крови // Биохимия. –1948. –Т. 13. –С. 421.
47. Шредер В.Н. Вопросы консервации крови доноров на основе изучения физико-химических и биологических свойств эритроцитов. // В кн.: Труды ин-та цитол., гистол., эмбриолог. – 1948. Т.2.–В.2.–С. 169-175.
48. Энельгардт В.А., Саков Н.Е. О механизме пастеровского эффекта // Биохимия. – 1943. – Т. 8. – С. 9.
49. A kinetic model of the hexokinase of human erythrocytes and its application to the cell / Gerber G., Preissler R., Hemrich R. [et. al.] // In: 7-th

International Berlin symposium on structure and function of erythrocytes.– Berlin. – 1973. – P. 20.

50. Absolute rates of globin chain synthesis in thalassemia / Bank A., Braverman A. S., O'Donnel J. V. [et. al.]// Blood, 1988. – Vol. 31. – P. 226.

51. Anti – HIV screening of blood donors / Cavalli P., Tanzi E., Villa D. et al. // Vox Sanguis. – 1994. – 67 (4). – P. 404.

52. Antonini E., Brunori M. Hemoglobin and myoglobin in their reactions with ligands. – Amsterdam. – 1971. – P. 14-32.

53. Baggiolini M. The enzymes of the granulocytes of polymorphonuclear leukocytes and their functions // Enzyme. – 1972. – Vol.13. – P. 132.

54. Benesch R., Benesch R. E. The effect of organic phosphate from the human erythrocyte on the allosteric properties of hemoglobin // Biochem. biophys. Res. Commun.– 1967. – Vol. 26. – P. 162.

55. Benesch R.E., Benesch R. Interaction of red cell organic phoshates with hemoglobin // Forsvar med. – 1969. – Vol. 5. – P. 154.

56. Benzakour O. Vitamin K-dependent proteins: Functions in blood coagulation and beyond / O. Benzakour // Thromb. Haemost. - 2008.- Vol. 100. - P. 527-529.

57. Berg J.M., Tymoczko J.L., Stryer L. Biochemistry. – New York: W H Freeman; 2002. 1515 p.

58. Bick M. The biochemical changes occurring during the storage of human blood // Austr. J. Exp. Biol. Med. – 1939. – Vol. 17. – 321-331.

59. Chanutin A., Curnish R. Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes // Arch. Biochem. – 1987. – Vol. 121. – P. 96.

60. Chernyak N.B., Timofeyeva L. M. Intermediate stages in anaerobic breakdown of carbohydrates in human thrombocytes. –In: Mezabolism and Membrane permeability of erythrocytes and thrombocytes. – Stuttgart. – 1968. – P. 239.

61. Danciger J. Vitamin K-dependent Proteins, Warfarin, and Vascular Calcification / J. Danciger // Clin. J. Am. Soc. Nephrol. - 2008. – Vol. 3. – P. 1504-1510.
62. Davie E.W. The coagulation cascade: initiation maintenamse, and regulation / E.W. Davie, K. Fujikawa, W. Kisiel // Biochemistry. – 1991. – Vol. 30, N 43. – P. 10363-10366.
63. Detwiller T.C., Zlvcovic E. Control of energy metabolism in platelets. A comparison of aerobic and anaerobic metabolism in washed rat platelets // Biochim.biophys. Acta. – 1970. – Vol. 197. – P. 117.
64. Doery J.C., H'irsh G., Cooper I. Energy metabolism in human platelets: interrelationship between glycolysis and oxidative metabolism // Blood. – 1980. Vol. 36. – P. 159.
65. Follow – up study of antihepatitis C virus antibodies in blood donors implicated in post – transfusion non – A, non – B hepatitis / Mazda T., Nakata K., Bannai M. [et al.] // Transfusion Medicine. – 1993. – 3 (2). – P. 149-151.
66. Gailani D. Intrinsic pathway of coagulation and arterial thrombosis / D. Gailani, Th. Renne // Arterioscler. Thromb. Vasc. Biol. –2007. – Vol. 27. – P. 2507-2513.
67. Gibson J.G. Approsches to redcellpreservation in the liquid state. In: The red blood cell. A comprehensive treatise. N. Y. – Lond.. Acad. Press. – 1984. – P. 477-489.
68. Gierer A. Function of aggregated reticulocyte ribosomes in protein synthesis // J. Mol. Biol. – 1963. – Vol. 6. – P. 148.
69. Goodnough L.T., Pespotis G. J. Establishing practice guidelines for surgical blood management // American J. of Sungry. – 1995. –170 (6A). – P. 154-175.
70. Green Colin Mammalian hibernation: Lessons for organ preservation: Pap. “Copin with cold: Natural Society for Low” Temperature Biology Symposium and Annual General Meeting, Cambridge, 17 Sept., 1999 // Crio-Lett. – 2000. –Vol. 21, №2. P. 91-98.

71. Greer J. Three-dimensional structure of haemoglobin Kansas and haemoglobin Richmond. // J. Mol. Biol. – 1971. – Vol. 59. – P. 99.
72. Harrington J.P, Hicks R.J. Spectral analysis of Fe (III) – complex reduction by hemoglobin: possible mechanisms of interaction // International J. of Biochemistry. –1994. – 26 (9). – P. 1111-1117.
73. Hedner U. Factor VIIa and its potential therapeutic use in bleeding-associated pathologies / U. Hedner // Thromb. Haemost. – 2008. – Vol. 100. – P. 557-562.
74. Hirsh J. Guide to anticoagulant therapy. Part 2: Oral anticoagulants. American Heart Association / J. Hirsh, V. Fuster // Circulation. – 1994. – Vol. 89. – P. 1469-1480.
75. Hepatitis C virus and transfusion: policy regarding the donation and the donor. Groupe Hepatites Virales de la Societe Nationale de Transfusion Sang sine / Moncharmont P., Jand C., Boudart D. [et al.] // Revue Francaise de Transfusion et de Hemobiologre. – 1992. –32 (3). – P. 205-210.
76. Hoffman B.M., Petering D.H. Cologlobins. Oxygencarrying cobaltreconstituted hemoglobin and myoglobin // Proc. nat. Acad. Sci. USA. – 1970. – Vol. 67. – P. 637.
77. (Identification of residues responsible for the alkaline Bohr effect in haemoglobin / Perutz M., Muirhead H., Mazzarella L. [et. al.] // Nature. – 1969. – Vol. 222. – P. 1240.
78. Karpatkin S., Charmatz A., Langer R.M. Glycogenesis and glyconeogenesis in human platelets. Incorporation of glucose, pyruvate and citrate into platelet glycogen: glycogen synthetase and fructose –l,6-diphosphatase activity // J. clin. Invest. – 1970. Vol. 49. – P. 140.
79. Karpatkin S., Langer R.M. Human platelet phosphorylase // Biochim. biophys. Acta. – 1969. – Vol. 185. – P. 350.
80. Kilmartin J., Rossi-Bernardi L. The binding of carbon dioxide by hose haemoglobin // Biochem. J. – 1991. – Vol. 124. – P. 31.
81. Lehmann H., Carrell R.W. Abnormal haemoglobin Philly // Brit. med. Bull. – 1969. – Vol. 25. – P. 14.

82. Lijnen H.R. Elements of the fibrinolytic system / H.R. Lijnen // Ann. N.Y. Acad. Sci. – 2001. – 936. – P. 226-2369.
83. Limbird T.J., Silver D. Sequential changes in blood preserved with citrate-phosphate-dextrose. Surg., Gynecol., Obstet. – 1974. – Vol. 138. – P. 401-405.
84. Linden J.V., Kaplan H.S. Transfusion errors causes and effects // Transfusion Medicine Reviews. – 1994. – 8 (3). – P. 169-183.
85. Mackman N. The role of tissue factor and factor VIIa in hemostasis / N. Mackman // Anesth. Analg. – 2009. – Vol. 108, N 5. – P. 1447-1452.
86. Maretzki D., Rapoport S. Glyzerinaldehyd-3-phosphatedehydro-genase aus Erythrozyten des Menschen. I. Isolierung und einige Eigenschaften // Acta biol. med. germ. – 1972. – Vol. 2. – P. 207.
87. Metabolism of macrophages / Karnovsky M.L., Simmons S., Glass E.A., Shaferm A.W., Darey Hart P. // In: Mononuclear phagocytes. Ed. Furth R. Philadelphia. – 1980. – P. 103.
88. Murphy J.R. Erythrocyte metabolism. I. The equilibration of glucose-C^{14} between serum and erythrocytes // J. Lab. clin. med. – 1960. – 55. – P. 281.
89. Nelson D.L., Cox M.M. Lehninger Principles of Biochemistry (Fourth Edition) – W. H. Freeman Publishers. 2004. –1124p.
90. Pauling L. Nature of the iron-oxygen bond in oxyhaemo-globin // Nature. – 1984. – Vol. 203. – P. 182.
91. Practical guidelines for process validation and process control of white cell – reduced blood components: report of the Biomedical Excellence for Safer Transfusion (BEST) Working Party of the International Society of Blood Transfusion (YSBT) / Dumont L.J., Dzik W.H., Rebulla P., Brandwein H. // Transfusion. – 1996. – 36 (1). – P. 11–20.
92. Rapoport S., Guest G. Distribution of acidsoluble phosphorus in the blood cells of various vertebrates // J. Biol. Chem. – 1941. - Vol. 138. – P. 268.

93. Rapoport S., Jacobasch G. Uber Regulation des Energiest off wechsels des Erythrocyten – In: Stoffwechsel und Membranpermeabilitat von Erythrocyten und Thrombocyten. – Stuttgart. – 1968. – 11 p.
94. Recombinant hemoglobin A produced in transgenic swine: structural equivalence with human hemoglobin / Rao M.J., Schneider K., Chait B.T. [et al.] // Artificial Cells, Blood Substitutes, & Immobilization Biotechnology. – 1994. – 22 (3). – P. 695-700.
95. Serbing E.D., Steinhardt J. Stabilization globin of bourse hemoglobin by protoporphirin IX and hemine // J. Biol. Chem. – 1970. – Vol. 245. – P. 5395.
96. Site – directed mutagenesis in hemoglobin: functional and structural study of the intersubunit hydrogen bond of threonine – 38 (C3) alpha at thealpha 1 – beta 2 interface in human hemoglobin / Hashimoto M., Ishimori K., Imari K. [et al.] // Biochemistry. – 1993. – 32 (49). – P. 1368-1369.
97. Steinberg A., Marks P.A. Substances stimulating glucose catabolism by the oxidative reactions of the pentose phosphate pathway in human erythrocytes // J. clin. Invest. – 1961. – Vol. 40. – P. 914.
98. Stjernholm R.L., Manak R.C. Regulation of pentose cycle activity and glycogen metabolism during phagocytosis // J. Reticuloendoth. Soc. – 1970. – Vol. 8. – P. 550.
99. Structure/function analysis factor XII using recombinant deletion mutants. Evidence for an additional region involved in the binding to negatively charged surfaces / F. Citarella [et. al.] // Eur. J. Biochem. – 1996.– Vol. 238, N 1.– P. 240-249.
100. The role of tissue factor in thrombosis and hemostasis / M.A. Malý [et. al.]// Physiol. Res. – 2007. – Vol. 56. – P. 685-695.
101. The regulation of clotting factors / M. Kalatalis [et. al.] // Eucaryotic gene expression.– 1997.– Vol. 7, N 3.– P. 241-280.
102. Transfusion medicine monitoring practices. A study of the College of American Pathologists. Centers for Disease Control and Prevention Outcomes

Working Group / Boone D., Steindel S.D., Herron R. [et al.] // Archives of Pathology Laboratory Medicine – 1995. –119 (II). – P. 999-1006.

103. Udem L., Ranney H., Bunn H.F. Some properties of Ub M Milwaukee // J. mol. Biol. – 1970. – Vol. 48. – P. 489.

104. Wakefield Th.W. Mechanisms of venous thrombosis and resolution / Th.W. Wakefield, D.D. Myers, P.K. Henke // Arterioscler. Thromb. Vasc. Biol. – 2008. – Vol. 28. – P. 387-391.

105. Пехименко Г. В., Кучмеровская Т. М. Особенности вторичной структуры сывороточного альбумина у некоторых представителей живых организмов // УБЖ . – 2011. – 83, № 3. – С. 65-75.

106. Гурина Н. М., Кучмеровская Т. М. Иммуносорбенты для лечения экспериментального аллергического энцефаломиелита у морских свинок // Біотехнологія. – 2012. – 5, № 5. – С. 45-53.

107. Гузик М.М., Дякун К.О., Яніцька Л.В., Кучмеровська Т.М. Вплив інгібіторів полі(ADP-рибозо)полімерази на деякі показники оксидативного стресу у лейкоцитах крові щурів за експериментального цукрового діабету // Укр. біохім. журн.– 2013. – 85, № 1. – С. 62 – 70.

108. Привроцька І. Б., Кучмеровська Т. М. Окислювальний стрес у лейкоцитах крові, про/антиоксидантний статус та жирнокислотний склад ліпідів підшлункової залози за експериментального гострого панкреатиту в щурів // УБЖ. – 2013. Т. 85, № 5. – С. 124–136.

109. Mosesson M.W. Fibrinogen and fibrin structure and functions. // J Thromb Haemost 2005. – Vol.3. P. 1894–1904.

PART 2
METHODICAL FUNDAMENTALS OF STUDYING THE METABOLIC PROCESSES IN HUMAN AND ANIMALS USING BLOOD INDICATORS

CHAPTER 2.1
THE USE OF BIOCHEMICAL ANALYZERS IN HUMAN AND VETERINARY MEDICINE

A biochemical blood test is a method of laboratory diagnosis that may be used for evaluation of the metabolism and the functioning of the internal organs of the animal and human body.

The biochemical blood test is performed using a biochemical analyzer [3, 27, 61]. The manufacturers of the CIS medical equipment market offer the following biochemical analyzers:

- spectrophotometers;
- semiautomatic biochemical analyzers;
- automatic biochemical analyzers.

The principle of a biochemical analyzer is based on a turbidimetric method: the measurement of the intensity of light in the analyzed suspension.

There are exist two types of spectrophotometers: single and multichannel. The principle of spectrophotometric devices is to register the magnitude of the optical density in the studied sample, with the following mathematical calculations of obtained data.

Laboratory assistant or operator does manually all preparatory operations: preparation of reagents, mixing and making samples, setting queues for tests, etc. The difference in spectrophotometers is the presence or absence of service functions, such as automatic sample calculation, display of results on the display or on a printed tape, setting queues, etc.

The semiautomatic biochemical analyzer is a system where only certain stages of clinical research are carried out - dosage of samples and reagents, measurement of

optical density. The device carries the study itself, only issuing requests for the addition of the next sample. The calculation of results in semiautomatic biochemical analyzers is carried out by the algorithm predefined by the operator and in predetermined units, after which the information, also, in automatic mode, is given on the display of the device.

An automatic biochemical analyzer is a system where the test sample moves along a conveyor system aboard automated devices that perform a certain stage of the analysis, being parts of a single analytical system. The laboratory assistant controls the process of test programming and establishes the sequence of determination of certain parameters. All other operations are carried out in completely automatic mode.

Biochemical analyzers are widely used in veterinary clinics and veterinary laboratories in Kyiv.

Semiautomatic analyzers *Stat Fax 1904+R* (fig. 2.1) and *Rayto RT-9200* (fig. 2.2). are used in the veterinary clinic “ZooLux”.

Fig. 2.1. Semiautomatic analyzer *Stat Fax 1904+R* [79].

The veterinary clinic "VetPro" uses a semi-automatic analyzer RaytoRT-1904 C (fig. 2.3).

The veterinary laboratory «BioSoft» uses the BioSystems A15 automatic analyzer (fig. 2.4).

Consequently, this suggests that the choice of a particular institution depends on the qualifications of the staff, the number of samples per day (the productivity of

the device), the list of tests to be carried out, the rules of reaction (methods of calculation at the endpoint, kinetics).

2.1.1. Biochemical analyzer Stat Fax® +1904 + R

This compact analyzer, which is controlled by a microprocessor, is a general purpose photometer with the possibility of two-wave measurement, with six filters and a temperature of incubation of 37 ° C (fig. 2.1) [79].

The device uses standard 12 mm - round vial. In accordance with the description, the analyzer can be used to measure optical density, calibration concentration or measurements of process velocity (kinetic characteristics).

The device is designed to measure and calculate results at the endpoint and using kinetic colorimetric methods. With this instrument, any test based on measuring the optical density can be performed on one of the filters with the corresponding or close wavelength. In addition, it is possible to enter data into the calculation formulas in existing programs for performing laboratory tests. These programs include the calculation of reaction velocity and single- or multipoint calibration. With the alphanumeric display, the device informs the operator that it is necessary to measure the sample rate in the corresponding test tube. After the measurement, it is carried out necessary calculation and the issuance of test results on the printer.

In addition to measuring on one of six filters, an operator can choose the wavelength for two-wave measurement in any operating mode. This corrects possible interference in test tubes and turbulence effects.

Each mode is independent to reduce errors and simplicity of management. In all modes, the device is nullified by air (absence of any solutions), the determination of the indicators is first measured by the blank solutions, then the experimental and finally automatically results are printed.

In Optical Density (Absorption) mode, the device measures and prints the absorption difference on user-selected filters. In the calculation mode by a factor (Factor) the concentration is determined by multiplying the optical density by the factor introduced by the user (coefficient). In the calculation mode for one standard

(Standard), the concentration calculation is performed according to the Beer law on the basis of measuring one standard (calibrator).

In multipoint calibration mode % absorption (Multipoint % ABS), the optical density of the sample is represented as a percentage of the first standard on the calibration curve. In multipoint calibration, the concentration calculation is processed according to Beer's law based on discrete-linear calibration according to several standards (up to 7). The use of an individual blank sample (blank) is available in all modes, including the measurement of optical density (Absorption) and kinetics (Relative). In a kinetic mode, the average change in absorbance per minute is measured, which is then multiplied by the factor (coefficient) entered by the operator, or calculated from a previously measured standard with known activity. Intermediate measurements of the optical density of the kinetic method can be printed with a built-in graphic printer to confirm the linearity of the reaction. The kinetic mode includes the option "Batch mode", with the possibility of simultaneous execution of several kinetic tests with conveyor sampling.

The device includes non-volatile memory that ensures the preservation of test parameters and calibration curve, and a quick test "call" on demand. The names of the 30 most commonly used techniques are written in the first 30 positions, which allow the users to program their tests under appropriate names. In addition, other tests can be stored in memory under the numbers in the "User menu" (and named by keyboard) with a total amount up to 69. The “Stat Fax® +1904 + R” also contains an incubation block (37 ° C) for 12 positions. The incubator is adapted to 12 mm round test tubes. A single, self-controlled incubation system is built into the measuring cell to maintain temperature for measurements in accordance with the methods in which it is required to incubate sample at 37 ° C. This is necessary to accurately determine the reaction rate.

Thus, Stat Fax® +1904 + R is designed to provide fast, accurate and reproducible results; no maintenance required, easy to operate, flexible and economical and has stable calibrated factors, reliable design, and lamp retention mode provides good performance.

2.1.2. *Rayto RT-9200*

This is a desktop semiautomatic biochemical analyzer for work in clinical laboratories with a small flow of patients (fig. 2.2). An analyzer is an open system that is suitable for working with biomaterials and reagents from any manufacturer [83].

Fig. 2.2. Semiautomatic analyzer Rayto RT-9200 [83].

Key specifications of Rayto RT-9200:

• Convenient keyboard, LCD display;

• Light filters from 300 to 800 nm, 5 standard filters, 3 additional filters (optional);

• The open type reagents system supports the cuvette (closed system under the order);

• Calculation modes: kinetics, two-point, bichromatic endpoint with or without blank reagent (blank) and blank sample form (control sample), linear/non-linear calibration;

• A large amount of memory - the ability to store up to 60 programs and 2200 research results;

• Quality control: 2 tests for the test;

• Built-in thermal printer;

• Compact design and easy to use;

• Multilingual software (on request).

Consequently, Rayto RT-9200 is mainly used in small clinics or for research purposes.

2.1.3. *Rayto RT-1904C*

It is an open type biochemical semiautomatic analyzer, which is a compact, compact microprocessor controlled photometric system, which allows conduct research on clinical biochemical parameters of blood, serum, urine, cerebrospinal fluid (fig. 2.3) [82].

Fig. 2.3. Semiautomatic biochemical analyzer Rayto RT-1904 C [82].

The Biochemical Semiautomatic analyzer Rayto RT-1904 C can be used to measure absorbance or concentration based on standard points or change of reaction velocity. Measurement on this device is carried out in a flow cuvette with a minimum volume of a sample of 200 μl. Rayto RT-1904 C is a completely open system that allows using any reagents.

Based on the manufacturer data [82], the following features of the semiautomatic biochemical analyzer Rayto RT-1904 C can be defined:

• Bichromatic optical system with 7 internal light filters 340, 405, 500, 540, 578, 620, 670 nm;

• The possibility of installing an additional the eighth filter with a wavelength of 450 nm (with a minimum sample size allows to carry out immuno-enzymatic techniques - hormones, infections);

• Possibility to use both single and two filters (main and differential) mono- and bichromatic modes;

• The preprogrammed calculation modes, both by the endpoint and by the change in the velocity of the reaction process;

• The following calculation modes: optical density measurement; measurement of concentration using the standard solution; concentration measurement using the conversion factor of optical density to concentration; multi-standard programmable mode; percentage absorption measurement; measurement in the dynamic mode of the reaction rate (kinetics mode);

• Thermostatic measuring cuvette with possibility of temperature change;

• Built-in printer;

• Full user support, including hints and messages on the display and warning sounds;

• The storing in memory up to 160 user tests with the possibility of their editing, deletion and input, as well as 500 test results;

• The result is given in the measurement units selected by the operator (25 units of measurement);

• Warning and error messages;

• Lamp self-preservation function;

• Availability of a clock and a calendar;

• Possibility to connect to a personal computer or to an additional printer.

Hence, due to a wide range of possibilities for measuring biochemical parameters, this device is used in both clinical and scientific-educational laboratories.

2.1.4. Open type automatic analyzer Random Access *A-15*

This automatic analyzer was created as a device that can help young beginner laboratories to avoid the use of semiautomatic photometers for analysis (fig. 2.4).

The Random AccessA-15 automatic analyzer allows you to become the automation of research, helping the lab to evolve and progress with greater efficiency by minimizing the quantity of routine processes and reducing the impact of the human factor. BioSystems has saved all advantages of the predecessor model A-25, reducing the cost of its maintenance, and most importantly - the price [84].

Fig. 2.4. Biochemistry Analyzer *Random Access A15* [84].

According to the "BioSystems" manufacturer data [84], the Random Access A15 biochemical analyzer has the following technical characteristics:

• a bandwidth of 150 tests in 1 hour;

• the possibility of conducting biochemical and turbidimetric analyzes;

• 4 independent stand positions for samples and reagents;

• 24 positions for samples in a tripod for samples (where the maximum quantity of samples is 72);

• 10 positions in a reagent stand (where the maximum quantity of reagents is 30);

• Possibility to install 20 and 50 ml bottle of reagents;

• the possibility of using samples for both primary and pediatric tubes;

• unlimited download of urgent samples;

• 3 fixed positions for flushing solutions or additional reagents;

• 24 positions in a tripod for samples, where the maximum number of samples is 72;

• high-precision dosage (deviation of 2% at a volume of 3 ml);

• low "dead volumes" of the reagent and sample;

• collision avoidance system;

• automatic adjustment of the dosing needle position;

• the possibility of using 5 types of specimens (serum, plasma, urine, cerebrospinal fluid, whole blood);

• reading time up to 15 minutes;

• multiplex methacrylic rotor;

• minimum reading capacity - 200 μl;

• the measuring range from 0.05 to 2.5 units of optical density;

• spectral range from 340 to 900 nm;

• basic interference filters 340, 405, 505, 535, 560, 600, 635, 670 nm.

Therefore, *Random Access A15* analyzer - very effective and easy to use provided sufficient funding of the appropriate laboratories.

2.1.5. Biochemical express analyzer FUJI DRI-CHEM 7000i

FUJI DRI-CHEM 7000i (FUJIFILM, Japan) is an innovative multifunctional high-performance automatic biochemical express analyzer on the slide-technology of “dry chemistry”, that defines 29 parameters (26 colorimetric tests and 3 electrolyte tests) with wide functional capabilities and fast results (fig. 2.5) [87].

Fig. 2.5. Biochemical express analyzer FUJI DRI-CHEM 7000i (FUJIFILM, Japan) [87].

The advantages of this compact high-performance (190 tests/hour) express analyzer on the slide-technology of "dry chemistry" are: automation of work for 5 patients; simple procedure consisting of 3 steps; convenient calibration with a

magnetic QC card; automatic breeding function; built-in function - electrolytes measuring.

The basic procedure on analyzer consists of three stages. Work on this device is completely automated for 5 samples, from pressing the "START" button, till printing the data. There is no need to enter the test program in advance, since the barcode on the back of the slide is automatically read, recognizing the parameter being analyzed (fig. 2.6).

There are three stages of the basic procedure on the FUJI DRI-CHEM 7000i analyzer:

1) installation of the slide;

2) installation of samples;

3) pressing the START button, for example, "The function of electrolytes measuring".

Fig. 2.6. Three stages of the basic procedure on the FUJI DRI-CHEM 7000i (FUJIFILM, Japan) [87] analyzer.

The multifunctional analyzer determines not only traditional biochemical parameters but also electrolytes. Determination of three electrolytes (Na, K, Cl) is carried out in 1 minute per slide.

According to the manufacturer's data [87], the measurement menu provides 26 colorimetric tests and 3 electrolyte tests. Due to the convenient device calibration using QC-cards, it is not necessary to do it every day (fig. 2.7).

The calibration for each batch of samples can easily be done by reading a QC card for each batch of slides. The device stores information on the last two batches of slides for each parameter in memory. Batch and slide type recognition is performed automatically. On the FUJI DRI-CHEM 7000i analyzer is provided the "Automatic

Dilution Function", i.e. labor-intensive dilution procedure is automated. The dilution of the investigated solutions is carried out on the panel in an automatic mode according to the established dilution factor. It is only necessary to establish mixing cups and solvent vial and press the "Dilution" button.

Fig. 2.7. An example of calibration and QC-card system on the FUJI DRI-CHEM 7000i (FUJIFILM, Japan) [87].

Measurement of C-reactive protein using this analyzer is presented in fig. 2.8. Calibration for the determination of C-reactive protein consists of measuring three calibration solutions and is carried out in an automatic mode: it is necessary to install the slides in the holder and the vials with calibrators in a special holder. High performance in the FUJI DRI-CHEM 7000i Analyzer is realized in capacity to install 5 samples at a time, that ensures high performance and efficiency in clinical practice.

Fig. 2.8. Example of measurement of C-reactive protein on the FUJI DRI-CHEM 7000i [87].

Exceptional stability of the slides is achieved through the application of chemical technology developed by FUJIFILM (Japan). The device is characterized by the excellent reproducibility of the received data and the absence of differences in the results of measurements of various operators.

In addition, the FUJI DRI-CHEM 7000i analyzer has the "Instant Test" function, that allows interrupting the current analysis at any time for immediate measurement. Thus, the FUJI DRI-CHEM 7000i analyzer is used in large clinical centers with a strong research base.

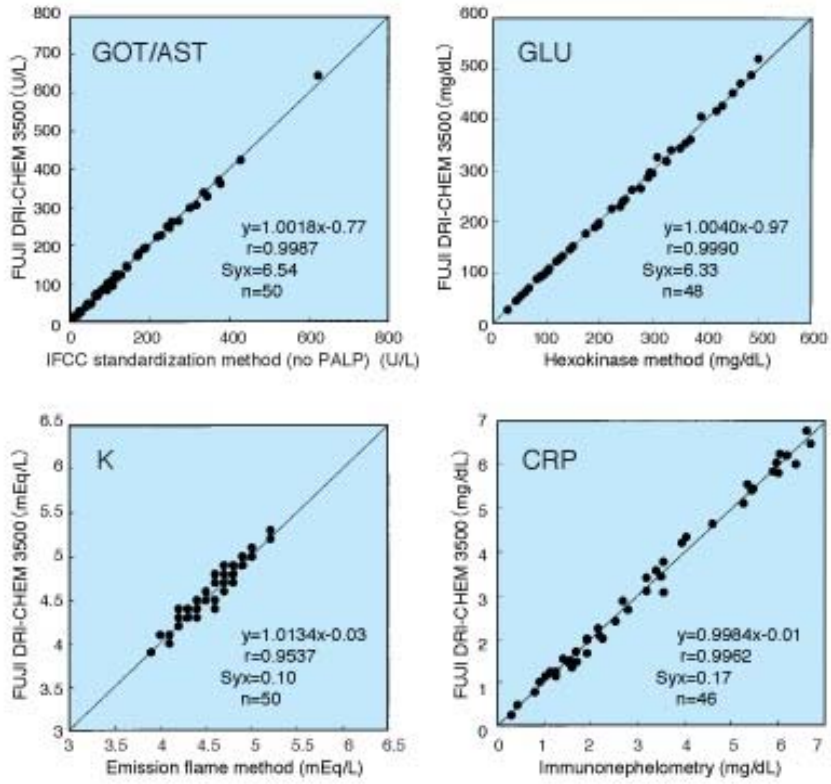


Fig. 2.9. High reliability of analyses performed on the FUJI DRI-CHEM 7000i [87].

2.1.6. Automated biochemical analyzer MINDRAY BS-120

This device (fig. 2.10, tab. 2.1) for the automatic determination of various indicators of biochemical analysis with high accuracy and reproducibility. This device carries out a wide range of measurements with low cost, saving reagent and labor resources. Detailed information about analyzer technical characteristics is given in tab. 2.1.

Table. 2.1

Technical Specifications of MINDRAY BS-120 [86]

Name (1)	Indicators (2)
Type of machine	Automatic biochemical analyzer
System type	open system
Analyzed liquids	serum, plasma, urine, cerebrospinal fluid
Productivity	100 tests per 1 hour (300 tests per 1 hour in determining ion-selective block)
The measurement principle	absorption photometry and turbidimetry
Types of performed tests	endpoint, kinetics, two-point kinetics, differential mode ("blind" sample on serum), monochromatic, bichromatic
Methods of calculation	Single-, double- and multi-point calibration four-point *Log-Logit,* five-point *Log-Logit,* five-point exponential, five-point polynomial, parabolic from 3 points, cubic spline (from 2 to 6 calibrators)
Types of sample containers	primary tubes, microcaps
Sample volume	from 3 to 45 µl, with step of 0,5 µl
Number of sample positions	8 positions
Dilution of samples	automatic dilution: from 2 to 150 times
Barcode "Reader"	built-in (available optionally)
Consumption of reagents	from 30 to 450 µL with step of 1 µL
Number of reagent positions	28 positions
Continued tab 2.1	
1	*2*
Probe sampling and reagents	Has integrated detector of liquid level,

	breakdown protection and previous warming of reagents in the probe manipulator
Probe cleaning	automatic internal and external rinsing, residual volume - less than 0,1%
Number of cells in the reaction disk	40 cells (8 segments by 5 cells)
The volume of the reaction mixture	180-500 µL
Reaction temperature	$37 \pm 0{,}1^{o}C$
Number of optical filters	8
Wavelengths	340, 405, 450, 510, 546, 578, 630, 670 nm
Measurement range	from 0.100 to 4.000 *ABS*
Measurement accuracy	0.0001 *ABS*
Urgent tests	are processed without interruption of the planned sample analysis
Water for washing	Deionized
Water consumption	2,5 L/hour
Operating System	*From Windows* 2000
Interface	RS-232
Size of the device	690 mm x 570 mm x 595 mm
Weight of the device	75 kg

There should be distinguished following characteristics of the device (that allows to determine the biochemical parameters at low cost): discrete random access, prioritization of urgent tests, 100 tests per hour (photometry); 28 reagents in a tripod of the device and 8 samples in one carousel; 40 reactionary cuvettes in a carousel (8 segments by 5 cells); minimum volume of reaction mixture - 180 µl; refrigerator

reagents on the panel; automatic cleaning of the sampler from the inside and the outside,

Fig. 2.10. Automated biochemical analyzer MINDRAY BS-120 [86].

automatic detection of the liquid level, protection against damage in the vertical movement; automatic dilution of the pathological specimen (pre- and post-, up to 150-fold); Russified control program; 8 light filters: 340, 405, 450, 510, 546, 578, 630, 670 nm; consumption of deionized water - 2.5 L/h.

Consequently, this device is used in powerful research centers and biochemical laboratories.

2.1.7. “CardioChek” Cholesterol blood analyzer

CardioChek, as well as CardioChek PA, are portable devices for a biochemical rapid blood test that allows a therapist to carry out a quick blood test in a real-time mode for several parameters (fig. 2.11). The Device CardioChek PA is included in the list of devices recommended for use in RF Health Centers according to a letter from the Ministry of Health [80].

CardioChek and CardioChek PA blood analyzers combine not only small size but also the ease of use, high accuracy of results up to 96% (some parameters prevail in many laboratory analogues). The cost of these blood analyzers makes them very attractive not only for medical institutions, but also for home use.

1 2

Fig. 2.11. Blood cholesterol analyzer CardioChek (1), as well as CardioChek PA (2) [80].

Here is a list of defining parameters: • Cholesterol (total); • High density lipoprotein (HDL); • Low density lipoprotein LDL (for CardioChek PA); • Creatinine (for CardioChek PA); • Ketones; • Triacylglycerols (triglycerides); • Glucose.

As seen from the devices of rapid analysis, the CardioChek PA blood analyzer has more advanced capabilities. There are main differences:

1. CardioChek works only with monobands;

2. CardioChek PA works both with monobands, and with multibands;

3. CardioChek PA, has a connector for connecting the printer to print the results and the ability of data transfering to computer.

The order of the analysis. The finger is first treated with a tampon and than punctured with a lancet. First drop of blood should be removed for purity of the analysis. Next you need to squeeze a large drop and to take blood using pipettes.

Pipettes are different in size, since different blood samples are required for different analyzes. To use CardioChek PA, as a home blood analyzer, it's best to carry out blood sampling with Vitrex Sterilance Press Series. A drop of blood is applied to the test strip and within 2 minutes you should expect the result on the device display.

Using the CardioChek PA blood biochemical analyzer, it can be tested several parameters at the same time. The device has specially built memory for storing the results of the study for measuring blood cholesterol and glucose levels, and in the package with test strips contains a code chip containing information about the name of the test, calibration, quantity of test strips batch, and expiration date.

Basically, this device is used in health centers and daily medical practice.

2.1.8. Semiautomatic analyzer, photometer Microlab 300 (VitalScientificN.V., The Netherlands)

The compact semiautomatic biochemical analyzer has a flow cuvette (fig. 2.12, tab. 2.2). This analyzer is economical and easy to operate for small laboratories [88].

Table 2.2

Specifications of the Microlab 300 photometer

Number of tests in the menu:	60 techniques
Minimum volume of the measuring mixture:	250 µl / analysis
Spectral range:	340, 405, 505, 546, 578, 620 nm

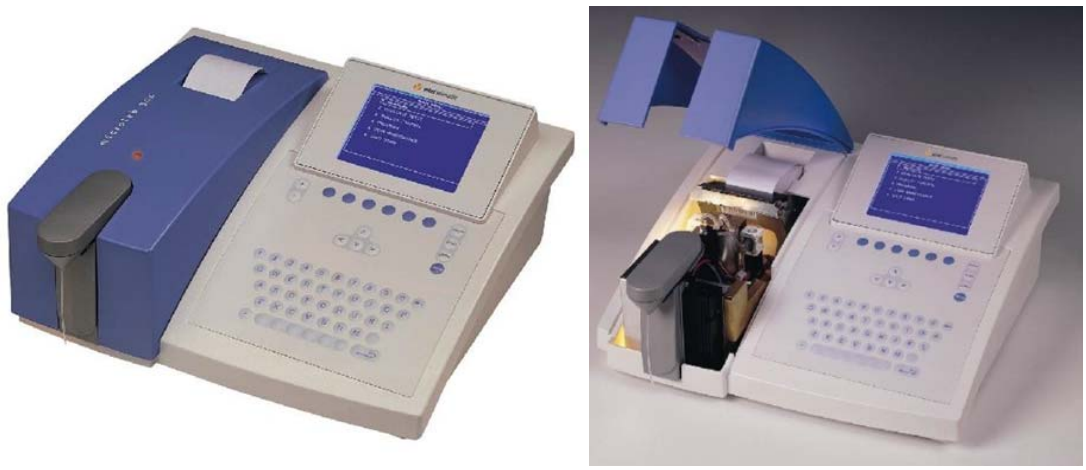


Fig. 2. 12. Semiautomatic analyzer, photometer Microlab 300 [88].

For the most part, such kind of analyzers are used in scientific research and small clinical laboratories.

This section provides information on typical broad-spectrum biochemical analyzers that are used for both scientific and educational purposes and for the purpose of biochemical analysis of biological samples in analytical laboratories.

CHAPTER 2.2
METHODICAL APPROACHES FOR THE ISOLATION OF BLOOD CELLS AND THEIR STUDIES

2.2.1. Obtaining blood samples

The blood of healthy donors for receiving blood plasma is taken on an empty stomach from the vein with an acute dry needle with a large diameter, without a syringe (to prevent hemolysis) [8].

At the beginning of manipulation, the first 5-6 drops are discarded, avoided tourniquet and do no massage of forearm to prevent the activation of blood clotting and fibrinolysis.

A 3.8% solution of sodium citrate is added to the polyethylene tube with help of dispenser and mixed with the blood at a ratio of 1: 9. The test tube with a mixture is tightly closed and slowly mixed several times with slow movements (not shaking). Deposition of uniform blood elements is carried out by centrifugation for 10 minutes. with an acceleration of 1200-1400 g. Blood plasma (supernatant) is transferred to a polyethylene tube.

2.2.2. Obtaining platelet-enriched plasma of human blood

Platelet-enriched plasma of human blood is obtained from whole blood, which contains 3.8% sodium citrate at a ratio 9:1 (blood: sodium citrate) by centrifugation for 160 g for 20 minutes at a temperature of 20 °C [58]. During centrifugation, the test tubes are not close, for oxygen supply of samples.

2.2.3. Obtaining the washed out human platelets

The washed out human platelets are obtained by gel-filtration chromatography of platelet-enriched plasma on Sepharose 2B (3 x 31 cm), balanced 4 mM HEPES buffer, pH 7.4, containing 0.137 M NaCl, 2.7 mM KCI, 1 mM $MgCl_2$; 5.6 mM glucose, 3 mM NaH_2PO_4, with 0.35 mg/ml of blood serum bovine albumin at a rate

of 1 ml/min. The rinsed platelets should be fractionated in the free volume of the column [92].

2.2.4. The isolation of protein C

Four alternative protocols can be used to isolate protein C [53, 107, 110]:

1) Ion-exchange chromatography on Sephadex DEAE A-50. To extract protein C from the fraction IV-1 by Konon-method, it is used Sephadex DEAE A-50 (column 2 x 23 cm) that is balanced using a 0.02 M tris-phosphate buffer with pH 6.0 containing 2 mM benzamidine and 0.15 M NaCl. Separation of the protein C-containing mixture (26 ml) is carried out in the same buffer with a linear gradient of 0.15-0.6 M NaCl at an elution rate of 20 ml/h. The obtained material is electrophoretically characterized [8, 41, 93].

2) Metal-affinity chromatography on the copper-containing sorbent. The method is based on the use of metal ions as a ligand, which binds proteins that have remains of histidine on the surface. 15 ml of a mixture of proteins are mixed with 15 ml of sorbent and incubated for 2 hours at 4 °C. The media is eluted with 0.02 M phosphate buffer pH 7.0 containing 15 mM imidazole (histidine competitor) at a rate of 10 ml/h. The obtained material is electrophoretically characterized.

3) Hydrophobic chromatography on Sorbent 1. Protein mixture (23 ml) in 0.1 M phosphate buffer pH 7.0 containing 1 mM benzamidine and 0.8 M ammonium sulfate is applied to the corresponding sorbent equilibrated with the same buffer and left on incubation overnight at 4 °C. The non-carrier material is eluted with 0.1 M phosphate buffer pH 7.0 containing 1 mM benzamidine and 1.1 M ammonium sulfate at a rate of 20 ml/h. The protein C elution is carried out with the same buffer, but without ammonium sulfate, at the same speed. The obtained material is electrophoretically characterized [8, 41].

4) Immune-affinity chromatography using monoclonal antibodies to protein C. To obtain protein C (protein content in the blood is 3-5 mg/L), activated BrCN-sepharose is used with the immobilized monoclonal antibodies 7D7B10 to protein C (column 1 × 10 cm). The sorbent is equilibrated with 0.05 M tris-HCl buffer pH 7.4

containing 0.15 M NaCl, 1 mM EDTA; a blood plasma (150 ml), which was previously dialyzed against the same buffer, is applied. The rate of application of the material and the rate of elution should be 0.65 ml/min. Elution of protein C is carried out with 0.05 M tris-HCl buffer pH 7.4, containing 0.15 M NaCl and 10 mM $CaCl_2$. The homogeneity of the resulting protein is checked by electrophoresis in SDS-PAGE [8, 41, 93]. For further studies, it is possible to use the preparations of protein C obtained by ion exchange chromatography [8, 35, 41, 93].

2.2.5. Obtaining concentrate of the vitamin K-dependent proteins

To obtain a concentrate of vitamin K-dependent proteins, 15 g fractions (IV-1, part 2.2.4, part II) are suspended in physiological solution in the presence of 1 mM benzamide, than add $(NH_4)_2SO_4$ to 40% saturation and incubated for 12 hours at 4 °C. The suspension is centrifuged for 40 minutes at 6000 g by Centrifuge Ependorf, Germany. Then to the supernatant, it is added $(NH_4)_2SO_4$ to 70% saturation, incubated for 1 hour at 4 °C, and centrifuged for 40 minutes at 6000 g.

The precipitate is dissolved in 0.02 M tris-phosphate buffer with pH 6.0 in the presence of 2 mM benzamide and centrifuged in suspension for 40 minutes at 6000 g. The supernatant is de-mineralized on the G-25. The resulting mixture of proteins is used to isolate protein C [53, 107, 110].

2.2.6. Extraction of prothrombin

Prothrombin is derived from the concentrate of vitamin K-dependent blood proteins [13]. The separation of vitamin K-dependent factors is carried out on a column of Q-Sepharose (2.5 x 11.8 cm), equilibrated with 0.02 M tris-HCl buffer, pH 7.4, containing 1 mM benzamide. Elution is carried out by step gradient NaCl 0,2 M; 0.4 M and 1 M NaCl at a rate of 10 ml/min · cm2. Prothrombin is eluted at 0.4 M NaCl concentration. The resulting prothrombin sample concentrates on Centriprep-30 at 1200-1400 g.

Additional purification of prothrombin is performed on Sephacryl S200 (2 x 115 cm) in 0.05 M tris-HCl buffer, pH 7.4, containing 1 M NaCl and 1 mM benzamide, at

a rate of 8 ml/min. The resulting prothrombin sample is purified from benzamidine *ex tempore* on PD-10 in 0.05 Tris-HCl buffers, pH 7.4, containing 0.15 M NaCl, at a rate of 1 ml/min [54, 99].

The purity of the sample is checked electrophoretically [67, 70, 90, 97, 102, 108]. The material is stored at -20˚C.

2.2.7. Obtaining of thrombin

Thrombin is received by activation of prothrombin separated from the fraction of vitamin K-dependent proteins on ecamulin-Sepharose [13, 15], by incubation for 1.5 h at 37 ° C.

Standardization of the sorbent is carried out by incubating the chromogenic substrate of calicin S2302 at 37 ° C. for 5 minutes in 0.05 M tris-HCl buffer, pH 7.4, containing 0.13 M NaCl, using 0.5 ml of ecamulin Sepharose 0.2 ml of 0.5 mM chromic substrate of kallikrein S2302.

The registration of substrate splitting is carried out at 405 nm and 492 nm on the Titertek Multiskan MC reader.

2.2.8. Precipitation of fibrinogen using sodium sulfate solution

Fibrinogen is obtained from human blood plasma by mixing with a 16% solution of sodium sulfate [14]. The portion of protein that is polymerized by the action of thrombin in the resulting protein sample is preferably 96-98%. [92] The purity of the fibrinogen is checked electrophoretically [67, 70, 90, 97, 102, 108]. Then this protein is stored at -20 ° C.

2.2.9. Analysis of the platelet aggregation

The platelet aggregation can be studied using the aggregometer (fig. 2.13) SOLAR AR2110 (Belarus). To 170 μl of rinsed platelet suspension (200,000 μl), in 5 mM HEPES buffer, pH 7.4, containing 0.145 M NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 5 mM glucose, is added 20 μl of 1.5 mg/ml of native fibrinogen at a final concentration in 0.05 M Tris-HCl buffer, pH 7.4, containing 0.13 M NaCl and 10^{-4} M $CaCl_2$ and

incubated for 5 min in the digest of the aggregometer at 37 ° C. Activation of the platelets is induced by the addition of 20 μl of collagen or ADP suspension at a final concentration of 2 mg/ml and 12.5 μM, respectively [69].

Fig. 2.13. Aggregometer SOLAR AR2110 (Belarus)

2.2.10. Determination of the prothrombin time

To determine the prothrombin time (PT), 0.1 ml of blood plasma was contributed and warmed for 1 min. at a temperature of 37 ° C in a glass cone. After that, 0.2 ml of thromboplastin containing 12.5 mM calcium chloride and blood plasma were added. Blood plasma concentration was measured by moderately shaking the test tube in a water bath at a temperature of 37 °C [5]. The time of blood plasma coagulation of donors in the test of prothrombin time determination was 18 seconds.

2.2.11. The activated partial thromboplastin time

The test of activated partial thromboplastin time is performed according to the methods [42]. The inhibitory correction test is performed by determining the time of blood plasma coagulation of patients with the addition of donors blood plasma in the ratio 1: 1. In the case of accumulation of inhibitors of clotting in blood plasma of the patient, normalization of time for blood plasma coagulation does not occur.

Normalization of the time of blood plasma concentration in an inhibitory correctional sample indicates a shortage of blood coagulation factors [26, 45].

2.2.12. Determination of echamolin time

To determine the echamolin time, 0.1 ml of blood plasma is contributed into a glass cone tube and warmed for 1 min at 37 ° C. Then, add 0.1 ml of 0.025 M calcium chloride and 0.1 ml of the echamolin solution. Mix thoroughly, and determine the time of blood plasma clotting, moderately shaking the test tube in a water bath at a temperature of 37 °C [45]. The timing of blood plasma in this test can be 120 seconds.

2.2.13. The content of soluble fibrin-monomer complexes

To determine the content of soluble fibrin-monomeric complexes (SFMC) [5] in a conical vial with 0.25 ml citrate blood plasma is contributed into 0.25 ml 0.1 M KH_2PO_4-NaOH buffer, pH 7.5 with content of 0.065 g of sodium chloride, 0.2% of ε-aminocaproic acid, 0.1% sodium citrate and mixed. Then add 0.4 ml 1 M KH_2PO_4-NaOH buffer, pH 7.5, mix and hold for 30 minutes at a temperature of 22 °C [46]. The quantitative values expressed in g/L scale for visual assessment: absence of visible dimness – 0; clear dimness – 0.03; filaments – 0.06; flakes – 0.09; gel-like sediment – 0.15.

2.2.14. The level of prothrombin according to the cleavage of the chromogenic substrate using echamolin and thromboplastin

The content of prothrombin is determined by activating it in the blood plasma physiological (thromboplastin) and non-physiological (echamolin) activators of prothrombin. Enzymatic activity in the test of thrombin determines by cleavage of a thrombin-specific chromogenic substrate [15]. For this, 10 µl of donors blood plasma put in the incubation medium, add 10 µl of 0.025 M calcium chloride, 10 µl of the activator of prothrombin (echamolin or thromboplastin), 195 µl 0.05 M Tris-HCl

buffer, pH 7.4 with a content of 0.13 M NaCl, 25 μl of 2 mM chromogenic substrate S2238. Incubation is carried out at 37 °C for 10 min.

The amount of split substrate is determined spectrophotometrically at a wavelength of 405 nm on the spectrophotometer microtablet (fig. 2.14), for example, Thermo Multiskan EX, Finland [32, 15].

Results of prothrombin and echamolin tests using chromogenic substrate are expressed as the prothrombin ratio (PR) and echamolin ratio (ER):

$$PR = (A_P/A_D)^{ISI} \ (1), \quad ER = A_P/A_D \ (2),$$

where: A_P – the amidolitic activity of blood plasma of the patient under the action of thromboplastin (1) and echamolin (2); A_D – the amidolitic activity of the blood plasma donor under the action of thromboplastin (1) and echamolin (2); ISI – the International Sensitivity Index of thromboplastin preparation [48].

Fig. 2.14. Microtablet spectrometer *Thermo Multiskan EX* (Finland) [5, 15].

The accumulation of functionally inactive forms of prothrombin (FIFP) in the blood plasma is determined by the difference of echamolin and prothrombin ratio: less prothrombin value than echamolin shows the accumulation of functionally inactive forms of prothrombin and activation of the coagulation system [15].

2.2.15. The activity of the protein C

The activity of the protein C in blood plasma is determined by activating the protein C with protein activator from the venom of the Copperhead ordinary (*Agkistrodonhalyshalys*) [21, 50].

For this, 30 μl of blood plasma, 100 μl of protein C activator, 85 μl 0.05 M Tris-NCI buffer, pH 7.4, with a content of 0.13 M NaCl and 35 μl of 2 mM chromogenic substrate S2236 incubated at 37 °C for 15 min.

The amount of split chromogenic substrate S2366 for protein C is determined spectrophotometrically at wavelengths of 405 and 492 nm on the spectrophotometer microtablet, for example, Thermo Multiskan EX, taking the absorbance of a 1M solution of pNA at 405 nm is equal to 10500 [73].

2.2.16. Antithrombin III level

The levels of antithrombin III can be defined according to the guidelines of the company "Renam" (Russia).

2.2.17. The content of functionally active factor X

The content of factor X determines by activating factor X in the blood plasma with activator from the venom of Russell's viper (*RVV*) [91].

For this, 10 μl of blood plasma, 5 μl RVV, 10 μl of 0.025 M $CaCl_2$ and 200 μl 0.05 M Tris-NCl buffer, pH 7.4, with a content of 0.13 M NaCl and 25 μl of chromogenic substrate S2765 are incubated at 37 °C.

The amount of split chromogenic substrate can be determined spectrophotometrically at wavelengths of 405 and 492 nm on microtablet spectrophotometer with a Thermo Multiskan EX.

The total content of factor X in the blood plasma can also be determined with method of immunoenzyme analysis, using polyclonal rabbit antibodies according to standard methods [103].

2.2.18. The content of fibrinogen in blood plasma

The content of fibrinogen in the blood plasma of patients is determined with the help of thrombine-like enzyme ancistone-N, obtained from the venom of the *Copperhead ordinary* (*Agkistrodonhalyshalys*) [52]. A spectrophotometric method for determination of fibrinogen: in a glass test tube add 0.2 ml of the investigated blood plasma and 1.7 ml of 0.1 M phosphate buffer pH 7.0, then added to the mixture of 0.1 ml of thrombin - 2 NIH and 0.1 ml of 0.04 M monoiodine acetic acid (if during the determination process instead of thrombin is used Ancestron-N, do not add monoiodine acetic acid to phosphate buffer, which prevents the activation of factor XIII, because Ancestron-N does not activate factor XIII). The mixture is thoroughly stirred with a glass wand with a large surface and place the tube in a thermostat (37°C), leaving the wand in. After 30 min of incubation, a clot of fibrin is removed by twisting the wand and the liquid is removed by pressing the tube wall. Next bundle on a stick need to be washed several times with cold sodium chloride solution, the liquid is removed from the surface with light touch to filter paper.

The clot is dissolved in 5 ml of 1.5% acetic acid solution. Protein concentration in the resulting solution is determined on a spectrophotometer by measuring the absorption at a wavelength of 280 and 320 nm (absorbance at 320 nm is determined to calculate a correction for the turbidity of the solution) [52].

The turbidimetric analysis is carried out by recording the light scattering of the fibrin cluster at a wavelength of 350 nm, which is formed in a spectrophotometric cuvette in which there were sequentially added 3 units of streptokinase, 0.7 μg of plasminogen, 1.3 units of ancestron, 80 μl of blood plasma and 0.01–0.05 mg/ml of the highly dispersed silicon dioxide in 0.05 M Tris-HCl buffer, pH 7.4, containing 0.13 M NaCl and 1 mM $CaCI_2$.

According to the data obtained the determination of the length of lag-phase (in seconds), which corresponds to the time of formation of protofibrils and which is not characterized by the increase of light scattering at 350 nm, and the rate of lateral aggregation protofibrils, which is defined as the tangent of the angle of inclination

drawn to the steepest part of the curve in light scattering. For the phase of lysis of fibrin clot, a decrease in light scattering at 350 nm is referred.

2.2.19. Obtaining blood serum

The blood serum of rats is obtained by centrifugation [8, 41, 93].

For the deposition of fibrin and whole blood elements, the blood is incubated at 37 C for an hour.

Then the clot is separated from the tube walls with a glass wand for better phase separation and to produce blood serum and centrifuge it for 15 min at speed of 1000 g. The supernatant (serum) is taken in a clean microtube type "Eppendorf" and frozen at -20 ºC for further use [104].

In general, besides metodical approaches of obtaining blood (including sampling, obtaining serum and platelet-rich plasma), its cells (e.g., platelets) study of individual elements of the coagulation system, including the isolation and/or characterization of prothrombin, thrombin, fibrinogen, fibrin-monomeric complexes, protein C, antithrombin, the vitamin K-dependent proteins and blood coagulation factor X .

CHAPTER 2.3
RESEARCH METHODS OF METABOLISM OF PROTEINS, LIPIDS, AND CARBOHYDRATES

2.3.1. Determination of the concentration of blood proteins

1) The concentration of proteins in solutions is determined spectrophotometrically by measuring the optical absorption in the region of 280 nm and subtracting the absorbance by 320 nm. To calculate the concentration, the following values of molar extinction coefficients are used: fibrinogen – 15.06 [82]; prothrombin - 14.0 [99]; echamolin - 10.3 [54]. The concentration of protein C in a solution with a neutral pH is determined by the Bradford method [70] in the modification of Sedmac [102].

2) Determination of protein concentration, using the Bradford method [70]. To determine the protein concentration, 10 µl of 10% NaOH, 70 µl of distilled water and 2 ml of the prepared solution are added to 20 µl of the sample. The prepared solution is being made by mixing 6 ml of the stock solution (containing 10 ml of 95% ethanol, 20 ml of 85% H_3PO_4, and 35 mg of Coomassie Brilliant Blue) with 3 ml of 95% ethanol and 6 ml of 85% H_3PO_4, then the volume is being increased by adding distilled water up to 100 ml.

The protein concentration in the sample is determined spectrophotometrically at a wavelength of 595 nm and compared to the control sample, which contains distilled water instead of the investigated sample. The quantity of protein is determined according to the calibration graph and measured in mg/ml.

2.3.2. Electrophoresis of blood proteins in a polyacrylamide gel

Electrophoresis in a polyacrylamide gel (PAAG), when used with SDS, is carried out, using the Laemmli method [90, 97] with a tris-glycine system on a device for vertical gel electrophoresis (BioRad, USA) in plates of 10% PAAG [41, 93].

To prepare the samples for electrophoresis, a sample buffer containing 5% sucrose or glycerol, 2% SDS and bromophenol is added to a solution with a protein

concentration of 1 mg/ml until the coloration. The samples are brought to boil. To restore disulfide bonds, 5% β-mercaptoethanol is being applied to the samples for 10 minutes. Declustering is carried out using an electric current of 19 mA for a concentrating gel and 36 mA for a distributive one.

The manifestation of gel to identify parts containing proteins is being carried out by staining with a coloring solution (0.125% of Coomassie G-250 in 25% isopropanol and 10% acetic acid) for 10 minutes. To remove the dye residues, 2-8% solution of acetic acid is used [8]. As markers, it is possible use the Low Molecular Weight Calibration Kit Pharmacia (97; 66; 43; 31; 21.5; 14.4 kDa) and Fermentas (170; 130; 95; 72; 55; 43; 34; 26; 17; 11 kDa).

2.3.3. Densitometry of electrophoregram of blood proteins

The obtained electrophoregrams are analyzed in the TotalLab TL100 (Phoretix) application to calculate the molecular weight of protein fractions and the percentage distribution of protein between the zones [67, 108].

2.3.4. Determination of concentration of high-density lipoproteins in blood serum

The concentration of HDL in the blood serum of, for example, rats, is measured using the spectrophotometric method with the Biochemical Analyzer MicroLab 300 (fig. 2.14). As part of the study, standard sets are used to calculate the amount of HDL produced by "PLIVA-Lachema Diagnostika" (Czech Republic).

The principle of the method is that the beta-lipoprotein-specific antibodies, contained in the R1 reagent, bind to lipoproteins, different from the HDL fraction.

Antigen-antibody complexes block the enzymatic reaction initiated by the addition of reagent R2.

HDL is determined quantitatively in a enzyme chromogenic mixture. The HDL level is expressed in mmol/L. The optical density of samples is measured at a wavelength of 600 nm using the Biochemical Analyzer Microlob 300 [44].

In addition, the study of the level of high-density lipoproteins is possible with other methodical approaches [16, 29, 31, 41, 55, 93].

2.3.5. Determination of concentration of low-density lipoproteins in blood serum

The concentration of LDL in blood serum (for example, rats) can be determined using a spectrophotometric method with the biochemical analyzer Microlob 300 (fig. 2.14). As a part of the study, standard sets are used to determine the amount of LDL produced by "PLIVA-Lachema Diagnostika" (Czech Republic).

The principle of the method is that the component of the reagent R1 protects LDL from its cleavage. Lipoproteins that do not belong to this group are broken up by cholesterol esterase and cholesterol oxidase. The hydrogen peroxide, formed in this reaction, is broken up the catalase of the reagent R1. Afterwards, the level of the LDL is calculated quantitatively in the presence of cholesterol oxidase and peroxidase. The level of LDL is expressed in mmol/L.

The optical density of the samples was measured at a wavelength of 600 nm using the Biochemical Analyzer Microlab 300 [44].

Along with this, the study of concentration of low density lipoproteins is carried out with the help of other methodical resources [16, 29, 31, 41, 55, 93].

2.3.6. Determination of the concentration of cholesterol in blood serum

Methods of determination of the total level of cholesterol are as follows: a) colorimetric (based on the reactions of the formation of colored complexes). There are about 150 of them; b) nephelometric methods, based on comparison of the degree of turbidity of the standard solution and the solution under study; c) titrimetric methods; d) fluorimetric methods that allow the calculation of cholesterol level in the microvessels of blood serum (0.01 ml); e) gas chromatographic and chromatographic methods (e.g. thin layer chromatography [41, 43, 71, 76, 81, 93, 101]); e) gravimetric methods [8, 41].

Method of measuring of total cholesterol level in blood serum based on the Lieberman-Burchard reaction (Ilko's method) [8]

Principle of the method: in a strongly acidic anhydrous environment, cholesterol interacts with a mixture of sulfuric and acetic acids and acetic anhydride. During the reaction, cholesterol is being consistently oxidized. In addition, on each stage of the reaction a molecule of cholesterol is formed. It has one additional double bond, as compared to the compound it was formed from. As a result of the final oxidation of the 3,5-'cholestadiene' ion, a colored compound is formed, which, after the dissolution in sulfuric acid, is measured at maximum absorption at 410 and 610 nm. Due to the instability of the compound color, the photometric time must be accurately kept on.

The reaction mixture with a standard cholesterol solution has an emerald color. However, blood serum samples can become green, blue and brown, which are caused by the production of endogenous heat due to the reaction of many components of blood serum. In addition, in the Lieberman-Burchard reaction, free cholesterol and cholesteride form color complexes with different molecular absorption coefficients. When cholesteride levels are high, the optical density is higher. Given that many factors influence the direct determination of cholesterol, its reaction with the Lieberman-Burchard mixture cannot be considered special.

The direct method of determination of cholesterol is relatively simple to perform and inexpensive. However, the toxicity and the ability to cause corrosion of the system in modern analyzers limit the application of the method. In large laboratories, researchers prefer to use enzymatic methods for the determination of cholesterol [81].

Concentration of cholesterol in blood serum (for example, rats) is measured using spectrophotometric method using Biochemical Analyzer MicroLab 300 (fig. 2.14). As a part of the research, standardized kits for calculation of the cholesterol level produced by "PLIVA-Lachema Diagnostika" (Czech Republic) are used. For determination of cholesterol, the enzymatic method is used. The cholesterides in the sample are hydrolyzed by cholesterol ecterase. Newly-formed free cholesterol is

oxidized by cholesterol oxidase with the simultaneous formation of a hydrogen peroxide (H_2O_2), which, oxidized, binds to 4- aminoantipyrine phenol in the presence of peroxidase, resulting in the formation of a chromophore.

The color intensity of the reaction mixture, measured at 540/560 nm, is a directly-proportional concentration of total cholesterol level in the sample. The level of cholesterol is expressed in mmol/L [44].

2.3.7. Determination of concentration of triacylglycerols in the blood serum

The concentration of triacylglycerols (triglycerides or TAG) in the blood serum is determined by the spectrophotometric method using the Biochemical Analyzer MicroLab 300 (fig. 2.14). In the course of the investigation, standard sets are used to determine the content of the TAG manufactured by "PLIVA-Lachema Diagnostika" (Czech Republic).

The principle of the method is based on numerous enzymatic reactions. The TAG sample is hydrolyzed with a mixture of bacterial lipases to form glycerol and fatty acids. Glycerol, in turn, is phosphorylated by glycerol kinase in the presence of ATP to form glycerol-3-phosphate, which further oxidizes with molecular oxygen in the presence of glycerophosphate cidase, which leads to the formation of hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate. Hydrogen peroxide is used in the oxidative cleavage reaction of p-chlorophenol and 4-amino-antipyrine, which catalyzes peroxidase and results in the formation of a chromophore whose optical density can be measured at 660/800 nm. The abortion value at 660/800 is directly proportional to the concentration of TAG in the sample. The concentration is expressed in mmol/L [44].

In addition to the above method, other biochemical analyzers and standard kits [19, 22, 30, 43, 55, 106, 71, 76, 101] are used to determine the concentration of triacylglycerols.

2.3.8. Determination of alanine aminotransferase activity in blood serum

Active alanine aminotransferase (ALT) in blood serum of rats can be determined using a spectrophotometric method using Biochemical Analyzer MicroLab 300 (fig. 2.14), measuring the absorbance values at 340 nm [25]. To test the activity of ALT it is possible to use the standard production kits of "PLIVA-Lachema Diagnostika" (Czech Republic).

The principle of the method is to transfer the amino group from the L-alanine to the α-oxoglutarate with the formation of such products as: pyruvate and L-glutamate by the alanine aminotransferase of the amino group. Next, the pyruvate with involvement of NADH is converted to L-lactate. The activity of ALT is determined by a direct proportional decrease in the optical density of the solution when NADH is oxidized to NAD^+. The activity of ALT is expressed in U/L.

In determining the activity of ALT, other biochemical analyzers and standard kits [7, 8, 22, 32, 38, 49, 75, 89, 96] are used.

2.3.9. Determination of aspartate aminotransferase activity in blood serum

Aspartate aminotransferase activity (AST) in a blood sample (for example, of rats) is measured by a spectrophotometric method using the Biochemical Analyzer MicroLab 300 (fig. 2.14) at a wavelength of 340 nm [25]. To test the activity of AST, use the standard production kits "PLIVA-Lachema Diagnostika" (Czech Republic).

The principle of the method is to transfer the amino group from the L-aspartate to the α-oxoglutarate to the aspartate aminotransferase of the amino group to form the oxaloacetate and L-glutamate. The resulting oxaloacetate with involvement of NADH is transformed to L-malate and NAD^+.

The activity of AST is determined by directly proportional decrease in the optical density of the solution when NADH is oxidized to NADAST activity is expressed in U/L.

Other active biochemical analyzers and standard kits [7, 8, 22, 32, 38, 49, 75, 89, 96] are used in the determination of ALT and AST activity.

2.3.10. Determination of γ-glutamyltranspeptidase activity in blood serum

Activity of γ-glutamyltranspeptidase (GGT) in blood serum of rats is determined by the spectrophotometric method on the biochemical analyzer Microlab 300 (fig. 2.14) at a wavelength of 400-430 nm [25]. To test the activity of GGT, it's used the standard production kits "PLIVA-Lachema Diagnostika" (Czech Republic).

For the action of gamma-glutamyltranspeptidase, the glutamic residue from γ-L - (+) - glutamyl-4-nitroanilide is converted to peptide acceptor-glycylglycine. This frees a chromogen-p-nitroaniline. The concentration of free p-nitroaniline is measured spectrophotometrically by inhibiting the enzyme reaction with acetic acid. The activity of GGT is expressed in U/L. In determining the activity of GGT, other biochemical analyzers and standard kits [7, 8, 22, 32, 38, 49, 75, 89, 96] are used.

2.3.11. Determination of α-amylase activity in blood serum

Activity of α-amylase in the blood serum of rats is measured using spectrophotometric method for biochemical analyzers Microlab300 (fig. 2.14) at a wavelength of 405 nm [25]. To test the activity of α-amylase, use the standard production kits "PLIVA-Lachema Diagnostika" (Czech Republic).

The principle of the method is the hydrolytic cleavage of starch with α-amylase to oligosaccharides, which do not give a color reaction with iodine.

The activity of α-amylase is determined by directly proportional decrease in the optical density when the intensity of coloration of the complex of iodine and starch in the sample is decreased. The activity of α-amylase is expressed in U/L.

Along with the above, to determine the activity of α-amylase, other types of biochemical analyzers and corresponding standard kits or methodical approaches [6, 8, 11, 18, 41, 49, 93, 105] are used.

2.3.12. Determination of alkaline phosphatase activity in blood serum

Activity of alkaline phosphatase in the blood serum of rats was measured using the spectrophotometric method of biochemical analyzer Microlab 300 (fig. 2.14) at a wavelength of 405 nm [25].

To test the activity of alkaline phosphatase use standard production kits of "PLIVA-Lachema Diagnostika" (Czech Republic).

The principle of the method is the hydrolytic cleavage (with the alkaline phosphatase of the serum) substrate of the reaction – p-nitrophenyl phosphate, to p-nitrophenol, which has a yellow color in the alkaline medium. The activity of alkaline phosphatase is determined by a directly proportional increase in the optical density with an increase in the color intensity of the sample, as a result of an increase in the content of p-nitrophenol. The activity of alkaline phosphatase is expressed in U/L. The activity of alkaline phosphatase can also be determined by the Fiske-Subbarow method [8, 75].

2.3.13. Determination of the creatinine concentration in blood serum

The blood serum concentration of creatinine is determined by spectrophotometric method on the biochemical analyzer Microlab 300 (fig. 2.14) at a wavelength of 500 nm [25]. Standard tests produced by "PLIVA-Lachema Diagnostika" (Czech Republic) are used to test the concentration of creatinine.

The principle of the method is based on interaction in the alkaline medium of the picric acid with blood serum creatinine, with the formation of a complex of orange-red color, concentration of which is measured photometrically. The concentration of creatinine is expressed in μmol/L.The concentration of creatinine is determined using other biochemical analyzers and standard kits [7, 22, 32, 38, 49, 89, 96].

2.3.14. Determination of the blood serum urea concentration

The blood serum urea concentration was determined by the spectrophotometric method using the biochemical analyzer Microlab 300 (fig.ure 2.14) at the wavelength of 340 nm [25]. The standard tests manufactured by PLIVA-Lachema Diagnostika (Czech Republic) were used to test the urea concentration.

The method is based on the hydrolytic cleavage of urea by urease to carbon dioxide and ammonium dioxide, which, in the presence of nitroprusside, reacts with

phenol and hypochlorite, forming a colored complex. The intensity of the color is proportional to the concentration of the urea of the analysed sample. The concentration of urea is expressed in mmol/L.

The concentration of urea can be determined using other biochemical analyzers and standard kits and methodical approaches [1, 7, 22, 32, 38, 49, 89, 96].

2.3.15. Determination of the blood serum uric acid concentration

The blood serum uric acid concentration in the sample examined is subjected to a spectrophotometric method using the biochemical analyzer Microlab 300 (fig. 2.14) at the wavelength of 550 nm. [25]. The standard sets manufactured by PLIVA-Lachema Diagnostika (Czech Republic) are used to test the urea concentration.

The principle of the method is based on the oxidation of uric acid under the action of uricase, with the formation of an equimolar amount of allantoin and hydrogen peroxide, the concentration of which is determined by reaction with the sodium salt of N-ethyl-N- (2-hydroxy-3-sulfopropyl) -m-toluidine and 4 -aminoantipyrine in the presence of peroxidase. The uric acid concentration is expressed in μmol/L.

2.3.16. Determination of the blood serum concentration of direct and total bilirubin

The blood serum concentration of direct and total bilirubin is determined by the spectrophotometric method using the biochemical analyzer Microlab 300 (fig. 2.14) at the wavelength of 564 nm [25]. The standard sets manufactured by PLIVA-Lachema Diagnostika (Czech Republic) are used to test the bilirubin concentration.

The principle of the method is based on the release of bilirubin from its complex with albumin under the action of the reagent R1. Direct bilirubin, reacting with diazotized sulfanilic acid containing in the reagent R2, forms a persistent nitrogen-containing complex of red color, which intensity of color is directly proportional to the concentration of direct bilirubin and can be determined photometrically. Concentration of direct and total bilirubin is expressed in μmol/L.

2.3.17. Determination of the content of proinflammatory and anti-inflammatory cytokines in the blood serum

The concentration of interferon-γ, interleukin-1β, 12, 10, and 4 in blood serum of rats is determined by enzyme-linked immunosorbent assay (ELISA) [62-66].

ELISA can be performed in microplates with sorption ability according to the common method for soluble proteins. The antigen, pre-diluted in 0.1 M NaHCO3 buffer, pH 9.6, to a concentration of 10 μg/ml, was incubated in a plate wells at 4 °C for 12 hours. Removal of the unsorted antigen was carried out by the three-fold washing of wells with a buffer for immobilization. The incubation was carried out in TBS (50 mM tris-HCl and 150 mM NaCl) pH 7.4, or in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) pH 7.4. Non-specific mice of the binding are blocked by incubation of wells with 5% skimmed milk or 3% bovine serum albumin solution for 60 minutes in a thermostat at 37 °C. Purification of plate wells is carried out by three times washing with working buffer (TBS or PBS) with 0.1% tween-20 addition. Primary antibodies are diluted with a working buffer to a concentration of 5-50 μg/ml, then are added to plate wells and incubated for 1 hour in a thermostat at 37 °C. After that the wells are washed three times with 0.1% tween-20 working buffer. Following this, a secondary antibody conjugate with visualization enzymes (alkaline phosphatase or horseradish peroxidase) is incubated for 1 hour in a thermostat at 37 °C. The plate wells are washed again three times with 0.1% tween-20 working buffer and incubated with sorbents for enzymes visualization (1 mg/ml para-nitrophenol phosphate (pNPP) in 10% diethanolamine pH 9.8 or 1 mg/ ml tetramethylbenzidine (TMB) in 50 mM phosphate citrate buffer pH 5.0 with 0.013% H_2O_2, respectively) for 1 h at 37 °C. Optical absorption is measured at a wavelength of 450 and 550 nm on a microplate reader. The optical error of the plate is determined by subtracting the indices at 550 nm from the index at 450 nm. The ability of cytokines is expressed in scores of control values that are taken for 100%.

2.3.18. Determination of the blood serum free amino acids concentration

The amino acids of blood serum composition is determined by the automatic amino acid analyzer T-339 ("Microtekno", Czech Republic) by ion exchange liquid chromatography in lithium citrate buffer in a one-column cycle [17].

The automatic analyzer of amino acids and its application is according to [8, 11, 18, 41, 93, 105].

1. *Application of ion exchange chromatography*.

Ion exchange chromatography on columns is used in numerous and various studies to obtain basic biochemical information, and as an auxiliary method in medicine in the diagnosis and treatment of certain diseases.

Ion exchange chromatography on columns is used in four important areas:

1) For qualitative and quantitative amino acids analysis of peptides and proteins (which gives a valuable characteristic of molecules) and possible use as a means of detecting certain specific differences in proteins.

2) To determine the amino acids composition of biological fluids, which provides information on the presence of free amino acids and allows to track changes occurring in the whole body under the influence of many factors such as the environment, physiological state, and genetic predisposition.

3) To determine the primary structure of proteins.

4) In the diagnosis and treatment of diseases associated with the alteration of metabolism or transport of amino acids.

2. *The Column ion exchange chromatography*.

In a case of the column ion exchange chromatography, fine-grained cation exchangers (resins), which are a copolymer of styrene and divinylbenzene of a spherical shape with a functional group-SO3-, are used for the separation of amino acids, and resins with a small grain size are required to shorten the analysis time.

Acidic-alkaline properties of amino acids are the basis of ion exchange column chromatography. Amino acids are organic compounds, which have at least one carboxylic acid group (acidic) and one amino group (basic) in the alpha-position relative to the carboxylic acid. In order for a mixture of amino acids on the column to

take place, the cation exchanger is pre-equilibrated with a buffer solution of sodium citrate or lithium citrate. Functional groups have, respectively, the form: - $SO_3^-Na^+$ or - $SO_3^-Li^+$. Amino acid molecules at pH 3 (and less) have a positive charge. When applied to a column of a mixture of amino acids at pH 2.2, the molecules of amino acids are attracted by ionic forces to the resin sulfo-group with their positively charged amino group and the ions of Na^+ or Li^+ are squeezed, distributed by column, depending on the size of the positive charge. The main amino acids of lysine, arginine, and histidine have the highest positive charge, so they immediately and firmly bind to the resin. The glutamic and asparagine amino acids have the least positive charge, so they pass through the entire column and connect with the last resin. On the process of distribution of amino acids on the column also affect the lateral radical amino acids. Further, the elution (washing out) of amino acids occurs under certain conditions: at high speed, at high pressure and temperature, and using five stages of change of the eluents. The sequence of the release of individual amino acids from the chromatographic column is determined not only by the properties of the cation exchanger but by the composition and temperature of the eluents.

The successful separation of amino acids in the core processes of ion exchange chromatography largely depends on the selection of conditions where this process takes place. These conditions include:

- quality and granulometric composition of ion exchangers;
- the size of the chromatographic column;
- the material from which the column is made;
- nature and properties of buffer solutions (eluents);
- temperature;
- the speed of the elution process;
- equipment and auxiliary devices for conducting chromatography;
- equipment for the detection of amino acids.

3. *Registration of amino acids.*

For registering amino acids in eluates, it is used the method of detection by ninhydrin. Ninhydrin (II), interacting with an amino acid on an amino group, forms a

compound of hydrindantin, which gives a coloring in the 560-nanometer area (except for a combination with proline and oxyproline, having a maximum absorption of 440 nanometers). In the normal mode of analysis, the ninhydrin reagent (a mixture of ninhydrin, a buffer solution and ninhydrin chloride in an argon atmosphere to prevent oxidation of ninhydrin and should be stored in a place protected from light and heat) is added to the fluid that is washed out of the column. The mixture is then heated to 100 °C in a reaction bath (it is a very important length of the reactor for the reaction to pass through).

4. *The principle of the amino acid analyzer*.

The basis of the amino acids automatic analyzer (developers Spekman, Stein and Moore) created a very sophisticated and simple principle of conducting all operations of analysis in a continuous flow of eluent. The principle of operation is that the reservoir eluent through the pump (which dosages) enters through the chromatographic column. At the output of the column to the eluate of the micro pressure, the ninhydrin reagent is continuously pumped in a certain ratio with the eluant. A mixture of ethylate of a ninhydrin reagent is sent to a reactor under a capillary tube, which is heated to a temperature of 95-98 °C and then sent to a flow cell. The intensity of the emergence of color is measured by photocolorimetry with the help of a photocell, in which light from the source passes through the walls of the cuvette. The signals of the photocell are amplified and recorded with a recording potentiometer in the form of a chromatogram. The peak area on the chromatogram is calculated and compared with the peak area of the amino acids with a known concentration. From the comparison of these areas, the calculation of the absolute amount of amino acids in the analyzed sample is made.

Recently, instead of the two-column method (when acidic and neutral amino acids are divided into large columns, and the main ones - on a small one), a single-column method of amino acids separation is widely used. This method allows reducing the consumption of reagents and the material to be studied, eliminating the quantitative differences in the dosage of samples for two columns.

The commonly accepted method for the separation of amino acids on ion-exchange columns is a method using sodium citrate buffers as eluents (a solvent that displaces amino acids from a chromatographic column). However, sodium citrate amide buffers (glutamine and asparagine) and non-proteinaceous amino acids (ornithine, citrulline, β-alanine and many other substances present in biological fluids) are not separated. Therefore, recently, lithium citrate buffers have been successfully used as eluents. It is believed that divergences in the distribution of amino acids in the use of lithium or sodium citrate buffer solutions are due to hydration. The last strongly bound most hydrated ions. Using lithium citrate buffer systems on ion-exchange columns, up to 60 ninhydrin-positive compounds can be separated. The analysis time increases.

The elution of amino acids from the ion exchange column is carried out alternately by lithium citrate buffer solutions pH 2.75; pH 2.95; pH 3.2; pH 3.8; pH 5.0 The ratio of ninhydrin reagent and eluent 1: 2; The temperature of the thermostating column is 38.5 ° C and 65 ° C. The sample is diluted in lithium citrate buffer pH 2.2 and applied to the ion exchange column using a dispenser.

5. *Calculation of qualitative and quantitative content of amino acids.*

In order to calculate the amount of amino acids in the test sample, a standard mixture of amino acids with a known concentration of each amino acid is pre-applied to the column of the auto analyzer of the amino acids. On the chromatogram, the peak area of each amino acid (or peak height) is calculated. The quantity of micromoles of each amino acid (X1) in the test solution is calculated according to the formula: X1 = S1/SO, where S1 is the peak area (or height) of the amino acid in the test sample, S0 is the peak area (or height) of the same amino acid in the solution of the standard mixture of amino acids, which corresponds to 1 μmol of the amount of each amino acid. The quantity in milligrams is obtained by multiplying the quantity of micromoles of an amino acid to its corresponding molecular weight. The qualitative composition of the amino acid mixture is determined by comparing the chromatograms of the standard and the studied mixture of amino acids.

6. *Preparation of samples for analysis*.

It is of great importance and is the first prerequisite for obtaining reliable and reproducible results when working on the automatic analyzer of the amino acids correctly selected method of preparation of the sample. The preparation of samples consists in the breaking of amino acids bounds in proteins, peptides that require hydrolysis, and the preparation of samples containing free amino acids (biological fluids, tissue extracts), which eliminate proteins and other substances that interfere with the analysis.

The most commonly used method is hydrolysis with hydrochloric (hydrochloric) acid. Hydrolysis is carried out as follows:

At the bottom of the test tube of refractory glass (Pyrex) place a carefully weighed sample with a dry protein content of about 2 mg or an equivalent amount of water solution of protein. To the dry weight of the protein in the test tube add 0.5 ml of distilled water and 0.5 ml of concentrated hydrochloric acid. To the water solution of the protein, add an equal amount of concentrated hydrochloric acid. The test tube is cooled in a mixture of dry ice with acetone or liquid nitrogen. After the contents of the test tube freeze, air is pumped off from it by means of a vacuum pump to prevent the oxidation of amino acids by hydrolysis. Then the test tube is sealed. The precipitated test tube is placed in a thermostat with a constant temperature of +106 °C for 24 hours. Upon completion of the hydrolysis, the test tube is exposed, pre-cooled to room temperature. The contents are quantitatively transferred to a glass beaker and placed in a vacuum - a desiccator over granular sodium hydroxide. Then air is removed from the desiccator using a water jet pump. After drying the sample, 3-4 ml deionized water is added to the weighing bottle and the procedure is repeated for drying. It is also possible to remove hydrochloric acid in a water bath under the fume hood. The sample prepared in this way is dissolved in 0.3N lithium citrate buffer pH 2.2 and applied to the ion exchange column of the amino acid analyzer.

To determine the tryptophan, alkaline hydrolysis is used, which can not be used to identify other amino acids, because in the case of alkaline hydrolysis quantities of amino acids undergo changes.

Deproteinization (protein precipitation) of samples for the preparation of an extract of free amino acids and low molecular weight compounds (peptides) can be carried out by the following methods:

1) Sulphosalicylic acid.

2) Picric or trichloroacetic acid (used very rarely, because before drawing a sample thus obtained on the device, the sample must be thoroughly cleaned from the acid residues).

3) Gel filtration.

4) Ultracentrifugation.

5) Hot ethanol (mainly for samples of vegetable origin).

6) Acetic Acid with Acetone.

7) Acetone.

More often than not others use the method of deproteinization of samples with sulfosalicylic acid (it is used for virtually all proteins, except for acid-soluble ones). To precipitate the protein, add 1 ml of biological fluid or tissue extract to a pure centrifuge tube, add 1 ml of 3% water solution of sulfosalicylic acid and mix thoroughly. After that, the released protein is separated by centrifugation at 3500-4500 rpm within 30 minutes. The supernatant thus obtained is applied to the ion exchange column of the amino acid analyzer. The supernatant, in addition to free amino acids, naturally contains numerous peptides of low molecular weight, which often carry very important information, because biologically active substances released from animal tissues and tissues of plant origin are most often peptides. To determine the amino acids composition of these peptides, they must be subjected to hydrolysis using the method described above.

2.3.19. Determination of the contents of serotonin and tryptophan in the brain, duodenum and blood serum

Blood serum and tissue homogenates are mixed with 0.4 M perchloric acid for protein precipitation in a ratio of 1: 5 (w/v). Samples are maintained at 4 °C for 1 hour. Further samples are centrifuged for 5 minutes at 800 g in a centrifuge with

cooling at 4 °C. After separating the phases, a supernatant is taken out, in which the pH is adjusted to 5-6 using 2M KOH. Samples are re-precipitated in a centrifuge with cooling at 4 °C. The supernatant is applied on a column of KM-sepharose, which is equilibrated with 0.01 M Na-phosphate buffer pH 6.2. The elution is carried out at room temperature with buffer I (0.01 M Na-phosphate buffer, pH 6.2) and buffer II (0.03 M Na-phosphate buffer, pH 6.2). Buffer I elutes tryptophan, and buffer II - serotonin. The tryptophan measurements are carried out on a spectrofluorophotometer at a wavelength of excitation of 295 nm and the absorption wavelength of 550 nm against a “blind” sample that suppresses distilled water [39, 77]. Measurement of the serotonin is carried out on a spectrofluorophotometer at an excitation wavelength of 359 nm and an absorption wavelength of 485 nm against the "blind" sample with pouring distilled water [109].

2.3.20. Determination of monoamine oxidase activity of blood serum

The monoamine oxidase (MAO, EC 1.4.3.4) activity in the blood serum can be determined by the Balakleievsky method and others [4]. The basis of this method is the formation under the action of monoamine oxidase benzaldehyde from benzylamine hydrochloride. Benzaldehyde interacts with 2,3-dinitrophenylhydrazine and forms an insoluble hydrazone that can be precipitated by centrifugation. The hydrazone, in turn, in the alkaline medium forms a stable colored compound of raspberry color, the content of which can be determined spectrophotometrically.

The test tubes contain incubation medium which is prepared by mixing 0.5 ml of 0.2 M phosphate buffer, pH 7.4, 2 ml of distilled water and 0.1 ml of 1% solution of benzylamine hydrochloride. "Blind" sample does not contain benzylamine hydrochloride. The reaction is started by applying 0.5 ml of blood serum to the incubation medium. Samples were incubated for 3 h in a thermostat at 37 ° C. The reaction is stopped by adding 1 ml of 20% trichloroacetic acid to the investigated samples. Precipitation of the proteins is carried out by centrifugation for 10 minutes at a speed of 600 g. Take the supernatant and add 0.5 ml of 0.1% solution of 2,3-dinitrophenylhydrazine (prepared previously on 2M HCl). Samples are mixed and left

for 25 minutes at room temperature. Hydrone precipitation is then carried out by centrifugation for 25 minutes at a speed of 700 g. To the hydrazone is added 5 μl of 3M NaOH and stirred. Then 1 ml of 96% ethanol is added to the samples, and the development of raspberry color is observed.

The ability of hydrazone is determined spectrophotometrically at a wavelength of 460 nm versus ethanol.

The activity of monoamine oxidase is expressed as a percentage of the control value, calculating the units of extinction per 1 ml of blood serum for 1 hour.

2.3.21. Determination of the glucose concentration in the blood

The concentration of glucose in the blood is determined in the adolescent animals [6, 8], which are hungry for at least 2 hours. Blood is taken from the diseased vein with a catheter. The concentration of glucose is determined using the glucose oxidase method, using a glucometer "GLUKOPHOT-II" (Ukraine), which is used according to the procedure [57].

On test strips containing a complete set of reagents for glucose oxidase reaction, apply drop of whole blood and maintain for 30 seconds at room temperature.

Next, the strips are washed with distilled water and placed in a test cell displaying a mini-photometer.

A concentration of glucose is expressed in mmol/L.

2.3.22. Determining the amount of insulin in the blood serum

The ability of insulin in the blood serum of rats is determined by the immunoenzyme method. ELISA is carried out in microplates with sorbent ability according to the common method for soluble proteins. The antigen, pre-diluted in 0.1 M $NaHCO_3$ buffer, pH 9.6, to a concentration of 10 μg/ml, was incubated in a well of plates at 4 °C for 12 hours. Removal of the unsorbed antigen is carried out by the three-fold washing of the wells with a buffer for immobilization. The incubation was carried out in TBS (50 mM tris-HCl and 150 mM NaCl) pH 7.4, or in PBS (137 mM

NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) pH 7.4. Non-specific mice of the binding are blocked by incubation of wells with 5% skimmed milk or 3% bovine serum albumin solution for 60 minutes in a thermostat at 37 °C. Purification of wells is carried out by three times washing with working buffer (TBS or PBS) with 0.1% tween-20 addition. Primary antibodies are diluted with a working buffer to a concentration of 5-50 μg/ml, then they are added to plate wells and incubated for 1 hour in a thermostat at 37 °C. After that the wells are washed three times with 0.1% tween-20 working buffer. From this, a secondary antibody conjugate with visualization enzymes (alkaline phosphatase or horseradish peroxidase) was incubated for 1 hour in a thermostat at 37 ° C. The wells are again washed three times with 0.1% tween-20 working buffer and incubated with the absorbance for imaging enzymes (1 mg/ l para-nitrophenol phosphate (pNPP) in 10% diethanolamine pH 9.8 or 1 mg/ml tetramethylbenzidine (TMB) in 50 mM phosphate citrate buffer pH 5.0 with 0.013% H_2O_2, respectively) for 1 h at 37 °C. Optical absorption is measured at a wavelength of 450 and 550 nm on a microplate reader. The optical error of the plate is determined by subtracting the indices at 550 nm from the index at 450 nm. The ability is to indicate the value of the counts from the control value, which is taken as 100%.

2.3.23. Determination of the concentration of glycosylated hemoglobin in the blood

The concentration of glycosylated hemoglobin in the blood of animals is determined by a spectrophotometric method, using standard "Pliva-lachema diagnostic" kits (Czech Republic) [20]. To obtain hemolysate erythrocytes, 1 ml of whole blood is centrifuged twice, washing 3 ml with a 0.9% solution of NaCl and distilled water. Hemolysate of erythrocytes is mixed with 85% H_3PO_4 and incubated in a boiling water bath (at boiling temperature) for 30 minutes.

Next, 250 μl of 5% trichloroic acid is added to the samples and centrifuged at 1000 rpm for 15 minutes. A 16.6% solution of thiobarbiturate is added to the supernatant and the tubes are incubated for 40 minutes in a thermostat at 37 ° C. The

optical density of the samples was measured spectrophotometrically at a wavelength of 443 nm against distilled water. The content of glycosylated hemoglobin was calculated by the formula:

G (μmol fructose/g hemoglobin) = $\frac{A_1 - (A_2 + A_3)}{C \times K}$;

where *G* is glycosylated hemoglobin, A_1 is an extinction of the test-sample, A_2 is an extinction of control sample with reagents, A_3 is an extinction of sample with positive control, *K* - tangent of angle of inclination, calculated according to the calibration graph for fructose, *C* - concentration of total hemoglobin (g/L), determined using the standard set of reagents. The concentration of glycohemoglobin was expressed in μmol fructose/g hemoglobin.

In this chapter, there are presented numerous methods for studying the exchange of such biomolecules in the blood as: proteins, amino acids, amines (determination of the concentration of lipoproteins, creatinine, urea, free amino acids and proteins, their electrophoretic separation in PAAG, electrophoretic densitometry, AST, ALT, γ-glutamyltranspeptidase activity, serotonin, tryptophan, and cytokines content), lipids (determination of cholesterol concentration, TAG, alkaline phosphatase activity, bilirubin concentration), and carbohydrates (determination of α-amylase activity, the concentration of glucose, glycosylated hemoglobin, uric acid, insulin content).

CHAPTER 2.4
BLOOD RESEARCH WITH METHODS OF MOLECULAR DIAGNOSTICS

2.4.1. Methodical approaches to molecular diagnostics using the reaction of interaction of antigen with an antibody

At present, methodical approaches to molecular diagnostics are rapidly developing, since traditional methods of immunoassay, such as serological reactions, agglutination reaction, complement fixation and hemolysis, and others. are less sensitive and have a numerous shortcomings [9, 10, 12, 33, 94]. Thus, serological reactions differ in their ability to detect individual antibody classes; the agglutination reaction well detects IgM antibodies, but less sensitive to the detection of lgG antibodies, complement fixation and hemolysis (which require the participation of the complement). They do not detect antibodies that do not attach the complement, for example, IgA-antibodies and IgE-antibodies, and in the neutralization reaction of viruses but detect only those antibodies that are directed against the antigenic determinants of the vibrational pathogen-related surface.

The sensitivity of immunological research methods exceeds all other methods of antigens and antibodies studying, for example, radioimmune and immunoassay analyze that allow us to capture the presence of protein in amounts measured in nanograms and even in picograms [12].

Using immunological methods of research, it is possible to determine the group of blood system, to check it for the safety of the bioprocess (for example, for the presence of hepatitis B or HIV infection).

In transplanted organs and tissues immunological research methods allow determining the compatibility of tissues and their testing for inhibiting their incompatibility.

Forensic medicine uses the Castellani reaction to determine the specificity of the protein and the agglutination reaction to determine the group of blood systrem.

The development of immunological methods of research is carried out both through the improvement of reagents (purity of antigens and antibodies), as well as through the creation of automated systems for the formulation of reactions and their instrumental accounting.

Depending on their mechanism and the results of the record, immunological research methods can be divided into

- reactions based on the phenomenon of agglutination;
- reactions based on the phenomenon of precipitation;
- reactions with the complement;
- neutralization reaction;
- reactions using chemical and p

hysical methods.

As an example, reactions based on the phenomenon of agglutination can be considered. Agglutination is the gluing of cells or individual particles - carriers of antigen using immune serum to this antigen.

The reaction of agglutination of bacteria using an appropriate antibacterial serum is related to the simplest serological reactions.

A suspension of bacteria is added to various dilutions of the tested serum and after a certain period of time at t = 37 ° C, the agglutination occurs at the highest dilution of the blood serum.

Immunological methods are widely used in laboratory diagnostics of infectious diseases.

The etiology of the disease is also established on the basis of the increase of antibodies as response to the pathogen in the re-convalescent blood serum compared with the breakdown taken in the early days of the disease.

On the basis of immunological research methods, the immunity of the population is studied in relation to the spread of infections during mass gathering, for example, crowding, influenza, and also for assessment the effectiveness of prophylactic vaccinations.

The development of immunological methods of research contributed to the creation of monoclonal antibodies produced by hybridoma, resulting from the fusion of the immunocompetent cell of B-lymphocyte and cells of myeloma mice.

Immunogenicity test or immunoenzyme methods are based on the use of antibodies conjugated to enzymes, mainly horseradish peroxidase or alkaline phosphatase. To detect the combination of labeled antibodies with the antigen, adding a substrate that cleaves the enzyme (attached to lgG), staining in a yellow-brown (peroxidase) or yellow-green (phosphatase) color [9, 10, 12, 33, 94].

It is also used the enzymes that break down not only the chromogenic but also the luminogenic substrate. In this case, with a positive reaction, a glow appears.

Like immunofluorescence, the immune enzyme method is used to detect antigens in cells or titration of antibodies in antigen-containing cells. The most popular variant of the immune-enzyme method is immunoabsorption [9, 10, 12, 33, 94].

On a solid carrier, this can be cellulose, polyacrylamide, dextran and various plastics, antigen is sorbed. Often, the carrier is the surface of the wells of micro plates In the wells with a sorbent for tesing entered antigen in blood serum is labeled with an antiserum and substrate enzyme. Positive results are taken into account when the color of the liquid medium is changed.

To detect antigens on the carrier, the antibodies are sorbed, then the studied material is added into the wells and the reaction of the enzyme-labeled antimicrobial blood serum occurs. Increasing the sensitivity of immunofluorescence immunoenzyme methods contributes to avidin and biotin which are added to reaction medium.

The radioimmunoassay method is based on the use of the radioisotope tag (label) of antigens or antibodies. It is the most sensitive method for detecting antigens and antibodies, used to identify hormones, drugs, and antibiotics, for the diagnosis of bacterial, viral, rickets, protozoal diseases, blood protein studies, and tissue antigens.

Initially, it was developed as a specific method for measuring the level of circulating blood hormones.

Test system: Radionuclide-labeled hormone (antigen) and antiserum to it. If anthrax spores is added to the antiserum containing the desired hormone, then they will bind by portion of the antibodies under the subsequent addition of the labeled titrated hormone with the antibodies as result binding will be reduced compared with its amount in the control.

The result is evaluated by comparing the curves of the bound and unbound radioisotope.

This kind of method is based on a competitive reaction. There are other modifications of the radioimmunoassay.

Immunohistological methods are used to detect antigens on the surface or inside the cell, for example, to detect lymphocyte markers and immunocomplexes in glomerulonephritis and other kidney diseases.

This method for the detection of antigens uses either immunofluorescence or immunoenzyme conjugates with peroxidase.

The numerous specific antigens is determined by the intensity of staining. Sometimes for auto-registration a spectrophotometer is used.

Immunoblotting is used to detect antibodies to individual antigens or to "recognize" antigens from known sera (fig. 2.15).

The method consists of 3 steps: 1) separation of biological macromolecules (for example, a virus) into separate proteins by electrophoresis in a polyacrylamide gel; 2) the transfer of separated proteins from gel to a solid substrate (blot) by placing a plate of polyacrylamide gel on activated paper or nitrocellulose (electroblotting); 3) detection of the substrate of the desired proteins by direct or indirect immunoenzyme reaction.

Immunoblotting is used as a diagnostic method at HIV infection. Diagnostic value has the detection of antibodies to one of the proteins of the outer skin of the virus.

In order to determine the concentration of immunoglobulins in separate classes, several methods are used, more often use the method of radial immunodiffusion in the gel (according to Manchin) - a kind of reaction of precipitation and ELISA [12].

Definition of antibodies of different classes is important for the diagnosis of infectious diseases. Detection of antibodies to antigens of microorganisms in blood serum is an important criterion when the diagnosis is made - serological method of diagnosis. Antigens of the IgM class appear in the acute period of the disease and disappear relatively quickly, antibodies of the IgG class are detected at a later date and longer (sometimes-years) are stored in blood serum of the sick, they are in this case called anamnestic antibodies.

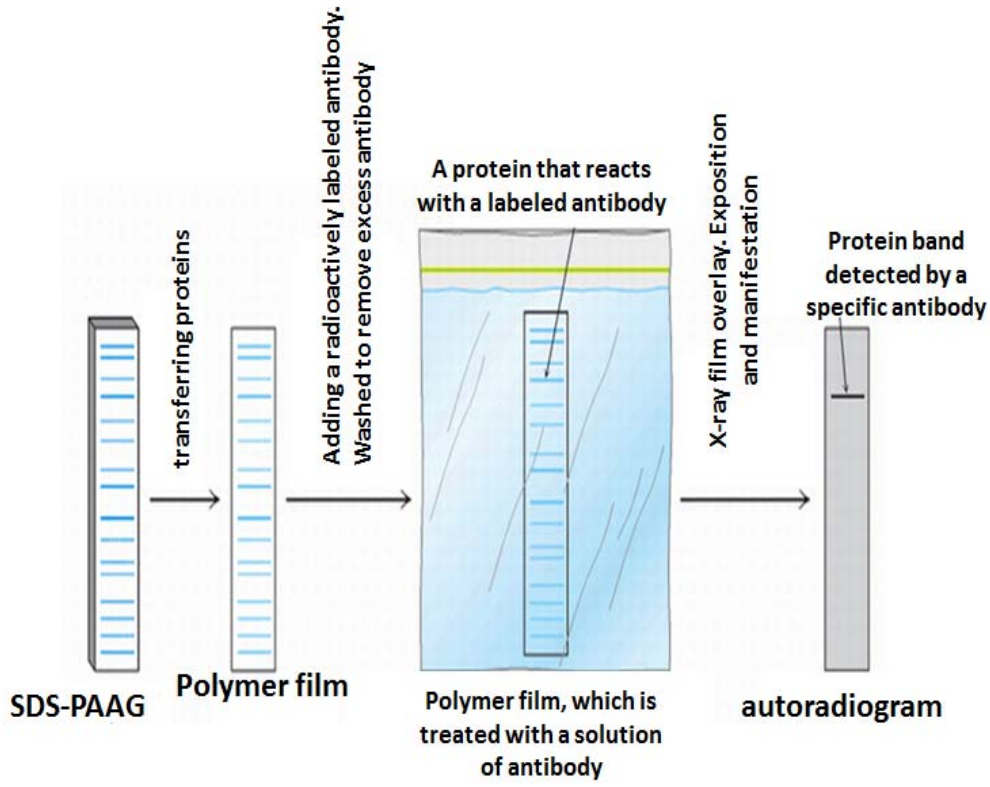


Fig.2.15. "Western"-blotting. Proteins from SDS-PAAG are transferred to a polymeric film and stained with a radiolabeled antibody. A strip corresponding to the protein to which the antibody is bound is detected on an autoradiogram, SDS (Sodium dodecyl sulfate) [68].

Allocate the concept: antibody titer, diagnostic titer, the study of a pair of serums. The greatest significance is the detection of IgM antibodies and the fourfold increase in antibody titers (or seroconversion - antibodies are detected in the second test with negative results from the first blood serum) in the study of paired - taken in the dynamics of the infectious process with intervals of several days-weeks of sampling.

"Antigen-antibody" reaction. Reactions with the use of labeled antibodies and antigens.

The reaction "antigen-antibody" (Ag-AT) is a specific interaction of antibodies with the corresponding antigens, resulting in complexes of antigen-antibodies (immune complexes) [9, 10, 12, 33, 94]. Often the final result of this reaction is the binding of toxins, immobilization of virulent bacteria and neutralization of viruses. The "antigen - antibody" reaction takes place in two stages, differing in their mechanism and velocity. The first stage is a specific binding of the active center of antibody with the corresponding groups of antigen or hapten; the second - the non-specific stage is actually a visually observable reaction. By interaction of antibodies with simple haptens, the second stage may be absent. In the absence of salts, the first stage can be realized, while the second can’t. Usually the first stage of the reaction occurs quickly but the second - sometimes very slowly.

The binding (or association) of the antigen with the antibody is reversible, and the strength of their binding is called an affinity, which can be quantified by determining the association constant.

The formation of insoluble complexes "antigen-antibody" due to the binding of soluble antigen with specific antibodies, followed by their sediments forming is called the phenomenon of precipitation. A special case of precipitation is the reaction of immune flocculation, which takes place only in a relatively narrow range of antigen concentrations, and soluble complexes are formed for a small excess of antibodies and antigen. The reaction of precipitation is used for the quantitative determination of antigens and antibodies, the concentration of immunoglobulins of

different classes in human blood, in forensic medical examination to determine the specificity of blood serum proteins using the Chistovich-Ulengut reaction.

The ability of antibodies to bind antigenic particles to large conglomerates (agglutination of bacterial and other cells, precipitation of dissolved antigens) is conditioned by the presence of at least two active centers in the antibody molecule. One specific group binds with one antigenic determinant, the second - with a similar determinant of another antigenic particle. Such antibody bivalence provides the possibility of binding an unlimited quantity of antigenic particles into conglomerates. By the different amounts of antigenic determinants on the antigen molecule, the nature of the structure of the conglomerates of the antigen-antibody complex may be different. Due to the excess of antigen or antibodies, large conglomerates do not appear because of the "overlapping" of reacting regions of molecules with an excessive amount of the second component. Hence, the "antigen - antibody" reaction manifests itself to a maximum extent only in a certain range of concentration of both reagents, which is called the zone of equivalence.

Numerous biological (effector) antibody functions are manifested by the interaction of the antigen with the antibody. Such functions include phenomena of binding of the complement, lysis, antibody-dependent cytotoxicity and opsonization.

The addition of the complement to the antigen-antibody complex is called the phenomenon of the complement binding that is able to self-assembling a multicomponent system of blood proteins, which possess one of the key roles in maintaining immune homeostasis. By activating the complement system caused by the "antigen-antibody" reaction, there are numerous disturbances of homeostasis due to cells damage or a change in their functions. This activation is provided only by two classes of antibodies: IgG and IgM. Because of the specificity of antibodies, the type of target cells and the nature of the participants in the reaction of the components of the complement there are possible irreversible damage of the cell membrane, increased susceptibility to phagocytosis, the release of pharmacological agents such as histamine, directed cell migrations (chemotaxis). The complement binding reaction

is used in the diagnosis of syphilis (Wasserman's reaction), a numerous viral infections, in the study of anti-tissue antibodies and autoantibodies [12].

The specific "antigen-antibody" reaction is the basis of several techniques that identify and determine the protein in the sample with the antigen-antibody complex.

ELISA (Enzyme-Linked Immunosorbent Assay) is a solid-phase immunosorbent assay in which, for identification of a substance, typically an antigen, in a liquid or wet sample antibodies (primary and secondary) are used observation of colour changing as a result of substrate transformation into a product by the action of the enzyme that is linked to the secondary antibody [9, 10, 12, 33, 35, 94].

ELISA is used as a diagnostic tool in medicine and pathology of plants, as well as a quality control test in various industries.

The antigens of the sample attached to the surface of the microtiter plate are also applied with specific antibodies (primary antibodies) so that they can contact the antigen. Then the secondary antibodies bound to the enzyme are applied. At the final stage, an enzyme substrate (bound to a secondary antibody) is added which converts it into a color product, which is then determined visually and/or using the device.

It should be noted that between each "step of experiment", that is, adding an appropriate component of the ELISA test system: antigen, primary and secondary antibodies; the plate is usually washed with a weak solution of detergent to remove any proteins or antibodies that are not bound. After the last stage of the plate washing a substrate is added which, by action of the enzyme, causes the change in the color of solution in the wells, the intensity of which indicates the amount of antigen in the sample.

By performing the "indirect" ELISA method, there are distinguished such stages:

1) a buffer solution of the test antigen is added to each well of the microtiter plates, where it maintains a certain time for attachment to the plastic surface due to interaction with the surface of the microplate wells;

2) to block any plastic surface of the well that remains uncovered by the antigen, a solution of carrier protein (which does not interact specifically with antibody) is added, for example, a blood serum albumin of a bull or casein;

3) a primary antibody that specifically binds to the antigen attached to the surface of the well, is added. This primary antibody may also be present in the blood serum of the donor's blood and can be tested for reactivity to the antigen;

4) a secondary antibody that binds to the primary antibody, is added. An enzyme is often attached to such a secondary antibody, and it has little effect on the binding properties of the antibody. In some cases, the primary antibody is specifically conjugated to the enzyme;

5) the substrate for this enzyme is then added. Often, this substrate changes the color when it reacts with the enzyme. Change of the color indicates the binding of the secondary antibody to the primary, that is, the corresponding donor immune response to the antigen-test, which is often used in clinical and scientific studies;

6) the higher concentration of the primary antibody present in the blood serum, the more intense the color change of the reaction mixture, therefore the spectrometer is often used to determine the quantitative values of the colour intensity.

ELISA can be used both in qualitative and quantitative form. Qualitative results provide a simple positive or negative result (yes or no) for the sample. The difference between a positive and a negative result is determined analytically and can be statistically counted. A two-or three-time standard deviation (inherent to a test error) is often used to distinguish positive from negative samples. The quantitative determination using the ELISA test system is based on measurement the optical density (OD) of the sample which then compared to the standard curve, obtained as the result of serial dilution of a known solution concentration of the target molecule. For example, if the test sample refers to OD = 1.0 on standard curve, then the concentration of the analyzed substance should be within the same range as this sample.

The less common version of the "sandwich" - ELISA method is used to detect a specific antigen sample:

1. A "ready" surface in advance, in which there is a known amount of immobilized antibodies.

2. Any sites of non-specific binding on the surface are blocked.

3. A sample with antigen is applied to plate.

4. The plate is washed to remove the unbound antigen.

5. The specific antibodies that bind to the antigen (hence the name "sandwich": the antigen is "located" between two antibodies) are added.

6. Solid-phase secondary antibodies are used to detect antibodies that also specifically bind to the Fc region of the antibody (non-specific).

7. The plate is washed to remove the unbound antibody-enzyme conjugates.

8. Chemical substances are added to convert them with an enzyme that is detected by color change, fluorescently, or by electrochemical signal.

9. The color product, fluorescence or electrochemical signal (for example, potential) is measured in the wells of the tablet to determine the presence and amount of antigen.

For example, ELISA "sandwich" is used for home pregnancy testing.

The radioimmunological method is based on the use of radioisotope labels of antigens or antibodies. It is the most sensitive method for detecting antigens and antibodies, used to identify hormones, drugs and antibiotics, for the diagnosis of bacterial, viral, rickets, protozoal diseases, blood protein studies, and tissue antigens. Initially, it was developed as a specific method for measuring the level of circulating blood hormones.

Test system: the radionuclide-labeled hormone (antigen) and antiserum to it. If such an antiserum is added to a material containing the desired hormone, then it will bind a part of the antibodies, with the subsequent adding of the labeled titrated hormone with antibodies binds reduced, comparing with the control. The result is evaluated by comparing the curves of the bound and unbound radioactive labels.

This kind of method is based on a competitive reaction. There are other modifications of the radioimmunological method.

Immunohistological methods are used to detect antigens on the surface or inside the cell, for example to detect markers of lymphocytes and immunocomplexes with glomerulonephritis and other kidney diseases.

In this reaction, for the detection of antigens use either immunofluorescence or immunoenzyme conjugates with peroxidase. The quantity of specific antigens is determined by the intensity of staining. Sometimes for auto-registration a spectrophotometer is used.

Immunoblotting is used to detect antibodies to individual antigens or to "recognize" antigens from known sera (Fig. 2.15).

To identify specific proteins in cell or tissue extracts (which have been pre-fractionated by electrophoresis and transferred to a nitrocellulose or PVDF membrane), western blot is used. Transferring proteins from the gel to the membrane allows to incubate them with a specific antibody and to carry out further visualization of the resulting bands.

Preparation of samples may be different depending on the physical and chemical properties, functional and structural characteristics of the protein being studied. Preferably, the tissues (from which the protein is obtained) are mechanically crushed and homogenized in the presence of powerful detergents (β-mercaptoethanol or Triton X-100). It is important to measure the total protein concentration (by the Lowry or Bradford method) and equalize it in different samples by dilution to facilitate comparison of final results.

High efficiency of the method is achieved by electrophoretic separation and specificity of monoclonal antibodies (antigen can be detected in quantities less than 1 nm).

Stages of western blot [35]:

1. **Separation of proteins** by gel electrophoresis in SDS-PAGE.

2. **Transfer of proteins to the membrane**. In the electroblotting method, transferring gel proteins to nitrocellulose or polyvinylidene fluoride (PVDF, Eng.) membrane occurs under the action of an electric current. Proteins move from gel to

membrane with preservation of its location. As a result of this process (Blotting - ingestion, Eng.), proteins are found in a thin surface layer of the corresponding membrane and are available for further binding to antibodies. Both types of membranes are used due to their properties to bind proteins nonspecifically. The binding of proteins is based on hydrophobic and electrostatic interactions between the membrane and the protein. The effectiveness of electroblotting significantly increases using Trans-blot Turbo system, which significantly reduces the time to transfer proteins from the gel to the membrane (7 minutes versus 2 hours).

In the binding of the protein complex-SDS to the nitrocellulose membrane, the main forces of the electric nature are involved, and this interaction is multicellular and leads to the "disintegration" of proteins on the surface of the membrane. Thus, after the electrical transfer to nitrocellulose membrane, there is a replica of the gel with proteins, which are arranged in the same order as in the polyacrylamide gel.

The effectiveness of transferring proteins from gel to membrane can be verified by staining the membrane with Coomassie blue or Ponceau S. The Ponceau S dye, which is more sensitive and better soluble in water, makes it easier to launder and apply antibodies.

3. **Blocking**. Blocking nonspecific bonds is achieved by incubating the membrane in a dilute protein solution - usually a blood serum albumin of a bull or skimmed milk powder with a small percentage of detergent type Tween 20 or TritonX-100. The protein from the diluted solution binds to the membrane in those places where there is no protein to be studied. As a result, the antibody can only bind to specific binding sites on the proteins investigated when added to them. Blocking allows you to achieve a clean area and exclude receiving incorrect results.

4. **Detection** after blocking, the membrane is washed three times with a buffer. The tested proteins are then detected using antibodies by the sandwich method: first, the proteins bind to primary (mono or polyclonal) antibodies, which in turn bind to secondary antibodies conjugated to enzymes (horseradish peroxidase or alkaline phosphatase). Visualization of the investigated proteins is achieved by an appropriate biochemical reaction with the formation of a product, which is determined by

colorimetric, chemiluminescent, fluorescence detection methods. Quantitatively, the protein can be estimated using densitometry.

Chemiluminescent detection has a higher sensitivity in comparison with the colorimetric method. The interaction of luminol and peroxide in the presence of horseradish peroxidase, conjugated with secondary antibodies, leads to the formation of an oxidized luminol, which is luminescent at 425 nm. The intensity of chemiluminescence is proportional to the amount of protein to be studied (antigen). Detection of chemiluminescence (as well as fluorescence) is carried out using a gel - documented system ChemiDoc MP, which has high sensitivity. The ChemiDoc MP system is equipped with CCD high-sensitivity special cameras and a powerful cooling system. These advantages allow you to accurately record fuzzy, vague bands. Availability of the software allows carrying out qualitative and quantitative analysis of the received data and comparing them with standard proteins.

Depending on the investigated protein, primary and secondary antibodies and their corresponding reagents are selected, taking into account the presence of a particular detector.

As a diagnostic method, immunoblotting is used to determine HIV infection. This diagnostic method allows to detect antibodies to one of proteins of the virus outer envelope [35].

In order to determine the concentration of immunoglobulins of separate classes, as indicated above, several methods are used, more often, the method of radial immunodiffusion in a gel (by Manchin) which is based on reaction of precipitation and ELISA.

Definition of antibodies of different classes is important for the diagnosis of infectious diseases. As noted above, the detection of antibodies to antigens of microorganisms in blood serum is an important criterion in the diagnosis - the serological method of diagnosis. Antigens of the IgM class appear during the acute period of the disease and disappear relatively quickly, antibodies of the IgG class are detected at a later period and are more prolonged (over the years) stored in the blood serum of the sick, their name in this case are anamnestic antibodies.

2.4.2. Using polymerase chain reaction in blood studies

At present, the polymerase chain reaction (PCR) is widely applicated in laboratory practice [8, 35, 59]. It is characterized by high specificity, sensitivity, versatility and short study time.

Most often, PCR is used to diagnose infectious diseases caused by agents that are difficult to cure, to determine the resistance of microorganisms to antibiotics, prenatal diagnosis and diagnosis of hereditary diseases, testing of donor blood for viral pathogens, for different types of genotype, identification of paternity, for the detection of mutations, etc.

In clinical diagnosis, PCR is used for: early diagnosis of infectious diseases in seronegative patients, when treatment is most effective; detection of persistent, latent and recurrent forms of infections; control the effectiveness of treatment; epidemiological research; the diagnosis of opportunistic infections, which often occurs in the presence of an immunodeficiency, so that it is difficult to diagnose using only the results of serological studies. PCRs use sanitary and epidemiological organs to control the microbiological contamination of the environment and food products and to identify genetically modified sources of nutrition.

The principle of the method is based on the detection of specific DNA fragments (mRNA) in a material of a variety of bioobjects, their selective synthesis according to concentration, in which they can be easily detected and further determination of the amplification reaction products - amplicons [59].

DNA is a unique carrier of genetic information in all existing organisms with the exception of RNA-containing viruses. The unique property of DNA is its ability to double after the spiral splice and the special difference in DNA strands. DNA doubling (replication) is carried out (on the principle of complementarity) with the enzyme - DNA polymerase. In order for an enzyme to begin its functioning, the presence of an initial double-stranded DNA fragment is required. Such a fragment is formed by the interaction of a short single-strand DNA fragment, called the primer, with the complementary part of the corresponding chain of parental DNA. Replication occurs on two chains of DNA, but they extend in opposite directions. As

a result of replication from one double-stranded DNA molecule, two double-strands, each of them contains one chain from the parental DNA molecule and the second, a subsidiary, are newly synthesized. This implies that the DNA replication cycle includes three main stages: 1) dispersion of the DNA helix and chain divergence (denaturation); 2) the accession of primers and 3) the completion of the subsidiary DNA chain. In the PCR, the indicated processes are carried out *in vitro* in cyclic mode. The transition from one stage of the reaction to another is achieved by changing the temperature of the incubated mixture [8, 35]. For PCR, one must have: 1) two synthetic oligonucleotide primers (approximately 20 nucleotides long) that are complementary to the DNA regions of opposite chains that flank the target sequence (the primers limit the DNA fragment that will be copied millions of times by the enzyme Taq-DNA- polymerase attaching to the 3'-ends of the primers for completing their predetermined length (in several hundreds of base pairs); 2) the target DNA; 3) thermostable DNA polymerase, which does not lose activity at a temperature of 95 °C; 4) four deoxyribonucleotides and 5) a buffer system for the effective operation of DNA polymerase, which necessarily contains magnesium ions.

There are necessary three main stages of the PCR: 1) preparation of a biomaterial test, that is, the release of DNA or RNA; 2) polymerase chain reaction itself (PCR amplification) and 3) PCR product (amplified nucleic acid) detection [8, 35, 59].

Preparation of samples (DNA and RNA from biological material). Samples of a bioobject are specially treated for the course of cell lysis, removal of protein, polysaccharide and lipid components. Various methods are used for this purpose, including sorbent, in which sorption of DNA (RNA) on sorbent occurs after cell lysis, multiple washing of nucleic acids (NA) and subsequent elution of DNA (RNA) buffer solution, etc., is used. As a result of this processing, a solution containing a DNA (RNA) of the investigated object is obtained.

The kit for the isolation of DNA (RNA) is selected depending on the type of bioobject. Detailed methods for extracting DNA (RNA) from bioobjects are described in the instructions, which are added by the manufacturer to the kit of

reagents for the isolation of NA. The resulting solution of DNA can be stored for a week at a temperature of 2 - 8 °C and up to a year (at a temperature of -60 °C). A solution of purified RNA can not be stored. It should be used immediately in research. Samples can only be stored in the form of reverse transcription of complementary DNA (cDNA) solutions.

Typical polymerase chain reaction. At this stage, the following three reactions are repeated many a time: denaturation, renaturation, and synthesis (fig. 2.16).

The first stage of PCR is denaturation of a DNA sample by holding it at a temperature 94-95 ° C. Exept from DNA, in the reaction mixture, should be in excess two primers, a thermostable DNA polymerase Taq (isolated from *Thermusaquaticus*) and four deoxyribonucleotides.

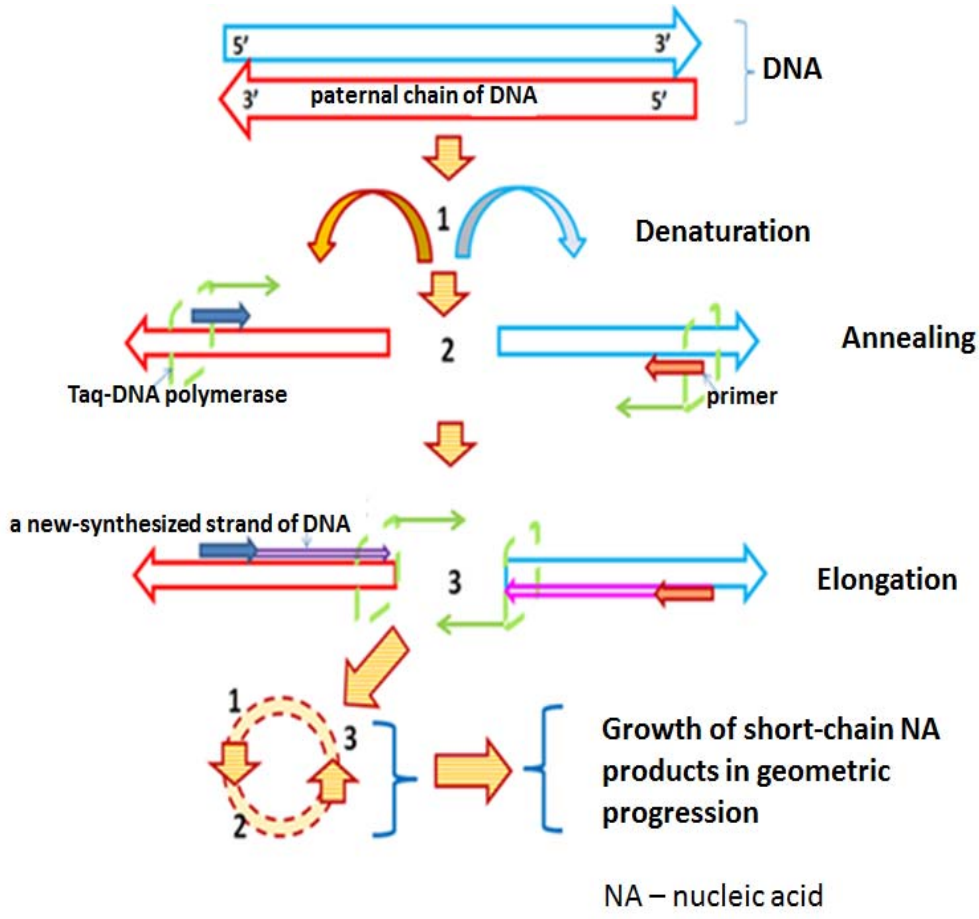


Fig. 2.16. Stages of Polymerase Chain Reaction (PCR) [8,35,59].

The second stage of the PCR is DNA renaturation, which occurs at a reduced temperature of 50 - 60 ° C. During renaturation occur annealing of primers, and they are hybridized with the complementary DNA sequences (molten DNA matrix) with form short double-stranded DNA fragments that are required to function the polymerase enzyme. Each of the primers hybridizes on one of the two chains of the DNA matrix so that the ends of the primers that can extend are directed toward each other. By joining the opposite chains of the DNA molecule, the primers limit their area, which in the future will be multiplied twice (amplified). The length of such a fragment, called the amplicon, is usually several hundred nucleotides.

The third stage of PCR is the synthesis of a fragment of the complementary daughterly chain of DNA (at 70 - 72 °C, that is, the optimum temperature for the activity of the DNA polymerase Taq). As a base for the synthesis, four deoxynucleotide- triphosphates are used in the mixture. Synthesis of fragments of DNA daughterly chains occurs simultaneously on both chains of the parental DNA. Then the cycle repeats again.

The fragments of the DNA chains generated in the first amplification cycle are matrices for the second cycle. Fragments formed in the first two cycles are matrices for the third, etc. Thus, the newly synthesized fragments serve as matrices for the synthesis of new DNA strands in the next amplification cycle, that is, there is a chain reaction. In general, the quantity of copies of a fragment increases in geometric progression and there is accumulation of amplicons in the solution according to the formula: $2 \cdot n$, where n is the quantity of amplification cycles. All reactions are carried out in test tubes using the thermostat. The development of programmable thermostats (thermocyclers or amplifiers), which, according to a given program, carry out cyclic temperature changes, has created the prerequisites for the wide application of the PCR method into the practice of laboratory clinical diagnostics. These devices perform automatic temperature change modes and their maintenance. The duration of each one is several minutes.

To synthesize primers that are specific to a particular DNA target, it is needed to know the nucleotide sequence of the DNA of the corresponding pathogenic

microorganism. The main criterion for selecting primers is the complementarity of the matrix. In this case, only a specific portion of the DNA, whose length is equal to the total length of the two primers and the DNA fragment between them, will be amplified during the PCR. The selection of the optimal temperature, the annealing of the primers on the matrix is an important factor that affects the efficiency and specificity of the amplification.

Modification of the PCR method or reverse transcription on the RNA matrix. Reverse transcription is a two-stage process (performed in both test tubes and in one without loss of sensitivity). In the first stage, an appropriate chain of complementary DNA (cDNA) is obtained on the RNA matrix. For example, reverse transcription is used to detect the genetic material of RNA-containing pathogens (hepatitis C virus). An enzyme that synthesizes cDNAs on an RNA matrix is called reverse transcriptase (revertase). During the second stage, the DNA copy of the viral RNA is amplified.

Detection of PCR amplification products

There are various methods to detect PCR reactions. Specific PCR products (amplicons) are determined by electrophoretic separation of the amplification blend in an agar-colored gel, stained with bromide. Bromide ethidium binds to fragments of double-stranded DNA, which appear in the gel in the form of light bands for UV irradiation (λ = 290-330 nm). To visualize such bands in a gel, using a special instrument – transiliminator and the obtained results are documented by photographing. DNA fragments are separated by molecular weight in agarose gel. The specificity of the amplified DNA bands is confirmed by their placement relative to the molecular weight markers and the placement of a positive control amplification fragment. Additional evidence of the specificity of the amplicon can be obtained by methods of restriction analysis, hybridization and direct sequencing. The technologies that are an alternative way of detecting amplification products include the modified analysis systems using fluorescent stains. In this case, the products of the reaction are analyzed with the help of fluorometry. Test tubes from the amplifier enter the detector, where, without opening their fluorescence will be registered. The levels of fluorescence are proportional to the quantity of generated specific PCR products. In

these variants of the analysis, the fluorescence is recorded after the reaction is completed and therefore the method is not considered quantitative. It is important that the results of the PCR are captured by the presence of fluorescence in closed tubes. Hence one of the important problems of PCR is solved - contamination by amplicons.

Real-time PCR.

Real-time PCR is one of the most modern method options. The base of this option is the quantitative detection of fluorescence signal, which increases in proportion to the amount of PCR product. The result of the reaction can be registered on the screen of the computer monitor directly in the process of PCR amplification. For quantitative research variants in PCR mode, it is necessary to have appropriate standards that are used to construct gauge curves after polymerase chain reaction [90-100].

Using the these curves, it is possible to count the unknown initial amount of copies of DNA (RNA) in the samples. The following are the main advantages of real-time PCR: 1) the registration of fluorescence intensity indicates the amount of infectious agent in the sample; 2) the main reason for “false positive” (incorrect) results is eliminated - scattering of products of amplification with aerosols and personnel in the process of gel electrophoretic detection; 3) it takes only several hours to receive the result (the rate of analysis); 4) the presence of an additional specific probe (which is complementary to the internal region of the amplified fragment) reduces the risk of obtaining incorrect results and increases the sensitivity of the analysis; 5) it is possible to carry out multiple PCRs, that is, to register in the same vial of the presence of several infectious agents using different fluorescent dyes, and 6) appearance of the possibility of identifying single nucleotide substitutions in the product of amplification [8, 35, 59].

Of particular note are the following benefits of PCR as a method of diagnosing infectious diseases.

Direct determination of the presence of pathogens.

Detection of a specific DNA-pathogen region by PCR method is a direct proof of the presence of an infectious agent. Numerous traditional diagnostics, including

immunosensory analysis, detection of protein-markers, that are products of the life of infectious agents. And this only indirectly indicates the presence of infection.

High specificity. In the investigated material, an unique DNA fragment is found to be unique to one of the this pathogen. Specificity is given by the nucleotide sequence of primers, which minimizes the risk of obtaining incorrect results, in contrast to immunological methods of analysis, which often encounter errors due to cross-reactive antigens.

High sensitivity. The PCR-analysis allows to detect pathogens of infectious diseases in cases where other methods (immunological, bacteriological, microscopic) can not perform this [2, 24, 28, 47, 51, 56]. The sensitivity of the PCR assay approximates 10 to 100 cells in the sample.

The versatility of the procedure for detecting various pathogens. The material for research by using PCR is the DNA of the pathogen. This allows you to diagnose several pathogens in one sample. After all, according to the similarity of the chemical composition of all NA determined unified methods for the allocation of DNA/ RNA. For a PCR diagnosis of practically all infectious diseases, one set of equipment, universal procedures for preparation of samples and analysis can be used.

High speed of the result. For the PCR analysis it is not necessary to isolate the pathogen from culture. This significantly reduces the time of research. Unified methods for the isolation of nucleic acids, automation of amplification and detection of reaction products give an opportunity to conduct a complete analysis within one working day.

Ability to diagnose not only acute but also latent infections. Of particular importance is the PCR method for diagnosing heavily cultured and persistent microorganisms, which are often encountered in latent and chronic infections. PCR diagnosis is also very effective in relation to pathogens with high antigenic variability (for instance, influenza viruses).

Limitation of the PCR method can be only due to the fact that, firstly, DNA is amplified as living and non-living microorganisms. This leads to requirements for interpretation and timing of research when controlling the effectiveness of treatment.

Control must be carried out within a certain period of time, during which the complete elimination of the pathogen occurs. Secondly, theoretically there is the possibility of cross-reactions (for example, as a result of inadequate selection of primers). This can lead to incorrect results.

Taking clinical material

The taking material, its pre-processing, storage and transportation, transfer to other organizations is carried out in accordance with instructions and methodological documents that regulate the implamentation of investigation for each type of infectious agent, instructions for kits of reagents and in accordance with the current Sanitary Rules.

In order to prevent the decomposition of nucleic acids, it is recommended to use special transport media, which are developed and recommended by manufacturers of kits of reagents, depending on the type of material.

Transportation and storage of biomaterials should be carried out in a cold chain with the control of the established temperature regime with the help of thermo indicators. Depending on the transport environment, the type of biomaterial and the temperature regime, the terms of transportation and storage of the sample may vary. Considering the possibility of collapse of DNA or RNA in clinical material, its storage and transportation times should be minimal. In case of impossibility to deliver samples to the PCR laboratory for the required time, they are frozen (-20 ° C). Only one-time short-term freezing-defrosting of biomaterial is allowed. Each biological sample can be a biomaterial for PCR. Blood samples (plasma) are used in qualitative and quantitative studies, and blood serum samples only for qualitative PCR analysis. In order to exclude the contamination, the clinical material should be removed using sterile disposable instruments (syringes, appropriate probes, sampling devices, etc.) in disposable sterile plastic containers (for example, Ependorf tubes). Clinical specimens should be stored separately from reagents [59].

Modification of the PCR method or reverse transcription on the RNA matrix. The principle of the method is that the nucleotide sequence of the RNA molecule is

transcribed into the nucleotide sequence of complementary DNA (cDNA) by the reverse transcriptase enzyme (RT), which is amplified in the usual PCR (fig. 2.17, 2.18, [100]).

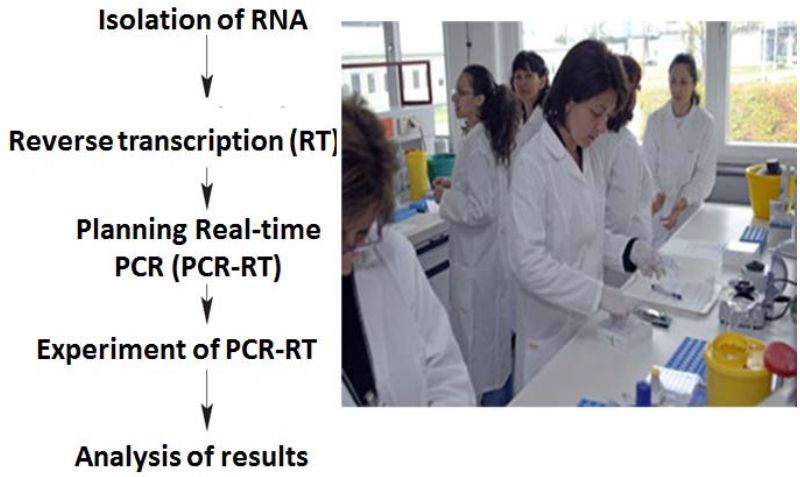


Fig. 2.17. Scheme of analysis of RNA samples using PCR-RT [100].

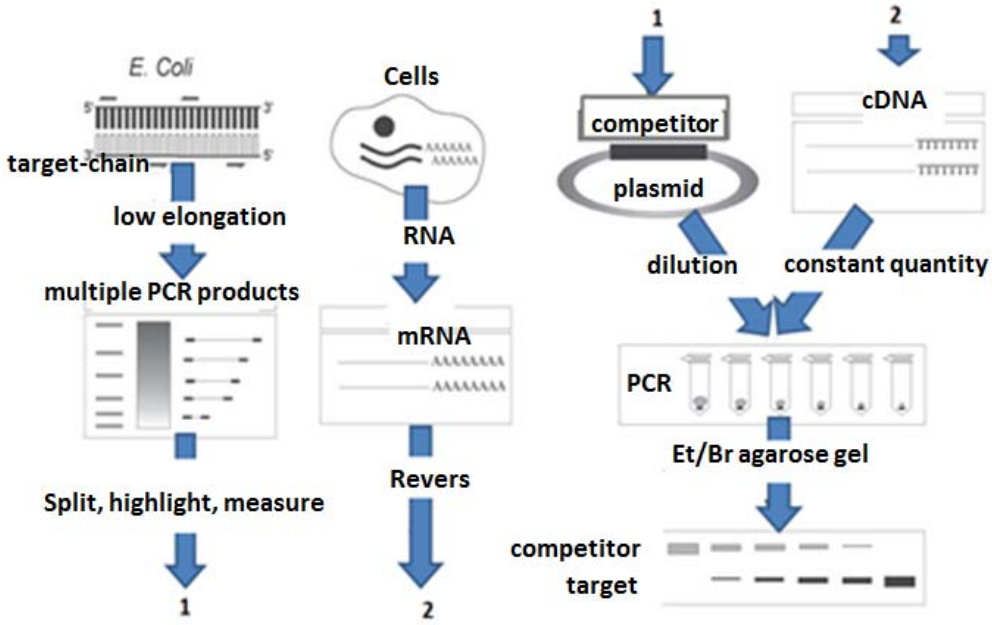


Fig. 2.18. Scheme of competitive reverse transcription polymerase chain reaction (RT-PCR; [100]).

Reverse transcription is a two-stage process (performed in both test tubes and in one without loss of sensitivity).

In the first stage, an appropriate chain of complementary DNA (cDNA) is obtained on the RNA matrix. For example, reverse transcription is used to detect the

genetic material of RNA-containing pathogens (hepatitis C virus). An enzyme that synthesizes cDNAs on the RNA matrix is called reverse transcriptase (revertase, RT).

During the second stage, the DNA copy of the viral RNA is amplified.

Consequently, the methods of studying blood with molecular diagnostic methods were presented above, namely methodical approaches using the reaction of interaction of antigen with an antibody and a polymerase chain reaction, which has also recently been improved, for example, in the molecular-genetic identification of a bovine leukosis virus it is used “nested” PCR (two-stage PCR) [23, 72, 74, 98] which has higher sensitivity than one stage PCR and at the same time it is a disadvantage due to high risk of contamination by the products of amplification.

Hence, the improvement of the two-stage PCR method for reducing contamination and preventing the incorrect results of research is still relevant.

REFERENCES
(PART 2)

1. Активність ключових ензимів орнітинового циклу у разі порушень метаболізму в клітині / Л. Калачнюк, Д. Мельничук, Г. Калачнюк // Вісник Львівського університету. Серія Біол. 2007. – Вип. 44. – С.37-42.
2. Антонов Б.И. Использование метода ПЦР при диагностике острых инфекционных болезней животных // Ветеринарный консультант. – 2002. – №16-17. – С. 22.
3. Бажибина Е.Б. Методический подход к интерпретации результатов биохимических исследований / Е.Б. Бажибина // Российский ветеринарный журнал. Мелкие домашние и дикие животные. - 2012. - № 2. - С. 28-34.
4. Балаклеевский А.И. Колориметрический способ определения активности моноаминоксидазы в сыроватки крови / Балаклеевский А.И. // Лабораторное дело. – 1976. – 3. – С. 151-152.
5. Баркаган З.С. Диагностика и контролируемая терапия нарушений гемостаза / З.С. Баркаган, А.П. Момот. – М.: Ньюдиамед, 2001. – 2-е издание – С. 296.
6. Біологічна і продуктивна дія інсуліну залежно від чистоти ін’єкцій та згодовування сорбенту / Л.Г. Калачнюк, М.Баран, М.І. Грабовенський [та ін.] // Наук. вісник ЛНУВМтаБТ ім. С.З. Ґжицького, Львів, 2010. Т.12, №2(44), Ч.2. – С.104-109.
7. Біохімічні показники крові собак при нирковій недостатності / А.В. Козловська, Л.Г. Калачнюк // Збірник матеріалів І(68) Міжнародної студентської наукової конференції "Здобутки студентської молоді у вирішенні науково-практичних питань ветеринарної медицини" (присвяченої 20-річчю набуття університетом статусу Національного), Київ, 2014- С. 95-96.

8. Біохімія (практикум). Навчальний посібник / Д.О. Мельничук, С.Д. Мельничук, Л.Г.Калачнюк, М.В. Шевряков, Г.І.Калачнюк. К: Видавничий центр НУБіП України, 2013. – 528 с.
9. Биохимия человека / Р.Марри, Д.Греннер, П.Мейс, В Родуэлл. – М.: Мир, 2009. – Т. 1. – 381 с.
10. Биохимия человека / Р.Марри, Д.Греннер, П.Мейс, В Родуэлл. – М.: Мир, 2009. – Т. 2. – 414 с.
11. Буренок О.В. Вплив екзогенного етанолу на вміст глюкогенних амінокислот у крові / О.В. Буренок, Л.Г. Калачнюк // II (69) міжнародна студентська науково-практична конференція «Здобутки студентської молоді у вирішенні науково-практичних питань ветеринарної медицини», присвяченої 95-річчю факультету ветеринарної медицини : Збірник матеріалів (Київ 20-21 травня 2015 р.) – К.: НУБіП України, 2015.- С. 43 – 44.
12. Бурместер Г.-Р. Наглядная иммунология / Г.-Р.Бурместер, А. Пецутто. 2-е изд.: Пер. с англ. М.: БИНОМ, 2009. – 320 с.
13. Варецька Т.В. Мікрогетерогенність фібриногену. Кріофібриноген / Т.В.Варецька // Укр. біохім. журн. – 1960. – N 32. – С. 13-24.
14. Варецька Т.В. Одержання фібрин-мономеру та вивчення його властивостей / Т.В.Варецька // Укр.біохім. журн. –1965. – N 37. – С.194-206.
15. Використання екамуліну із отрути ефи багатолускової в клінічній лабораторній діагностиці / Д.С. Корольова [та ін.] // Лаб. діагностика. – 2006. – Т. 37, №3. – С. 18-22.
16. Використання ліпосом на основі фосфоліпідів молока у гепатології. / за ред.. Д.О. Мельничука. Монографія. К: Вид. центр НУБіП України, 2010. - 400 с.
17. Вміст амінокислот у сироватці крові щурів при хронічній алкогольній інтоксикації / В.В. Конопельнюк, В.В. Войтенко, О.М.Савчук, Л.І. Остапченко // Лабораторна діагностика. - 2013. - № 1. - С. 44-47.

18. Вміст глюкогенних амінокислот у крові за дії екзогенного етанолу / Л.Г. Калачнюк, О.В.Буренок, В.О. Прис-Каденко // Наук. вісник ЛНУВМтаБТ ім. С.З. Ґжицького, Львів, 2015. –Т.17, №1(61), Ч.2 – С.35-38.
19. Вміст домінуючих високомолекулярних жирних кислот у крові та печінці телят за діареї / Л.Г. Калачнюк, І.М. Басараб, М.С.Калачнюк, Г.І.Калачнюк // Наук. вісник НУБіП України. – 2012. – Вип. 172, Ч. 1. – С. 59-63.
20. Гликозилированый гемоглобин / Диагностический набор // Pliva-lachema diagnostika. – 2008. – 10003258.
21. Горницкая О.В. Выделение и свойства активатора протеина С из яда Щитомордника обыкновенного (*Agkistrodon halys*) / О.В. Горницкая, Т.Н.Платонова // Биомедицинская химия. – 2003. – Т. 49, № 5. – С. 470–478.
22. Дествие экзогенного этанола на биохимические показатели крови / Л.Г.Калачнюк, М.С. Калачнюк, И.М.Басараб [и др.] // Рос. журнал гастроэнтерологии, гепатологии, колопроктологии. Приложение № 42. Материалы 19-ой Российской Гастроэнтерологической Недели (30 сентября-2 октября 2013 г., Москва).– 2013. – Т. XXIII, № 5. – С. 61(226).
23. Діагностика лейкозу великої рогатої худоби методом двостадійної полімеразної ланцюгової реакції (Методичні рекомендації) / В.Г.Спиридонов, Л.М.Іщенко, Д.Ю. Рибальченко [та ін.] – К.: Вид. центр НУБІП України, 2010. – 29 с.
24. Діагностика лейкозу у тільних корів і телят методом двостадійної ПЛР в реальному часі /Л. Іщенко, В. Спиридонов, В. Бусол, [та ін.] // Тваринництво України . – 2011. – №6. – С. 22-25.
25. Диагностический набор // Pliva-lachema diagnostika. – 2008.
26. Долгов В.В. Лабораторная диагностика нарушений гемостаза / В.В. Долгов, П.В.Свирин. – М.Тверь: «Издательство «Триада», 2005. – 227с.

27. Дылдин Д.Р. Методы измерения в клинической биохимии / Д.Р.Дылдин, А.Н.Шибанов. 2009 Электронный ресурс. Режим доступа: http://unirnedao.rU/articles/6826/9672/iterri/l 44?print=l
28. До питання валідації зажиттєвих методів діагностики лейкозу великої рогатої худоби / В.О. Бусол, С.Д. Мельничук, А.П. Блажко [та ін.] // Ветеринарна медицина 92. Міжвідомчий тематичний науковий збірник. – Харків. – 2009. – С. 118 – 121.
29. Зміни біохімічних показників крові за екзогенної дії алкоголю та фосфоліпідвмісних комплексів / Л.Г.Калачнюк, І.М. Сидір, Н.І. Руснак [та ін.] // Наук. вісник НАУ, Київ, 2008. – Вип. 127. – С. 117-120.
30. Зміни у складі домінуючих високомолекулярних жирних кислот у сироватці крові телят за аліментарної діареї / І. Басараб, М. Калачнюк, Л. Калачнюк, Г.Калачнюк // «Молодь і поступ біології»: Збірник тез VIII Міжнар наук конф студентів і аспірантів (3–6 квітня 2012 року). – Львів: ЛНУ ім. І. Франка, 2012 р. – С. 32 – 33.
31. Калачнюк Л. Аполіпопротеїн В: особливості метаболізму в клітинах печінки телят при діареї / Л. Калачнюк, О. Савка, Д.Мельничук, Г. Калачнюк // Вісн. Львів. ун-ту. Серія Біол. – 2004. – Вип. 38. – С. 57–66.
32. Калачнюк М.С. Дія екзогенного алкоголю на активність маркерних ензимів у крові / М.С.Калачнюк, Л.Г. Калачнюк, Г.І.Калачнюк // Збірник праць за підсумками II Міжнар. науково-практ. конф. мол. вчених, аспірантів і студентів «Наукові здобутки молоді у вирішенні актуальних проблем виробництва та переробки сировини, стандартизації і безпеки продовольства» (19 – 20 квітня 2012 р.), Частина 1. – Київ: НУБіП України, 2012 р. – С. 409 – 410.
33. Кольман Я. Наглядная биохимия / Я. Кольман, К.-Г.Рем.3-е изд.: Пер. с нем. М.: Мир, 2009. – 469 с.
34. Кузнецова Н.В. Использование полимеразной цепной реакции для выявления инфицированных вирусом лейкоза крупного рогатого скота /

Н.В. Кузнецова, Н.В. Кузнецов, Г.А. Симонян // Ветеринария. – 1997. – №5. – С. 12-15.

35. Курс лекцій і методичні рекомендації до виконання лабораторних робіт з дисципліни: «Спеціальна біохімія», частина 3: «Основи методичних підходів молекулярної діагностики» для студентів факультету ветеринарної медицини / С.Д. Мельничук, Л.Г. Калачнюк, Г.І. Калачнюк. К: Вид. центр НУБіП України, 2014. – 196 с.
36. Лабораторні методи досліджень у біології, тваринництві та ветеринарній медицині : довідник / В.В. Влізло, Р.С. Федорок, І.Б.Ратич [та ін.]. – 2012. – Львів: СПОЛОМ. – 764 с.
37. Лившиц В.М. Биохимические анализы в клинике / В.М. Лившиц, В.И. Сидельникова. – 2006. – Москва: Триада-Х. – 212 с.
38. Лугова Є.С. Стадійність хронічної ниркової недостатності у дрібних тварин / Є.С.Лугова, Л.Г. Калачнюк // Наук. вісник ЛНУВМтаБТ ім. С.З. Ґжицького, Львів, 2015. –Т.17, №1(61), Ч.2 – С.89-91.
39. Максименко Е.Г. Уровень триптофана и серотонина в условиях судорожной активности головного мозга. / Е.Г.Максименко, В.Н. Савченко // Вісник Харьківського нац. університета им. В.Н. Каразина. Медицина. – 2000. – 1, № 494. – С. 40-43.
40. Маршалл В.Дж. Клиническая биохимия / В.Дж. Маршалл, С.К. Бангерт. – 2011. – Москва: Изд-во БИНОМ. – 408 с.
41. Методичні рекомендації до виконання лабораторних робіт з дисципліни: «Біохімія тварин з основами фізичної і колоїдної хімії» для студентів факультету ветеринарної медицини / С.Д.Мельничук, Л.Г.Калачнюк, Г.І.Калачнюк, Л.В.Кліх // Методичні рекомендації. К: Видавничий центр НУБіП України, 2013. – 148 с.
42. Момот А.П. Принципы, методы и способы лабораторной диагностики патологии системы гемостаза на современном этапе / А.П. Момот // Лабораторная диагностика. – 2004. – № 2. – С. 52–70.

43. Новицкая Г.В.Методическое руководство по тонкослойной хроматографии липидов / Г.В.Новицкая. –Москва: Наука, 1972. –64 с.
44. Общие липиды / Диагностический набор // Pliva-lachema diagnostika. - 2008. – 10003157.
45. Определение общего уровня протромбина и выявление его функционально неактивных форм с помощью фермента экамулина, выделенного из яда эфы многочешуйчатой / Т.Н. Платонова, Е.А. Сушко, А.В. Петров, Д.А. Соловьев // Укр. биохим. журн. – 1995. – Т. 67, № 4. – С. 75–79.
46. Определение растворимого фибрина в плазме крови / Т.В. Варецкая, Л.И.Михаловская, Л.А. Свитальская, Я.М.Ена // Клин. лаб. диагностика.– 1992.– №7/8.– С. 10-14.
47. Особливості прижиттєвої діагностики лейкозу великої рогатої худоби при використанні полімеразної ланцюгої реакції / Л.М. Іщенко, В.Г. Спиридонов, С.Д.Мельничук [та ін.] // Ветеринарна медицина 92. Міжвідомчий тематичний науковий збірник. – Харків. – 2009. – С. 118 – 121.
48. Оцінка ефективності корекції білоксинтезувальної функції печінки при експериментальному гепатиті / В.А.Грищенко, В.А. Томчук, О.М.Литвиненко [та ін.] // Укр. біохім. журн . – 2011. – 83, № 1. – С. 63-68.
49. Павлюкова А.О. Порушення вуглеводного і ліпідного обмінів за цукрового діабету у собак / А.О. Павлюкова, Л.Г. Калачнюк, Г.І.Калачнюк // Наукові праці Міжнар. наук студ. конф. факультету вет. медицини (19 – 20 квітня 2012 р.). Львів: ЛНУВМтаБТ імені С.З. Гжицького, 2012. – С. 196 – 197.
50. Платонова Т.М. Застосування активатору протеїну С з отрути щитомордника звичайного (*Agkistrodonhalyshalys*) для визначення активності протеїну С у плазмі крові за різних патологій / Т.М.

Платонова, О.В. Горницька, Є.Д. Мороз // Лаб. діагностика. – 2001. – №3. – С. 28-31.

51. Порівняльна ефективність діагностики лейкозу великої рогатої худоби при використанні різних методів дослідження / А.В. Абрамов, Д.М.Король, С.Д. Мельничук [та ін.] // Ветеринарна медицина України. – 2007. – №2. – С. 33 – 34.

52. Порівняльна характеристика методів визначення вмісту фібриногену в плазмі крові / А.С. Соколовська [та ін.] // Експериментальна та клінічна фізіологія і біохімія. – 2002. – № 3. – С. 82-86.

53. Синтез металоафінного сорбенту з іммобілізованими іонами міді для виділення препарату протеїну С із плазми крові людини / О.К. Маркарян, Г.К. Березницький, Д.Д. Жерносєков, Л.К. Забава // ІІІ Всеукраїнська наук. Конференція студ. асп. і молодих учених, «Хімічні проблеми сьогодення". – 2009, Донецьк. – С. 103.

54. Соловьев Д.А. Выделение и характеристика экамулина – активатора протромбина из яда эфы многочешуйчатой (Echismultisquamatus) / Д.А. Соловьев, Т.Н. Платонова, Т.П.Угарова // Биохимия. – 1996. – Т. 61, № 6. – С. 1094–1105.

55. Структурно-функціональні зрушення в ліпідах гепатоцитів і крові неонатальних телят за діареї та лікувальної дії ліпосом на основі фосфоліпідів молока / Л.Г. Калачнюк, І.М. Басараб, Д.О. Мельничук [та ін.]// Наук. вісн. ЛНУВМтаБТ ім. С.З. Ґжицького, Львів, 2011. –Т.13, №2 (48), Ч.1. – С.383 – 390.

56. Сучасні біохімічні методи діагностування лейкемії / А.В. Дудко, М.С. Калачнюк, Л.Г.Калачнюк, Г.І. Калачнюк / 67 студентська науково-практична конференція ННІ ветеринарної медицини та якості і безпеки продукції тваринництва «Роль молоді у забезпеченні сталого розвитку галузі ветеринарної медицини» (до 115-річчя НУБіП України): Тези доповідей (Київ, 11-12 березня 2013 р.). – К.: НУБіП України, / Редкол.: Н.М. Рідей (відп.ред.) та ін.. – К., Ч.1. – 2013. – С. 47-49.

57. Тест медицинский “Глюкофот-II”: [инструкция по использованию комплекса “Глюкофот-II – Гемоглан”. – Киев: Норма, 2008. – 12 с.
58. Ультраструктурные изменения тромбоцитов при ADP-стимулируемой агрегации (в присутствии фибриногена) / Н.В. Белицер, М.Г.Анищук, Т.М.Познякова, О.В. Горкун // Цитология и генетика. – 1989. – Т. 24, № 3. – С. 3–7.
59. Умови проведення полімеразної ланцюгової реакції у лабораторній практиці (методичні аспекти) / М.С. Калачнюк, Л.Г. Калачнюк, Д.О. Мельничук [та ін.] // Біологія тварин. – 2012. – Т.14, № 1-2. – С. 660-668.
60. Чиркин А.А. Клинический анализ лабораторных даннях / А.А. Чиркин. - 2012.-Т.1. - Москва: Медицинская литература.- 384 с.
61. Шибанов А.Н. Выбор биохимического анализатора / А.Н.Шибанов, О.В.Силкин // Оснащение современной лаборатории Электронный ресурс. Режим доступа: http://unimedao.ru/files/Vybiraembiohimicheskiy analizator 05 2009fl1.pdf
62. Amersham Interferon Gamma [(r)IFNγ], Rat Biotrak ELISA System. // Product booklet. – 2009. – GE Healthcare. – RPN2741 – 28 p.
63. Amersham Interleukin-10 [(r)IL-10], Rat Biotrak ELISA System. // Product booklet. – 2009. – GE Healthcare. – RPN2746 – 28 p.
64. Amersham Interleukin-1β [(r)IL-1β], Rat Biotrak ELISA System. // Product booklet. – 2009. – GE Healthcare. – RPN2743 – 28 p.
65. Amersham Interleukin-12 [(r)IL-12], Rat Biotrak ELISA System. // Product booklet. – 2009. – GE Healthcare. – RPN2744 – 28 p.
66. Amersham Interleukin-4 [(r)IL-4], Rat Biotrak ELISA System. // Product booklet. – 2009. – GE Healthcare. – RPN2747 – 28 p.
67. A red line not to cross: evaluating the limitation and properness of gel image tuning procedures / H.C. Wu, C.C. Yen, W.H. Tsui, H.M. Chen // Anal Biochem. – 2010. – Vol. 396, N1. – P.42-50.
68. Berg J.M. Biochemistry / J.M.Berg, J.L.Tymoczko, L.Stryer – New York: W H Freeman; 2002. 1515 p.

69. Born, G. V. R. Changes in the distribution of phosphorus in platelet-rich plasma during clotting / G. V. R. Born // Biochem. J. – 1958. – Vol. 68. – P. 695.
70. Bradford M. A rapid and sensitive method for the quintitation of microgram quantities of protein using principle of protein-due binding / M. Bradford // Anal. Biochem. – 1976. – Vol. 78, N2. – P. 248-254.
71. ChristlieW.W.Lipidanalysis / W.W.Christlie. –Oxford: Pergamon Press., 1979. –338 p.
72. Development of a polymerase chain reaction and its comparison with agar gel immunodiffusion test in the detection of bovine leukemia virus infection / M.F. Camargos, D.Stancek, L.M. Lessa [et al.] // Braz. J. Vet. Res. Anim. Sci. – 2003. – V.40 (5). – P. 341-348.
73. Erlanger B. The action of chymotrypsin on two new chromogenic substrates / B. Erlanger // Arch. Biochem. Biophys. – 1966. – Vol. 115. – P. 206-218.
74. Evennann J.F. A Look at How Bovine Leukemia Virus Infection is Diagnosed / J.F.Evennann // Vet. Med. Assoc. – 1992. – V.175. – P. 705-708.
75. Fisher H.F. Methods of enzymology / H.F. Fisher – 1985. – Vol.113.–P.16–27.
76. Folch J. A rapid method for isolation and purification from animal tissues total lipid / J.Folch, M. Lees, C. Sloane-Stanley // J. Biol. Chem. – 1957. – Vol. 226, № 1. – P. 497–511.
77. Gaitonde M.K. A fluorimetric method for the determination of trypophan in animal tissues / M.K. Gaitonde // Biochem. S. – 1974. – Vol. 139. – P. 625-631.
78. https://ahdc.vet.cornell.edu/sects/clinpath/reference/chem.cfm106
79. http://doc.westmedica.com/54RU/%D0%B8%D0%BD%D1%81%D1%82%D1%80%D1%83%D0%BA%D1%86%D0%B8%D0%B8_%D0%BF%D0%BE%D0%BB%D1%8C%D0%B7%D0%BE%D0%B2%D0%B0%D1%82%D0%B5%D0%BB%D1%8F/sf1904+_ru.pdf
80. http://holesterinanet.ru/analizator-krovi-na-xolesterin-cardiochek.html

81. http://pan-ta-pani.com/90528-metodi-viznachennya-vmistu-holesterinu-sirovatki-krovi.html
82. http://ukrdiagnostika.ua/laboratory-equipment/biochemical-analyzers-semi-automatic/rt-1904c.html
83. http://ukrdiagnostika.ua/laboratory-equipment/biochemical-analyzers-semi-automatic/rt-9200.html
84. http://www.biosystems-sa.ru/?page=production&type=1&id=2
85. http://www.ed.ac.uk/polopoly_fs/1.19330!/fileManager/reference%20intervals.pdf
86. http://www.protech-solutions.com.ua/category_41/object_89
87. http://www.stormoff.ru/catalog_656_2140.html
88. http://www.transmed.com.ua/cat/obladnannja-dlja-laboratorii/vital-scientific-nv-niderlandi/analizator-napivavtomatichnii-fotomert-microlab-300-101.html
89. KalachniukM. Biochemical indices of blood of animals depending on age / M. Kalachniuk, L.Kalachnyuk, G. Kalachnyuk // Youth and Progress of Biology: Book of Abstracts of X International Scientific Conference for Students and PhD Students (Lviv, 8 – 11 April 2014). – Lviv, 2014. –P.53-54.
90. Laemli R.V. Cleavage of structural proteins during of bacteriophage T4 / R.V. Laemli // Nature. – 1970. – Vol.227. – P.680-685.
91. Marsh N.A. Diagnostic uses of snake venom / N.A. Marsh // Haemostasis. – 2001. – Vol. 31, N 3-6. – P. 211–217.
92. Medved L. Conformational changes upon conversion of fibrinogen into fibrin. The mechanisms of exposure of cryptic sites / L.Medved, G. Tsurupa, S. Yakovlev // Annals of the New York academy of sciences. – 2000. – Vol. 936. – P. 185–204.
93. Methodical recommendations to laboratory classes on «Biochemistry of animals with fundamentals of physical and colloidal chemistry» for students of Faculty of Veterinary Medicine; Part I; Physico-chemical methods in biochemical investigation / С.Д.Мельничук, Л.Г. Калачнюк // Методичні рекомендації. К: Вид. центр НУБіП України, 2013. – 149 с.

94. Nelson D.L. Lehninger Principles of Biochemistry / D.L.Nelson, M.M.Cox (Fourth Edition) – W.H.Freeman Publishers. 2004.-1124p.
95. Nestlé PURINA Biochemical Profiling in the Dog and Cat. 2004. - P.100-101.
96. Oliynyk O.V. Changes of some biochemical indices of blood in animals with chronic renal failure / O.V. Oliynyk, M.S. Kalachniuk, L.G.Kalachnyuk // Наук. праці Міжнар. наук. студ. конф. факультету вет. медицини (з нагоди 230-річчя від часу відкриття кафедри ветеринарії у Львів. університеті), 10-11 квітня 2014 р. - С.251.
97. Ostapchenko L. Enzyme electrophoresis method in analysis of active components of haemostasis system / L. Ostapchenko, O.Savchuk, N. Burlova-Vasilieva. // ABB. – 2011. – Vol. 2. – P. 20-26.
98. Polymerase chain reaction (PCR) for detection of BLV proviruses a practical complement BLV diagnostics / P. Blankenstein, H. Fechner, A. Looman [et al.] // Berl. Munch. Tierarztl. Wochenschr. – 1998. – V. 111. – P. 180-186.
99. Primary structure of human prethrombin 2 and α-thrombin / R.J. Butkowski, J. Elion, M.R.Downing, K.G. Mann // J. Biol. Chem. – 1977. – Vol. 252, N 14. – P. 4942–4957.
100. Real-Time PCR Applications Guide. Bio-Rad Laboratories, 2006.
101. Šaršunova M. Chromatografia na tenkych vrstvach vo farmaciia v klinickej biochemii / M.Šaršunova, V. Schwarz, Č.Michalec. – Bratislava: Osveta, 1977. – 624 s.
102. Sedmac K. Use of perchloric acide solution on the Coomassi BBG-250 Bredford staining method / K. Sedmac, S. Grossberg // Anal. Biochem. – 1977. – Vol. 79, N3. – P. 544-552.
103. Selected methods for antibody and nucleic acid probes / Cold Spring Harbor Laboratory Press, USA, 1993. – P. 680.
104. Selective proliferation ofrathepatocyte progenitor cells in serum-free culture. / Chen Q., Kon J., Ooe H. [et al.] // Nat. Protoc. – 2007. – Vol. 2, № 5. – P. 1197-205.

105. The influence of exogenous factors on the glucogenic amino acids content in the blood / A.M. Stetsenko, I.M. Basarab, L.G. Kalachnyuk, G.I. Kalachnyuk // Наук. Вісн. НУБіП України. Серія «Ветеринарна медицина, якість і безпека продукції тваринництва». – К.: 2011. – Вип. 167, Ч.1.– С. 237 – 240.

106. Toxic and protective effect of some compounds on the level of triacylglycerols and cholesterol in the liver cells and in the blood / V.A. Dubchak, I.M. Basarab, L.G. Kalachnyuk, G.I. Kalachnyuk // «Актуальні проблеми наук про життя та природокористування». Тези доп. міжнар. наук.-практ. конф. молодих вчених 26 – 29 жовтня 2011 р., Київ: Видавн.центр НУБіП України. – С. 49-50.

107. Wang C.-C. Synthesis of chelting resins with iminoacetic acid and its waste water treatment application / C.-C. Wang, C.-Y.Chen, C.-Y. Chang // J. Appl. Polym. Sci. – 2002. – Vol. 84. – P. 1353-1362.

108. Webb E.C. Enzymes / E.C. Webb // Academic Press Inc., Publishers. – 1964. – New York. – P. 950.

109. Weissbach H. A simplified method for measuring serotonin intissue; simultaneous assay of both serotonin and histamine / H.Weissbach, T.P. Waalkes, S. Udenfriend / J. Biol. Chem. // – 1957. – Vol. 230, № 2. – P. 865-871.

110. Wu M. Process scale-up studies for protein C separation using IMAC / M. Wu, D.F.Bruley // Adv. Exp. Med. Biol. – 2005. – Vol. 566. – P. 243-248.

APPENDICES

Annex A

Indicators of blood (normal state) of animals and their characteristics

Table A.1

Biochemical parameters of blood for different animals [Part 2, 36, 78, 85]

Indexes	Dogs	Cats	Cattle	Sheep	Horses
Albumin (g/L)	26 - 35	28 - 39	28 - 36	24 - 35	23 - 35
Ammonium (μmol/L)	25 - 73	59- 110			15 - 90
Bile acids (μmol/L)	0 - 7	0 - 7	13 - 67	1 - 33	1,0 - 15
Total bilirubin (μmol/L)	0 – 6.8	0 – 6.8	0 – 6.8	0 – 6.8	17 - 34
Conjugated bilirubin (μmol/L)	0 – 4.5	0 - 45			
Cholesterol (mmol/L)	3.8 – 7.0	2.0- 3.4	1.0 – 3.1	1.0 – 2.6	2.3 – 3.6
Cortisol (nmol/L)	20 - 230	10- 250	25 - 140	25 - 140	25 - 140
Creatinine (μmol/L)	40 - 132	40- 177	44 - 165	40 - 150	40 -150
Fibrinogen (g/L)	2.0 – 4.0	2.0- 4.0	4.5 – 7.5		2.0 – 4.0
Fructosamine (μmol/L)	49 - 225	100 - 350			
Glucose (μmol/L)	3.0 – 5.0	3.3- 5.0	2.0 – 3.0	2.0 – 3.0	2.8 – 5.5
Globulins	18 - 37	23 - 50	27 - 50	34 - 55	30 - 50
Triglycerides (mmol/L)	0.57 – 1.14	0.57 - 1.14	0.17 – 0.51	0.15 – 0.79	0.12 – 0.35
Total protein (g/L)	58 - 73	69 - 79	59 - 73	73 - 89	58 - 75
Urea (mmol/L)	1.7 – 7.4	2.8- 9.8	2.0 – 6.6	2.6 – 6.6	2.5 – 8.3

Table A. 2

The activity of enzymes for different animals in the blood [Part 2, 36, 78, 85]

Enzymes	Cats	Dogs	Cattle	Sheep	Horses
Amylase (U/L)	225- 990	525-960			
ALAT (U/L)	21 - 102	6 - 83	17 - 27	3 - 16	5 - 20
ASAT (U/L)	15 - 65	15 -50	53 - 105	45 - 134	258 - 554
Creatine phosphokinase (U/L)	50 - 200	50 - 200	82 - 196	34 - 190	150 - 385
γ-glutamyltransferase (U/L)	2 - 8	1 - 5	20 - 46	34 - 100	13 - 44
Glutamate dehydrogenase (U/L)	0 - 5	0 - 5	2 - 23	1 - 12	1 - 12
Lactate dehydrogenase (U/L)	50 - 320	80 - 360	508 - 956	200 - 600	192 -482
Lipase (U/L)	13 - 200	0 - 83			

Table A. 3

Blood serum proteins for different animals [Part 2, 36, 78]

Proteins	Cats	Dogs	Cattle	Sheep	Horses
Albumin (g/L)	27 - 37	27 - 41	28 - 37	26 - 36	24 - 34
α-globulins (g/L)	5 - 20	6 - 22	8 - 11	11 - 17	11 - 18
β-globulins (g/L)	11 - 20	5 - 14	11 - 15	4 - 10	10 - 22
γ-globulin (g/L)	5 - 10	12 - 20	8 - 17	19 - 33	9 - 18

Table A. 4

Electrolytes in blood of different animals [Part 2, 36, 78]

Electrolytes/ microelements	Dogs	Cats	Cattles	Sheeps	Horses
Calcium (mmol/L)	2.3 – 3.0	2.1 – 2.9	2.0 – 3.0	2.1 – 2.8	2.6 – 3.3
Chlorides (mmol/L)	99 - 115	117 - 140	94 - 11	98 - 115	98 - 118
Cuprum (mmol/L)	15.7 – 19.0		9.4 – 19.0	9.4 – 18.8	19.1 – 27.9
Inorganic phosphate (mmol/L)	0.9 – 2.0	1.4 – 2.5	1.2 – 2.3	0.9 – 2.5	0.8 – 1.8
Magnesium (mmol/L)	0.69 – 1.18	0.82 – 1.23	0.7 – 1.23	0.65 – 1.23	0.6 – 1.0
Potassium (mmol/L)	3.6 – 5.6	4.0 – 5.0	3.6 – 5.6	4.0 – 5.5	2.7 – 5.9
Selenium (GSHPx) (U/ml×cells)			>40	>40	
Sodium (mmol/L)	139 - 154	145 – 156	136 - 145	142 - 160	134 - 150
Zinc (μmol/L)	9.0 – 18.0		9.0 – 14.0	9.0 – 14.0	10.0 – 20.0

Table A. 5

Biochemical blood indicators of small animals [Part 2, 95]

Indicators	Units	Dogs	Cats
Nitrogen of urea in blood	mg/100 ml	7 - 32	15 – 35
Creatinine	mg/100 ml	0.5 - 1.5	0.9 - 2.3
Glucose	mg/100 ml	67 - 132	75 – 134
Total bilirubin	mg/100 ml	0.1 - 0.8	0.1 - 0.4
Total protein (TP)	g/100 ml	4.8 - 6.9	5.5 - 7.1
Albumin	g/100 ml	2.3 - 3.9	2.8 - 3.9
ALT	IU/L	3 - 69	20 - 108
Alkaline phosphatase (ALP)	IU/L	20 - 157	23 - 107
γ glutamyltransferase (GGT)	IU/L	5 – 16	5 – 16
Amylase	IU/L	378 - 1033	440 - 1264
Lipase	IU/L	104 – 1753	148 - 1746
Sodium	mmol/L	138 - 148	148 - 157
Potassium	mmol/L	3.5 - 5.0	3.5 - 5.1
Chloride	mmol/L	105 - 117	115 - 128
Calcium	mg/100 ml	9.7 - 12.3	9.0 - 11.7
Phosphorus	mg/100 ml	2.2 - 7.0	2.6 - 8.8
Cholesterol	mg/100 ml	125 - 301	45 - 274
Triacylglycerols	mg/100 ml	21 - 120	21 - 81
Total CO_2(TCO_2)	mmol/L	13 - 24	16 - 25
The difference between the measured concentrations of cations and anions	mmol/L	9 -18	10 - 23

Table A. 6

General blood indices of small animals [Part 2, 95]

Indicators	Units	Dogs	Cats
1	2	3	4
Hematocrit	%	37 – 55	30 – 45
Hb	g	12 – 18	8 – 15
Red blood cells	µl	5.5 – 8.5	5.0 – 10.0
Total protein [plasma]	g/100 ml	6,0 – 8,0	6.0 – 8.0
White blood cells	µl	6000 – 17000	6000 – 18000
Immature neutrophils	µl	0 – 300	0 – 300
Neutrophils	µl	3000 – 12000	3000 – 12000
Lymphocytes	µl	1000 – 5000	1500 – 7000
Monocytes	µl	150 – 1350	50 – 850
Eosinophils	µl	100 – 1250	100 – 1500
Mean corpuscular volume (MCV)	Uncia (1 oz = 29.6 ml)	60 – 75	40 – 55
Mean concentration of hemoglobin in erythrocytes (MCHC)	g/100 ml	32 – 36	30 – 36
Fibrinogen	mg/100 ml	200 – 400	150 – 300
Thrombin	µl	2 – 9	3 – 7
Thrombin time (TT)	s	5.5 – 7.9	6.4 – 9.6
Partial time of thromboplastin formation	s	11.4 – 16.4	9.3 – 18.7
FSPs (fibrin/fibrinogen splitting products)	g/ml	<10	<10

Addition B

Indicators of human blood and their characteristics

Table B. 1

Indicators of general analysis of human blood [Part 2, 60]

Indicators	Normal state in SI units
Red blood cells (RBC): for female for male	3.8-4.5×10^{12}/L 4.5-5.0×10^{12}/L
Hemoglobin (Hb) HGB: for female for male	120.0-140.0 g/L 130.0-160.0 g/L
Hematocrit (Hct) HCT: for female for male	0.36-0.42 0.40-0.52
Color index	0.8-1.1
Mean content of hemoglobin in 1 erythrocyte (MCH)	25.4-34.6 pg
Mean corpuscular hemoglobin concentration (MCHC)	30-38g/L
Mean corpuscular volume (MCV)	80-100 μm ^{3} (Uncia, 1 oz = 29,6 ml)
Mean diameter of erythrocytes	7.2-7.5 μm
Red cell Distribution Width (RDW)	11.5-14.5 %
Reticulocytes (Rt)	2-12‰
White blood cells (WBC)	4.0-9.0×10^9/L
Rod-core neutrophils	1-6 %
Segmented nuclear neutrophils	47-72 %
Eosinophils (EO):	0.5-5.0 %
Basophiles (BASO):	0-1 %
Monocytes (MONO):	3-11 %
Lymphocytes (LYMPH):	19-37 %
Erythrocyte sedimentation rate (ESR): for female	 2-15 mm/h

for male	1-10 mm/h
Platelets (PLT)	180.0-320.0×10^9/L

Explanation for table B.1. [Part 2, 60]

Red blood cells

↑ absolute (6.5) erythrocytosis: erythremia, increased production of erythropoietin (chronic lung disease, congenital heart disease, Itsenko-Cushing's syndrome);

Relative erythrocytosis: ↓ volume of blood circulating (dehydration), arterial hypertension, smoking and alcohol, highlands;

↓ anemia.

Hemoglobin

↑ erythremia (polycythemia), dehydration (burns, vomiting, diarrhea, intestinal obstruction), Highlands;

↓ anemia, hyperhydration.

Hematocrit

↑ dehydration (blood clotting), erythremia, kidney tumor;

↓ blood loss, starvation, hemodilution (intravenous infusion in large quantities), pregnancy, hyperproteinemia.

Color Indicator

where Hb - hemoglobin concentration, g/L;

Red blood cells (RBC), * the first three digits of the number of erythrocytes and if the quantity of erythrocytes is less than 1.0 × 10^{12} / L, then the first two digits.

↑ hyperchromic anemia (B_{12}, folic acid), gastric polyposis, after gastrectomy;

↓ hypochromic anemia: fetal deficiency, pregnancy, menstruation, lead poisoning.

Mean content of hemoglobin in 1 erythrocyte (MCH)

↑ hypertriglyceridemia, liver disease, multiple myeloma;

↓ alcoholism.

Mean corpuscular hemoglobin concentration (MCHC)

↑ RBC sphericity anemia;

↓ enzyme deficiency anemia.

Mean corpuscular volume (MCV)

↑ macrocrital and megaloblastic anemia, hemolytic anemia, liver disease;

↓ ferrum deficiency anemia, thalassemia.

Mean diameter of erythrocytes

normocytic (≈ 7.1 - 7.9 μm)

microcytic (<7.1 μm)

macrocytic (> 7.9 μm)

megalocytic (> 12 μm)

Red cell Distribution Width (RDW)

RDW more than 15.0% indicates the presence of heterogeneous cells(micro-, normo-, macro- and helmet erythrocytes).

Reticulocytes (Rt)

↑ acute blood loss (reticulocytic crisis at 3-5 days), B_{12}-deficiency anemia (reticulocytic crisis 5-9 days after starting treatment), hemolytic anemia, O2;

↓ hypoplastic anaphylaxis, deficiency anemia (Fe, B12, folio), radiotherapy, cytostatics.

White blood cells (WBC)

↑ stress, work, post-eating, bacterial infections, tissue necrosis (myocardial infarction), intoxication (uremia, diabetic acidosis, gout), chronic myelogenous leukemia, allergy (bronchial asthma), brain hemorrhage;

↓ bacterial infection (abdominal typhus, paratyphoid, tularemia, brucellosis, miliary tuberculosis), viral infection (hepatitis, influenza, measles, rubella), oncology, collagenosis.

Eosinophils (EO):

↑ allergies, parasites, scarlet fever;

↓ large surgery, after nicotinic acid and adrenaline.

Basophils (BASO):

↑ acute leukemia, polycythemia, Hodgkin's lymphoma, colitis, allergies;

↓ acute infections, hyperthyroidism, pregnancy, stress.

Monocytes (MONO):

↑ infections, granulomatosis (tuberculosis, syphilis, brucellosis), acute monocytic leukemia;

↓ aplastic anemia, action of glucocorticoids.

Erythrocyte sedimentation rate (ESR):

↑ acute and chronic infection, connective tissue diseases, oncology, kidney disease, trauma, chemical poisoning;

↓ polycythemia, chronic circulatory failure.

Platelets (PLT)

↑ polycythemia, chronic myelogenous leukemia, acute blood loss, oncology;

↓ immune thrombocytopenia, systemic lupus erythematosus, splenomegaly, DIC syndrome (disturbance of blood clotting due to massive release of thrombolytic substances from tissues), antitumor drugs

Table 2. B

Biochemical analysis of human blood [Part 2, 37, 60]

Indicators	Normal state in SI Units
1	2
Residual nitrogen	14-28 mmol/L
Alanine transaminase (ALT)	0.10 – 0.68 mmol/(h×L) (7-40 U/L)
Aspartate transaminase (AST)	0.10 – 0.45 mmol /(h×L) (10-30 U/L)
Albumin	35-55 g/L
α-amylase pancreatic amylase	28—100 U/L 0—50 U/L
BNP (B-type natriuretic peptide) for male for female	0-100 pg/ml 200-420 pg/ml 140-340 pg/ml
Total protein	65 – 85 g/L

Bilirubin: total unconjugated conjugated	8.55-20.52 mmol/L 1.7-17.1 mmol/L 0.86-5.3 mmol/L
Vitamin B12	208-963.5 pg/ml
Glucose (by Hagedorn-Jensen) - in whole blood by the orthotoluidine method	4.4-6.6 mmol/L 3.3-5.5 mmol/L
γ-glutamyltransferase: for male for female	to 49 U/L (11-69) to 32 U/L (9-39)
Total Ferum binding ability of serum: for male for female	 14,32-25,06 μmol/L 10,74-21,48 μmol/L
Bile acids	54-72 μmol/L
Pottasium	2.5-6.8 μmol/L
Calcium (in blood serum)	3.4-5.3 μmol/L
Creatinine: for female for male	44.0-97 μmol/L 44.0-115.0 μmol/L
Creatine phosphokinase (CPK) for male for female CPK-MV (isoenzyme of the heart)	 25-200 U/L 25-175 U/L 0-20 U/L
Lactate dehydrogenase	120-240 U/L
Lipase	0-190 U/L
Magnesium	0.7-1.07 μmol/L
Copper	11-22 μmol/L
Myoglobin: for male for female	Not higher than 95 ng/ml 19-92 μg/L 12-76 μg/L

Continuation of tabl. B 2	
1	2
Uric acid: for male for female	 180-420 μmol/L 180-340 μmol/L
Urea	2.5-8.3 mmol /L
Sodium serum of blood	130-157 mmol /L
Rheumatoid factor (blood serum)	undetectable (10 U/ml)
Antistreptolysin-O	to 200 IU/L
C-reactive protein (CRP)	negative
Transferrin	2-4 g/L
Triacylglycerols	0.55-1.65 mmol /L
Troponin T Troponin I	0-0.1 ng/ml 0-0.5 ng/ml
Ferritin: for male for female	 21-235 μg/L 12-128 μg/L
Folic acid	7.2-15.4 ng/ml
Alkaline phosphatase: for male for female	 до 270 U/L; до 240 U/L
Phosphates	0.65-1.3 mmol /L
Chloride ions (chlorine)	95.0-110.0 mmol /L
Cholesterol (total) HDL cholesterol LDL cholesterol	3.9-5.2, increased more than 6.5 mmol /L 0.9-1.9 mmol /L До 3.5 mmol /L

Explanation for table B.2. [Part. 2, 37, 40, 60]

Residual Nitrogen

↑ acute and chronic renal insufficiency, severe heart failure;

↓ liver failure.

Alanine transaminase (ALT) - is an enzyme of the liver, which is involved in the exchange of amino acids. ALT can be found in liver, heart muscle, kidneys, skeletal muscle. In pathological processes in these organs ALT is transported to blood and analysis shows increased levels of the enzyme.

↑ necrosis of liver cells (hepatitis, cirrhosis, toxic lesion), chronic alcoholism, heart muscle damage (heart failure, myocarditis, myocardial infarction), burns, muscle injuries;

↓ final stage of liver cirrhosis, vitamin B6 deficiency, hemodialysis, pregnancy.

Aspartate transaminase (AST). AST can be founded in many tissues of the liver, kidneys, heart, skeletal muscles and other organs;

↑ necrosis of liver cells (hepatitis, cirrhosis, toxic lesion), chronic alcoholism, heart muscle damage (heart failure, myocarditis, myocardial infarction), burns, muscle injuries;

↓ rupture of the liver, deficiency of vitamin B6;

↑ ALT> AST → diseases of the liver and biliary tract;

↑ AST> ALT→ myocardial disease.

Albumin

↑ dehydration;

↓ insufficient supply of protein with food (fasting, malnutrition), disturbance of protein absorption in the gastrointestinal tract (enteritis, surgical removal of the part of the stomach and intestines), reduced synthesis of albumin in the liver (toxic liver

damage, liver cirrhosis), increased protein loss (ulcerative colitis, peritonitis, large burns), kidney damage (nephrotic syndrome with protein in the urine).

α-amylase

Alpha amylase (diastase) is formed in the pancreas and salivary glands, pancreatic amylase - in the pancreas.

↑ diseases of the pancreas or salivary glands, liver; alcoholism, aspirin or glucocorticosteroids;

↓ insufficiency of pancreas or liver function, pregnancy toxicosis.

B-type natriuretic peptide (BNP - B-type Natriuretic Peptide)

↑ heart failure, left ventricular hypertrophy, inflammatory heart disease (myocarditis, allograft rejection), acute and chronic renal insufficiency, acute coronary syndrome and myocardial infarction, ascites, liver cirrhosis, paraneoplastic disease (small cell lung cancer), old age.

Total protein

↑ cirrhosis of the liver (compensated, in the early stages), dehydration;

↓ in low income or protein synthesis in the body (hunger, malnutrition, disruption of the gastrointestinal tract, inhibition of protein synthesis in the liver (hepatitis, cirrhosis, poisoning), inborn errors of blood protein synthesis (analbuminaemia, Wilson disease), increased breakdown proteins in the body (tumors, large burns), increased thyroid function, urinary protein excretion in kidney diseases, prolonged treatment with corticosteroids, bleeding, pregnancy, hemodilution.

Bilirubin

Total:

↑ liver damage (hepatitis, liver cirrhosis), vitamin B12 deficiency, cholelithiasis, pregnancy, right ventricular failure.

Unconjugated (indirect):

↑ hemolysis, extensive bleeding

Conjugated (direct):

↑ hepatic jaundice (hepatitis, cirrhosis, toxic lesion), hemopoietic jaundice

Vitamin B12

↑: liver disease (acute and chronic hepatitis, cirrhosis, hepatic coma, metastasis of malignant tumors in the liver), leukemia, elevated transcobalamin levels, chronic renal failure;

↓: insufficient vitamin B12 intake in the body: strict vegetarian diet, low vitamin content in female milk (cause of anemia in infants), alcoholism, malabsorption syndrome malabsorption (celiac disease, celiac), excision of the different parts of the gastrointestinal tract (stomach, small intestine), chronic inflammatory diseases and anatomical defects of the small intestine, atrophic gastritis, parasitic diseases (Diphyllobothriasis).

Glucose

↑ diabetes mellitus, glucocorticosteroids, adrenaline;

↓ insulin overdose, insulinoma, angiotensin converting enzyme inhibitors.

γ -glutamyltranspeptidase (GGT) is found in the kidneys, pancreas and liver.

↑ illness of the liver or pancreas, gallstone disease, myocardial infarction;

↓ pregnancy and hypothyroidism.

Ferum of blood serum

↑ hypo- and aplastic anemia, hemochromatosis, hemolytic anemia, deficiency of vitamins B6 and B12;

↓ iron deficiency anemia, acute and chronic infectious diseases, leukemia, bleeding gastritis, parasitic diseases (diphyllobothriasis).

Total Ferum-binding ability of blood serum

↑ hypochromic anemia, acute hepatitis, late pregnancy;

↓ prolonged fasting, oncological diseases, anemia (non-iron deficiency associated), chronic infection, cirrhosis, hemochromatosis.

Bile acids

↑ hepatitis, cirrhosis, cholestasis.

Potassium

↑ increased potassium intake (usually during therapy), potassium redistribution in the body (hemolysis, massive tissue damage, severe acute fasting, convulsive activity), decreased potassium renal elimination (all types of renal failure, shock, hemodialysis), medicinal products (adrenaline, ascorbic acid (high doses), β-adrenoceptor blocking agents (rarely), heparin, digoxin, levodopa, mannitol, angiotensin-converting enzyme inhibitors, antitumor and cytostatics, spironolactone, glucocorticosteroids;

↓ decrease in potassium intake (hunger, lack of potassium in food), potassium loss (vomiting, diarrhea, uropoiesis disruption, Cushing's syndrome, burn disease), medicinal products (aminoglycosides, aspirin, sodium bicarbonate, bisacodyl, glucose, insulin, diuretic).

Calcium (in serum)

↑ hyperparathyroidism, oncological diseases that involve bone affection, vitamin D excess, dehydration, acute renal failure;

↓ hypoparathyroidism, hypovitaminosis D, chronic renal failure, rickets, prolonged treatment with anticonvulsants, hypoalbuminemia, prolonged fasting, prolonged vomiting or diarrhea.

Creatinine

This is the final protein exchange product. It is formed in the liver, secreted into the bloodstream and is involved in energy metabolism in the muscle tissue. Creatinine is

excreted from the body by the kidneys with urine. Deviation of creatinine level, as a rule, indicates kidneys and skeletal muscle disorder.

↑ acute and chronic renal failure, excessive physical activity, massive destruction of muscle tissue, excessive consumption of meat products, burns, dehydration, heart failure;

↓ fasting, muscle mass decrease, vegetarians, muscle atrophy.

Creatinphosphokinase (CPK):

↑ injuries, surgeries, myocardial infarction, dermatomyositis;

↓ muscle mass reduction, sedentary lifestyle.

CPK-MB (isoform):

↑ heart disease.

Lactate dehydrogenase

↑ myocardial damage (LDH_1), skeletal muscle damage, thromboembolism of pulmonary artery and lung damage (LDH3), hemolysis (LDH1 and LDG5), liver damage (LDH5).

Lipase

↑ pancreatic diseases, pancreatic duct obstruction, peritonitis;

↓ pancreatic atrophy, cachexia.

Magnesium

↑: acute and chronic renal failure, dehydration, magnesium medicinal products overdoses;

↓: malnutrition, incoercible vomiting (by cannabis), prolonged diarrhea, diabetic acidosis, acute and chronic pancreatitis, 2nd and 3rd trimesters of pregnancy, vitamin D deficiency (children rickets, bone softening).

Copper

↑: hemohromatosis, cirrhosis, obstructive jaundice, pregnancy;

↓: Wilson-Konovalov's disease, malnutrition, enteropathy, malabsorption syndrome.

Myoglobin

↑ myocardial infarction, pain and skeletal muscle damage;

↓ rheumatoid arthritis.

Uric acid. Urinary acid is synthesized in the liver and is excreted from the body by kidneys.

↑ gout, kidney failure, leukemia, skeletal muscle damage;

↓ Wilson-Konovalov's disease, low protein diet.

Urea. The main product of protein breakdown. It is made by the liver from ammonia. It is excreted from the body by kidneys.

↑ acute and chronic renal failure, protein foods, urolithiasis, burns;

↓ non-protein food, decompensated liver cirrhosis, pregnancy, renal failure.

Sodium of blood serum

↑ a) Hyperosmolar hyperhydration: excess salt intake, hyperosmolar NaCl solution, hyperaldosteronism, acute renal failure, osmotic diuresis enlargement (glucosuria).

b) Hyperosmolar dehydrotation: diabetes insipidus, weeks, water intake.

↓ a) Hypoosmolar dehydrotation: the stage of polyuria gastroesophageal renal failure, hypoaldosteronizm, vomiting, diarrhea, burns, diuretics.

b) Hypoosmolar hyperhydration: excessive water intake and hypotonic solutions, excessive antidiuretic hormone secretion, generalized edema (heart failure, nephrotic syndrome, cirrhosis), chronic renal failure, oligohydruria stage of acute renal failure.

Rheumatoid factor

↑ rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, Waldenstrom's disease.

C-reactive protein

↑ inflammation, necrosis, injuries and tumors, parasitic infections, rheumatoid arthritis, myocardial infarction, pregnancy.

Transferrin

↑ chronic iron deficiency anemia, estrogen and oral contraceptives intake;

↓ hemochromatosis, chronic inflammatory processes, chronic renal failure, burns, nephrotic syndrome.

Triacylglycerols (triglycerides)

↑ dyslipidemia, ischemic heart disease, arterial hypertension, myocardial infarction, aortic stenosis, chronic renal failure, diabetes mellitus;

↓ fasting, hyperthyroidism, burn disease, ischemic stroke.

Troponin

↑: myocardial infarction, myocarditis, chronic heart failure, postoperative heart failure, chronic renal failure (final stage).

Ferritin. This is the main indicator of iron level in the body. The level of ferritin is used to diagnose anemia, as a result of oncological, rheumatic and infectious diseases.

↑ fasting, hemochromatosis, acute and chronic infectious, inflammatory diseases (osteomyelitis, pulmonary infections, burns, systemic lupus erythematosus, rheumatoid arthritis); liver pathology (including alcoholic hepatitis); oral contraceptives intake, breast tumors;

↓ iron deficiency anemia.

Folic acid

↑: vegetarian diet, pernicious anemia, small intestine distal segment diseases;

↓: malabsorption, bowel resection, alcoholism, lack of food, aspirin or anticonvulsants intake, cytostatics, pregnancy, liver diseases.

Alkaline phosphatase. The level of alkaline phosphatase in the blood allows to diagnose liver, osseous system, kidney and bile ducts diseases.

↑ cholestasis, liver damage, bone affection;

↓ hypothyroidism, vitamins C or B12 deficiency.

Phosphates

↑: kidney failure, pregnancy toxicosis, fracture healing (a good sign!), increased muscular work, excessive vitamin D intake, Addison's disease, Itsenko-Cushing's disease, magnesium deficiency (!);

↓: spasmophilia, rickets, fasting, exhaustion, prolonged use of medicinal products containing aluminum, vomiting or diarrhea, malabsorption syndrome.

Chloride ions

↑ dehydration, acute renal failure, salicylate poisoning, non-diabetes mellitus, elevated adrenal cortex function, metabolic acidosis during diarrhea;

↓ excess sweating, prolonged vomiting, metabolic acidosis associated with loss of organic anions, respiratory acidosis.

Cholesterol or Cho (total cholesterol)

↑ hypercholesterolemia, aortic stenosis, ischemic heart disease, diabetes, obesity;

↓ diet, acute infections, diabetes, chronic renal failure, liver dystrophy.

High Density Lipoprotein (HDL) Cholesterol

↑ high and regular physical activity, under the influence of medicinal products that reduce total lipids level, under conditions of alcoholism.

↓ aortic stenosis, myocardial infarction, diabetes, acute infections.

Low-Density Lipoprotein (LDL) Cholesterol

↑ obesity, liver diseases, medicinal drugs intake (β-blockers, diuretics, glucocorticosteroids), alcoholism;

↓ fasting, malignant neoplasms.

Table B.3.

Examination of hemostasis system [Part 2, 60]

Hemostasis	Tests	Normal range
I Vascular component in hemostasis	Pinch test.	No petechiae
	Bandage test (Konchalovsky-Rumpel-Leede test)	≤ 10 petechiae
	Nesterov's test	≤ 40 petechiae
II Platelet component in hemostasis	Duke bleeding time	2-5 min
	Platelet count	180.0-320.0×10^9/L
	Platelet adhesiveness (retention) test	20 - 55%
	Platelet aggregation test	55-145%

Table B.4.

Human hemostasiogram [Part 2, 60]

Phases	Tests	hypo		hyper
1	*2*	*3*	*4*	*5*
1st phase of coagulation (prothrombinase formation)	Lee-White whole blood clotting time	↑	8-12 min	↓
	Activated partial thromboplastin time (APTT)	↑	24-34 sec (60 for heparin)	↓
	Autocoagulation test (autocoagulogram)	↑	7-11 sec (20-25 for heparin)	↓
	Plasma recalcification time	↑	60-150 sec	↓
2nd phase of coagulation (thrombin formation)	Quick's prothrombin time (PT)	↑	11-15 sec	↓
	Prothrombin ratio (PR)	↑	70-120% (0.7-1.1)	↓
	International normalisation ratio (INR)	↑	0.9-1.3 (2.5-4.5 for indirect anticoagulants)	↓
3rd phase (fibrin formation)	Thrombin time	↑	10-20 sec	↓
	Fibrinogen A (factor I)	↓	2-4 g/L	↑
	Ethanol gelation test	-	Negative	+
	Plasma protamine paracoagulation test	-	Negative	+

1	*2*	*3*	*4*	*5*
4th phase (post-coagulation)	Clot retraction	↓	50-70%	↑
	Spontaneous euglobulin fibrinolysis (fibrinolytic activity)	↓	180-240 min (10-20%)	↑
	Antithrombin III	↑	80-120%	↓
	Soluble fibrin monomer complexes	↓	≤ 4 mg/100 mL	↑
	D-dimer		0.25-0.5 μg/mL (250-500 ng/mL)	↑
	Lupus anticoagulant	-	0.8-1.2 U	-
	Plasminogen	↑	75-140%	-
	Protein C	-	70-130 % 3-6 mg/L	-
	Protein S	-	70-140% 418-600 mg/L	-
	Fibrin degradation products (FDP)	-	до 5 mg/L	↑

***Explanation to Table B.4*[Part 2, 37, 40, 60]**

I Vascular component in hemostasis

Capillary resistance

Capillary resistance is a capacity of capillaries to protect the integrity of the vascular wall from the mechanical effects. Due to the presence of resistance disorder,

there is capillary fragility observered. Capillary resistance is determined by the pinch sign, bandage sign (Konchalovsky-Rumpel-Leede test) and Nesterov's test.

Pinch test. In the case of capillary resistance disorder, in the place of the pinch of the skin made under the clavicle, the smallest hemorrhages (petechiae) or bruises appear.

Bandage sign (Konchalovsky-Rumpel-Leede test). In healthy people, when pressure according to a tonometer is increased up to 100 mm Hg for 5 minutes on the forearm at 2 cm from the cubital fossa in a circle with a diameter of 2.5 (5) cm, no petechiae or no more than 10 petechiae (with a diameter up to 1 mm) are formed.

Nesterov's test. This analysis is based on the formation of negative pressure in cuvettes with a diameter of 1.5 cm, which is imposed under the collarbones.

In healthy people, for at least three minutes, due to the presence of blood thinning up to 30 mm Hg, there are no more than 20 small petechiae observed.

Increased capillary fragility is observed at: sepsis, vitamin C deficiency, DIC syndrome, overdosing with anticoagulants, deficiency of prothrombin complex factors.

II Platelet component in hemostasis

1) Duke bleeding time – determination of the bleeding duration from the surface microcirculatory vessels of the earlobe after the disruption of their integrity with the help of scarificatum. Every 30 seconds, a drop of blood is mopped up with the help of a piece of paper, as long as the blood outflow is stopped (N - 2-5 min). An increase in time indicates the presence of thrombocytopenia, thrombocytopathy, Willebrand disease, DIC syndrome, the effects of heparin, disaggregants and salicylates, vitamin C deficiency.

Reduced rate: in the case of an increased spastic capacity of peripheral capillaries, as well as due to a technical errors.

2) Determination of platelet count - N (180 - 320) × 10^9/L.

↑ polycythemia, chronic myelogenous leukemia, acute blood loss, oncology

↓ thrombocytopenic purpura, systemic lupus erythematosus, splenomegaly, DIC syndrome, antineoplastic drugs.

3) Platelet adhesiveness (retention) test - N - 20 - 55%.

Platelet adhesiveness, or retention, is the property of platelets to adhere to the damaged vascular wall and leukocytes.

↑ ischemic heart disease, as well as in the postpartum and postoperative period.

↓: thrombocytopathy, uremia, leukemia.

4) Platelet aggregation test

Platelet aggregation is a property of platelets to be adhered together with the formation of aggregates.

Spontaneous aggregation is observed at: DIC syndrome, thrombosis, diabetes mellitus, acute cerebrovascular abnormalities.

III Blood plasma (coagulation) hemostasis

1) Indicators characterizing the first phase of blood coagulation (the formation of prothrombinase):

a) *Lee-White whole blood clotting time* - N 8 - 12 min (depends on F XII)

This is the time elapsed from the time of blood sampling to the moment of its coagulation, which depends on the activity of plasma factors (XII) and platelets, as well as the state of the vascular wall.

↑ at thrombocytopenia, thrombocytopathy, overdosing with anticoagulants, coagulation factor deficiency, post-hemorrhagic anemia.

↓ (shortening) – at hypercoagulable syndrome, DIC syndrome, thrombosis.

b) *Activated partial thromboplastin time (APTT):*

• Prolonging of APTT – hypocoagulation, congenital or acquired deficiency of blood coagulation factors II, V, VIII, IX, X, XI, XII (with the exception of the factors VII

and XIII), fibrinolysis, 2nd and 3rd phases of DIC syndrome, treatment with the heparin preparations, acute hepatic diseases;

• Shortening of APTT – hypercoagulation, 1st phase of DIC syndrome, contamination of tissue at thromboplastin test in blood collection.

c) *Activity of the factors:* V, VII, VIII, IX, X, XI, XII

d) *autocoagulation test* (autocoagulograms): 7-11 sec (20-25 for heparin)

e) *Plasma recalcification time.*

This is the time of blood coagulation after adding a certain amount of calcium chloride.

↑ at thrombocytopenia, the presence of anticoagulants, lack of coagulation factors.

↓ at thrombosis, DIC syndrome, erythrocytosis.

2) Indicators characterizing the 2nd phase of blood coagulation (thrombin formation):

a) *Quick's prothrombin time (PT)* – N 12-15 sec

PT depends on the content of the factors I, II, V, VII, X.

↑ at liver disease, vitamin K deficiency, DIC syndrome, hereditary deficiency of coagulation factors II (prothrombin), V, VII, X, a decrease in the fibrinogen level (fibrinogen level less than 50 mg/100 mL) or its lack, coumarin treatment, the presence of anticoagulants;

↓ at thrombosis, hypercoagulability (at Disseminated intravascular coagulation (DIC) syndrome), increased activity of the factor VII;

b) Prothrombin ratio (PR) – N 70-120% (0.7-1.1)

↑ at deficiency of coagulation factors, liver damage, vitamin K deficiency, treatment with anticoagulants, heparin treatment, final stages of DIC syndrome;

↓ at thrombosis, increased blood coagulation in women during childbearing, DIC syndrome.

c) *International normalisation ratio* (INR) N 0.9-1.3

Main indications for use: monitoring with anticoagulant therapy with anticoagulants of indirect action – Warfarin (Warfarin Nycomed, Warfarex), Acenocumarol (Sincumar) and other analogues.

INR is calculated when determining the prothrombin time (PT).

↑ at liver disease, vitamin K deficiency, intravascular coagulation, hereditary deficiency of the factors II (prothrombin), V, VII, X; afibrogenogenesis, coumarin treatment.

↓ at the initial stages of thrombosis of the deep veins of the lower extremities, polycythemia, the last months of pregnancy.

3) Indicators characterizing the third phase of blood coagulation (fibrin formation):

a) *Thrombin time* N 10-20 sec

The indicator depends on the concentration of fibrinogen and the processes of its polymerization.

↑ at hypofibrinogenemia associated with DIC syndrome or other pathology; liver damage, increased concentration of fibrinogen/fibrin degradation products, the presence of blood coagulation inhibitors.

↓ at hyperfibrinogenemia, a decrease in the activity of blood plasma antithrombin, paraproteinemia.

b) Fibrinogen A (factor I) N 2-4 g/L

Fibrinogen, a blood coagulation factor, is produced by parenchymatous cells of the liver and under thrombin action, converts into fibrin (insoluble protein), the main substrate of the thrombus or clot.

↑ at (hypercoagulation status): inflammatory processes in kidney diseases, peritonitis, pneumonia, myocardial infarction, systemic connective tissue diseases (collagenoses), acute stage of infectious diseases, injuries, burns, surgical

interventions, pregnancy, menstruation, malignant tumors (especially lung cancer), heparin usage, oral contraceptives, postoperative period;

↓: hereditary deficiency, DIC syndrome, use of a numerous drugs (e.g. phenobarbital, streptokinase), post-bleeding condition, liver disease, burn, injury, shock;

c) Ethanol gelation test N negative.

Ethanol test indicates an increased quantity of soluble fibrin-monomeric complexes. In N, it is negative. Positive is an increase in the activity of the fibrinolytic system (with DIC syndrome, thrombosis, treatment with streptokinase and other thrombolytics).

d*) Protamine test* (protamine sulfate test)

N negative.

Positive in hypercoagulation.

4) Indicators characterizing the 4th phase of coagulation (postcoagulative):

a) *Retreatment of blood clot* N 50-70%

↑ at: anemia, hyperfibrinogemia.

↓ at: erytmia, increasing hematocrit, thrombocytopenia.

b) *Spontaneous euglobulin fibrinolysis* (fibrinolytic activity)

This is the time it takes to completely dissolve the clot.

↑ (extension of time - suppression of the fibrinolytic system)

at: thrombosis, hemorrhagic vasculitis, aplastic processes of hematogenesis.

↓ (reduction of time is an index of fibrinolysis activation)

at: DIC-syndrome, cirrhosis, surgical lungs operations, uterus, prostate; complications after childbirth; shock and stress states; acidosis; hypoxia; strong physical activity.

c) *Antithrombin of* III N 80-120%

↑: inflammatory processes in the body, acute hepatitis, lack of vitamin K, treatment with indirect anticoagulants.

↓: congenital deficiency, DIC syndrome, severe liver disease, ischemic heart disease, last trimester of pregnancy, thromboembolism, sepsis, heparin treatment.

d) *Soluble fibrin-monomeric complexes* (SFMC) N up to 4 mg/100 ml

SFMC is a marker of intravascular coagulation of blood at thrombosis, thromboembolia, and DVS syndromes of different genesis.

↑ at: complications of pregnancy (placental abnormalities, eclampsia), chronic renal failure, large traumas, burns, infectious diseases, chronic forms of DVS syndrome. Increased SFMC level → ↑ risk of intravascular thrombus formation.

The effect of heparin therapy shows up a decreasing in the previously elevated index.

e*) D-dimers* N 0.25-0.5 μg/ml (250-500 ng/ml)

D-dimer - the most reliable sign of blood clots appearance in vessels of different thicknesses.

↑: DVS syndrome, thrombosis of different localization (pulmonary embolism, major pulmonary thrombosis, myocardial infarction as a result of coronary thrombosis), pregnancy, tumors, inflammation.

↓: does not have a diagnostic value.

f) *Lupus anticoagulant* N 0.8-1.2 unit

The presence of lupus anticoagulant in the blood can be a symptom of autoimmune pathologies: systemic red wolf, antiphospholipid syndrome, ulcerative colitis, malignant formations, etc.

h) *Plasminogen* N 75-140%

Enhancement: pregnancy (especially 3rd trimester), DIC syndrome, treatment with fibrinolytics (streptokinase), severe liver disease, thrombocytopenia.

i) *Protein* C N 70-130% 3-6 mg/L

↓ innate deficit, liver disease, warfarin therapy, DIC syndrome, deficit of vitamin K.

j) *Protein* S N 70-140% 418-600 mg/L

↓ innate deficit, (manifested by relapses of venous thrombosis up to 40 years

old), liver disease, warfarin therapy, DVS syndrome, vitamin K deficiency.

k) Products of degradation of fibrin (PDP) N up to 5 mg/L

↑: myocardial infarction, thrombosis, pulmonary artery thromboembolism, fibrinolysis, thrombolytic or fibrinolytic therapy, DVS syndrome, malignant tumors, infections, liver diseases.

Table B.5

Acid-alkaline state of human blood [Part 2, 37, 60]

Number	Indicators	Normal state
1	pH: - arterial blood - venous blood	 7.35-7.45 unit 7.32-7.42 unit
2	pCO_2 – partial pressure of carbon dioxide - arterial blood - venous blood	 36-44 mmHg 46-55 mmHg
3	pO_2 - partial pressure of oxygen - arterial blood - venous blood	 90-100 mmHg 35-45 mmHg
4	*SB* (*Standart Bicarbonate*) - plasma - blood	 21-24 mmol/L 24-28 mmol/L
5	AB (Actual Bicarbonate)	AB = SB.
6	TCO_2 is the concentration in the blood (plasma) of total carbon dioxide	22.7 – 28.6 mmol/L
7	BB (Buffer Base)	44-54 mmol/L
8	BE (Base Excess) – deficit/excess of bases	± 2.5 mmol/L
9	Anion deficiency (interval) $=[Na^+] - ([Cl^-] + [HCO_3]^-)$	12±4 mmol/L
10	Potassium	3.6-5.4 mmol/L
11	Sodium	130-157 mmol/L
12	Clorides	95-110 mmol/L

13	Calcium	2-2.5 mmol/L
14	Lactic acid (lactate)	0.33-0.78 mmol/L
15	Glucose	3.3-5.5 mmol/L

Explanation to the table B.5. [Part 2, 37, 40, 60]

1) pH:

pH <7.35 = acidosis

pH> 7.45 = alkalosis

2) pCO 2 - partial pressure (tension) of carbon dioxide.

This is respiratory index of acid-base balance, reflecting the functional state of the respiratory system.

↑ hypercapnia, caused by hypoventilation of the lungs.

↓ hypocapnia, due to hyperventilation of the lungs.

3) pO2 - partial pressure (tension) of oxygen

Characterizes oxygenation and is not related to the 'Acid-Base Equlibrium State' In general, it is a marker of the severity of lung diseases, but is not interpretable at an unknown value of FiO_2.

In the rough approaching the predicted PaO2 value can be calculated as FiO2 (%) x 6 mmHg (for example, when ventilating a patient with FiO_2 = 40% PaO_2 is 6 x 40 = 240 mm Hg). If the real value is lower than calculated one, there is intrapulmonary blood shedding (blood does not pass through the ventilated alveoli and enters the aorta being non oxidized). The more severe the lung damage, the lower the value of PaO_2 at this level FiO_2.

4) SB (Standart Bicarbonate), it is [HCO_3^-].

Renal (metabolic) component of acid-base balance compensation.

↑ metabolic alkalosis

↓ metabolic acidosis

5) AB (Actual Bicarbonate)- bicarbonate (none "potential").

The AB index reflects the degree of respiratory and metabolic disorders.

6) TCO_2 - concentration in blood (plasma) of total carbon dioxide.

7) BB (Buffer Base)

This sum of all anions concentrations of buffer systems (HPO_4^{2-}, HCO_3^-, $Protein^-$, Hb).

BB indicator reflects the degree of metabolic disorders and almost does not change with respiratory disturbances of acid-base balance.

8) BE (Base Excess) - excess/deficit of base

It is the difference between the blood pressure index of the examined blood and the BB in the normal range.

BE = BB - Nbb

It develops when accumulation of the base or deficiency of non-volatile acids. One of the best indicators of metabolic disturbances.

BE = BD (Base Deficit) - a deficiency of bases developing at accumulation of non-volatile acids or a loss of bases (a metabolic acidosis).

9) Anion deficiency is a measurable indicator that characterizes the available amount of organic anions in the blood plasma (protein, lactate, ketone bodies, etc.). It increases with an increase in the content of non-volatile acids. The meaning of uncertain anions can be displayed.

Interpretation of results

1) Estimation of pH

Acidosis <(pH = 7.35-7.45)> Alkalosis

2) Estimation of the mechanism of disturbances (respiratory or metabolic)

a) if pH and $PaCO_2$ are changed in opposite directions – primary disturbances of respiratory character.

b) if pH and PaCO2 are changed in the same direction - primary disorders of metabolic nature.

3) Estimation of correlation of $PaCO_2$ and HCO_3^-.

If $PaCO_2$ and HCO_3^- are changed in opposite directions, the disturbance is in both directions.

4) In metabolic processes, $PaCO_2$, HCO_3^- (AB, SB), BE, BB vary in the same direction, while in respiratory processes, some of them may be normal.

5) Hints.

↑ Anionic interval: metabolic acidosis

↑ Lactate: metabolic lactate acidosis

Hyperglycemia + ketone bodies in the urine: diabetic ketoacidosis

Hypokalaemia and/or hypochloremia: metabolic alkalosis

Hypochloremia: metabolic acidosis with a normal anionic interval

↑ Kreatinine and urea: metabolic acidosis as a result of renal insufficiency.

6) Determination of causes of disturbance of acid-base balance:

a) Respiratory acidosis.

Reasons:

1) high concentration of CO2 in inhaled air;
2) oppression of the brainstem respiratory center (brain trauma, infection, cerebral edema, overdose of narcotic and sedatives drugs);
3) disturbance of the passage of the respiratory tract (bronchospasm, laryngospasm, aspiration, chronic obstructive pulmonary disease, asthmatic status, respiratory distress syndrome);
4) disturbance of the respiratory function of the lungs (severe pneumonia, atelectasis, pneumothorax, hematorax, pulmonary edema, tumors, emphysema, pulmonary edema);
5) disturbance of blood circulation (heart failure, massive thromboembolism);
6) neuromuscular disorders (myasthenia, poliomyelitis, multiple sclerosis, damage of the spinal cord, lateral amyotrophic sclerosis and other);
7) Deformation and damage to the chest (kyphoscoliosis, fracture of the ribs, obesity).

b) Respiratory alkalosis

Causes:

1) Stimulation of the brainstem respiratory center (trauma of the brain with involvement of the respiratory center, infection, neoplasm of the brain, stroke, intake of adrenomimetic drugs).

2) Metabolic disorders (hepatic insufficiency, gram-negative sepsis, overdose with salicylates, fever).

3) Pulmonary disorders (status asthmaticus of the stage I, pulmonary artery thromboembolism of the initial stage).

4) Hyperventilation with hypoxia (pneumonia, congestive heart failure, marked anemia, staying on top of the mountains).

5) Prolonged artificial ventilation of the lungs in the mode of hyperventilation.

c) Metabolic acidosis

Causes:

1) Accumulation of acidic products under the metabolic disorders:

a) Ketoacidosis (diabetes mellitus, prolonged fasting, hypoxia, liver pathology, alcohol intoxication, prolonged fever);

b) Lactic acidosis (heart failure, shock conditions, severe infections, liver pathology, hypoxia);

c) Accumulation of acids at the activation of catabolism (massive injuries, burns, inflammatory processes).

2) Accumulation of acidic products in the body at disorder of their excretion (acute and chronic renal insufficiency, shock conditions).

3) Significant loss of bicarbonate with intestinal juice (with diarrhea).

4) Increased dietary intake of acidic products in the body, poisoning by acids and some drugs (salicylates, ethanol, methanol, ethylene glycol, paraldehyde).

d) Metabolic alkalosis

Causes:

1) Loss of gastric juice at frequent and irregular vomiting, frequent rinsing of the stomach.

2) Excessive administration of sodium bicarbonate in the treatment of metabolic acidosis.

3) Accumulation of HCO_3^- in the body as a result of oxidation of excess amounts of salts of organic acids (with food, when transfusion of citrate in blood).

4) Isolation of a large quantity of H^+ at the intake of diuretics (especially under treatment with thiazide and other).

5) Marked hypokalemia, which causes the transition of H^+ to the cell and the elevation of their excretion with urine (glucocorticoid therapy, primary aldosteronism, liver failure, long-term use of diuretics, insulin therapy, hypercorticism).

6) Secondary hyperaldosteronism due to hypovolemia of different etiology.

7) Disorder of the allocation of HCO_3^- (milk-alkaline syndrome).

8) Chloride diarrhea (congenital metabolic alkalosis caused by pathology of the intestine and loss of H^+ and Cl^-).

Table B. 6.

Normal blood state characteristics and deviation in the case of acidosis or alkalosis [Part 2, 37]

Indicators		pH	pCO_2	BE	BB	AB	SB	Electrolytes				Lactic acid
								Cl^-	Na^+	K^+	Ca^+	
		units	**mm Hg**	**mM**								
Acidosis	**Metabolic**	<	<	<	<	<	<	>	>=	>	>	>
	Respiratory	<	>	>=	>=	>	N	<	>	>	>	<=
Normal range		7.35-7.45 (art) 7.32-7.42 (ven)	36-44 (art) 46-55 (ven)	± 2.5	44-54	24-28		95-110	130-157	3,6-5.4	2-2.5	0.33-0.78
Alkalosis	**Metabolic**	>	>	>	>	>	>	<	<=	<	<	<
	Respiratory	>	<	<=	<=	<	N	>	<	<	<	>=

Table B.7.

Blood hormone tests [Part 2, 37, 60]

Indicators	Normal range
Adrenal hormones	
Adrenaline (epinephrine)	2-2.5 nmol/L
Noradrenaline (norepinephrine)	3.84-5.31 mmol/L
Dopamine	<30-40 ng/L
Corticosteroids (17-OCS)	0.14-0.56 μmol/L
Cortisol	140-640 nmol/L
Aldosterone	15-70 nmol/L
Sex hormones	
Testosterone: Male Female	 19.85 ± 4.68 nmol/L 1.28 ± 0.35 nmol/L
Pituitary hormones	
Adrenocorticotropic hormone (ACTH)	16.4-32.8 nmol/L
Antidiuretic hormone (vasopressin)	<1.5 ng/L
Thyroid-stimulating hormone (TSH)	0.4-4 mU/L
Thyroid glands	
Thyroxine (T_4)	65-155 nmol/L
Triiodothyronine (T_3)	1.77-2.43 nmol/L

Kidneys	
Angiotensin I Angiotensin II	11-88 ng/L 10-60 ng/L
Renin Female Male	 0.96± 0.13 μg/(h×L) 1.16 ± 0.13 μg/(h×L)

Explanation to Table B.7. **[Part 2, 37, 40, 60]**

Blood catecholamines (adrenaline or epinephrine and norepinephrine, dopamine, DOPA - 3,4-dioxyphenylalanine, vanillylmandelic acid):

These hormones increase blood pressure, glucose and cholesterol levels in blood, increase the rhythm of heart contractions, narrow peripheral vessels, inhibit intestinal motility and increase the intake of fatty acids in bloodstream.

↑ at physical and emotional load, pheochromocytoma, hypertonic crisis, neuroblastoma, myocardial infarction, long-term pain syndrome, manic-depressive states, Itsenko-Cushing syndrome, stress, hyperplasia of the brain.

↓ at myasthenia, defeat of the hypothalamus.

Blood ketosteroids (17-ACS)

↑ at hyperplasia, adenoma, adrenal carcinoma, tumors of the testicles and ovaries.

↓ at Addison's disease, adrenal hypoplasia, administration of glucocorticosteroids.

Cortisol

Cortisol enhances the formation of glucose from amino acids and proteins, limits the synthesis of antibodies, as well as reduces allergic reactions.

↑ at Itsenko-Cushing disease, adenomas and adrenal cancer, stress.

↓ at Addison's disease, chronic adrenal insufficiency.

Blood aldosterone (Aldosterone regulates the water-salt metabolism in the body.)
↑ at Conn's syndrome (primary hyperaldosteronism), secondary aldosteronism (diarrhea, laxatives), adrenal hyperplasia, heart failure, edema with delayed sodium excretion, hepatic cirrhosis, treatment with potassium containing preparations, arterial hypertension;
↑ at Addison's disease, hypocorticism (hypofunction of the adrenal glands), diabetes mellitus, acute alcohol intoxication, eclampsia, excessive administration of glucose solutions.

Testosterone (affects the development of genital organs, growth of muscles and bones, formation of secondary sexual characteristics).
↑ at tumors and hyperplasia of adrenal cortex, early puberty, testicular tumors.
↓ at anorchia, disorder of products of gonadotropic hormones, deficiency of the testicles.

Adrenocorticotropic hormone (a stimulator of adrenal cortex hormones synthesis).
↑ at Addison's disease, Itsenko-Cushing disease, stress, tumor, or hyperplasia of the anterior pituitary gland.
↓ at secondary adrenal insufficiency, adrenal tumors.

Antidyuretic hormone (vasopressin)
↑ at tumor of the brain, vascular damage, pneumonia, congestive heart failure
↓ at non-sugar diabetes (pituitary form), nephrotic syndrome.

Thyroid-stimulating hormone (TSH) (stimulates the processes of tyrosine iodization and thyroglobulin degradation in the thyroid gland).
↑ at primary hypothyroidism, thyroiditis.

↓ at secondary hypothyroidism (disorder of the hypothalamic-pituitary system), thyroid adenoma, stress.

Thyroxine (T4) and triiodothyronine (T3) (regulate the intensity of carbohydrates, protein and lipid metabolism, affect the functions of the respiratory, cardiovascular, immune and nervous systems.

↑ at thyrotoxicosis, deficiency of iodine in the body, a state from ↑ thyroid-stimulating hormone level.

↓ at hypothyroidism, dexamethasone action, a state of ↓ thyroid-stimulating hormone level (nephrotic syndrome, chronic liver disease).

Angiotensin I and II

↑ at hypertonic disease, renin-secreting tumors of the glomerular kidney system.

↓ at Conn's syndrome.

Table B. 8

Basic laboratory screening tests [Part 2, 37, 60]

No.	Laboratory tests	Organs
1.	AST (aspartate aminotransferase)	Myocardium
2.	LDH (lactate dehydrogenase)	Myocardium
3.	ALT (alanine aminotransferase)	Myocardium / Liver
4.	Bilirubin	Liver
5.	Urea	Kidneys
6.	Creatinine	Kidneys
7.	GGT (γ- glutamyl transpeptidase)	Bile ducts
8.	AP (alkaline phosphatase)	Bile ducts
9.	α-amylase	Pancreatic gland
10.	Cholesterol	Lipid metabolism
11.	α-cholesterol (cholesterol-HDL)	Lipid metabolism
12.	Triacylglycerols	Lipid metabolism
13.	Glucose	Carbohydrates metabolism
14.	Proteins	Hydratation
15.	Albumin	Protein secretion
16.	Uric acid	Purine metabolism, antioxidants
17.	C-reactive protein	Inflammation

18.	Calcium	Metabolism, bones, parathormone, calcitonin
19.	Phosphates	
20.	Sodium	Water-salt metabolism
21.	Potassium	

Explanations to Table B. 8

Blood tests for the detection of abnormal states of the human body [Part 2, 37, 40, 60]

Anemia:

- blood test (*Complete Blood Count*) with white blood cell count;
- erythrocyte sedimentation rate (*ESR-erythrocytes sedimentation rate*);
- total protein;
- reticulocytes;
- color index (hemoglobin content in one erythrocyte);
- blood serum iron;
- blood serum iron-binding capacity;
- folic acid;
- vitamin B12;
- ferritin;
- erythropoietin;
- transferrin.

Blood analysis and study of abnormal states of the human body [Part 2, 37, 40, 60]

Anemia:

- blood test (CompleteBloodCount) with leukocyte formula;
- erythrocyte sedimentation rate (ESR-erythrocyte sedimentation rate);
- total protein;
- reticulocytes;
- color index (the number of hemoglobin that is in one erythrocyte);
- iron in blood serum;
- iron-binding capacity;
- folic acid;
- vitamin B12;

- ferritin;
- erythropoietin;
- Transferrin.

Arterial hypertension:
- total cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL);
- leptin;
- prothrombin time;
- prothrombin index;
- fibrinogen;
- renin;
- angiotensin;
- aldosterone;
- K, Na, Cl ions in serum of blood;
- Magnesium
- cortisol;
- epinephrine;
- norepinephrine;
- adrenocorticotropic hormone (ACTH);
- thyrotropic hormone (TTG);

Atherosclerosis:
- total cholesterol, HDL, LDL;
- lipoprotein;
- homocysteine
- leptin;
- Triacylglycerols (triglycerides).

Diseases of the adrenal glands:
- adrenaline, norepinephrine, dopamine;

- adrenocorticotropic hormone (ACTH);
- aldosterone;
- angiotensin;
- DHEA-S (dehydroepiandrosterone sulfate);
- cortisol;
- renin;
- 17-hydroxyprogesterone (17-OHP)) together with the determination of 17-ketosteroids (KS) in urine;

Liver Disease:
- blood test;
- erythrocyte sedimentation rate (ESR) with leukocyte formula;
- total protein;
- protein fractions;
- total bilirubin;
- conjugated, or direct bilirubin;
- alkaline phosphatase;
- lipase;
- cholinesterase;
- prothrombin time, the International Normalized Ratio (INR), which means the ratio of the prothrombin time of the patient to the prothrombin time of normal blood plasma, is reduced to the degree of the International Thromboplastin Sensitivity Index;
- total cholesterol;
- alpha-fetoprotein (AFP);
- glycosylated hemoglobin;
- Triacylglycerols (triglycerides);
- alanine aminotransferase;
- aspartate aminotransferase;
- erythropoietin;

- D-dimer.

Pancreatic disease:
- amylase of blood, together with the definition of amylase in urine and pancreas;
- lipase;
- blood glucose;
- total cholesterol;
- gastrin;
- C-peptide;
- insulin-like growth factor 1;
- antibodies to β-cells of the pancreas;
- carbohydrate antigen 19-9 (CA 19-9), an oncogenic marker.

Kidney disease:
- general blood test with leukocyte formula and rate of erythrocyte sedimentation (ESR).

Vascular Disease:
- general blood test;
- total cholesterol, HDL, LDL;
- triacylglycerols;
- leptin;
- hydroxybutyrate dehydrogenase (1st and 2nd fraction of LDH);
- K, Na, Cl ions in serum;
- bleeding time;
- time of curtailment;
- prothrombin time;
- fibrinogen;
- D-dimer;
- creatine kinase;

- homocysteine
- C-reactive protein;
- epinephrine (adrenaline);
- norepinephrine

Diabetes:
- blood test with leukocyte formula and erythrocyte sedimentation rate (ESR);
- blood glucose;
- total cholesterol, HDL, LDL;
- triacylglycerols;
- glycosylated hemoglobin;
- Fructosamine
- insulin;
- C-peptide;
- antibodies to insulin;
- antibodies to β-cells of the pancreas;
- insulin-like growth factor 1;
- creatinine;
- urea
- leptin;
- prothrombin time.

PART 3

MATHEMATICAL MODELLING METHODS APPLICATION FOR CHARACTERIZATION OF BIOCHEMICAL PROCESSES IN THE BLOOD

3.1. General concepts and basic principles of mathematical modeling

Apparently, nowadays, there is no such field of knowledge wherever the achievements of mathematics would be used. Physicists and chemists, astronomers and biologists, geographers and economists, even linguists and historians use the mathematical apparatus. Mathematization of a large number of applied and fundamental sciences has allowed modeling the behavior of the parts of the considered system, their interaction, taking into account the factors affecting this system. Modeling can greatly simplify the planning and execution of experiments [3].

The branch of mathematics, which deals with the construction and study of mathematical models, is called mathematical modeling.

Mathematical modeling as a method of scientific knowledge began to be used by humanity many centuries ago, from the moment when the foundations of the differential and integral calculus were laid. Italian mathematician Fibonacci developed the first mathematical model in the XII century. Attempts to use mathematical modeling in biomedical directions began in the 80's of XIX century. The idea of correlation analysis, put forward by Galton and improved by biologist and mathematician Pearson, arose because of attempts to process biomedical data. Since the 1940s, mathematical methods have penetrated medicine and biology through cybernetics and computer science. Therefore, in XX century, in addition to technical specialties and natural sciences, mathematical modeling began to be widely used in medicine and pharmacy [2, 4, 11].

The use of modern modeling methods is due to:

• the general tendency to expand and deepen the study of processes in the real physical world;

• a significant length of a number of processes;

• Practically impossible to obtain the necessary information by studying the object - the original (objects of micro and macro);

• incomplete authentic data about a physical object that really exists;

• the complexity of the real processes and the high cost of experimental research of the object - the original, when for economic reasons it is more expedient to move them to the object model;

• presence of critical modes of operation of the object, when the research in some limits of the change of exogenous parameters can be dangerous, and research results are not predictable (such situations are very characteristic of many processes and aggregates of power engineering, mechanical engineering, etc.);

• lack of proper conditions, and sometimes insufficient qualification of personnel to study the object - the original;

• need for a large number of experiments with the following generalization of results and multi-factor optimization, when a long study of a real object becomes economically inexpedient;

• absence of the physical object itself, when its manufacturing is at the stage of a design or design project, in the creation of technical objects (machines and apparatus) using mathematical modeling in automated design systems (ADS) [10].

The model (from Latin modus – a copy, image, outline) can be defined as any system, mentally representable or actually existing, which is in a certain relation to another system (the original or object).

According to well-known methodologist V. A. Shtoff, in the process of mathematical modeling must necessarily fulfill the following conditions:

1. Between the model and the original there is the relation of similarity, the form of which is clearly expressed and accurately fixed *(the condition of reflection or refined by analogy)*.

2. The model in the process of scientific knowledge is the deputy of the investigated object *(the condition of representation)*.

3. The model study allows you to get information about the original *(the condition of extrapolation)*. [19].

With the basics of mathematical modeling, as a means of scientific research and the possibilities of processing data using a computer, future specialists will be acquainted with the courses of medical and biological physics, higher mathematics, medical informatics, information technologies in pharmacy. The programs of the above-mentioned educational disciplines include the study of the method of mathematical modeling of the kinetics of chemical reactions, the processes of dissolution of medicinal substances from tablets, the study of population development processes, the theory of epidemics, immunological processes, pharmacokinetics, and others [1, 2, 7].

Mathematical models shall be understood to be the systems of mathematical relations, logical expressions representing certain aspects of the object being searched. Typically, models use differential equations that describe the dynamic processes characteristic of living nature, as well as systems of linear and nonlinear algebraic equations or inequalities [2].

Today, mathematical methods are widely used in biophysics, biochemistry, genetics, immunology, epidemiology, physiology, pharmacology, medical instrumentation, in the creation of biotechnical systems, etc. The development of mathematical models and methods contributes to the expansion of the field of cognition in medicine, the emergence of new highly effective methods of diagnosis and treatment, the creation of medical technology. In recent years, the active introduction of methods of mathematical modeling and the creation of automated, including computer systems, in medicine has considerably expanded the possibilities of diagnosis and therapy of diseases [4, 17, 21].

The method of mathematical modeling eliminates the necessity of making bulky physical models associated with material costs; to shorten the time of definition

of characteristics (especially when calculating mathematical models using computer technologies and efficient computing methods and algorithms) [18].

The mathematical model is constructed only with the help of quantitatively strictly determined values, which in the course of research may change or remain constant. Therefore, before constructing a mathematical model or applying already known mathematical methods and models, it is necessary to separate the object of research into those elements (components) that characterize the most significant properties of this object (process, phenomenon). Each element of the theme formed in this way corresponds to a certain quantitative value. So we get an abstract system of interconnected elements (components) representing (modeling) the real system or object that we are exploring. The process (procedure) for constructing such an abstract simplified system is called the mathematical formalization of a real object, a phenomenon, or a system. But this is not a mathematical model yet. It is also necessary to establish links between individual elements of the system and between the elements of the system and the environment in which this system operates [3].

Features of the mathematical models. For proper understanding of mathematical models, it is advisable to note some of their features:

- Approximation of the description. The mathematical model describes the real object or process is always approximate, with some precision. The proximity of the mathematical model is explained by the assumptions and limitations adopted during its construction, the purpose of which is to simplify the model, make it convenient for use and calculations, and facilitate computational work.

Inaccuracy of the measurements obtained from the experimental data used in the model is also the reason for the approximation of the mathematical model;

- Considering only the main factors. When developing a mathematical model of an object or process try to take into account only the most important factors that influence the results of modeling. Insignificant factors that cause little influence on the behavior of the object under study or the process proceeding from the point of view of the task, as a rule, are not taken into account in the mathematical model. Create a simple model, be able to highlight and take into account the main thing –

this is the art of modeling. Among experts in the field of mathematical modeling with the use of the ECM, it is believed that the degree of understanding by the researcher of the essence of the investigated object is inversely proportional to the number of variables used in the mathematical model;

- A compromise between simplicity and completeness of the description. Excessive simplification of the model can lead to loss of accuracy, and sometimes even make the model unnecessary. The desire to get a more detailed model, take into account the greater number of factors leads to the complication of the mathematical model and the rise in price of the numerical experiment on the computer. Therefore, the researcher must find a reasonable compromise between the requirements of the simplicity of the model, the completeness of the main factors and the accuracy of the model;

- Limited use. The fourth feature of mathematical models is the limited use of them. This is due to accepted assumptions, the rejection of secondary factors for the task, which can be fair in one case and not permissible in the other. The mathematical model is developed for certain purposes and can be used under certain conditions and requires its refinement for use in other conditions;

- The difference between the mathematical models from the law. The law of fundamental science has the character of some absolute category at a certain level of knowledge. It can be either unconditionally true, or definitely false, and then discarded. The mathematical model is not such an absolute category. The same aspects of the phenomena or processes studied can be described by various mathematical models. One of them may be better, and the second one is worse from some point of view under certain conditions and vice versa in others. In other words, mathematical models of the same process may have little in common if they are created for different purposes;

- Adequacy of the mathematical models. Efficiency, success of using the results of mathematical modeling mainly depend on the quality of the mathematical model, on how well it is constructed. Therefore, the main of the basic requirements is to test the mathematical model for the adequacy of the task set before the researcher.

Adequacy of the mathematical model shall be understood to mean: a) the correct qualitative description of the object (process) in the selected state characteristics; b) the correct quantitative description of the object (process) in the selected characteristics with some reasonable degree of accuracy.

Adequacy of the model should not be equated with the accuracy of the model. The concept of accuracy is wider, because it also includes the degree of completeness of the physical factors involved in the process.

Adequacy of the mathematical model of the set task of the research should be verified by practice using the data of the physical experiment [1, 5, 9, 10, 12].

Modeling is done according to the defined scheme. First, the purpose of the modeling is formulated, and then the hypothesis, representing a qualitative description of the system, is selected, the type of model and the mathematical methods of its description, depending on the purpose and kind of information, are chosen. The final stage is to create a model and compare it with the object-system for identification [3].

Mathematical modeling and its main stages

In scientific and engineering practice, the following common terms exist: mathematical modeling, numerical modeling, computer modeling, simulation modeling, statistical modeling, and others.

Mathematical modeling as one of the most effective methods of scientific research, is a complex study of the properties of a physical object with the help of its created mathematical model on a computer.

Mathematical modeling includes the following series of steps:

Stage I. Physical formulation of the research problem. At this stage, the object of research is determined. However, this is not enough, because every object of research, every process is inexhaustible in its properties and relationships (connections). Therefore, in accordance with the research objectives and specific conditions, it is necessary to distinguish from them the most significant objectives, the solution of which should lead to the achievement of the desired goal.

Stage II. Creating a mathematical model. The process of constructing a mathematical model (a mathematical description) is a creative process that depends on a number of factors: on the degree of completeness of information about the object being studied, its internal mechanisms, the goals and tasks of modeling, computational resources, the degree of reliability of the expected results, the intellectual level, the mathematical training and experience of the researcher and others. Mathematical models of objects with a high degree of completeness of information should be constructed using ordinary differential equations and differential equations in partial derivatives. This allows you to study the behavior of the object with a change in time and in spatial (geometric) coordinates. For models of objects with a low degree of information about them using regression methods;

Stage III. Choosing or developing a method for constructing a model solution, and its algorithmization and program implementation on the ECM. The choice or development of a method for constructing a mathematical solution of a model and its subsequent algorithmization and program implementation depend on the complexity of the model itself, which mathematical apparatus is used to construct a mathematical model. If a mathematical model has a deterministic view, that is, it is constructed in the form of a system of differential equations, and then it would be more appropriate to construct a solution of such a model in an analytical way. In this case, we obtain the analytical dependence of the distribution fields of the main parameters of the model (pressure, temperatures or concentrations) relative to the time variable and spatial coordinates, which satisfies the main constraints of the model (initial and boundary conditions). If in this way it is impossible to construct a solution of the model, then they use different approaches;

IV stage. Checking the mathematical model for adequacy. The verification of a mathematical model for the adequacy of a physical object is an important and time-consuming stage of scientific research, on which the quality of the results of modeling and their practical use depends. Therefore, it is necessary to carry out a comprehensive assessment of the correspondence of the results of numerical simulation on the computer and data of physical experiments in a wide range of

changes in input parameters of the model, using the methods of mathematical theory of the experiment;

Stage V – research on a mathematical model. All computational experiments are carried out in advance of the planned plan on the developed and tested mathematical model adequacy;

Stage VI. Transmission of data obtained on a mathematical model to a physical object, studying and using the received information in practical activity. The results of modeling are used to automate the design of technical systems and objects created, the search for optimal modes of technological processes, industry, ecology, etc. [1, 5, 9, 10, 12, 15].

Mathematical modeling makes sense if a certain analogy is maintained between the model and the process being studied. It is best if the mathematical model is identical to the original in the form of equations and relations between variables in the equations. But such identity can only be achieved for the simplest, often technical, objects.

Experimental researches allow us to gain a definite set of facts, which (aggregate) in turn becomes the object of study. Generalization of the facts finds expression in a certain system of hypotheses, explanations of the functioning of the investigated object. The experimenter, proceeding to study, usually has a certain hypothesis and builds a scheme of experiment in accordance with this hypothesis [3].

By the degree of complexity for mathematical models of biological objects and phenomena, there is a division into:

- functional models that reproduce a certain relationship between known and unknown values;

- models represented by a system of equations with many unknowns, which requires for their research the use of powerful computers and the availability of appropriate software tools;

- models of the optimization type represented by systems of equations or inequalities with respect to unknown quantities whose purpose is to find a solution that would give the optimal value of a certain indicator;

- simulation models used for the analysis of complex systems, characterized by accurate reproduction of the biological process or phenomenon, require special calculations on the computer;

- more complex systems and complexes of interconnected models of the listed types [2; 3; 7].

Let's consider more in detail some techniques of mathematical modeling, in particular simulation and static modeling.

Imitation modeling. Often, the method of mathematical modeling is used - imitation modeling. The essence of imitation is that the model of the real system is first constructed verbally, conceptually, and then formalized for mathematical description of the model, including methods of informatics, system analysis and mathematical modeling. The main condition for constructing an imitation model is the use of modern computers. It should be noted that when constructing an imitation model, the requirement for a complete mathematical description is not mandatory. When constructing an imitation model, a significant place is allocated to various additional information about the actual object of research, which is obtained by studying the object with laboratory and other non-mathematical methods. Often, it can not be represented by exact mathematical expressions or equations. It is the incompleteness of a mathematical description of a real object that makes an imitation model different from a strict mathematical model in the traditional sense. In imitation, the intuition of a scientist, researcher or specialist and their work in dialogue mode with a computer are widely used. In concurring with the exact mathematical description of individual elements of a real theme, the imitation model, as a rule, should have an advantage in informative and practical use. Therefore, every mathematical model that is successfully used to solve complex practical problems and problems can be called the imitation model or imitation mathematical model with full right.

It is difficult to draw a clear line between different types of imitations, since in mathematical (analytical) models often it is necessary to use a numerical experiment

with the use of computers, and in imitation (system) models it is impossible to conduct analytical solving of the task. The development of specific mathematical models, including analytical ones, is the basis (base) for successfully constructing a good imitation model that can help in the study of complex systems in order to identify the most common and important patterns of the development of organisms, populations and groups as the main elements of these systems.

We also note that models can not be simultaneously and sufficiently adequate (realistic) and rather general (theoretical). Of the most common mathematical models describing a wide class of processes and phenomena, partial mathematical models are derived (which are obtained), which describe more concrete, narrower sets that are characterized by additional connections. So models of different levels are built. Each model of the lower level should be agreed with the model of the higher level (it should not contradict it). A classic example of high-level mathematical models is known conservation laws in mechanics and physics [3, 8, 18, 19]

Static modeling. In order to simplify the process of mathematical formalization and to provide a fairly clear interpretation of the results of the simulation, often the relationships between the output and the input quantities are described by linear functions with a certain degree of approximation. The quality of the models, that is, their adequacy to the actual processes, determine not only the set of input data but also the reciprocal form of communication. There are no models that could reflect all the variety of conditions, factors, and interrelations of the real phenomenon. Therefore, consider only the most significant of them. Studying and identifying patterns of change in productive variables in a model is an important condition for ensuring that its real conditions are adequate.

One of the tasks of static modeling is to find out the quantitative measure of the influence of one or another factor (or complex thereof) on the results. In static modeling of processes most often have to deal with the correlation of signs, when the connection between them is only based on the study of mass phenomena. In this case, the regularity of the connection between the signs is manifested as a tendency that is disturbed by the influence of the set of random processes, that is, the change in the

factor characteristic to a certain value is not accompanied by strictly defined changes in the resultant trait. Of course, there is some distribution of the values of the latter. Most often this distribution is normal. In all cases, with a sufficient number of observations, one can identify a pattern that, on average, characterizes the parameters of the relationship.

When studying the relationship between the signs solve the following tasks:

- Determine whether there is a connection;
- determine which quantitative measure of communication;
- what analytical form of expression of communication;
- the reliability of the found pattern and the possibilities of using the equation parameters for solving practical problems in the development of optimization models [3; 7].

3.2. Correlation analysis of biochemical processes of blood

The correlation connection is found not in each individual case, but with a large number of observations when comparing the mean values of interrelated attributes. It is based on the law of large numbers, which manifests itself in the mass process as a tendency to increase or decrease the result of the characteristic depending on the corresponding change in the factor. From the mathematical point of view, the correlation dependence is a functional relationship only between the mean values of the studied features.

In the direction of the relationship between the correlating values can be direct and inverse. In direct communication, the change in factor character causes the change of the resultant trait in the same direction. If the resultant sign decreases with the decrease of the factor's characteristic, or, on the contrary, with the decrease of the factor characteristic the resultant increases, then such a connection is called inverse.

In the form of communication distinguish rectilinear and curvilinear correlation dependencies. The straight-line correlation is characterized by a uniform growth or reduction of the resultant characteristic under the influence of the corresponding change in the factor's characteristic. Analytically, it is determined by the equation of

the straight line. In the curvilinear correlation relationship, the same changes in the mean values of the factor characteristic correspond to various changes in the mean values of the resultant trait. Analytically, the curvilinear relationship is determined by the equation of the curve of the line.

Depending on the number of investigated features, there is a pair (simple) and multiple correlations. For pair correlations, the relationship between factor and performance is analyzed; for multiple correlations the dependence of the resultant attributes on two or more factor characteristics.

With the help of correlation analysis, the following tasks are solved: the presence and choice of the form of communication of the result of a trait with one or a combination of factors is revealed; Quantify the changes in the dependent variable from the factors affecting it; establish the tightness of communication of the effective indicator with one factor or their complex; analyze the total volume variation of the dependent variable and determine the influence of individual factors in this variation; statically evaluate the sample correlations.

The direction and form of communication and the choice of the type of mathematical equation most clearly determine the interdependence of factor and performance characteristics. The direction and form of communication are established using static groupings, as well as graphs constructed in a system of rectangular coordinates based on empirical data.

With the help of the graph, the ratio of the studied features makes a conclusion about the choice of the type of mathematical equation for the quantitative assessment of the connection. The equations that determine the static relationship between correlated values are called regression equations (correlation equations), and lines built on their basis - regression lines [6].

Consider the scheme of calculations. First of all, we need to find out what the general form of dependence y from x. Here it is expedient reception is the construction of the schedule. On the graph, it is possible to visually identify the alleged form of communication. Standard regression analysis programs, which are almost in any software and mathematical support of modern computers, allow you to

find the best communication form for this case. In practice, most studies begin with the consideration and evaluation of linear communication as the simplest to interpret the parameters. Therefore, the form of communication is expressed by the equation:

$$y = ax + b,$$

where: y - the value of the resultant sign, x - the value of the factor sign, and, b - the desired parameters.In the direct connection between the correlating signs, the coefficient of regression a will have a positive value, with the inverse – negative

If the values of the resultant characteristic y were changed strictly in proportion to the changes in the factor characteristic x, then the expected values of y_i could be accurately calculated by the given values of x_i. In real terms, the observed values of x_i will differ from the expected values of $\overline{y}_i$ by the value of ε_i , that is, we obtain some distribution of deviations:

$\varepsilon_i = y_i - \tilde{y}_i$.

The smaller this difference, the more clearly the regularity of the connection between the signs appears. Therefore, in determining the parameters, you should find a form of communication that would provide a minimum of deviations. And since the deviation has different characters, then the requirement is to provide a minimum of the sum of squares of deviations:

$$\varepsilon = \sum_{i-1}^{n} (y_i - \tilde{y}_i)^2 \to \min,$$

where n - number of investigated values, $n > 2$

The method in which unknown parameters are selected to meet the requirements are called the least squares method.

If for the value ε we apply the theory of extremums, then we obtain the necessary conditions for determining the unknown parameters a and b:

$$\frac{\partial \varepsilon}{\partial a} = 0,$$

$$\frac{\partial \varepsilon}{\partial b} = 0.$$

The resulting equations are called normal equations (the number of normal equations must be equal to the number of parameters).

We will determine the parameters of the equation of linear dependence *a, b* by the least squares method. To determine the unknown parameters of the equation $y = ax + b$, we need to construct a system of two equations and solve it with respect to the unknown *a, b.*

The system of normal equations will have the following form:

$$\begin{cases} \sum_{i=1}^{n} y_i = nb + a\sum_{i=1}^{n} x_i, \\ \sum_{i=1}^{n} y_i x_i = b\sum_{i=1}^{n} x_i + a\sum_{i=1}^{n} x_i^2. \end{cases} \quad (1)$$

The solution of the system (1) has the form:

$$a = \frac{n\sum_{i-1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2},$$

$$b = \frac{1}{n}\left(\sum_{i=1}^{n} y_i - a\sum_{i=1}^{n} x_i\right).$$

Now let's solve this system of equations with respect to unknown parameters *a, b* by example of changes in glucose concentration in plasma of canned blood of bulls, whose prototypes were preserved by bicarbonate-carbon dioxide environment, and control - glucose citrate preservative "Glucurium" [13; 14].

As it can be seen from tab. 3.1, on the day of blood selection (first day of study), glucose levels in the control and experimental samples were the same and were 4.9 ± 0.18 mmol/L and 4.7 ± 0.07 mmol/L, respectively.

In the process of storing content it is gradually reduced. On the tenth day between the control and the trial there was a significant difference between its

average indicators: 3.7 ± 0.13 mmol/L (control) and 4.5 ± 0.05 mmol/L (experiment), respectively; ($p<0.05$). In relation to the baseline data, glucose levels in control samples decreased by 24.4%, whereas in research only 4.3%. Such a trend in falling glucose levels was observed throughout the whole period of preservation of canned blood. At the end of the experiment, (thirty days of storage), the amount of glucose in the control samples is decreased by 89.9% compared to the first day, whereas in the experiments only 55.3% (tab. 3.1).

Table 3.1

Concentration of glucose in plasma of canned blood of bulls when stored under artificial carbon dioxide hypobiosis, mmol/L, (M ± m, n = 5)

Blood samples	Term of storage, days						
	1	5	10	15	20	25	30
Control	4.9 ±0.18	4.6 ±0.04	3.7 ±0.13	3.1 ±0.07	2.4 ±0.02	1.4 ±0.05	0.5 ±0.09
Experimental	4.7 ±0.07	4.6 ±0.11	4.5 ±0.05*	4.1 ±0.33*	3.4 ±0.09*	2.9 ±0.23*	2.1 ±0.08*

Note: * $p<0.05$ in comparison with control samples.

These data indicate that the intensity of glucose utilization in the blood, which was stored in the modified carbonic acid preservative, is significantly lower compared to the control indicating inhibition of glycolysis as the main energy process in erythrocytes.

It is necessary to find the equation of relation between the concentration of glucose in the plasma of canned blood of bulls when it is stored under artificial carbon dioxide gipobiosis and the shelf life, days.

We calculate the amounts that are included in formula (1) - (2). For convenience, the results of all calculations are placed in the following tables 3.2, 3.3.

Table. 3.2

Output and calculation data for calculating the equation of relation between the concentrations of glucose in plasma of canned blood of bulls from the term of storage (control group)

	x_i	y_i	$x_i y_i$	x_i^2	y_i^2
	1	4.9	4.9	1	24.01
	5	4.6	23	25	21.16
	10	3.7	37	100	13.69
	15	3.1	46.5	225	9.61
	20	2.4	48	400	5.76
	25	1.4	35	625	1.96
	30	0.5	15	900	0.25
Σ:	106	20.6	209.4	2276	76.44

Table. 3.3

Output and calculation data for calculating the equation of relation between the concentrations of glucose in plasma of canned blood of bulls from the term of storage (experimental group)

	x_i	y_i	$x_i y_i$	x_i^2	y_i^2
	1	4.7	4.7	1	22.09
	5	4.6	23	25	21.16
	10	4.5	45	100	20.25
	15	4.1	61.5	225	16.81
	20	3.4	68	400	11.56
	25	2.9	72.5	625	8.41
	30	2.1	63	900	4.41
Σ:	106	20.6	337.7	2276	104.69

The system (1) for the control group has the form:

$$\begin{cases} 106\,a + 7b = 20,6, \\ 2276\,a + 106\,b = 209,4, \end{cases}$$

its solution

$$a \approx -0,153,$$

$$b \approx 5,257,$$

so the linear equation will have the form:

$$\tilde{y} = -0,153\,x + 5,257.$$

Similarly, we compile the system (1) for the experimental group:

$$\begin{cases} 106\,a + 7b = 26,3, \\ 2276\,a + 106\,b = 337,7, \end{cases}$$

find its solution:

$$a \approx -0,09,$$

$$b \approx 5,12,$$

consequently, the linear equation will have the form:

$$\tilde{y} = -0,09\,x + 5,12.$$

The obtained equations describe the nature of the connection between the signs and are called regression equations. Regression equations are used to predict the expected levels of effective attributes at the established values of factor characteristics. The expected values of the concentration of glucose in the plasma of canned blood of bulls, calculated using the equation of communication, will be as follows tab.3.4:

Table 3.4

Expected values of concentration of glucose in plasma of canned blood of bulls when stored under artificial carbon dioxide hypobiosis, mmol/L, (M ± m, n = 5)

Blood samples	Term of storage, days						
	1	5	10	15	20	25	30
Control	5.1	4.5	3.7	3.0	2.2	1.4	0.7
Experimental	5.0	4. 7	4.2	3.8	3.3	2.9	2.4

Fundamentals of the analysis of regression equations. The equation of regression allows us to assess the role of factor in the formation of a productive one. To do this, relying on the method of decomposition of variation by factors, it is necessary to determine the proportion of the factor in the overall variation (of variability) of the resultant trait.

An important task of analyzing the regression equation is to determine the covariance between the result and the factor put into the model. A quantitative indicator of the directness of the correlation of the result with one factor is the coefficient of the pair correlation, which is calculated by the formula:

$$r = \frac{\overline{xy} - \bar{x}\bar{y}}{\sigma_x \sigma_y},$$

$$\overline{xy} = \frac{\sum_{i=1}^{n} x_i y_i}{n}, \quad \bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}, \quad \bar{y} = \frac{\sum_{i=1}^{n} y_i}{n},$$

$$\sigma_x = \sqrt{\frac{\sum_{i=1}^{n} x_i^2}{n} - \bar{x}^2}, \quad \sigma_y = \sqrt{\frac{\sum_{i=1}^{n} y_i^2}{n} - \bar{y}^2},$$

where: r - linear correlation coefficient; σ_x - mean square deviation of factor mark; σ_y - mean square deviation of the resultant trait.

Absolute value of the correlation coefficient $|r| \le 1$. The closer the correlation coefficient to ± 1, the closer the link between *y* and *x* and, conversely, the closer the correlation coefficient to 0, the weaker link between the productive and the factor characteristics. If $|r| < 0{,}3$, there is no connection, if $|r| = 0{,}3 - 0{,}5$ - the connection is weak, if - $|r| = 0{,}5 - 0{,}7$ the link is average and if $|r| > 0{,}7$ - the connection is tight. If $|r| = 1$, then the quantities *x* and *y* are bound by a linear functional dependence. The correlation coefficient has the same sign as the coefficient of regression in the equation of communication.

On the basis of data tab. 3.2 we calculate the coefficient of correlation dependence of the concentration of glucose in the plasma of canned blood of bulls from the shelf life (control group).

We calculate all the values necessary for calculations:

$$\bar{x} = 15{,}14\text{ , }\bar{x}^2 = 229{,}22\text{, }\overline{x^2} = 325{,}143\text{,}$$

$$\bar{y} = 2{,}943\text{ , }\bar{y}^2 = 8{,}661\text{ , }\overline{xy} = 29{,}914\text{ ,}$$

so,

$$r = \frac{29{,}914 - 15{,}14 \cdot 2{,}943}{\sqrt{325{,}143 - 229{,}22} \cdot \sqrt{10{,}92 - 8{,}661}} = 0{,}995.$$

Similarly, based on the data in Table. 3 we calculate the correlation coefficient of the concentration of glucose in the plasma of canned blood of bulls from the shelf life (experimental group):

We calculate all the values necessary for calculations:

$$\bar{x} = 15{,}14\text{ , }\bar{x}^2 = 229{,}22\text{, }\overline{x^2} = 325{,}143\text{,}$$

$$\bar{y} = 3{,}757\text{ , }\bar{y}^2 = 14{,}115\text{ , }\overline{xy} = 48{,}243\text{ ,}$$

so,

$$r = \frac{48{,}243 - 15{,}14 \cdot 3{,}757}{\sqrt{325{,}143 - 229{,}22} \cdot \sqrt{14{,}956 - 14{,}115}} = 0{,}959.$$

The correlation coefficient shows that there is a close correlation between the concentration of glucose in the plasma of canned blood of bulls and the shelf life for both groups.

Other types of communication can be used in the analysis of research data and in the preparation of information for optimized models.

For nonlinear forms, a simpler and often used one factor quadratic function of a parabolic type is used:

$$y = a_0 + a_1 x + a_2 x^2.$$

Dependencies, expressed by more complex equations, can take place, for example:

$$y = a_0 + a_1 x + a_2 x^2 + ... + a_n x^n,$$

$$y = a + \frac{b}{x},\ y = ax^b,\ y = ab^x.$$

Single-factor static models are fairly simple and easy to interpret. But in real life, the performance indicators depend on a set of factors. Therefore, more reliable information about their role can be obtained from the study of multi-factor static models.

Linear multi-factor regression model has the form:

$$y = a_0 + a_1 x + a_2 x_2 + ... a_n x_n,$$

Where, a_0, a_1, ..., a_n – the desired parameters of the equation of communication; x_1, ..., x_n – factors included in the model.

The multifactorial stroke model has the form:

$$y = a_0 x_1^{a_1} ... x_n^{a_n}.$$

The parameters of multi-factor models are also determined by the method of least squares.

In general, other forms of equations, such as polynomials, can be used to describe complex interconnections:

$$y = a_0 + a_1 x_1 + ... + a_n x_n + a_{11} x_1^2 + ... + a_{1n} x_n^2 + a_{21} x_1 x_2 + ...$$. [3, 6, 7, 16].

The mathematical model of any process, including biochemical, allows obtaining the necessary information without carrying out a cumbersome experiment, often due to the complexity of the methodology and the high cost of experimental research. At present, mastering modern methods of collecting, processing and analyzing static information is an integral element of constructing a mathematical model. In static modeling of processes most often have to deal with the correlation of signs, when the connection between them is only based on the study of mass

phenomena. The correlation method involves quantifying the mutual relationships between the static characteristics that characterize the phenomena under study. In practice, this problem coincides with the task of selecting empirical formulas according to experimental data and is most often solved by the method of least squares. The second task of correlation analysis is to estimate that the correlation between random variables is so close (strong). The constructed equations of communication allow predicting changes that occur in a given biochemical system with a high degree of probability.

REFERENCES

(PART 3)

1. Антонов Ю.Г. Моделирование биологических систем. Справочник. – К.:Наук. думка, 1977. – 260 с.
2. Беликов В.Г., Пономарев В.Д., Коковкин-Щербак Н.И. Применение математического планирования и обработка результатов эксперимента в фармации. - М.: Медицина, 1973. - 232 с.
3. Вергунова І.М. Основи математичного моделювання для аналізу та прогнозу агрономічних процесів. –К.: «НОРА-ПРІНТ», 2000. – 145 с.
4. Гельфанд И.М., Розенфельд М.А., Шифрин М.А. Очерки о совместной работе математиков и врачей. К.: Наука, 1989. - 272 с.
5. Глушков В.М., Иванов В.В., Яненко В.М. Моделирование развивающихя систем. – М.: «Наука», 1983. – 349 с.
6. Горковий В.К., Ярова В.В. Математична статистика. – К.: ВД «Професіонал», 2004, - 384 с.
7. Ермаков С.М., Михайлов Г.А. Курс статического моделирования. – М.: «Наука», 1977. – 350 с.
8. Максимей И.В. Имитационное моделирование на ЄВМ. – М.: «Колос», 1981. – 319 с.
9. Мари Д. Нелинейные задачи в биологии. Лекции о моделях. – М.: «Мир», 1983. – 398 с.
10. Методы математической биологии в 5 кн.: Кн.2 «Методы синтеза алгебраических и вероястностных моделей биологических систем». – К.: «Вища школа», 1981. – 215 с.
11. Микшина В.С., Алмазова Е. Г. Математические модели управления в здравоохранении. // Матем. моделирование, 2009, том 21, - №4, - С. 111–121.
12. Молчанов А.М. Предисловие редактора. В кн.: «Математическое моделирование биологических процессов». М.: «Наука», 1979.
13. Патент 65175 А Україна, А61К31/01. Бікарбонат-вуглекислотний розчин для консервування донорської крові / Мельничук С.Д., Арнаута О.В., Мельничук Д.О. Національний аграрний університет.
14. Типовий технологічний регламент виготовлення розчину «Глюгіцир» для консервування донорської крові. – К.: 1997. – 44 с.
15. Розен В. Принцип оптимальности в биологии. – М.: «Мир», 1969. – 169 с.
16. Суліма І.М., Яковенко В.М. Вища математика. Теорія ймовірностей. Математична статистика. – К.: НАУ, 2004. – 238 с.
17. Ташкинов А.А., Вильдеман А.В., Бронникков В.А. Модели классификации в задачах прогнозирования двигательного развития у детей с

церебральным параличом // Системный анализ и управление в биомедицинских системах.- 2010. Том 9. - №1. – С. 142-149.
18. Тиманюк В.О., Кокодий М.Г., Пенкин Ю.М., Рыжов А.А., Жук В.А. «Компьютерное моделирование в курсах физики и биофизики». – В-во Запорізького державного медичного університету, 2011. – 520 с
19. Шеннон Р. Имитационное моделирование систем. – М.: «Мир», 1978. – 417 с.
20. Штоф, В.А. Введення в методологію наукового пізнання / В.А. Штоф. - Л.: В-во ЛДУ, 1972.
21. Shidlovskiy N.P. Theorey and methodology development of a system mobiled pharmacy - complexed// Alphabit medical.-2005.-№ 8. - P.24-26.

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Theoretical and methodical fundamentals of the study of metabolic processes in human and animals using blood indicators

MANUAL

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