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CLINICAL AND DIAGNOSTIC SIGNIFICANCE OF THE DETERMINATION  
BLOOD PROTEIN FRACTIONS. ELECTROPHORESIS. ANALYSIS  
PROTEINOGRAM

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Proteins are high-molecular nitrogen-containing organic compounds, which include more than 20 types of amino acids. Simple proteins consist only of amino acids, complex proteins (lipoproteins, glycoproteins, nucleoproteins, chromoproteins, etc.), in addition to amino acids, contain various non-protein components: lipids, carbohydrates, nucleic bases, chromogens and other substances.

Proteins are central to the metabolism of the human body, performing a number of important functions:

- 1) structural (structural basis of cells, organelles, fibrillar proteins);
- 2) transport (lipoproteins, hemoglobin, albumin);
- 3) contractile (muscle proteins - actin, myosin);
- 4) catalytic (enzymes);
- 5) regulatory (hormones);
- 6) protective (immunoglobulins, antibodies, interferon; proteins of the blood coagulation system and fibrinolysis);
- 7) energy (the breakdown of amino acids provides up to 18% of the energy consumed).

Protein metabolism is an extremely complex process that provides in a healthy adult a dynamic balance between the biosynthesis (anabolism) of proteins, which occurs with the use of energy, and the breakdown (catabolism) of proteins, accompanied by the release of energy.

The normal intensity of protein biosynthesis in tissues and organs, corresponding to the needs of the body, is determined by the action of several factors:

1. Sufficient intake of food protein (at least 100 g / s), containing the required amount of essential amino acids.
2. Complete digestion of proteins in the organs of the gastrointestinal tract, which is provided primarily by the enzymes of the stomach (pepsin, gastrin), pancreas (trypsin, chymotrypsin, carboxypeptidase A and B, elastase) and the small intestine (enteropeptidase).
3. Absorption of products of protein hydrolysis (amino acids) in the small intestine, which makes serious demands on the state of the mucous membrane of the small intestine, its motility and the presence of the corresponding transport proteins - carriers of amino acids.

4. Adequate energy supply (ATP, GTP) of protein biosynthesis in all tissues and organs (primarily in the liver) and its regulation by anabolic hormones (sex hormones, insulin, STH of the pituitary gland) and vitamins (C, B6, etc.).

Violation of the action of any of the listed factors can lead to inhibition of protein biosynthesis in the body and the development of protein deficiency.

The concentration of proteins in the plasma of a healthy person ranges from 65 to 85 g / l. The bulk of the total protein in blood plasma (about 90%) is albumin, globulins and fibrinogen.

Albumin is the most homogeneous fraction of simple proteins, synthesized almost exclusively in the liver. About 40% of albumin is in plasma, and 60% is in the extracellular fluid. The main functions of albumin are the maintenance of colloid-osmotic (oncotic) pressure, as well as participation in the transport of many endogenous and exogenous substances (free fatty acids, bilirubin, steroid hormones, magnesium ions, calcium, antibiotics, cardiac glycosides, barbiturates, acetylsalicylic acid, etc. ).

Serum globulins are represented by four fractions ( $\alpha_1$ ,  $\alpha_2$ ,  $\beta$  and  $\gamma$ ), each of which is not homogeneous and contains several proteins that differ in their functions.

The composition of  $\alpha_1$ -globulins normally contains two proteins that are of the greatest clinical importance:

1.  $\alpha_1$ -antitrypsin, which is an inhibitor of a number of proteases (trypsin, chymotrypsin, kallikrein, plasmin);

2.  $\alpha_1$ -glycoprotein involved in the transport of progesterone and testosterone and binds small amounts of these hormones.

1.  $\alpha_2$ -macroglobulin - an inhibitor of a number of proteolytic enzymes (trypsin, chymotrypsin, thrombin, plasmin, kallikrein), is synthesized outside the liver;

2. haptoglobin - a protein that binds and transports free hemoglobin A to the cells of the reticuloendothelial system;

3. ceruloplasmin - has oxidase activity and oxidizes ferrous iron into trivalent, which ensures its transport by transferrin;

4. apoproteins A, B and C, which are part of lipoproteins.

The  $\beta$ -globulin fraction also contains several proteins:

1. transferrin - a protein involved in the transport of ferric iron;

2. Hemopexin - a carrier of free heme and porphyrin, binds hemin-containing chromoproteins (hemoglobin, myoglobin, catalase) and delivers them to the liver RES cells;
3. lipoproteins;
4. part of immunoglobulins;
5. some protein components of complement.

$\gamma$ -globulins are immunoglobulins, which are characterized by the function of antibodies produced in the body in response to the introduction of various substances with antigenic activity; modern methods make it possible to distinguish several classes of immunoglobulins (IgG, IgA, IgM, IgD and IgE).

Fibrinogen is an essential component of the blood coagulation system (factor I). It forms the basis of the blood clot in the form of a three-dimensional network in which blood cells are trapped.

#### Research methods

The standard set of biochemical parameters reflecting the state of protein metabolism is often limited to determining the content of total protein, protein fractions (albumin,  $\alpha$ 1-,  $\alpha$ 2-,  $\beta$ - and  $\gamma$ -globulins) and fibrinogen. If necessary, also determine C-reactive protein (CRP), the content of seromucoid and other serum proteins.

**Total protein.** The most common method for determining total serum protein is the so-called biuret method. The biuret reagent contains copper sulfate, which in an alkaline medium reacts with serum proteins and forms violet-colored compounds. The color intensity, depending on the concentration of protein in the serum, is determined on a photometer and compared with the intensity of a calibration solution with a known concentration of albumin (photometric method).

There is also a refractometric method for determining total protein, based on the principle of measuring the refractive indices of light passing through colored compounds.

**Albumen.** The amount of albumin in the blood serum is determined photometrically using the dye bromocresol green, which, when interacting with albumin in a weakly acidic medium, forms a colored blue complex.

**Protein fractions.** In clinical practice, electrophoresis is often used to determine various protein fractions (including albumin,  $\alpha$ 1-,  $\alpha$ 2-,  $\beta$ - and  $\gamma$ -globulins). The speed of movement of individual protein fractions towards the anode (+) depends on their electric charge and other physicochemical properties. The fraction of albumin moves most rapidly to the anode, then  $\alpha$ 1-,  $\alpha$ 2-,  $\beta$ - and  $\gamma$ -globulins. After the end of the electrophoretic separation of protein fractions, the acetate films or strips of paper are

dried in air and in an oven and stained with one of the dyes (bromophenol blue, crimson C, etc.).

Thus, pieces of paper tape or cellulose acetate films are obtained, containing individual protein fractions - electrophoregrams. The color intensity corresponding to the concentration of individual protein fractions is measured with a photometer or densitometer. The results are expressed as a percentage of the total serum protein content. For clarity, graphs of the quantitative ratio of protein fractions are plotted.

For the separation of serum proteins, other, more subtle research methods are also used (ultracentrifugation, immunoelectrophoresis, etc.), which make it possible to identify individual serum proteins that are part of a particular protein fraction, as well as to determine their physicochemical properties.

#### Interpretation of results

The content of total protein in blood serum in a healthy person ranges from 65 to 85 g / l, and albumin - from 35 to 50 g / l. Normal values of protein fractions, expressed as a percentage in relation to the total protein content. In a healthy person, fluctuations in these values are observed within a fairly wide range, which is partly due to the method for determining protein fractions, in particular, the type of dye used to dye paper or cellulose acetate films with electrophoregrams.

It is also important to calculate the albumin-globulin coefficient (A / G), which is normally about 1.5.

Table 1.4 presents the main causes of hypo- and hyperproteinemia, as well as some mechanisms of impairment of the protein content in the blood serum.

#### Table 1.4

The main causes of changes in the content of total protein and albumin in serum.

Изменение содержания белка	Механизмы	Заболевания и синдромы
Гипопротеинемия: общий белок < 65 г/л, альбумин < 35 г/л	Нарушения синтеза белка	1. Болезни печени: гепатиты, циррозы, липоидоз печени, первичный рак печени, метастазы рака в печень, амилоидоз 2. Злокачественные новообразования 3. Длительные заболевания, лихорадки, интоксикация 4. Лучевая болезнь 5. Застойная сердечная недостаточность
	Усиление процессов катаболизма белка	1. Тиреотоксикоз 2. Гиперсекреция глюкокортикоидов (болезнь Иценко-Кушинга и др.) 3. Осложнения терапии глюкокортикоидами
	Значительные потери белка	1. Нефротический синдром 2. Острые и хронические желудочно-кишечные инфекции, злокачественные новообразования желудка, кишечника 3. Обширные термические ожоги, распространенная экзема с экссудативным процессом 4. Парацентез с удалением большого количества асцитической жидкости 5. Острые и хронические массивные кровопотери
	Недостаток белка в пище	1. Алиментарное белковое голодание 2. Осложнения лечения голодом
	Нарушения переваривания белков и всасывания продуктов их распада	1. Болезни органов пищеварения (язвенная болезнь, стеноз привратника, панкреатиты, рак поджелудочной железы, атрофический гастрит и др.) 2. Синдром мальабсорбции (энтериты, панкреатиты и др.)
	Относительная гипопротеинемия (гипергидратация организма)	1. Выраженный отечный синдром любого происхождения 2. Введение больших количеств жидкости в сосудистое русло
	Увеличение иммуноглобулинов	1. Парапротеинозы: миеломная болезнь, макроглобулинемия Вальденстрема 2. Хронические заболевания, сопровождающиеся активацией иммунной системы
Гиперпротеинемия: общий белок > 85 г/л, альбумин > 50 г/л	Увеличение белков острой фазы	1. Острые воспалительные процессы (редко)

It should be borne in mind that in many pathological conditions, these disorders are based on a combination of several mechanisms at once. For example, in malignant neoplasms of the gastrointestinal tract, hypoproteinemia may be based not only on inhibition of protein synthesis at the cell level due to cancer intoxication, a decrease in the activity of anabolic hormones, a lack of vitamins and impaired liver function in its metastatic lesion, but also insufficient intake of protein with food. due to the anorexia characteristic of these diseases, impaired digestion and absorption of food proteins in the gastrointestinal tract, an increase in protein catabolism and their loss through the gastrointestinal tract.

Any reasons leading to protein deficiency (for example, protein starvation) naturally cause an increase in the catabolism of their own proteins during their breakdown, which affects the function of all organs and systems.

The most important clinical consequences of hypoproteinemia of any origin are: 1) weight loss, up to cachexia, 2) anemia, 3) hypoproteinemic edema (with a decrease in albumin below 20 g / l), 4) dysfunction of various organs and systems.

Table 1.5 shows the main reasons for the change in the content of globulin fractions and the proteins of these fractions responsible for violations.

Table 1.5

Фракция глобулинов	Изменение содержания фракции	Заболевания и синдромы	Белки, входящие в состав фракции и ответственные за изменения
$\alpha_1$	Увеличение	Острые воспалительные процессы	Белки острой фазы: $\alpha_1$ -антитрипсин, $\alpha_1$ -гликопротеид, серомукоид, С-реактивный белок и др.
		Повреждение и распад тканей	$\alpha_1$ -антитрипсин, $\alpha_1$ -гликопротеид, С-реактивный белок и др.
	Уменьшение	Тяжелые деструктивные процессы в печени (болезнь Боткина, гепатолентикулярная дегенерация, циррозы печени, хронический активный гепатит и др.) Первичная деструктивная эмфизема легких	$\alpha_1$ -гликопротеид (нарушение синтеза в печени) Врожденный дефицит $\alpha_1$ -антитрипсина
$\alpha_2$	Увеличение	Острые воспалительные процессы	Белки острой фазы: $\alpha_2$ -макроглобулин, гаптоглобулин, церулоплазмин
		Повреждение и распад тканей	$\alpha_2$ -макроглобулин и др.
		Болезни соединительной ткани (коллагенозы)	Гаптоглобулин
		Нефротический синдром	$\alpha_2$ -макроглобулин
		Беременность	Церулоплазмин
	Уменьшение	Гемолиз, гемоглинурия	Гаптоглобулин (связывание со свободным гемоглобином)
		Повреждение ткани поджелудочной железы (панкреатит, сахарный диабет)	Дефицит $\alpha_2$ -макроглобулина
$\beta$	Увеличение	Первичные и вторичные гиперлипопротеидемии	$\beta$ -липопротеиды
		Хронические заболевания с активацией иммунной системы: хронические инфекции, цирроз печени, болезни соединительной ткани, злокачественные новообразования, аутоиммунные и аллергические заболевания	Иммуноглобулины: G, A, M, E, D
	Уменьшение	Абеталипопротеинемия	$\beta$ -липопротеиды
		Атрансферринемия	Дефицит трансферрина
$\gamma$	Увеличение	Заболевания с активацией иммунной системы: хронические инфекции, заболевания печени, аутоиммунные и аллергические заболевания	Иммуноглобулины
		Парапротеинемии: миеломная болезнь, макроглобулинемия Вальденстрема, болезнь «тяжелых цепей»	Образование патологических парапротеинов
	Уменьшение	Длительные хронические заболевания с истощением иммунной системы (хронические инфекции, злокачественные новообразования, болезни печени и др.)	Дефицит иммуноглобулинов
		Лечение цитостатиками, иммунодепрессантами, глюкокортикоидами, лучевые воздействия	Дефицит иммуноглобулинов
		Иммунодефицитные заболевания с поражением В- и Т-лимфоцитов: агаммаглобулинемия Брутона, атаксия-телеангиоэктазия (синдром Луи-Бар)	Дефицит иммуноглобулинов
		Избыточная потеря белка (энтериты, обширные ожоги, нефротический синдром)	Дефицит иммуноглобулинов

The most common causes of changes in serum globulin fractions

The table shows that an increase in the content of  $\alpha_1$ - and  $\alpha_2$ -fractions of globulins is observed in several pathological conditions:

1. In acute inflammatory processes, since  $\alpha$ -globulins contain the so-called acute phase proteins ( $\alpha_1$ -antitrypsin,  $\alpha_1$ -glycoprotein,  $\alpha_2$ -macroglobulin, haptoglobulin, ceruloplasmin, seromucoid, C-reactive protein).

2. In case of significant damage and decay of tissues (dystrophic, necrotic processes), accompanied by cell destruction and release of tissue proteases, kallikrein, thrombin, plasmin, etc., which naturally leads to an increase in the content of their natural inhibitors ( $\alpha$ 1-antitrypsin,  $\alpha$ 1- glycoprotein,  $\alpha$ 2-macroglobulin, etc.). Tissue damage also leads to the release of pathological C-reactive protein, which is a product of cell degradation and is part of the  $\alpha$ 1-fraction of globulins. Significant tissue destruction is observed in malignant neoplasms, tumor metastasis, severe trauma, after surgery, with heart attacks of various organs (ischemic stroke, pulmonary, intestinal infarction, etc.).
3. In diseases accompanied by depolymerization of glycoproteins of the main substance of connective tissue (collagenoses), mainly due to an increase in the content of haptoglobin and other proteins.
4. With nephrotic syndrome, in particular due to an increase in  $\alpha$ 2-macroglobulin.
5. During pregnancy - mainly due to the stimulating effect of estrogens on the synthesis of ceruloplasmin, which is part of the  $\alpha$ 2-globulin fraction.

A decrease in the content of  $\alpha$ -globulins in clinical practice is quite rare. Sometimes it is observed:

1. With severe destructive processes in the liver parenchyma: with acute infectious hepatitis, hepatolenticular degeneration of the liver (Wilson-Konovalov disease), hepatocerebral degeneration syndrome (Westphal-Strumpfel-Wilson disease), with cirrhosis and liver cancer, chronic active hepatitis. In these cases, a decrease in the content of  $\alpha$ -globulins is associated with a decrease in the synthesis of haptoglobin, ceruloplasmin,  $\alpha$ 1-glycoprotein.
2. With hemolysis of erythrocytes, hemoglobinuria, which is explained by the increased binding of haptoglobin to free hemoglobin.
3. With congenital deficiency of  $\alpha$ 1-antitrypsin, which is part of the  $\alpha$ 1-fraction of globulins and is an inhibitor of tissue proteases, including elastase, which destroys elastic fibers. Deficiency of  $\alpha$ 1-antitrypsin can lead to damage to the lung tissue and the development of primary diffuse destructive pulmonary emphysema.
4. With a decrease in the content of  $\alpha$ 2-macroglobulin - an inhibitor of some proteolytic enzymes of the pancreas (trypsin, chymotrypsin). It is believed that in some cases (rarely) a deficiency of  $\alpha$ 2-macroglobulin can contribute to damage to the pancreas (pancreatitis, diabetes mellitus).

An increase in the fraction of  $\beta$ -globulins is observed:

11. In primary and secondary hyperlipoproteinemia, especially type II (see below for more details), since most of the complex lipoprotein proteins are part of the  $\beta$ -fraction of



globulins. Secondary hyperlipoproteinemias are more common in atherosclerosis, diabetes mellitus, hypothyroidism, and nephrotic syndrome.

2. With long-term chronic diseases, accompanied by an increase in the content of immunoglobulins in the blood (usually simultaneously with an increase in the content of  $\gamma$ -globulins): chronic infections, cirrhosis of the liver, diseases of the connective tissue (rheumatism, RA, SLE, etc.), malignant neoplasms, autoimmune and allergic diseases.

A decrease in the fraction of  $\beta$ -globulins is rare:

1. With abetalipoproteinemia.

2. With atransferrinemia - a rare disease caused by a deficiency of the main transport protein - transferrin, which leads to the development of iron deficiency anemia and causes a picture of hemosiderosis (iron deposits in internal organs) with the development of hepatosplenomegaly.

An increase in the  $\gamma$ -globulin fraction is detected in the following pathological conditions:

1. In diseases accompanied by an intensification of immune processes, since the  $\gamma$ -globulin fraction consists mainly of immunoglobulins: in chronic infections, chronic liver diseases (chronic hepatitis and liver cirrhosis), autoimmune diseases (including connective tissue diseases - RA, SLE and etc.), chronic allergic diseases (bronchial asthma, recurrent urticaria, drug disease, atopic dermatitis, eczema, etc.).

2. With paraproteinemia - diseases accompanied by the formation of pathological proteins (paraproteins) belonging to one of the classes of immunoglobulins (G, A, less often D and E): multiple myeloma (plasmacytoma), Waldenström macroglobulinemia, heavy chain disease, etc.

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A decrease in the fraction of  $\gamma$ -globulins occurs:

1. With long-term chronic diseases, accompanied by depletion of the immune system and a decrease in the formation of immunoglobulins (chronic infections, malignant neoplasms, diseases of the kidneys, liver, lymphocytic leukemia, lymphogranulomatosis, etc.).

2. When treating with cytostatics, immunosuppressants, glucocorticoids, as well as as a result of X-ray irradiation, the use of radionuclides or radiation therapy.

3. With a variety of immunodeficiency states associated with damage to B- and T-lymphocytes (Bruton's agammaglobulinemia, ataxia-telangiectasia - Louis-Bar syndrome, etc.).

4. With excessive loss of protein (enteritis, nephrotic syndrome, extensive burns).

The data presented indicate that in each specific case, the interpretation of the proteinogram is extremely difficult and should take into account not only the possible nosological diagnosis, but also the individual characteristics of the clinical picture of the disease, its duration, the effectiveness of treatment, the fact of taking certain medications, the use of radiation therapy.

the state of the immune system, the presence of concomitant diseases, etc. Nevertheless, in most cases, a practitioner should be guided by the following fundamental provisions:

1. The most common causes of an increase in the content of  $\alpha$ -globulins are acute inflammatory diseases and diseases accompanied by significant damage and decay of tissues. 2. An increase in  $\beta$ -globulins (especially when combined with hypergammaglobulinemia) more often indicates the activation of the body's immune system in patients with various acute or chronic diseases of internal organs, although it is also necessary to take into account the possibility of the influence of hyperlipoproteinemia, especially in patients with atherosclerosis, diabetes mellitus, hypothyroidism and nephrotic syndrome. 3. An increase in the content of  $\gamma$ -globulins may also be due to a significant activation of the immune system or the so-called paraproteinemia (myeloma or Waldenstrom's macroglobulinemia). 4. A decrease in  $\alpha$ -globulins can often be associated with severe destructive processes in the liver, occurring with impaired protein synthesis. 5. Hypogammaglobulinemia indicates the presence of an immunodeficiency state caused by the depletion of the body's immune system during long-term chronic diseases, long-term treatment with cytostatics, immunosuppressants, glucocorticoids and radiation exposure. 6. Excessive loss of protein by the body (enteritis, massive burns, nephrotic syndrome), in addition to hypoalbuminemia, may be accompanied by a decrease in the content of  $\gamma$ -globulins.

Methods for the determination of serum fibrinogen, seromucoid, C-reactive protein and protein sediment samples, as well as their diagnostic value, are described in other sections of this manual.

## Enzymes

Enzymes are substances of a protein nature that ensure the normal course of all chemical reactions in the body. They are distinguished by high specificity in relation to the substrates on which they act, which is explained by their protein nature and the unique three-dimensional spatial organization of the protein molecule. The geometric shape of the active center of enzymes, that is, that part of the protein molecule that directly interacts with the substrate, must fully correspond to the shape of the substrate molecule. That is why the slightest violation of the secondary or tertiary structure of the enzyme molecule leads to a significant decrease in its activity.

The activity of the enzyme is also influenced by other factors: 1) the presence of a temperature optimum; 2) pH of the medium, its ionic composition; 3) the concentration of reacting substrates; 4) the effect on enzymes of special substances - enzyme effectors (inhibitors or activators), as well as the presence in the active center of some cofactor enzymes - non-protein parts of the enzyme (vitamins, nucleotides, metal ions), which are also necessary for the manifestation of enzyme activity.

Changes in the activity of enzymes in some cases can be considered as a consequence, in others - as the cause of various pathological conditions.

The main factors determining the activity of blood serum enzymes are: 1) the rate of enzyme synthesis in the cell; 2) the rate of release of enzymes from the cell, which can increase, for example, with an increase in permeability and damage to the cell membrane caused by inflammation, ischemia, dystrophy, necrosis, autoimmune damage to cells, etc .; 3) the rate of removal of enzymes from the extracellular fluid by their inactivation, destruction and / or excretion in urine, bile, etc .; 4) changes in the activity of natural inhibitors and activators of enzymes.

Classification and nomenclature of enzymes. Depending on the type of catalyzed reactions, all enzymes are divided into six classes and several subclasses and sub-subclasses (Table 1.6). In accordance with this classification, each enzyme is designated with a cipher, including the numbers of the class, subclass, sub-subclass and the serial number of the enzyme in the sub-subclass. They also use trivial ones, usually used names of enzymes (for example, alanine aminotransferase).

Table 1.6

Classification of enzymes (according to A.Sh.Byshevsky and O.A. Tersenov) Class  
Types of catalyzed reactions

No. Name

1. Oxidoreductases Redox reactions
2. Transferases Intermolecular transfer reactions:  $A - B + C = \text{further} = A + B - C$
3. Hydrolases Hydrolytic cleavage reactions
4. Lyases Non-hydrolytic cleavage reactions with the formation of double bonds
5. Isomerases Reactions of changes in the geometric or spatial configuration of the molecule
6. Ligases, or synthetases Reactions of combining two molecules, accompanied by hydrolysis of macroergs

Aminotransferase

Aminotransferases (transaminases) - enzymes that play an important role in nitrogen metabolism, are involved in the breakdown of amino acids that are not used in the processes

biosynthesis. They catalyze the transamination reaction, in which there is, as it were, an exchange of the amino group ( $\text{NH}_2$ ) between the amino acid and the keto acid (Fig. 1.51 and 1.52). The mediator in this transfer of the amino group is vitamin B6 (more precisely, its derivative - pyridoxal phosphate), which is part of the active center of aminotransferases (coenzyme). Pyridoxal phosphate (vitamin B6) interacts with the amino acid, taking on the amino group ( $\text{NH}_2$ ), and then transferring it to the keto acid. Transamination reactions are reversible and can proceed in both directions, depending on the ratio of the concentrations of the reacting components and the need for them.

All amino acids, with the exception of lysine and threonine, are specifically affected by aminotransferases. Two of them are of the greatest importance: aspartate aminotransferase and alanine aminotransferase.

Aspartate aminotransferase (ASAT) - catalyzes the transamination reaction between aspartic and  $\alpha$ -ketoglutaric acid. As a result of this reaction (Fig. 1.51), aspartic acid (aspartate), having lost its amino group ( $\text{NH}_2$ ), turns into oxaloacetic acid (oxaloacetate), and  $\alpha$ -ketoglutaric acid, acquiring an amino group, turns into glutamic acid. The reverse course of the reaction leads to the formation of aspartic and  $\alpha$ -ketoglutaric acid by the interaction of glutamic acid and oxaloacetate.

Alanine aminotransferase (ALT) catalyzes a similar reaction between alanine and  $\alpha$ -ketoglutaric acid to form glutamic and pyruvic acids (pyruvate). In the reverse course of the reaction (from left to right), alanine and  $\alpha$ -keto acid are formed from glutamic acid and pyruvate (Fig. 1.52).

The main participants in these two reactions are glutamic and aspartic acids, which are then included in the ornithine cycle of urea biosynthesis described above - the main mechanism for neutralizing ammonia in the body.

Aminotransferases are found in almost all organs, but the highest aminotransferase activity is found in the liver, skeletal muscle, heart and kidneys. The activity of aminotransferases in erythrocytes is 6 times higher than in blood serum.

Normally, a relatively small amount of aminotransferases is present in the blood serum. An increase in the activity of these enzymes in the serum is of diagnostic value, due to damage to the tissue of the liver, heart, skeletal muscles, kidneys and the entry of enzymes into the general bloodstream. Damage to liver, heart, skeletal muscle and / or kidney tissue is usually accompanied by a significant release of aminotransferases into the bloodstream. The immediate causes of increased serum aminotransferase activity are:

1. necrosis or damage to liver cells of any origin (acute viral hepatitis, chronic hepatitis, liver cirrhosis, liver tumors, alcohol intoxication, obstructive jaundice, taking certain hepatotoxic drugs);
2. acute myocardial infarction (MI), acute myocarditis;
3. injury or necrosis of skeletal muscles;
4. hemolysis of erythrocytes.

In clinical practice, the ratio of AST / ALT activity in blood serum (de Ritis coefficient) is of great importance.

1. In acute myocardial infarction, the activity of AST is higher than that of ALT (de Ritis coefficient is more than 1.3). 2. In acute viral and chronic hepatitis, especially in the early stages, the activity of ALT is higher than that of AST (the de Ritis coefficient is less than 1.0). Severe liver damage can change this ratio. 3. In alcoholic hepatitis, the activity of AsAT is often higher than ALT (the de Ritis coefficient is more than 1.3).

#### $\gamma$ -glutamyl transpeptidase (GGTP)

$\gamma$ -glutamyl transpeptidase ( $\gamma$ -glutamyl transferase GTP), like aminotransferase, belongs to the class of transferases and is actively involved in nitrogen metabolism. The enzyme catalyzes the transfer of a glutamine group from a  $\gamma$ -glutamine residue to an acceptor peptide or L-amino acid.

The greatest activity of GGTP is found in the tissue of the liver, kidneys, and pancreas. Normally, the activity of the enzyme in the blood serum does not exceed 66-106 IU.

An increase in the activity of GGTP is observed in the following pathological conditions:

1. obturation of the intrahepatic and extrahepatic biliary tract (especially a significant increase in GGTP, going in parallel with an increase in the activity of alkaline phosphatase - see below);
2. liver diseases (hepatitis, cirrhosis of the liver, tumors and metastases in the liver), especially occurring with the phenomena of cholestasis;
3. pancreatitis and pancreatic tumors;
4. intoxications with ethanol (even with moderate alcohol consumption), drugs and sedatives (drug intoxication).

It should be emphasized that an increase in the activity of GGTP is one of the most sensitive, albeit nonspecific, biochemical tests, indicating the presence of damage to the liver parenchyma, biliary system and alcohol intoxication. The activity of this enzyme

responds especially well to alcohol-toxic liver damage, which is used to objectively monitor the effectiveness of alcoholism treatment.

### Creatine kinase (creatine phosphokinase, CPK)

Creatine phosphokinase catalyzes the reversible reaction of creatine phosphorylation to form creatine phosphate (CP). The latter is a kind of reserve high-energy compound, which can be quickly used as an "emergency" source of energy, mainly in tissues, the functioning of which makes increased demands on energy supply (nervous tissue, muscles).

In the normal (sparing) mode of functioning of these tissues, the energy needs of muscles and nerve tissues are met due to the formation of ATP during the oxidation of carbohydrates or acetoacetate. At the same time, a large amount of creatine phosphate (CP) accumulates here due to the direct reaction catalyzed by CPK:

Creatine + ATP is converted to CF + ADP.

In this case, the concentration of CF is 5–8 times higher than the concentration of ATP.

With the enhanced functioning of nerve tissues and muscles, the reaction equilibrium shifts in the direction of the disintegration of CP, which makes it possible to maintain a constant concentration of ATP for some time. It is not surprising, therefore, that the highest CPK activity is found in skeletal muscles, heart and brain.

There are three fractions (isoenzymes) of CPK, which have a relatively high organ specificity:

- 1) MM-fraction (muscle);
- 2) MV fraction (cardiac);
- 3) BB-fraction (cerebral).

Normally, the activity of CPK in blood serum does not exceed 66.6 mmol / (h x L), and 94–96% of this activity is due to the MM-fraction of CPK (muscle). The share of the MV fraction (cardiac) normally accounts for only 2–4% of the CPK activity, and the BB isoenzyme (cerebral fraction) is absent in the blood serum.

An increase in serum CPK activity is usually associated with damage to the heart muscle or skeletal and, much less often, smooth muscles. The immediate reasons for the increase in the activity of CPK and its isoenzymes are:

1. Acute myocardial infarction (the CF fraction of CPK increases especially significantly).
2. Acute myocarditis, trauma and heart surgery (mainly CF-CFK fraction).

3. Some clinical variants of unstable angina pectoris (prolonged severe attacks of angina pectoris, Prinzmetal's angina). In these cases, a moderate and short-term increase in predominantly MV-

fraction of CPK, or its values are at the upper limit of the norm.

4. Damage to skeletal muscles: polymyositis, dermatomyositis, muscular dystrophies, any trauma and surgery.

5. Intravenous and intramuscular injections.

6. Less commonly - generalized convulsions, severe physical activity, pulmonary embolism, prolonged hypothermia, congestive heart failure, severe arrhythmias, etc.

The decrease in the activity of CPK in the blood serum is not of great clinical significance, since it may be associated with a decrease in physical activity (physical inactivity) and the size of muscle mass.

#### Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is one of the most important cellular enzymes involved in the process of glycolysis, and catalyzes the reversible reduction reaction of pyruvic acid (pyruvate) to lactic acid (lactate).

As you know, pyruvate is the end product of glycolysis (Fig. 1.53). Under aerobic conditions, pyruvate, undergoing oxidative decarboxylation, is converted to acetyl-CoA and then oxidized in the tricarboxylic acid cycle (Krebs cycle), releasing a significant amount of energy. Under anaerobic conditions, pyruvate is reduced to lactate (lactic acid). This latter reaction is catalyzed by lactate dehydrogenase. The reaction is reversible: in the presence of O<sub>2</sub>, lactate is re-oxidized to pyruvate.

With electrophoresis or chromatography, it is possible to detect 5 LDH isozymes, differing in their physicochemical properties. Two isoenzymes are of greatest importance - LDH1 and LDH5. Fraction LDH1 more actively catalyzes the reverse reaction of the conversion of lactate into pyruvate. It is mostly localized in the heart muscle and some other tissues that normally function under aerobic conditions. In this regard, myocardial cells, which have a rich mitochondrial system, oxidize not only pyruvate in the tricarboxylic acid cycle, formed as a result of the process of glycolysis occurring in them themselves, but also lactate formed in other tissues.

Fraction LDH5 more effectively catalyzes the direct reaction of the reduction of pyruvate to lactate. It is localized mainly in the liver, in skeletal muscles. The latter are often forced to function under anaerobic conditions (with significant physical exertion and rapidly approaching fatigue). The resulting lactate with the bloodstream enters the liver, where it is used for the process of gluconeogenesis (glucose resynthesis), as well as in the

heart and other tissues, where it is oxidized into pyruvate and involved in the tricarboxylic acid cycle (Krebs cycle).

The isoenzyme LDH1 is predominantly localized in the heart, while LDH5 is located in skeletal muscles and liver. Most organs contain a complete set of LDH isoenzymes, including the LDH2,3,4 fractions.

Any damage to tissue cells containing a large amount of LDH (heart, skeletal muscle, liver, erythrocytes) leads to an increase in the activity of LDH and its isoenzymes in the blood serum. The most common causes of increased LDH activity are:

1. Heart damage (acute myocardial infarction, myocarditis); in these cases, an increase in the activity of LDH1 and / or LDH2 usually predominates.
2. Damage to the liver (viral hepatitis, cirrhosis of the liver, cancer, obstructive jaundice), when the isoenzyme LDH5 predominantly increases.
3. Damage to skeletal muscles, inflammatory and degenerative diseases of skeletal muscles (mainly an increase in the LDH5 isoenzyme).
4. Diseases of the blood, accompanied by the breakdown of blood cells: acute leukemia, hemolytic anemia, B12-deficiency anemia, sickle cell anemia, as well as diseases and pathological conditions accompanied by the destruction of platelets (massive blood transfusion, pulmonary embolism, shock, etc.). In these cases, an increase in LDH2,3,4 activity may prevail.

It should be remembered that many diseases and injuries of the heart, skeletal muscles, liver and blood are accompanied by an increase in the activity of total LDH in the blood serum without a clear predominance of any of its isoenzymes.

#### Glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase is the most important enzyme of the pentose phosphate pathway for the conversion of carbohydrates. It catalyzes the initial reaction of this pathway - the oxidation of glucose-6-phosphate to 6-phosphate gluconolactone. The greatest activity of the enzyme is determined in erythrocytes.

The biological meaning of the functioning of the pentose phosphate pathway in erythrocytes lies primarily in the fact that it is the most important source of NADPH, which is subsequently used for the biosynthesis of various organic substances, as well as to maintain a normal concentration of glutathione in its reduced SH-form. The latter protects hemoglobin and erythrocytes from denaturation and decay under the action of various agents with oxidizing properties. Such oxidizing agents include antimalarial agents, PASK, sulfonamides, phenacetins, large doses of vitamin C, as well as viral



infections and certain foods - mushrooms, legumes, etc. These agents promote the oxidation of glutathione in erythrocytes. With a deficiency of glucose-6-phosphate dehydrogenase in cells, the pentose phosphate pathway of glucose breakdown and the release of a sufficient amount of NADPHN necessary for the return of oxidized glutathione to its SH-form are blocked. A decrease in the concentration of reduced glutathione leads to the deposition of denatured hemoglobin in the membrane of erythrocytes (Heinz bodies) and its deformation, which is the main reason for the increased decay (hemolysis) of erythrocytes in RES cells.

Deficiency of glucose-6-phosphate dehydrogenase is one of the most common hereditary defects leading to the development of hemolytic anemia. The disease may not manifest itself for a long time. Hemolytic crisis

occurs when taking the drugs described above, with infections, diabetic acidosis.

Aldolase (fructose-1, 6-diphosphate aldolase) (K.F.4.1.2.13)

Fructose diphosphate aldolase (aldolase) is an enzyme involved in the glycolytic breakdown of glucose. Aldolase catalyzes the formation of 1 molecule of fructose-1, 6-diphosphate of two molecules of 3-phosphoglyceric aldehyde (triose phosphate). The enzyme is present in all tissues and organs, but the greatest activity is found in muscle tissue, heart, liver and brain.

An increase in aldolase activity is observed in many pathological conditions, accompanied by damage and destruction of cells:

1. Damage to the liver and pancreas (viral or toxic hepatitis, metastatic liver cancer, liver cirrhosis, necrosis of various tissues, acute pancreatitis);
2. Acute myocardial infarction, infarction of the lungs, intestines, gangrene of the extremities, etc .;
3. Diseases accompanied by damage to muscle tissue (muscle injury, dermatomyositis, muscular dystrophy);
4. Malignant neoplasms of various localization (liver cancer, melanoma, central nervous system tumors, tumors of the stomach, intestines);
5. With some blood diseases (leukemia, megaloblastic anemia, hemolytic anemia, etc.).

Alkaline Phosphatase (ALP)

Alkaline phosphatase (phosphomonoesterase) is an enzyme that hydrolyzes orthophosphoric acid esters in an alkaline medium. Alkaline phosphatase is found in almost all organs, but its maximum activity is detected in the liver, bone tissue, intestines and placenta. There are several ALP isoenzymes that differ in their physicochemical properties and relative organ specificity: hepatic, bile, bone, intestinal, placental

isoenzymes. Normally, electrophoretic studies on cellulose acetate films reveal only two alkaline phosphatase fractions in the  $\alpha_2$ -globulin zone. For some diseases of internal organs, their number may be greater.

An increase in the activity of alkaline phosphatase and the corresponding isozymes is most often observed in the following pathological conditions:

1. Diseases of the liver and biliary tract: obstructive jaundice (the most significant increase in activity), cholangitis, hepatitis, liver cirrhosis, especially accompanied by intrahepatic cholestasis, liver cancer and liver metastases.
2. Bone diseases accompanied by an increase in osteoblast activity: osteitis deformans (Paget's disease), rickets, malignant bone neoplasms (osteosarcomas), osteomalacia, bone metastasis, myeloma, bone fracture healing, hyperparathyroidism with bone involvement, etc.
3. Diseases accompanied by intestinal lesions: ulcerative colitis, regional ileitis, intestinal bacterial infections, etc.
4. When using certain drugs that have a hepatotoxic effect and / or increase cholestasis: barbiturates, indomethacin, dopegit, preparations of nicotinic acid, methyltestosterone, salicylic acid, sulfonamides, some antibiotics, etc.
5. During pregnancy.

The most significant increase in the activity of alkaline phosphatase in the blood serum is observed in diseases of the bones and obstruction of the biliary tract.

### Acid phosphatase

Acid phosphatase is the second enzyme involved in the hydrolysis of phosphoric acid esters, but in an acidic environment. Just like alkaline phosphatase, acid phosphatase is found in almost all organs and tissues, but its greatest activity is detected in the prostate gland. CP is also found in the liver, spleen, erythrocytes and platelets, kidneys and bone marrow.

In men, about 50% of the activity of CP in the blood serum falls on the prostatic fraction of the enzyme, and the rest is associated with phosphatase produced in the liver, erythrocytes and platelets. In women, serum CF is produced by the liver, erythrocytes, and platelets.

A significant increase in the activity of CP in the blood serum, especially its prostatic fraction, is used almost exclusively for the diagnosis of prostate cancer. It should be remembered that with metastasis of cancer of this localization in the bone, the activity of not only acidic phosphatase (AP), but also alkaline phosphatase (ALP) increases. In

contrast, other bone injuries are accompanied by an increase in alkaline phosphatase alone.

A moderate increase in CP activity is also detected in some inflammatory diseases of the prostate gland (prostatitis), especially after the use of certain diagnostic and therapeutic procedures (prostate massage, urinary tract catheterization, cystoscopy, after rectal examination, etc.).

### $\alpha$ -Amylase

$\alpha$ -Amylase catalyzes the degradation (hydrolysis) of starch, glycogen and some other polysaccharides to maltose, dextrins and other oligosaccharides (see below for more details). Partial digestion of these polysaccharides begins in the oral cavity under the action of salivary gland amylase (S-type enzyme) and ends in the small intestine under the influence of pancreatic amylase (P-type).

Serum  $\alpha$ -amylase consists mainly of two isoenzymes: pancreatic and salivary.

1. About 60–70% of the total activity of serum  $\alpha$ -amylase is accounted for by the salivary isoenzyme (S-type), and only 30–40% - by the pancreatic (P-type). 2. Unlike most enzymes,  $\alpha$ -amylase is filtered in the glomeruli of the kidneys and excreted in the urine.

An increase in the activity of  $\alpha$ -amylase occurs in the following diseases:

1. mumps;
2. pancreatitis, pancreatic cancer, diabetic ketoacidosis;
3. renal failure (due to a decrease in the excretion of  $\alpha$ -amylase in the urine);
4. Other diseases: bronchogenic lung cancer, ovarian tumors, obstructive intestinal obstruction, peritonitis, acute appendicitis, burns, cholecystitis, etc.

### Lipase

Lipase is an enzyme produced in the pancreas and secreted in large quantities into the duodenum with pancreatic juice.

Unlike other cellular lipases involved in the process of lipolysis in the liver and fat cells, pancreatic lipase is one of the secreted enzymes that break down triacyl-glycerol, which is formed in the small intestine after emulsification of fats from food to mono- and diacylglycerols and free fatty acids, which are then absorbed into the bloodstream (see below for more details).

The most common method for determining the activity of pancreatic lipase in blood serum is the spectrophotometric measurement of changes in the turbidity of an olive oil suspension caused by lipase. Normal values of the enzyme activity may differ in different

laboratories, however, in a healthy person, the activity of pancreatic lipase in the blood serum is minimal and does not exceed 0-28  $\mu\text{mol} / (\text{min.l})$ .

The reasons for the increase in serum lipase activity can be:

- 1) Acute pancreatitis of any origin, in which a particularly significant increase in enzyme activity is found.
- 2) Other diseases of the digestive system, in which the presence of reactive changes in the pancreas cannot be ruled out: biliary colic, intestinal obstruction, peritonitis, intestinal infarction, perforation of the stomach or intestines. In these cases, a moderate increase in lipase activity is usually observed.