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CHANGES IN THE STRUCTURE AND FUNCTIONS OF RED BLOOD CELLS IN CALVES WITH BRONCHOPNEUMONIA

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This monograph presents the results of experimental studies on the biochemical structure and functional properties of red blood cells in clinically healthy calves and those suffering from catarrhal bronchopneumonia, as well as changes in these properties during treatment with antibacterial drugs. It presents the authors' research findings, as well as data from other researchers on the etiology, pathogenesis, and specific diagnostic methods for catarrhal bronchopneumonia, along with the treatment of calves using effective treatment regimens, taking into account the impact of drugs on the structure and functional properties of red blood cells. This monograph will be useful for students, researchers, and practicing veterinary professionals working in the field of cattle husbandry.

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LIST OF ABBREVIATIONS

NRI – Research Institute

SVK – Agricultural Production Cooperative

OJSC – open joint-stock company

MHC – mean hemoglobin content per red blood cell

AU – activity units

AST – aspartate transaminase

ALT – alanine transaminase

T/L – tera per liter – erythrocyte count in the International System of Units (SI)

2,3-DPG – 2,3-diphosphoglycerate

P – phosphorus

Total P – total phosphorus

Inorganic P – inorganic phosphorus

Er – erythrocytes

Hb – hemoglobin

Hi – methemoglobin

subscript (v) or (a) – indicator or ratio in venous or arterial blood

INTRODUCTION

Respiratory diseases in animals are a major problem in veterinary medicine, causing significant economic losses in livestock farming, comprising a decline in the productive and breeding qualities of animals and their death [1, 2].

Bronchopneumonia in young farm animals is widespread and ranks second in the disease incidence profile after gastrointestinal diseases [1–3]. Nearly one in two animals contracts bronchopneumonia at an early age. The disease occurs throughout the year, and in hot climates, it is most common in June–August [4]. Bronchopneumonia causes stunted growth and developmental delays in young animals, increased feed costs, and animal mortality.

The widespread prevalence of bronchopneumonia in calves is due not only to known primary (unsanitary housing conditions, high indoor humidity, sharp fluctuations in daily temperature) and secondary (pathogenic and opportunistic microflora) [5–7], but also by immune deficiencies, which occur in nearly 60–70 % of newborn calves [4, 8–10].

In cases of bronchopneumonia in calves, pathological processes develop not only in the organs of the respiratory system, but also throughout the entire body, as all types of metabolism are disrupted. This also leads to the suppression of the functions of all vital organs and systems. Therefore, to achieve the desired outcome, it is important to adopt a comprehensive approach to the selection of medicinal products for the treatment of calves with bronchopneumonia [11].

In recent years, the incidence rate of respiratory disease in animals has not decreased but, on the contrary, has shown a tendency to increase [3, 11, 12]. Protracted and recurrent forms of pneumonia are more frequently recorded, as are cases of a sluggish course of the disease, which is due to reduced immunological reactivity of the animals' bodies [13].

The most effective approach is a comprehensive treatment programme for animals with bronchopneumonia, which should be aimed at rectifying any shortcomings in husbandry and feeding practices, boosting the animals' resistance,

and involves the simultaneous use of various agents: antimicrobials, pathogenetic agents, symptomatic treatments, stimulants and vitamin preparations [9].

The course of pneumonia is accompanied by the development of hypoxic hypoxia, which is complicated by circulatory factors; therefore, studying the erythrocyte system is important in these diseases. Quantitative changes in erythrocytes and hemoglobin content have been fairly well studied; however, questions regarding the biochemical structure and functional properties of erythrocytes in calves with bronchopneumonia, as well as their changes under the influence of medicinal substances, particularly antibacterial drugs, remain unresolved. Therefore, despite the large number of studies conducted, the problem of pneumonia remains relevant both in terms of further studying its pathogenesis and in the search for and experimental validation of new effective therapeutic agents.

Unit 1

LITERATURE REVIEW ON BRONCHOPNEUMONIA IN CALVES

1.1. Classification and Pathogenesis of Pneumonia

Currently, there are several classifications of pneumonia [4, 14].

In human medicine, this disease is classified according to the following criteria: clinical-morphological, etiological, pathogenetic, and synthetic. A number of foreign authors consider classification based on the etiological principle to be promising [15].

The first classification of pneumonia, developed by K. Rokityansky in 1842, was based on a morphological principle, and pneumonia was classified according to the location and size of the pneumonic infiltrate. In 1937, G.M. Bullowa proposed a classification of pneumonia based on the etiological principle [16]. Abroad, the classification developed by Hegglin (Hegglin R., 1969), which was based on the etiopathogenetic principle, was widely used. According to this classification, primary and secondary pneumonias are distinguished, with the etiology of the disease specified [17].

In 1962, M.S. Molchanov proposed a classification of pneumonias, which was approved by the Congress of Internists. O.V. Korovina and E.V. Gembitsky supplemented and refined it in 1983. According to this classification, pneumonia is classified by etiology, pathogenesis, symptoms, and course [18]. Based on etiology, pneumonia is divided into bacterial, viral, ornithosis, rickettsial, mycoplasmal, fungal, mixed, allergic, and pneumonia of unknown etiology. Based on pathogenesis, pneumonia is classified as primary and secondary (hypostatic, contact, aspiration, traumatic, postoperative, occurring against the background of exacerbation of chronic non-infectious lung diseases, toxic, thermal, and resulting from infectious diseases).

Based on clinical and morphological characteristics, authors distinguish parenchymal (croupous and focal) and interstitial pneumonia; by localization and extent – unilateral (left- or right-sided), including total, partial, segmental, lobar, and bilateral – also indicating the extent of the process. By severity of the process – into

very severe, severe, moderate, mild, and abortive forms; by course – into acute and protracted.

V.V. Erohin (1927) notes three types of disease course: acute, subacute, and protracted.

The most optimal classification of secondary pneumonias, reflecting the current state of scientific knowledge, is the classification proposed by E.V. Gembitsky et al. (1989). It distinguishes the main types based on pathogenesis, such as hypostatic, atelectatic, intoxication, burn, septic, radiation, and others [19].

In clinical veterinary practice, a classification based on the anatomo-pathogenetic principle – which takes into account the spread and mechanism of the pathological process – has become widely used (Domrachev G.V., 1959). According to this principle, pneumonia is divided into lobar and lobular types. Lobular (segmental) pneumonia includes catarrhal (bronchopneumonia), atelectatic, metastatic, aspiration, and hypostatic forms; lobar pneumonia includes croupous pneumonia, epidemic pneumonia of cattle, and contagious pleuropneumonia of horses. Based on pathomorphological criteria, pneumonia is classified into serous, catarrhal (in cases of bronchial involvement), purulent, fibrinous, hemorrhagic, as well as mixed forms – serous-catarrhal, catarrhal-purulent, and purulent-necrotic.

V.A. Alikaev (1985) distinguishes bronchopneumonia, aspiration pneumonia, pneumonenteritis, and pneumonia in young animals during the post-weaning period.

Based on the course of the disease, pneumonia is classified as acute, subacute, chronic, latent, or recurrent [20].

The most practical classification is based on the etiological-epizootological principle [21]. According to this classification, all pneumonias are divided into three groups: non-contagious, symptomatic, and infectious (specific). Infectious pneumonias include mycoplasmosis, chlamydiosis, Haemophilus pleuropneumonia, Bordetella pneumonia, and adenoviral pneumonia. Symptomatic pneumonias are diseases in which pneumonia is only one of the symptoms of the disease (salmonellosis, streptococcosis, influenza, tuberculosis, pasteurellosis, plague, Aujeszky’s disease, and certain helminthiases). Pneumonias resulting from the

development of associations of microorganisms acting in symbiosis against a background of reduced host resistance are considered nonspecific. Currently, scientists do not classify symptomatic pneumonias into a separate group but combine them with infectious pneumonias [22].

P.N. Korikov (1990), depending on the cause, classified all pneumonias into nonspecific (non-infectious), specific (bacterial, viral), and mixed (viral, complicated by nonspecific microflora); by localization – small-focal, large-focal, and confluent; by course – acute, subacute, and chronic. A number of other authors classify pneumonia depending on its origin into specific, helminthic, and nonspecific (non-contagious) [23].

According to I.I. Tarasov (1977), pneumonia in young farm animals should be classified as primary or secondary. Primary pneumonia includes cases arising from adverse environmental factors and intrauterine developmental disorders, while secondary pneumonia develops as a complication of infectious or parasitic diseases (respiratory viruses, salmonellosis, dictyocaulosis, etc.). In bronchopneumonia, not only the lungs and bronchi are affected, but changes also occur in other organs [24–26].

There is a multifaceted set of causes underlying the pathological condition in the lungs. Accordingly, their pathogenesis varies, indicating a multitude of mechanisms leading to damage to the structural components of the respiratory segment of the lungs and the air–blood barrier. In some cases, certain factors result in direct toxic damage to the endothelium of blood capillaries; in others – local damage to the walls of the blood capillaries, as, for example, in the case of immune complex deposition; and in still others – the formation of a traumatic defect in the walls of the alveoli, for example, in experimental pneumoconiosis. However, in both the first and second cases, the structure of the blood capillaries in the alveolar walls of the lungs is disrupted, their permeability increases, and tissue edema develops. Exudate and formed elements of the blood fill the alveoli, and an inflammatory reaction develops. The nature of the inflammation and its progression are determined by the characteristics of the pathogenic factor and the tissue response [27–29].

In the etiology and pathogenesis of bronchopneumonia, pathogens of acute respiratory diseases play a significant role – viruses such as parainfluenza-3, infectious rhinotracheitis, adenovirus, respiratory syncytial virus, and the mucosal disease virus, etc. Complicating viral infections, and often causing the disease on their own, are Chlamydia, Mycoplasma, Pasteurella, Staphylococcus and Streptococcus, Klebsiella, Pseudomonas, Salmonella, and others. In real-world conditions, in most cases, associations of various microorganisms are isolated from sick animals [22, 24, 25, 30, 31].

Microorganisms enter the lungs primarily through the upper respiratory tract. The pathogens initially colonize and multiply on the mucous membranes of the nose, oral cavity, and pharynx, and only after some time do they infect the bronchi and lungs [17, 18].

Some scientists believe that the lungs of healthy animals are free of microflora [31], while according to others, microorganisms – including pathogenic ones – constantly enter the lungs; however, they are low-virulence and incapable of causing disease under normal conditions [6, 7, 30]. This is due to the action of protective mechanisms, among which the ciliated epithelium of the upper respiratory tract, the mucous layer covering it, surfactant, interferon, lysozyme, and especially macrophages play an important role [32, 33]. The body’s first line of defense against various environmental agents is the ciliated epithelium and the mucus-secreting cells that line the surface of the airways and act as a coarse filter. The mucous membrane of the airways prevents the penetration of microbes into the body’s internal environment, and the secretion covering its surface has a bactericidal effect on them, since the mucus contains secretory IgA, transferrin, and lysozyme, which provide anti-infective protection [9, 34, 35].

Improper housing and feeding conditions, the development of immunodeficiency and acidosis, exposure to chemicals, irrational medication use, stress, the impact of adverse environmental factors, and other factors contribute to the disruption of the anatomical or physiological defense mechanisms of the lungs [17, 36]. These include: impaired bronchial drainage function, reduced microbial

clearance, decreased mucociliary index, impaired pulmonary surfactant system, suppression of the cough reflex, damage to the epithelium of the tracheobronchial tree, insufficient phagocytic activity of neutrophils and alveolar macrophages, impaired innervation of the diaphragm, impaired lung motility, and reduced local and systemic defenses. A particular role in the pathology of the respiratory organs is played by the disruption of surfactant integrity within the structural units of the lungs. As the pathology develops, surfactant is gradually washed out of the alveoli; in healthy animals, this substance prevents alveolar collapse and the adhesion of their walls, and acts as a nonspecific protective factor. The combination of all these adverse factors contributes to the proliferation of pathogenic microorganisms and the onset of a pathological process in the lungs [22, 31, 37].

Since, in addition to their gas exchange function, the lungs actively participate in lipid metabolism, produce a number of amines that regulate blood clotting processes, inactivate serotonin, and participate in water-salt metabolism and the homeostasis of blood cell composition, especially leukocytes, any disruption in the synchronization of these functions leads to a decrease in the activity of the air-blood barrier and the surfactant covering it [17, 37–39].

Under the influence of etiological factors, neural and humoral reactions are disrupted [2, 24]. In the submucosal layer of the bronchi, there is initially a spasm, followed by paresis of the capillaries (pulmonary arterioles) and venous stasis. This results in the development of hypertension in the pulmonary circulation, leading to hemorrhages and edema in the lung tissue [39–43].

In blood serum, the concentration of lysozyme and histamine decreases, while the content of large-molecular-weight globulin protein fractions [2, 24], which irritate lung tissue, contribute to blood stasis in the lungs, and promote edema in the mucous membranes of the bronchi and bronchioles [15, 44]. The phagocytic activity of leukocytes decreases, and the function of T- and B-lymphocytes is suppressed [45, 46].

The course of the acute stage of the disease depends on the functional state of the tracheobronchial tree [4, 22]. Inflammation of the bronchial mucosa leads to an

increase in the amount of bronchial secretions, disruption of its structure – replacement of ciliated epithelial cells with goblet cells that produce mucus – changes in the physicochemical properties of the secretions formed, as well as difficulty in their clearance [47]. As a result of this process, the physiological mechanism of bronchial tree clearance is completely disrupted. Viscous secretions accumulate in the airway lumen, losing their protective antibacterial properties, which creates conditions for the spread of inflammation to the lung tissue [48].

Some researchers believe that the state of the body's nervous system plays an important role in the pathogenesis of bronchopneumonia, as does the influence of adverse environmental factors; their combined effect can lead to disruption of nervous system function and a decrease in the body's overall resistance and reactivity. The microflora present in the respiratory organs of healthy animals, under favorable conditions resulting from a reduction in the barrier function of the epithelium, acquires virulent properties and multiplies intensively, leading to an increase in its virulence and the development of an inflammatory process in the lungs [49–51].

Depending on the predominance of pathogenic microflora – mycoplasmas or viruses – primary changes may be mild or maximally pronounced. The predominant accumulation of bacteria leads to the formation of an exudative process and leukocytic reactions, manifested by the rapid accumulation of initially serous and then catarrhal exudate. This process is associated with bacterial proliferation exclusively within the lumen of the bronchi and alveoli, in contrast to mycoplasmas and viruses, which lead to changes in the mucous membranes of the respiratory tract [68]. The nature of the inflammation varies depending on the etiological factor and the body's degree of defense. The accumulation of serous, serous-catarrhal, and catarrhal exudate – whose components include leukocytes, mucin, bronchial epithelium, and microbes – leads to the filling of the bronchial and alveolar lumens. The inflammatory exudate consists of desquamated alveolar epithelium at various stages of dystrophy – ranging from protein and lipid degeneration to necrosis – as well as a certain number of red blood cells [52]. Airflow into the affected alveolar

segments is limited or ceases entirely [53, 54]. Not only inhalation but also exhalation is impeded, as confirmed by measurements of airflow velocity using a PT-2 pneumotachometer (Grigoryan S.M., Manasyan A.V., 1998). The respiratory surface of the lungs decreases. Oxygen diffusion through cell membranes slows down due to their thickening and changes in their physicochemical properties. At the same time, the concentration of carbon dioxide increases. Disturbances in external and internal gas exchange develop, contributing to the onset of acidosis and impaired functioning of the cardiovascular and digestive systems, as well as liver function. As a result of the absorption of toxins secreted by microbes and tissue breakdown products from the sites of inflammation into the blood and lymph, irritation and then suppression of the function of the cerebral cortex and the autonomic nervous system occur. A disturbance in the thermoregulatory center sets in, accompanied by an increase in body temperature, and respiratory and vascular failure develop. Therefore, bronchopneumonia is a systemic disease [55–60].

As mentioned above, in animals, during an acute inflammatory process, gas exchange in the lungs is disrupted, leading to changes in tissue respiration. As a result, all types of metabolism in the body are disrupted [2, 61]. Factors of cellular and humoral defense (bactericidal and lysozyme activity, among others) are reduced [22].

In calves, the metabolism of glycoproteins, particularly “acute-phase proteins,” is disrupted, and the blood oxyprolin content increases, which is explained by enhanced collagen breakdown at the onset of the disease and subsequently its synthesis [62–64]. In affected animals, the concentration of glucose, pyruvic, lactic, sialic acids, and glycoproteins increases [59]. Similar changes have been observed in humans [65, 66].

In cases of pneumonia, hypoxia creates conditions for increased oxidation of cell membrane lipids, activation of endogenous phospholipases, and inhibition of antioxidants [13, 67, 68]. As a result, toxic products accumulate in tissues: peroxides, hydroperoxides, fatty acids, and lysophospholipids, which damage the structure and disrupt the function of cell membranes [47]. In addition, a close relationship has been

identified between lipid peroxidation, the antioxidant system, and nucleoprotein metabolism in patients with acute pneumonia. Moreover, in patients with a viral etiology of the disease, these indicators change more markedly than in the presence of bacterial flora [68].

In cases of acute pneumonia, a pronounced copper deficiency is observed. This is explained by the fact that copper acts as a catalyst for many biological processes and is intensively utilized for the synthesis of ceruloplasmin molecules, the activity of which is elevated in acute pneumonia. An increase in carbonic anhydrase activity in the blood and an increase in zinc levels in the body should be considered as one of the compensatory reactions that promote the removal of carbon dioxide from the blood and an increase in its respiratory activity. The increase in cobalt levels in patients with acute pneumonia involves a complex compensatory-protective mechanism aimed at stimulating immunogenesis. The decrease in potassium concentration in the cytoplasm and reduced excretion of potassium in the urine is explained by an increase in its content, as well as that of sodium and magnesium, in red blood cells, indicating significant changes in red blood cells in patients with acute pneumonia [69–71].

During the period of pronounced disease progression, iron concentration in the body is elevated. Evidently, part of this trace element accumulates in the liver (depot), where complex iron-containing organic compounds are synthesized and used to normalize disrupted redox processes [4, 22]. However, serum iron levels in patients decrease depending on the severity of the disease [72]. As a result of impaired iron metabolism, the number of hemoglobin molecules and red blood cells decreases [21], which further exacerbates the state of hypoxia in affected animals.

Blood erythrocytes, due to the presence of hemoglobin molecules in their cytoplasm, are capable of forming temporary complexes with gases, thereby performing gas transport functions and ensuring the delivery of oxygen to tissues and the removal of carbon dioxide from them [73, 74].

The functional capacity of blood erythrocytes depends on the condition of the lungs. Since the lungs have high metabolic activity, the membranes of erythrocytes

passing through the pulmonary circulation undergo certain changes: the amount of lipid components increases, energy processes intensify, and so on. All of this affects the functions of erythrocytes and the duration of their stay in the bloodstream [75–79].

During circulation in the blood, erythrocytes pass through the stages of "young," "mature," and "aging" cells. Changes in morphological structure that occur during the terminal differentiation of a red blood cell (loss of the nucleus, mitochondria, and ribosomes) ensure the maximum functional activity of the "mature" cell – the transport of oxygen from alveolar air to tissues and the removal of carbon dioxide from them [74, 80], regulation of acid-base and water-electrolyte balance, and the adsorption and transport of certain biologically active substances and xenobiotics [81, 82]. As a result of the degradation of intracellular organelles, erythrocytes lose their ability to synthesize proteins, which over time leads to a gradual decline in metabolic processes and disruption of many vital cellular functions, causing their aging. In aging erythrocytes, the capacity for oxygen transport, synthesis of macromolecular compounds, and reception of bioregulators and complement [83–87], and the intensity of lipid peroxidation increases, leading to destructive processes in plasma membranes and disruption of the cation and amino acid transport system [88, 89]. Due to increased water permeability, the buoyant density of cells rises, and their resistance to hemolysis decreases [90, 91]. There are reports of changes in the basic composition of membranes and the appearance of specific antigens in the membranes of aging erythrocytes [92–94]. Old erythrocytes are eliminated from circulation in the reticuloendothelial system of the spleen and liver [93].

It is believed that one of the factors determining the duration of a red blood cell's function in the bloodstream is the number of recirculations performed by the cell, which depends on the intensity of metabolism in the body. Consequently, in small mammals characterized by a high metabolic rate, the lifespan of erythrocyte cells is significantly shorter, ranging from 40–60 days in rabbits, guinea pigs, and rats, and 20–40 days in mice [95], whereas in dogs and cattle it is 97–117 days, and

in Karakul sheep it is 130 days [81, 96, 97]. The lifespan of a red blood cell in the bloodstream is limited and varies throughout an organism's individual development and during various physiological and pathological processes [76, 77].

In bronchopneumonia, not only does the hemoglobin concentration change, but significant structural changes also occur in the cells themselves and their membranes. The state of hypoxia in the body leads to more active circulation of red blood cells. This, in turn, affects the increase in oxygenation and deoxygenation of cells, and the metabolic activity of the erythrocytes themselves changes. Anaerobic glucose breakdown intensifies, which in turn leads to an increase in the level of 2,3-diphosphoglycerate in the erythrocyte cytoplasm. This phosphoric acid ester has the ability to reduce hemoglobin's affinity for oxygen, i.e., to promote the faster release of oxygen from hemoglobin; therefore, an increase in 2,3-DPG indicates oxygen deprivation in the body [95–97].

A number of researchers have noted that in bronchopneumonia, lipid peroxidation intensifies, including in erythrocytes [98–99]. Such changes cannot fail to affect the structural composition of cell membranes and, consequently, their functional properties [100, 101]. An increase in the levels of cholesterol and certain phospholipids (sphingomyelin and others) leads to membrane thickening and a significant reduction in their permeability [102, 103–106]. The membranes lose the elasticity necessary to pass through small blood vessels, resulting in the destruction of red blood cells in the capillary bed [103, 107, 108].

Thus, bronchopneumonia is a disease in which all types of metabolism are disrupted, resulting in the involvement of all organs and systems, indicating a systemic disease.

1.2. Treatment and Prevention of Bronchopneumonia in Calves

The effectiveness of treating calves with bronchopneumonia depends on many factors, the most important of which are: accurate and timely diagnosis [2, 4, 22, 24, 58], elimination of the causes of the disease (optimization of housing and feeding practices, increasing the body's resistance, and the proper prescription of

antimicrobial drugs based on the sensitivity of the microflora) [30, 109], as well as the use of pathogenetic, replacement, and symptomatic therapies to restore the functions of the bronchi, cardiovascular, and respiratory systems [22, 24, 109].

Therapeutic efficacy depends on the stage of the disease and the degree of lung damage. Treatment of calves with bronchopneumonia is most effective and cost-effective in the early stages of the disease, when the respiratory tract is predominantly affected and the exudative process is of a serous-catarrhal nature. Such lesions are observed in the first 3–7 days from the onset of the disease. During this period, purulent-necrotic and indurative changes do not occur in the lungs, and with effective therapy, animals recover in more than 90% of cases [24].

A prerequisite for any drug treatment is the elimination of the etiological factors of the disease. The success of etiotropic therapy for bronchopneumonia depends on the concentration of the drug at the site of inflammation. In acute and subacute disease courses, antimicrobial agents penetrate the histohematological barrier well; however, in chronic cases with the development of edema, cellular infiltration, and sclerosis around the focus, the capillaries in the lungs become obliterated. In this case, the penetration of drugs into the site of inflammation is impeded. Therefore, drugs should be administered in a timely manner [4, 22, 24].

One of the first antibiotics to be widely introduced into veterinary practice was penicillin [58–60]. Since then, its efficacy has been noted by many researchers. Based on this drug, extended-release antibiotics and semisynthetic penicillins were developed [60, 21].

Among new drugs for the treatment of bronchopneumonia, egocin is recommended; it is injected twice at 4–5-day intervals, with a survival rate of 90% in young animals [110].

I.A. Malenky [111] notes the high antibacterial effect and low toxicity of Pharmazin-200 to the body, recommending it as a therapeutic and prophylactic agent.

The macrolide antibiotic roxithromycin provides a good therapeutic effect in cases of pneumonia in humans. It is low-toxicity and well-tolerated, remaining in

alveolar macrophages for two hours after complete elimination from blood serum [112].

In recent years, a new antibiotic – Mycothyl – has attracted attention. The use of Mycothyl in calves with bacterial bronchopneumonia in the early stages is characterized by a 100% recovery rate [113]. G.G. Chusova and A.I. Zolotarev note the positive effect of quinoxidine and furacon, the efficacy of which is 89% and 90%, respectively [21].

A 5% solution of enroxil at a dose of 0.5 ml per 10 kg of body weight, administered at 24-hour intervals, is effective; the course of treatment is 2–3 days [114–116]. Sodium cyftiofur can be administered intramuscularly or via aerosol [117]. It is advisable to administer ampicillin intramuscularly with simultaneous subcutaneous administration of the immunostimulant Sub D4 [118], as well as the antibiotic loracarbaf, which has an efficacy of 97.6% [119]. J. Schauer et al. [120] recommend the use of nifedipine (Corinfar), while B. Fischer and R. Ferlinz [121] practice the injection of corticosteroid preparations. Indomethacin and salbutamol are quite effective in pulmonary pathology [122].

V.A. Lochkarev (1999, 2000) recommends intravenous administration of streptomycin sulfate (10 mg per kg of body weight), after adding 2 mL of a 10% calcium chloride solution, 1 mL of a 20% sodium caffeine benzoate solution, and 17 mL of an isotonic sodium chloride solution.

Intravenously administered streptomycin has antimicrobial and anti-inflammatory effects, reduces dyspnea in animals with acute bronchopneumonia, and, 2–4 hours after injection, brings calves out of a critical condition. Hyperemia and pulmonary edema decrease and then disappear, which facilitates a more intense effect of the antibiotic on the pulmonary microflora [21].

In cases of bronchopneumonia in calves, intrapulmonary administration of tetraolean into the upper thirds of the left and right diaphragmatic lobes of the lungs, in the area of the anterior margin of the 7th–8th ribs, 10–12 cm above the shoulder joint line, to a depth of 2.5–3 cm, is effective (Sakhatsky I.M., 1998).

In the late 20th century (Chumakov V.Yu., 1997), it was proposed to administer drugs into the lymphatic system (endolymphatic therapy), since under these conditions their concentration in tissues is higher than with traditional methods. In this case, the drugs directly affect pathogenic factors – microorganisms and metabolic products. Endolymphatic delivery of medicinal substances makes it possible to enhance the immune response if necessary. Antibiotics administered into the lymphatic system accumulate in the lymph nodes and affect pathogenic factors; some of them may bind to lymph and exhibit lymphotrophic properties. In cattle, their intranodular endolymphatic administration into the prescapular, superficial cervical, or popliteal lymph nodes is indicated.

Intratracheal administration of isoniazid and tetracycline-class antibiotics, as well as intraperitoneal oxygen administration, are used (Mustakimov R.G., 1989; Kvyatkovsky V.N., 1990). In cases of diffuse localization of the pathological process, animals with bronchopneumonia are periodically administered a 3% solution of sodium norsulfazole intratracheally, benzylpenicillin with streptomycin intramuscularly, and trimethosul, trimethazoline in recommended doses, and a 20% glucose solution intravenously. The treatment efficacy according to this regimen is 97.2–100% for acute catarrhal bronchopneumonia and 91.7% for purulent-catarrhal bronchopneumonia.

One of the widely used methods in veterinary medicine for treating respiratory diseases is aerosol therapy (Nagashyan O.Z., 1986; Goloviznin Yu.V., 1980, 1982; Usachenko A.A., 1991; Drobot, 2021) [123]. Antibiotics are successfully used in aerosol therapy (penicillin, streptomycin, dihydrostreptomycin, neomycin, oxytetracycline, erythromycin, kanamycin, gentamicin, pharmazin-50, morphocycline, monomycin, oleandomycin, tetracycline, novoimamine) (Brilin A.P., 1983; Karput I.M., 1985; Pisarenko N.I., 1982; Totikov Z.D., 1987; Tregubov V.I., 1981; Shaikhanov M.Kh., 1982; Yashin A.V., 1987); norsulfazole [123]; etazol, cobalt chloride (Rusakov A.V., 1988); ftivazid, tubazid, lincomycin hydrochloride (Afonin F.S., 1980); tylosin tartrate (Antipov V.A., 1988); isatizone (Zharova V.Ya., 1986); immunostimulants (Sokolov A.P., 1994); oxycan (1982) [21].

In cases of catarrhal and catarrhal-purulent bronchopneumonia, it is recommended to use the aerosol complex preparation PVENTI from the triiodide group (Amirbekov M., 1996).

For the treatment of calves with respiratory diseases, the use of aerosol preparations with bactericidal, virucidal, sporicidal, and fungicidal effects, as well as those that have a detrimental effect on the pathogenic microflora of the respiratory organs, is effective. Of these, the following deserve special attention: glutaraldehyde, resorcinol, turpentine oil, lactic acid, hydrogen peroxide, camphor alcohol, iodine-triethylene glycol in combination with turpentine oil or lactic acid, a 5% chloramine solution, iodinol, a 5% alcohol solution of thymol, iodine-aluminum, chlorine turpentine, and others [22, 125].

To combine antibacterial, stimulatory, and symptomatic therapy, it is recommended to use rovatazin, which consists of pharazine, fumaric acid, and terpene hydrate. The therapeutic efficacy of this drug was 86.8%. Its pharmacological activity is due to a broad spectrum of antimicrobial action, the ability to increase the body's nonspecific resistance, and its pathogenetic effect [21, 126–128].

Rusakov A.V. (1988) described that to eliminate hypoxia and improve bronchial patency, bronchodilators are administered parenterally (euphyllin, theobromine, theophylline, atropine sulfate, ephedrine hydrochloride, platyphylline hydro tartrate, epinephrine hydrochloride) are administered parenterally. In addition, the first three drugs stimulate the cardiovascular system, increase diuresis, and have an antitoxic effect.

To restore respiratory function, improve gas exchange, thin, and facilitate the removal of exudate from the bronchi, expectorants are administered orally concurrently with chemotherapeutic agents: ammonium chloride, terpin hydrate, potassium iodide, and sodium bicarbonate [2].

To correct the functional state of the cardiovascular, central nervous, and respiratory systems, preparations of caffeine, camphor, cordiamine, corazol, corglycol, strophanthin tincture, digitalis leaves, gitalen, adonizide, and glucose solution are used [129].

For the treatment of calves with bronchopneumonia, antibiotics are used in combination with immunomodulators: thyrotropin, levamisole, and sodium nucleinate (Krasnikov G.A., 1989). Against the background of antibiotic therapy, the highest efficacy was observed with the use of sodium nucleinate – 91.6% – and with the use of timotropin and levamisole – 84.7% and 80%, respectively. Levamisole and potassium orotate stimulate cellular and humoral immunity and increase the animals' resistance (Pakhmutov M.A., 1986).

For respiratory diseases, the following groups of immunomodulators capable of stimulating the immune response are used: preparations of microbial origin (pyrogenal, prodigiozan, anabol, zimosan), substances of natural origin (ginseng, lemongrass, aralia, thymus and nucleic acid preparations), synthetic chemicals (levamisole, diuciphon, zixorin), and lymphokines (interferon).

Enzyme preparations (trypsin, lysozyme), bacterial polysaccharides (pyrogenal, prodigiozan), yeast polysaccharides (zymosan, glucans, propermil, dextrans), nucleic acid preparations (sodium nucleinate), purine and pyrimidine derivatives (methyluracil, pentoxil), imidazole derivatives (levamisole, dibazol), thymus preparations (T-activin, thymosin, humoral factor), the antibody-producing stimulator B-activin, interferons, and interleukins. Measures and methods of immunoprophylaxis also include hemosorption, plasmapheresis, and the administration of blood plasma and immunoglobulins [130–132].

Vitamins have a pronounced stimulatory effect on the mechanisms of natural resistance [123, 133, 134]. Ascorbic acid has a positive effect on blood clotting and tissue regeneration. It possesses exceptional redox capacity and participates in the vital process of cellular respiration. Vitamin C has a positive effect on the recovery of animals suffering from bronchopneumonia [135–138].

The effects of vitamins A and E on the bodies of sick animals deserve special attention. Retinol promotes the regeneration of the respiratory tract epithelium and plays an important role in the synthesis and permeability of cell membranes. It participates in lipid, protein, energy, and other types of metabolism [139]. Tocopherols are the most active fat-soluble antioxidants and play a specific role in

selenium metabolism, thereby protecting biological membranes from the destructive effects of peroxyl radicals [140]. Maintaining the integrity of erythrocyte membranes prevents the worsening of oxygen deprivation in calves with bronchopneumonia and helps eliminate hypoxia and hypoxemia [141–143].

During the treatment of calves with bronchopneumonia, antibiotic therapy is combined with the administration of horse blood, hydrolysin (Abramov S.S., 1988), horse serum hemolysate (Gandbold D., 1994), a suspension of female placenta, and a stimulant according to E.A. Konkov's method (Ternovskaya K.A., 1989). In these cases, the duration of treatment is reduced, drug costs are lowered, and high calf survival rates are achieved [144].

There are methods of treatment using autologous blood irradiated with ultraviolet rays, which leads to an increase in the body's defenses and a reduction in recovery time [125, 145–147]. Irradiated blood in the form of an aerosol promotes the activation of erythro- and leukopoiesis, increases the bactericidal activity of blood serum, and improves tissue oxygen uptake [148–152].

Antiviral activity has been established for biostimulants derived from the spleen and placenta of cows; sodium huminate, cresacin, riboxin, inosine, tafcin, and samotomedin "B." Their effect depends on the dose and concentration of the preparations: low doses promote an increase in antiviral activity, while high doses lead to a decrease [153, 154].

Currently, special attention should be paid to the extraction of biologically active substances from bee products, which serve as the basis for the preparation of resistance-stimulating agents [155, 156].

Among the preparations possessing the ability to enhance the body's immunobiological reactivity are tissue polypeptides – cytomedins. Their advantage over other immunomodulators lies in the fact that they lack pronounced antigenic properties and exert a corrective effect on the organs from whose tissues they were derived [157–160].

Among herbal remedies for boosting the body's resistance, the following are recommended: ginseng tincture, zamaniha, Manchurian aralia, safflower-like levzeya,

Chinese magnolia vine, Eleutherococcus senticosus extract, white birch sap, 1% extract of birch sponge fungus, birch leaf infusion (1:20), infusions of coltsfoot, cow parsnip, spring primrose, tricolor violet, common anise, marsh cinquefoil, decoction of medicinal angelica, and anise oil. For comprehensive treatment of patients, medicinal plants containing a complex of vitamins are prescribed: infusion of stinging nettle leaves (1.5:20), infusion of meadow clover leaves and inflorescences (2.5:100), infusion of common rowan berries (1:10), decoction of pine needles (1:20), infusion of rosehip berries (1:20) [161–165].

Pathogenetic therapy plays a crucial role in the treatment of animals with bronchopneumonia. In addition to the aforementioned agents in this category (nonspecific stimulants, immunostimulants, vitamin and enzyme therapy, etc.), in cases of this pathology, novocaine blocks of the vagosympathetic trunk, stellate ganglion, thoracic internal nerves, and border trunks, as well as intrapleural and other blocks, are performed [166].

Physical methods are successfully used to treat sick animals. Artificial aeroionization of premises with negatively charged ions improves the morphological composition and biochemical properties of blood in calves with bronchopneumonia, enhances phagocytosis, lysozyme activity, and the bactericidal properties of blood serum [167]. Electrical stimulation restores the regulatory function of the central nervous system, promotes the normalization of metabolism, accompanied by a reduction in the inflammatory response and a shorter recovery time [168].

Prevention is a key factor in combating respiratory diseases in young animals [2, 22, 24, 169–171]. It encompasses a range of organizational, management, and veterinary-sanitary measures [172, 173]. It is believed that organizational and management measures should ensure the production of physiologically healthy offspring, an optimal indoor microclimate, and balanced feeding of calves at different stages of their lives [49, 174]. Year-round stall housing weakens the animals' gene pool. Calves born in the winter-spring period exhibit a pronounced immunodeficiency. In this regard, to ensure the production of healthy offspring, issues regarding the improvement of both housing conditions and the composition

and structure of feed consumed by the dairy herd must be addressed – by significantly increasing the production of hay and concentrated feeds while simultaneously reducing the consumption of preserved feeds.

The most important aspect of preventing bronchopneumonia in calves is maintaining an optimal microclimate in the facilities. In calf barns and outdoor pens, overheating of the animals must be prevented, as this is one of the factors contributing to the development of bronchopneumonia [175].

To prevent diarrhea and bronchopneumonia syndrome, calves are raised from the first days of life until two months of age in individual outdoor preventive housing units [175, 176].

When preventing animal diseases, including bronchopneumonia, population size should be taken into account: in large groups, there is an increase in the number of animals contracting bronchopneumonia [175, 176].

The complex of veterinary and sanitary measures includes preventive, routine, and, if necessary, emergency disinfection with formaldehyde, peracetic acid, lactic acid, and others [136, 177].

As a preventive measure, it is recommended to use a 6% solution of novarsenol at a dose of 0.01 g per 1 kg of body weight, administered intravenously or intraperitoneally [178]. This drug is adsorbed by lung tissue and has a bacteriostatic effect, activates the mononuclear system, stimulates hematopoiesis, and enhances certain other protective functions of the body [179].

Oxygumate, as a feed additive, helps increase the body’s overall resistance and reduce morbidity in young animals [180].

To prevent stress and enhance humoral resistance factors, the use of sodium succinate is recommended [181].

As immunostimulants for the prevention of catarrhal bronchopneumonia in veterinary medicine, levamisole, sodium nucleinate, sodium humate, thymus preparations, and prodigiozan were used more frequently than other drugs. For the same purpose, a single intramuscular injection of blood irradiated with ultraviolet rays is administered at a dose of 0.5 ml per 1 kg of body weight [182–187].

S.S. Abramov et al. (1982) recommend physical methods for the prevention of bronchopneumonia: daily, for six hours, conduct air ionization at a dose of 200,000 ions/cm3 of air, as well as irradiation with ultraviolet (at a dose of 120 mR/g/m2) and infrared rays. Under the influence of physiotherapeutic preventive procedures, the body's resistance increases while simultaneously improving the indoor microclimate [21, 182].

For group prevention, it is recommended to use aerosols of chloramine B, aluminum iodide, chloroskipidar, iodine monochloride, iodotriethylene glycol, formalin, and lactic acid [182].

One approach to pneumonia prevention is the stimulation of active immunity against pathogens causing chronic, and less commonly acute, inflammatory processes of microbial etiology. For this purpose, autovaccination is performed [188].

On farms with a history of respiratory infectious diseases, animals are immunized against parainfluenza-3, infectious rhinotracheitis, viral diarrhea, adenovirus infection, rotavirus and coronavirus diarrhea, pasteurellosis, streptococcosis, mycoplasmosis, and others [125].

An analysis of the literature shows that the problem of bronchopneumonia in calves in Ukraine and beyond is quite relevant. However, the pathogenesis of the disease requires further study. It can already be stated that pathological changes in the body cause disturbances in the molecular organization and function of cell membranes and the function of the cell as a whole [189–192]. Membranes not only separate cells from the surrounding environment, but important processes also occur within the membrane itself, on which their permeability to many substances depends.

Changes in metabolism during bronchopneumonia affect the structural organization and functional activity of the erythrocyte system [193], leading to a disruption of the primary function of erythrocytes – the supply of oxygen to various organs and tissues. Protein-lipid interactions play a significant role in the functional activity of erythrocytes. Quantitative and qualitative changes in lipids affect the functional activity and binding capacity of membrane proteins by altering the microviscosity in the region where they are localized [103, 107]. Proteins, in turn,

influence the structure of the lipid bilayer, thereby affecting the transport of substances across the membrane [194, 195]. Therefore, changes in protein biosynthesis or their conformational state in bronchopneumonia may also cause disturbances in membrane functional activity. This may be one of the causes of impaired synthesis of 2,3-diphosphoglycerate (2,3-DPG), which is responsible for hemoglobin's affinity for oxygen.

Questions regarding the diagnosis and treatment of this condition remain important. Despite the wide range of new broad-spectrum antibiotics available, treatment for bronchopneumonia is not always effective. When treating animals with this disease, it is necessary to take into account metabolic disorders in the body and the side effects of chemotherapeutic drugs on it. To date, the impact of antibacterial agents used to eliminate the microbial factor on the functional integrity of red blood cells and the body as a whole has not been fully elucidated. Answers to these questions will enable a more detailed study of the pathogenetic mechanisms of bronchopneumonia development in calves and the development of effective comprehensive treatment methods for this disease.

Unit 2

RESEARCH METHODOLOGY

2.1. Materials and Methods

The study was conducted at the laboratory of the Research Institute of Internal Animal Diseases, the laboratory for the diagnosis of infectious diseases in farm animals at the Bila Tserkva National Agrarian University, the laboratory of the Department of Internal Animal Diseases of the National University of Life and Environmental Sciences of Ukraine, and on livestock farms in the Kyiv region between 1997 and 2025, and on farms in the Kyiv region. A study was conducted on 120 calves aged 1.5–3 months, including 45 clinically healthy calves and 75 calves with bronchopneumonia. The 75 calves were treated using various regimens (Fig. 2.1).

Clinical examinations were conducted daily throughout the treatment course and at the end of the experiment. Blood samples were collected from the study animals in the morning (before feeding) from the jugular vein and the abdominal aorta, with two tubes taken from each vessel. One tube was pre-filled with a heparin solution (as an anticoagulant), while the other was used to collect blood for serum preparation.

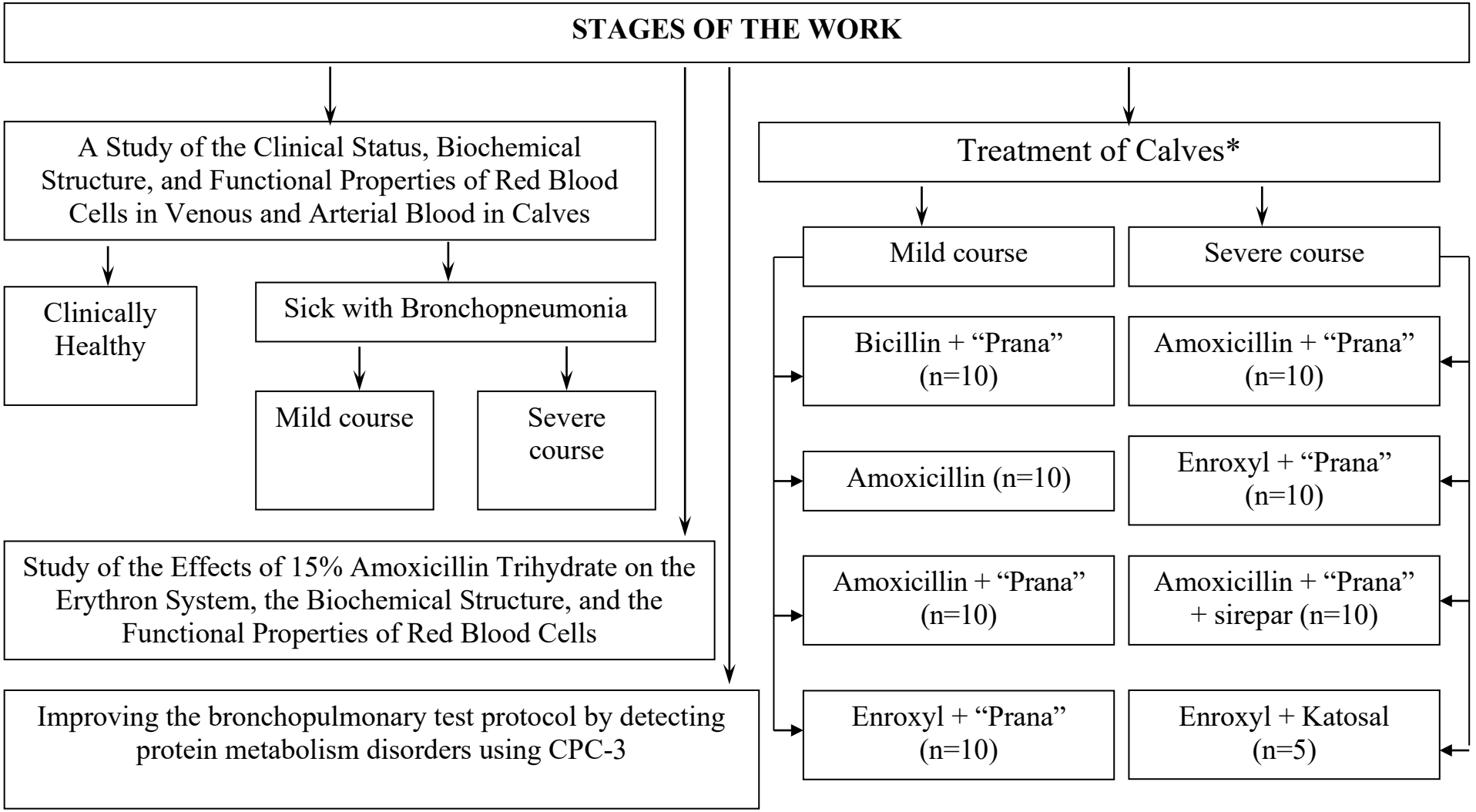
Total hemoglobin and methemoglobin levels were determined using the hemoglobin-cyanide method, with the concentration of the latter calculated according to the method of A.M. Goryachkovsky and K.V. Moiseeva, the total number of erythrocytes – by the Melanger method, and the hematocrit value – by microcentrifugation (according to Shklyar). Based on these data, the hemoglobin content per erythrocyte (HCE), the mean corpuscular volume, and the color index were calculated.

The biochemical structure and functional properties of erythrocytes were studied using the following indicators:

a) protein content in erythrocyte membranes according to Lowry;

b) phospholipid content in erythrocyte membranes [196];

c) total lipid content (using Lachema test kits, Czech Republic);



Note. * – in all treatment regimens, Trivitamin and sodium bicarbonate were used

Fig. 2.1. Protocol for Conducting Experimental Studies

d) cholesterol content in membranes (using Lachema test kits, Czech Republic); e) 2,3-diphosphoglycerate content in erythrocytes (according to Dyce, as modified by L.I. Apukhovskaya);e) acid resistance of erythrocytes followed by the construction of an erythrogram (according to Gitelzon I.I. and Terskov I.A., 1960) [197];

g) the population composition of peripheral blood erythrocytes – in a sucrose density gradient (according to Syzova I. et al., 1980) [197].

The erythrocyte suspension was obtained by centrifuging the animals' blood at 1,500 rpm for 20 minutes. The resulting cell suspension was washed three times with cold isotonic sodium chloride solution, followed by centrifugation under the same conditions. Under standard conditions, the number of erythrocytes in 1 μL of suspension is practically constant (16 × 109). To obtain erythrocyte membranes, the cell suspension, washed with isotonic solution, was hemolyzed for 30 minutes at 4 °C with a solution containing 20 mmol Tris-HCl, 14 mmol NaCl, 10 mmol KCl, 20 mmol sucrose, pH 7.4 (solution 1), in a 1:50 ratio. After centrifuging the hemolysate for 20 minutes at 7,000 rpm and washing the membrane suspension twice with Solution 1, the membranes were treated for two hours at 4 °C with a solution containing 5 mmol Tris-HCl, 7 mmol KCl, pH 7.4, in the same ratio, followed by centrifugation under the specified conditions and two washes with solution 1. The erythrocyte membranes isolated in this manner contain virtually no hemoglobin. Extraction of the lipid components of erythrocyte membranes was performed using the chloroform-methanol method [198]. All solutions were pre-purified and stored in accordance with Keist's recommendations [197].

To diagnose the course and predict the prognosis of bronchopneumonia in calves, a biochemical test was performed according to I.P. Kondrakhin and an improved method (Rozumnyuk A.V., Levchenko V.I.). Serum without traces of hemolysis was used for the study. The analysis was performed within the first two days after blood collection.

The results of morphological, biochemical, and immunological blood tests were analyzed using methods of variational statistics. The arithmetic mean (M), the

standard error of the arithmetic mean (m), the significance of the difference between the arithmetic means of two variation series according to the significance criterion (p) and Student's tables, and the correlation coefficient were determined. The difference between two values was considered significant at $p < 0.05$; 0.02, 0.01, and 0.001. In clinically healthy animals, the standard error of the mean (δ) was determined [199].

The following drugs were used to treat calves with bronchopneumonia:

a) Bicillin-3, intramuscularly, 10,000 IU/kg body weight, daily;

b) amoxicillin trihydrate 15%, intramuscularly, 1 mL/10 kg body weight (150 mg/10 kg), twice daily;

c) enroxil 5%, intramuscularly, 1 mL/20 kg body weight, for three consecutive days;

d) Trivitamin, intramuscularly, 5 mL, once every three days;

e) Sodium bicarbonate 0.5% solution, orally, 1 L, twice daily;

f) Prana, intramuscularly, 4 mL, three times daily;

g) Syrepar (liver hydrolysate), intramuscularly, 2.5 mL, once daily;

h) Katosal, intramuscularly, 8 mL, once daily (Table 2.1). Laboratory analysis was performed to determine the composition of the lung microflora and its sensitivity to antibacterial agents.

Table 2.1

Treatment regimens for calves with bronchopneumonia

Scheme of treatment	Medicines	Groupe of animals		
		Sick with bronchopneumonia*		Clinically healthy**
		Mild course	Severe course	
1-a	a, d, e, f	n=10	–	n=10
2-a	b, d, e	n=10	–	
3-я	b, d, e, f	n=10	n=10	n=10
4-a	c, d, e, f	n=10	n=10	n=10
5-a	b, d, e, f, g	–	n=10	n=10
6-a	c, d, e, h	–	n=5	n=5

Note. * – follow-up period of four weeks; ** – follow-up period of 7 days.

2.2. Improvements to a Biochemical Test (by Professor I.P. Kondrakhin) for the Diagnosis and Prognosis of Bronchopneumonia in Calves

Principle of the method. The method is based on the detection of dysproteinemia (a decrease in the albumin-globulin ratio), which is observed in bronchopneumonia (pneumonia).

Preparation of blood samples for analysis. In calves, blood is drawn from the jugular vein into dry chemical tubes, following aseptic and antiseptic procedures. To prevent hemolysis, blood is drawn along the wall of the tube and delivered to the laboratory on the day of collection. Immediately after collection, the blood tubes must be placed in warm water (37–38 °C) for 1–2 hours. If clotting has not occurred, a thin glass rod is used to separate the clot from the tube wall, and the blood is placed in a thermostat at 37–38 °C for 1–2 hours. Then, the serum is decanted into centrifuge tubes and centrifuged for 15 minutes at 3,000 rpm. Only serum without hemolysis is suitable for testing. The analysis must be performed no later than three days after blood collection.

Reagents: 0.85% sodium chloride solution (pharm. grade); 75 mg of zinc sulfate heptahydrate (pharm. grade) is dissolved in 100 mL of distilled water (0.075% solution). Store in a glass bottle with a ground-glass stopper for up to 30 days.

Equipment: test tubes, pipettes, CFC–3. *Procedure.* First, 2.0 mL of a 0.85% sodium chloride solution is added to a test tube, followed by 1.0 mL of blood serum, and the mixture is stirred. Add 2.0 mL of a 0.075% zinc sulfate solution. Mix and, after 15 minutes, measure the absorbance at a wavelength of 600 nm in cuvettes with a working solution path length of 10.0 mm. The control is a 0.85% sodium chloride solution. The results are evaluated by absorbance. However, to make the results easier to interpret, we converted the obtained values to the equivalent amount of zinc sulfate solution consumed in ml (according to I.P. Kondrakhin) based on the data in Table 2.2.

Clinical significance. Pneumonia is accompanied by dysproteinemia, which manifests as a decrease in the serum albumin-globulin ratio. The more severe the

course of the inflammatory process in the lungs, the more pronounced the dysproteinemia and turbidity of the test (precipitation of coarse-dispersed globulin proteins; Tables 2.2, 2.3).

Table 2.2

The relationship between the extinction of the sample and the amount of zinc sulfate solution used

$ZnSO_4 \cdot 7H_2O$, ml	Extinction	$ZnSO_4 \cdot 7H_2O$, ml	Extinction	$ZnSO_4 \cdot 7H_2O$, ml	Extinction
2,40	1,460	1,75	1,860	1,10	2,500
2,35	1,480	1,70	1,890	1,05	2,540
2,30	1,520	1,65	1,980	1,00	2,575
2,25	1,550	1,60	2,235	0,95	2,600
2,20	1,590	1,55	2,255	0,90	2,630
2,15	1,615	1,50	2,270	0,85	2,655
2,10	1,655	1,45	2,295	0,80	2,685
2,05	1,670	1,40	2,325	0,75	2,710
2,00	1,720	1,35	2,350	0,70	2,735
1,95	1,745	1,30	2,375	0,65	2,765
1,90	1,777	1,25	2,400	0,60	2,800
1,85	1,800	1,20	2,435		
1,80	1,830	1,15	2,470		

Clinical significance. Pneumonia is accompanied by dysproteinemia, which manifests as a decrease in the serum albumin-globulin ratio. The more severe the course of the inflammatory process in the lungs, the more pronounced the dysproteinemia and turbidity of the test (precipitation of coarse-dispersed globulin proteins; Tables 2.2, 2.3).

Table 2.3

Predicting the Course of Bronchopneumonia in Calves

Clinical status	Instrument readings, extinction (CPC–3)	Volume of zinc sulfate solution, mL
Clinically healthy	2,235 and less	1,6–1,8 and more
Mild and moderate course of the disease	2,270–2,375	1,5–1,3
Severe course of the disease	2,435 and more	1,2 and less
The prognosis is poor	2,630 and more	0,9–0,8 and less

A decrease in the biochemical test's extinction ratio indicates a positive therapeutic effect (the animal is recovering), while an increase indicates that treatment is ineffective and that the pathological process is worsening. In cases where the pathological process in the lungs does not change significantly, the test result remains at the same level (Fig. 2.1).

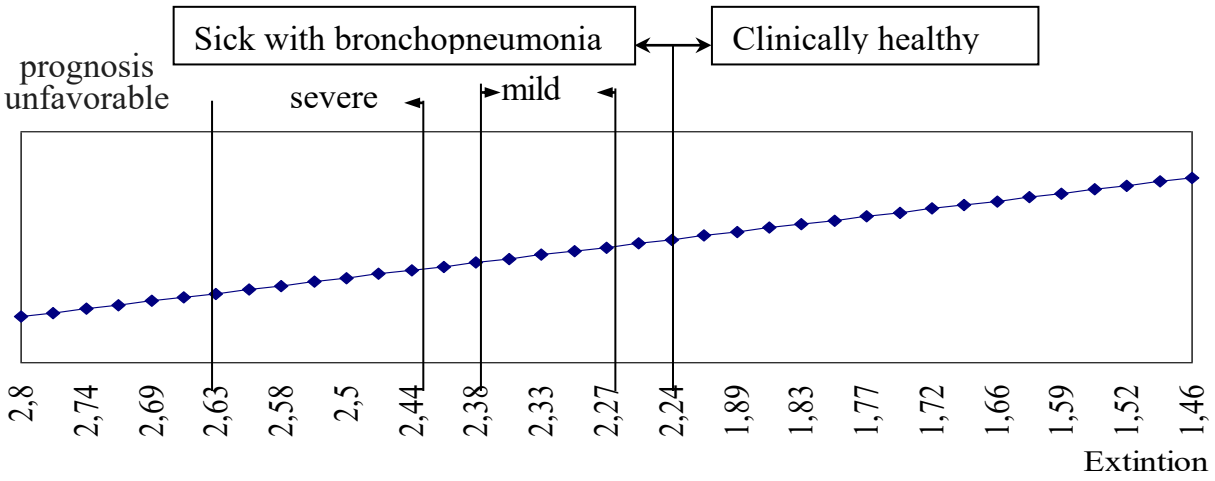


Fig. 2.1 – Changes in the optical density of the sample depending on the course of the disease

UNIT 3

THE STATE OF ERYTHRON, BIOCHEMICAL STRUCTURE, AND FUNCTIONAL PROPERTIES OF ERYTHROCYTES IN CLINICALLY HEALTHY CALVES

3.1. Erythropoiesis Parameters in Clinically Healthy Calves

Oxygen deprivation, which develops in cases of bronchopneumonia, leads to stimulation of the bone marrow. The state of erythropoiesis in this disease can only be characterized by determining normal reference values. The results of studies by foreign and a number of domestic authors regarding erythropoiesis parameters in the venous blood of clinically healthy calves are quite diverse. Indicators for arterial blood, however, are rarely reported. Therefore, we decided to determine hemoglobin concentration and red blood cell count in clinically healthy calves aged 1.5–3 months in both venous and arterial blood.

Clinically healthy calves aged 1.5–3 months were selected from various farms in Ukraine based on clinical signs and biochemical test results [10].

The results of the studies showed that in clinically healthy calves, the red blood cell count in venous blood was significantly higher than in arterial blood ($p<0.01$; Tables 3.1, 3.2). The limits of this indicator in the venous blood of clinically healthy calves were narrower compared to arterial blood, as evidenced by the smaller standard deviation (δ) and the range of $M \pm \delta$, within which 73.3% of the values fell, and $M \pm 2\ \delta$ (96.7% of the values). In arterial blood, 75% of the values fell within the range of $M \pm \delta$, and 100% within $M \pm 2\ \delta$. As can be seen from the data presented in Tables 3.1 and 3.2, all values fall within the range of $M \pm \delta$ in more than two-thirds of cases, and within $M \pm 2\ \delta$ in 95% of cases.

After analyzing the erythropoiesis indicators in venous and arterial blood, we found a significant difference between them. For convenience we compared the average values for venous and arterial blood (Table 3.3).

Table 3.1

Erythropoiesis parameters in venous blood of clinically healthy calves aged 1.5–3 months

Biometric index	Erytrocites, T/l	Hemoglobin, g/l	Hemoglobin		Hematocrit, %	MCH, pg	MCV, μm³
			g/l	%			
Lim	6,90 – 7,40	96,0 – 112,6	2,32 – 2,41	2,21 – 3,09	35,0 – 37,5	14,1 – 17,3	47,3 – 54,4
M±m	7,18 ± 0,20	107,8 ± 3,1	2,90 ± 0,18	2,69 ± 0,21	36,0 ± 0,8	15,0 ± 0,4	50,1 ± 2,1
δ	0,08	4,3	0,33	0,26	0,5	0,8	1,8
M±δ	7,10 – 7,26*	103,5 – 112,1*	2,57 – 3,23*	2,43 – 2,95*	35,5 – 36,5*	14,2 – 15,8*	48,3 – 51,9*
M±2δ	7,02 – 7,34**	99,2 – 116,4**	2,24 – 3,56**	2,17 – 3,21**	35,0 – 37,0**	13,3 – 16,6**	46,5 – 53,7**

Table 3.2

Erythropoiesis parameters in arterial blood of clinically healthy calves aged 1.5–3 months

Biometric index	Erytrocites, T/l	Hemoglobin, g/l	Hemoglobin		Hematocrit, %	MCH, pg	MCV, μm³
			g/l	%			
Lim	5,4–7,1	97,2–102,8	3,29–4,31	3,29–4,31	31,0–34,7	15,2–17,0	50,2–55,2
M±m	6,2±0,2	99,8±1,3	3,70±0,18	3,70±0,18	32,3±0,6	16,1±0,3	52,1±0,8
δ	0,6	1,6	0,32	0,32	1,2	0,5	1,7
M±δ	5,6–6,8*	98,2–101,4*	3,38–4,02*	3,38–4,02*	31,1–33,5*	15,6–16,6*	50,4–53,8*
M±2δ	5,0–7,4**	96,6–103,0**	3,06–4,34**	3,06–4,34**	29,9–34,7**	15,1–17,1**	48,7–55,5**

Note: * – included in > 2/3 of the indicators; ** – included in > 95% of the indicators

Table 3.3

The Status of Erythropoiesis in Clinically Healthy Calves Aged 1.5–3 Months

Index		Venous	Arterial	p<
Eritrocytes, T/l		7,2±0,2	6,2±0,2	0,01
Hemoglobin, g/l		107,8±2,1	99,8±1,3	0,01
Hemoglobin	%	2,69±0,21	3,70±0,18	0,01
	g/l	2,90±0,18	3,70±0,18	0,01
Hematocrit, %		36,0±0,8	32,3±0,6	0,02
MCH, pg		15,0±0,4	16,1±0,3	0,05
MCV, μm^3		50,1±1,1	52,1±0,8	0,1

Note. p< – comparison of venous and arterial blood

The red blood cell count and total hemoglobin concentration in arterial blood were significantly lower than in venous blood, a difference offset by a higher mean hemoglobin content per red blood cell ($p < 0.05$). The same mean red blood cell volume ($p < 0.1$) and lower red blood cell count in arterial blood led to a 3.7% decrease in hematocrit compared to venous blood ($p < 0.02$).

The concentration of methemoglobin in arterial blood was 1.5 times higher than in venous blood. This can be explained by the direct contact of the respiratory organs with the environment, and therefore the blood passing through the lungs is enriched not only with oxygen but also with other gases in the air. Since nitrogen accounts for about 60% of air, it can be assumed that the higher methemoglobin content in arterial blood is the result of the oxidation of erythrocyte iron by nitrogen compounds (in particular, nitrogen dioxide). Since arterial blood is the same in all vessels, and we took venous blood from the jugular vein, we attribute the higher number of red blood cells and hemoglobin in venous blood to blood thickening. After passing through the salivary glands, lacrimal glands, and other tissues, the blood loses its liquid component, which leads to a higher concentration of red blood cells and hemoglobin.

3.2 Population composition and acid resistance of peripheral blood red blood cells in clinically healthy calves

Analysis of the graph showing the acid resistance of erythrocytes in 1.5–3-month-old calves revealed that the main peak occurred at 3.5 minutes in both venous and arterial blood (Fig. 3.1).

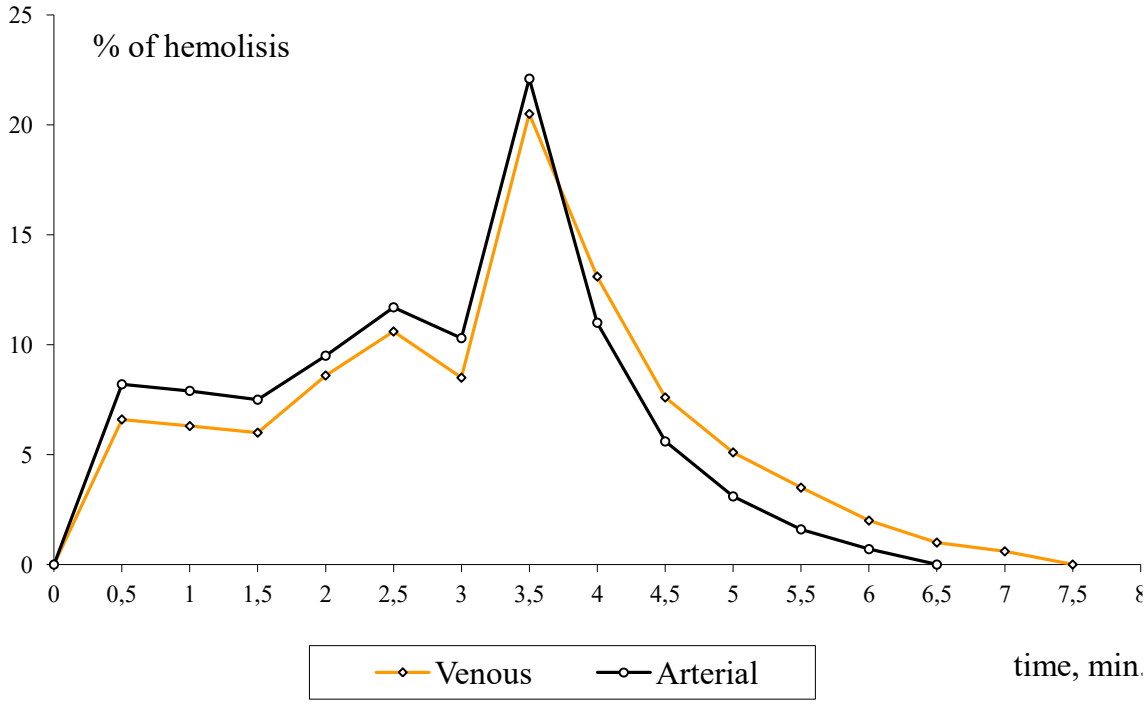


Fig. 3.1 – Acid resistance of red blood cells in venous and arterial blood from clinically healthy calves aged 1.5–3 months

The amplitude of the main peak was 20.5% in the venous waveform and 22.1% in the arterial waveform, and the width of their bases was 2 minutes. The left side of the graph showed additional peaks. The first peak rises at the 30-second mark; its height is 6.6% in the venous waveform and 8.2% in the arterial waveform, with a width of 1.5 minutes. The second additional peaks appeared at one and a half minutes and reached their maximum at 2.5 minutes; their height was 10.6% in venous blood and 11.7% in arterial blood, and their width was 1.5 min. Erythrocyte hemolysis was complete in venous blood at 7.5 minutes and in arterial blood at 6.5 minutes.

Analysis of the erythrocyte acid hemolysis graphs showed that the peaks on the left side of the arterial blood erythrogram were higher. Although all peaks on both graphs coincided in time, erythrocyte hemolysis in arterial blood was completed one minute earlier, indicating a decrease in their acid resistance compared to erythrocytes in venous blood. Since the acid erythrogram in most cases demonstrates a relationship between resistance and the age composition of erythrocytes [197], the determination of red blood cell populations allows for a better understanding of the processes occurring in the blood of clinically healthy animals and those suffering from bronchopneumonia. The results of fractionating peripheral blood from clinically healthy calves in a sucrose density gradient showed that in venous blood, more than half are “young” erythrocytes (52.0 ± 1.7%), slightly more than a third are “mature” (36.1 ± 1.3%), and only 11.9 ± 0.8% of the total number are “old” erythrocytes (Table 3.4). The same trend is observed in arterial blood (Table 3.5).

Table 3.4

Populations of red blood cells in venous blood from clinically healthy calves aged 1.5–3 months

Biochemical index	Eritrocyte population, %		
	“Old”	“Adult”	“Young”
Lim	8,2 – 14,6	31,7 – 40,7	48,6 – 58,2
M±m	11,9 ± 0,8	36,1 ± 1,3	52,0 ± 1,7
δ	1,4	2,3	2,6
M± δ	10,5 – 13,3*	33,8 – 38,4*	49,4 – 54,6*
M±2 δ	9,1 – 14,7**	31,5 – 40,7**	46,8 – 57,2**

Note: * – included in > 2/3 of the indicators; ** – included in > 95% of the indicators

However, when comparing the average values of red blood cell populations in venous and arterial blood in clinically healthy calves, we found some differences (Table 3.6). The concentration of “old” and “mature” populations in arterial blood was 2.1% and 2.7% higher, respectively, compared with the corresponding values in venous blood ($p<0.05$). At the same time, the concentration of “young” erythrocytes in arterial blood was 4.8% lower ($p < 0.02$).

Table 3.5

Populations of red blood cells in arterial blood from clinically healthy calves aged 1.5–3 months

Biochemical index	Eritrocyte population, %		
	"Old"	"Adult"	"Young"
Lim	13,3–14,6	36,7–41,2	43,8–52,1
M±m	14,0±0,2	38,8±0,6	47,2±1,0
δ	0,3	1,1	2,4
M±δ	13,7–14,3*	47,7–39,9*	44,8–49,6*
M±2δ	13,4–14,6**	36,6–41,0**	42,4–52,0**

Note: * – included in > 2/3 of the indicators; ** – included in > 95% of the indicators

Table 3.6

Population of red blood cells in clinically healthy calves aged 1.5–3 months: venous and arterial blood

Groups of animals	Eritrocyte population, %		
	"Old"	"Adult"	"Young"
Venous	11,9±0,8	36,1±1,1	52,0±1,7
Arterial	14,0±0,2	38,8±0,6	47,2±1,0
p<	0,05	0,05	0,02

Note: p< – comparison of venous and arterial blood

We attribute these changes to the intense oxidation of the lipid components of red blood cell membranes in the lungs, which leads to more rapid "aging" of arterial blood cells.

3.3. Lipid content in the erythrocyte membranes of clinically healthy calves

Results of studies on the total lipid content of erythrocyte membranes in clinically healthy calves aged 1.5–3 months showed that their concentration in venous blood ranged from 1.8 to 2.2, and in arterial blood – from 2.89 to 3.29 g/g of erythrocyte membrane protein (Tables 3.7, 3.8). The difference between their mean

contents was statistically significant ($p<0.001$).

Determining the content of total lipids in biological membranes is important for studying their biochemical structure. However, the phospholipid structure has the greatest influence on the functional state of membranes, as it ensures the membranes' negative charge, their permeability, the ability to transport substances, and the processes of interaction between various components and molecules within the membrane itself [191].

In the membranes of erythrocytes from clinically healthy calves aged 1.5–3 months, the phospholipid content ranged from 0.74 to 1.01 mmol/g of protein in erythrocyte membrane suspensions in venous blood and from 1.19 to 1.46 mmol/g in arterial blood.

To maintain the structural and functional integrity of erythrocytes, there is a constant exchange of cholesterol between erythrocytes and plasma lipoproteins in the body. Cholesterol is an important component of erythrocyte membranes, on which the stability of erythrocytes and their hemoglobin saturation depend. Results of studies on erythrocyte membranes from clinically healthy calves aged 1.5–3 months showed that its content ranges from 0.44 to 0.69 mmol/g of protein in erythrocyte membrane suspensions in venous blood and from 0.60 to 0.88 in arterial blood.

The variations in the lipid component indices of erythrocyte membranes in venous blood fell within the range of $M \pm \delta$ in 73.3–83.3% of cases and within the range of $M \pm 2\ \delta$ in 96.7–100% of cases; in arterial blood, these figures were 70–80% and 95–100%, respectively. The structural and functional properties of erythrocyte membranes depend not only on their lipid content but also on the ratio between these components. The cholesterol-phospholipid ratio in the erythrocyte membranes of venous and arterial blood from clinically healthy calves aged 1.5–3 months was the same (Tables 3.7, 3.8), despite the fact that the content of both components in erythrocytes of arterial blood was higher ($p < 0.001$; Table 3.9). However, the increase in both components – phospholipids and cholesterol – was proportional, with a correlation coefficient of +0.96.

Table 3.7

Lipid composition of erythrocyte membranes in venous blood from clinically healthy calves aged 1.5–3 months

Biometric index	Total lipids, g per g of erythrocyte membrane protein	Cholesterol	Phospholipids	Cholesterol / Phospholipids
		mmol/g erythrocyte membrane suspension protein		
Lim	1,8–2,2	0,44–0,69	0,74–1,01	0,51–0,58
M±m	2,10±0,10	0,50±0,03	0,92±0,04	0,54±0,01
δ	0,16	0,08	0,09	0,02
M±δ	1,94–2,26*	0,42–0,58*	0,83–1,01**	0,52–0,56*
M±2δ	1,78–2,42**	0,34–0,66**	0,74–1,10*	0,50–0,58**

Table 3.8

Lipid composition of erythrocyte membranes in arterial blood from clinically healthy calves aged 1.5–3 months

Biometric index	Total lipids, g per g of erythrocyte membrane protein	Cholesterol	Phospholipids	Cholesterol / Phospholipids
		mmol/g erythrocyte membrane suspension protein		
Lim	2,82–3,29	0,60–0,88	1,19–1,46	0,49–0,60
M±m	3,04±0,07	0,73±0,04	1,32±0,06	0,55±0,01
Δ	0,12	0,07	0,07	0,03
M±δ	2,92–3,16*	0,66–0,80*	1,25–1,39*	0,52–0,58*
M±2δ	2,80–3,28**	0,59–0,87**	1,18–1,46**	0,49–0,61**

Note: * – included in > 2/3 of the indicators; ** – included in > 95% of the indicators

Table 3.9

Lipid content of red blood cell membranes in clinically healthy calves: venous and arterial blood

Biometric index	Total lipids, g per g of erythrocyte membrane protein	Cholesterol	Phospholipids	Cholesterol / Phospholipids
		mmol/g erythrocyte membrane suspension protein		
Venous	2,10 ± 0,11	0,50 ± 0,03	0,92 ± 0,04	0,54 ± 0,01
Arterial	3,04 ± 0,07	0,73 ± 0,04	1,32 ± 0,06	0,55 ± 0,02
p<	0,001	0,001	0,001	0,1

Note. p< – comparison of venous and arterial blood

When comparing the average levels of lipid components in erythrocyte membranes, we found higher levels in arterial blood ($p < 0.001$ for all parameters). This is evidently due to the enrichment of blood with lipid compounds in the lungs, liver, and other organs, followed by their release to the body's cells and tissues. The identical lipid index (cholesterol–phospholipids) in venous and arterial blood indicates the stability of the ratio of lipid components in erythrocyte membranes in clinically healthy calves.

3.4. The content of total and inorganic phosphorus and 2,3-diphosphoglycerate (2,3-DPG) in the red blood cells of clinically healthy calves

After passing through the lungs, red blood cells become enriched with oxygen. In the tissues, oxygen is released from the hemoglobin in the red blood cells and absorbed by the cells of the organs. 2,3-diphosphoglycerate (2,3-DPG) plays an important role in these processes; it reduces the affinity of hemoglobin for oxygen and promotes the rapid transfer of oxygen into the body's cells and tissues [200]. The results of our studies showed that in the cytoplasm of red blood cells from clinically healthy calves aged 1.5–3 months, the total phosphorus content in venous blood ranges from 2.95 to 3.48 μmol/mL of red blood cell suspension, and in arterial blood,

from 3.98 to 4.63. This indicates a statistically significantly higher level ($p < 0.001$) in arterial blood cells. The inorganic phosphorus content in the erythrocytes of these calves was 1.24–1.33 and 0.91–1.10 μmol/mL of erythrocyte suspension, respectively, indicating a statistically significantly higher concentration ($p < 0.001$) in the cytoplasm of red blood cells from venous blood of clinically healthy animals (Tables 3.10–3.12).

Table 3.10

Phosphorus and 2,3-DPG concentrations in red blood cells from venous blood of clinically healthy calves aged 1.5–3 months; μmol/mL of red blood cell suspension

Biometric index	Common Phosphorus	Non-organic Phosphorus	2,3-DPG	2,3-DPG•100% / P common
Lim	2,95–3,48	1,24–1,33	1,73–2,21	57,8–64,1
M±m	3,30 ± 0,02	1,30 ± 0,04	2,00 ± 0,05	60,6 ± 1,6
δ	0,17	0,05	0,11	1,8
M±δ	3,13–3,47*	1,25–1,29*	1,89–2,11*	58,8–62,4*
M±2δ	2,96–3,64**	1,20–1,34**	1,78–2,22**	57,0–74,2**

Note. * – included in > 2/3 of the indicators; ** – included in > 95% of the indicators

Nearly two-thirds of the total phosphorus in red blood cells is accounted for by 2,3-diphosphoglycerate [201]. Given that this enzyme reduces the affinity of hemoglobin for oxygen, determining the 2,3-DPG content is important for assessing the degree of hypoxia and hypoxemia in calves with catarrhal bronchopneumonia. Studies have shown that the concentration of 2,3-diphosphoglycerate in clinically healthy calves aged 1.5–3 months was higher in the red blood cells of arterial blood compared to venous blood ($p < 0.001$; Table 3.12).

The ratio of 2,3-DPG to total cytoplasmic phosphorus in red blood cells (2,3-DPG × 100% / total P) indicates a more intensive utilization of the latter in the 2,3-diphosphoglycerate shunt of glycolysis in red blood cells of arterial blood compared to those of venous blood. This indicator was 15.2% higher in arterial blood ($p <$

0.001).

Table 3.11

Phosphorus and 2,3-DPG concentrations in red blood cells from arterial blood of clinically healthy calves aged 1.5–3 months; μmol/mL of red blood cell suspension

Biometric index	Common Phosphorus	Non-organic Phosphorus	2,3-DPG	2,3-DPG•100% / P common
Lim	3,98–4,63	0,91–1,10	2,91–3,46	69,8–80,1
M±m	4,22 ± 0,11	1,02 ± 0,02	3,20 ± 0,07	75,8 ± 2,0
δ	0,20	0,05	0,14	3,0
M±δ	4,02–4,42*	0,97–1,07*	3,06–3,34*	72,8–78,8*
M±2δ	3,82–4,62**	0,92–1,12**	3,92–3,48**	69,8–81,8**

Note: * – included in > 2/3 of the indicators; ** – included in > 95% of the indicators

Table 3.12

Phosphorus and 2,3-DPG concentrations in red blood cells of clinically healthy calves aged 1.5–3 months; μmol/mL of red blood cell suspension

Biometric index	Common Phosphorus	Non-organic Phosphorus	2,3-DPG	2,3-DPG•100% / P common
Venous	3,30±0,02	1,30±0,04	2,00±0,05	60,6±1,6
Arterial	4,22±0,11	1,02±0,02	3,20±0,07	75,8±2,0
p<	0,001	0,001	0,001	0,001

Note: p< – comparison of venous and arterial blood

As it circulates through the vessels of a clinically healthy organism, blood changes its composition, which is reflected in both the biochemical structure and the functional properties of red blood cells.

The thicker blood from the jugular vein in the right atrium is diluted by thinner blood from the caudal vena cava (due to the inflow of fluid from the gastrointestinal tract). However, the lower concentration of hemoglobin in this blood, compared to arterial blood, indicates the oxidation of iron by strong oxidizing agents in the lungs

and other organs, as well as the high antioxidant capacity of brain tissues. The oxidation of lipid components in erythrocyte membranes in the lungs leads to an increase in the number of “old” cells and a decrease in their stability (in the blood of arterial vessels, compared to erythrocytes in the jugular vein). Lipid synthesis in the liver and lungs increases their concentration in plasma and, accordingly, in the membranes of blood red blood cells. After these cells pass through the capillaries of organs that do not synthesize lipid compounds, their concentration in plasma and red blood cell membranes decreases.

An increase in the amount of lipids in the membranes of cells located in arterial vessels promotes an increase in the rate of anaerobic glucose breakdown, which in turn activates the 2,3-diphosphoglycerate shunt and increases the 2,3-DPG content in red blood cells. This results in easier oxygen delivery to cells and tissues by the hemoglobin of arterial blood red blood cells compared to that of venous blood red blood cells. An analysis of studies conducted on clinically healthy calves indicates that in both venous and arterial blood, the quantitative and qualitative composition of red blood cells, their biochemical structure, and functional properties depend on the lability of the bilayer and its interaction with blood plasma.

UNIT 4

CLINICAL STATUS, CHANGES IN THE STRUCTURE AND FUNCTIONAL PROPERTIES OF RED BLOOD CELLS IN CALVES WITH BRONCHOPNEUMONIA

4.1. Clinical status of calves with bronchopneumonia

Based on the results of clinical examinations and biochemical tests, we divided the affected calves into two groups: those with mild (first group) and severe (second group) cases of bronchopneumonia. In calves of the first group, body temperature ranged from 40.3 to 41.4 °C; in calves of the second group, it was on average 1.6 °C lower, ranging from 38.6 to 40.1 °C ($p<0.001$; Table 4.1). However, in calves from both the first and second groups, this indicator was significantly higher compared to clinically healthy animals ($p < 0.001$; $p < 0.05$).

Table 4.1

Results of a clinical examination of calves with bronchopneumonia

Index	Clinically healthy	Mild course	p<	Severe course	p<	p*<
Temperature, °C	38,7±0,16	40,8±0,14	0,001	39,2±0,19	0,05	0,001
Heart rate, bpm	78,0±3,6	108,0±4,0	0,001	95,0±3,5	0,01	0,05
Breath rate, rmpm	24,0±2,1	35,0±3,0	0,01	53,0±2,9	0,001	0,001
Biochemical test, ml (0,075 % $ZnSO_4 \cdot 7H_2O$)	1,89±0,12	1,34±0,09	0,01	1,02±0,08	0,001	0,02

Note. p< – comparison between sick and clinically healthy animals; p*< – comparison between mild and severe disease progression.

The heart rate in calves of the first group was also higher compared to that in animals of the second group ($p^*<0.05$). This can be explained by the development of cardiovascular insufficiency in animals with severe bronchopneumonia. In these animals, heart sounds were muffled, whereas in calves of the first group, they were amplified. We observed accentuation of the second heart sound at the “punctum

optimum" of the pulmonary artery in calves of both groups; however, this phenomenon was more pronounced in animals with a mild course of the disease. In calves of the second group, in addition to sinus tachycardia, we observed respiratory arrhythmia, characterized by an increase in heart rate during inspiration and a decrease during expiration. On average, the pulse rate in these calves was 17 beats per minute higher than in clinically healthy calves ($p < 0.01$), but 13 beats per minute lower than in animals in the first group ($p < 0.05$).

A reduction in the respiratory surface area of the lungs due to bronchopneumonia leads to an increase in respiratory rate. The more severe the disease process, the greater the amount of affected lung tissue, and consequently, the more pronounced the tachypnea. Depending on the severity of the disease, the number of respiratory movements increased: in calves of the first group – by 1.5 times ($p < 0.01$), and in calves of the second group – by 2.2 times ($p < 0.001$), compared with clinically healthy animals.

In calves with severe disease, we observed mixed dyspnea, and the respiratory rate in these animals was 1.6 times higher compared to calves in the first group ($p^* < 0.001$). In addition to changes in respiratory rate and rhythm, we observed wheezing in the lungs of animals with bronchopneumonia. Mucus accumulating in the bronchi causes coughing. In animals with a mild course of the disease, the cough was infrequent and dry; in calves with a severe course, it was frequent and muffled; and in most cases, it was wet.

The duration of the disease is one of the main reasons for the progression from a mild to a severe course. If calves with bronchopneumonia are not treated in the early stages, the disease becomes severe and can be fatal [10]. Therefore, in addition to objective indicators such as temperature, pulse and respiratory rates, and the presence of a cough and wheezing, additional factors must also be taken into account. In calves from the second group, we observed lethargy, loss of appetite, weight loss (in some cases, emaciation); the visible mucous membranes and skin were pale, and the coat was ruffled. A decrease in skin turgor indicates dehydration due to the accumulation of exudate in the lungs. In animals with a mild course of

bronchopneumonia, these changes were less pronounced, and some of them were not observed at all (pallor of the mucous membranes, lethargy).

Bronchopneumonia in calves causes disturbances in protein metabolism, manifested by a decrease in the albumin-globulin ratio. Biochemical test results showed that the more severe the course of the disease, the greater the decrease in this ratio. In affected calves with a mild course of the disease, this indicator was 1.4 times lower ($p<0.01$), and in calves with a severe course, it was 1.8 times lower ($p<0.001$), compared to clinically healthy animals.

Thus, it can be concluded that in the event of delayed diagnosis of bronchopneumonia, the disease in calves takes a severe course. This, in turn, is accompanied by the subsequent development of cardiovascular failure, disturbances in protein metabolism, and other vital processes in the body, which can lead to the rapid death of the animals.

4.2. Erythropoiesis Parameters in Calves with Bronchopneumonia

The peripheral blood of calves with mild bronchopneumonia was characterized by a statistically significant higher red blood cell count in both venous ($p < 0.01$) and arterial ($p < 0.001$; Table 4.2) samples. The difference in red blood cell counts between the venous and arterial blood of these animals was not statistically significant ($p < 0.1$). The ratio of red blood cells in venous to arterial blood (RBCv/RBCa) was 1.04, compared to 1.16 in clinically healthy animals. The erythrocyte count in venous blood of animals with severe disease did not differ from that in calves with mild bronchopneumonia ($p < 0.1$), but was significantly higher than in clinically healthy animals ($p < 0.05$). In arterial blood, although this parameter was still significantly higher compared to clinically healthy animals ($p < 0.01$), it was significantly lower compared to calves with a mild course of the disease ($p < 0.05$). The ratio (Er_v/Era) in these calves was 1.11, indicating a significantly lower number of red blood cells in the arterial blood of animals with a severe course of the disease ($p < 0.05$).

Table 4.2

Erythropoiesis Parameters in Calves with Bronchopneumonia

Group of animals			Eritrocytes, T/l	Hemoglobin, g/l	Methemoglobin		Hematocrit, %	MCH, pg	MCV, μm³
					%	g/l			
Clinically healthy		Venous	7,2±0,2	107,8±2,1	2,69±0,21	2,90±0,18	36,0±0,8	15,0±0,4	50,0±1,1
		Arterial	6,2±0,2	99,8±1,3	3,70±0,18	3,70±0,18	32,3±0,6	16,1±0,3	52,1±0,8
		p<	0,01	0,01	0,01	0,01	0,02	0,05	0,1
Sick animals	Mild course	Venous	8,0±0,2	114,2±2,2	4,51±0,18	5,14±0,17	32,0±0,7	14,3±0,3	40,0±1,2
		p'<	0,01	0,05	0,001	0,001	0,001	0,1	0,001
		Arterial	7,7±0,1	107,6±2,0	5,50±0,21	5,91±0,32	29,9±0,5	13,9±0,2	38,8±0,7
		p*<	0,001	0,01	0,001	0,001	0,01	0,01	0,001
		p<	0,1	0,05	0,01	0,05	0,05	0,1	0,1
	Severe course	Venous	8,0±0,3	102,0±2,0	6,64±0,23	6,77±0,28	32,0±0,6	12,7±0,3	40,0±0,8
		p'<	0,05	0,05	0,001	0,001	0,001	0,02	0,001
		p"<	0,1	0,001	0,001	0,001	0,1	0,001	0,1
		Arterial	7,2±0,2	90,3±1,4	7,43±0,27	6,62±0,18	28,0±0,7	12,5±0,2	38,8±1,2
		p*<	0,01	0,001	0,001	0,001	0,001	0,001	0,001
		p**<	0,05	0,001	0,001	0,1	0,05	0,001	0,1
		p<	0,05	0,001	0,05	0,1	0,001	0,1	0,1

Note: p< – comparison of venous and arterial blood parameters in the respective groups; p'< – comparison of venous blood parameters in sick and clinically healthy calves; p''< – comparison of venous blood parameters in sick calves with different disease courses; p*< – comparison of arterial blood parameters in sick and clinically healthy calves; p**< – comparison of arterial blood parameters in sick calves with different disease courses.

Hemoglobin concentration also increased in animals with mild bronchopneumonia: by 6.4 g/L in venous blood ($p < 0.05$) and by 7.8 g/L in arterial blood ($p < 0.05$). The ratio of hemoglobin in venous to arterial blood (Hbv/Hba) in these animals was almost indistinguishable from that of clinically healthy animals (1.06 vs. 1.08), indicating a statistically significantly lower concentration of this pigment in the arterial blood of calves with a mild course of the disease ($p < 0.05$).

In calves with severe disease, hemoglobin concentrations decreased sharply in both venous and arterial blood compared to both clinically healthy animals ($pv < 0.05$; $pa < 0.001$) and animals with mild bronchopneumonia ($pv < 0.001$; $pa < 0.001$). The ratio (Hbv/Hba) in these animals was 1.16, indicating a more pronounced decrease in hemoglobin in the arterial blood of calves with severe bronchopneumonia ($p < 0.001$).

The relative methemoglobin level (in percent) in animals with bronchopneumonia increases depending on the course of the disease in both venous and arterial blood. The absolute methemoglobin level (g/L) is less indicative, since the total hemoglobin level fluctuates significantly in affected calves.

The percentage of methemoglobin (Ni) in venous blood increases 1.7-fold ($p < 0.001$) in cases of mild bronchopneumonia, in arterial blood – by 1.5 times ($p < 0.001$), and in severe cases – by 2.5 ($p < 0.001$) and 2.0 ($p < 0.001$) times, respectively, compared with clinically healthy animals. After analyzing the ratio of hemoglobin in venous to arterial blood (Hv/Ha) – clinically healthy animals: 0.73; patients with mild bronchopneumonia – 0.82; and those with severe bronchopneumonia – 0.89 – it can be concluded that, depending on the severity of the disease, the hemoglobin concentration increases more rapidly in venous blood compared to arterial blood.

Mild bronchopneumonia is characterized by a significant decrease in hematocrit: in venous blood – by 4.0% ($p<0.001$), and in arterial blood – by 2.4% ($p<0.01$). In severe cases of the disease, the hematocrit value is also lower compared to clinically healthy animals ($pv < 0.001$; $pa < 0.001$). When compared with sick calves with a mild course of the disease, this parameter decreases only in arterial

blood – by 1.9% ($p < 0.05$).

After performing statistical analysis of red blood cell parameters, we found a decrease in the mean hemoglobin content in erythrocytes (MCH), particularly in severe cases of the disease (in venous blood – by 2.3 pg; $p < 0.02$; in arterial blood – by 3.6 pg; $p < 0.001$), compared with clinically healthy animals. In cases of mild bronchopneumonia, MHC in erythrocytes was significantly reduced only in arterial blood – by 2.2 pg ($p < 0.01$). A comparison of the ratio of MCV in venous to arterial blood (MCVv/MCVa: clinically healthy – 0.93; sick – 1.02 on average) revealed a more pronounced decrease in VGE in the arterial blood of sick animals.

The decrease in hematocrit and the higher number of red blood cells in the blood of sick animals led to a reduction in the mean red blood cell volume: for both mild and severe courses, it was 40.0 μm3 in venous blood (± 1.2 – mild course; ±0.8 – severe course), and in arterial blood – 38.8 μm3 (±0.7 – mild course; ±1.2 – severe course). Thus, in the venous blood of sick animals, the mean red blood cell volume decreased by 10 μm3 ($p < 0.001$) compared to clinically healthy animals, and in arterial blood, it decreased by 13.5 μm3 ($p < 0.001$). A more pronounced decrease in hematocrit and an increase in red blood cell count in the arterial blood of animals with mild bronchopneumonia also affect the ratio of mean corpuscular volume between venous and arterial blood (0.96 in clinically healthy animals; in patients – 1.03). In severe cases of the disease, this ratio does not change compared to that in animals with mild cases of the disease.

4.3. Population Composition and Acid Resistance of Peripheral Blood Erythrocytes in Calves with Bronchopneumonia

The literature contains data on the relationship between red blood cell stability and their number. The greater the number of red blood cells, the faster they undergo hemolysis. However, this phenomenon does not hold true for all pathological conditions of the body [197].

The acid resistance of red blood cells in animals with mild bronchopneumonia

is characterized by a shorter hemolysis time and slightly higher peaks on the erythrogram (Fig. 4.1). Hemolysis of red blood cells in these animals was complete by 6.5 minutes. In calves with severe bronchopneumonia, acid hemolysis ended at 5.5 minutes. A shorter hemolysis time indicates reduced erythrocyte resistance. The more severe the disease, the lower the resistance of red blood cells.

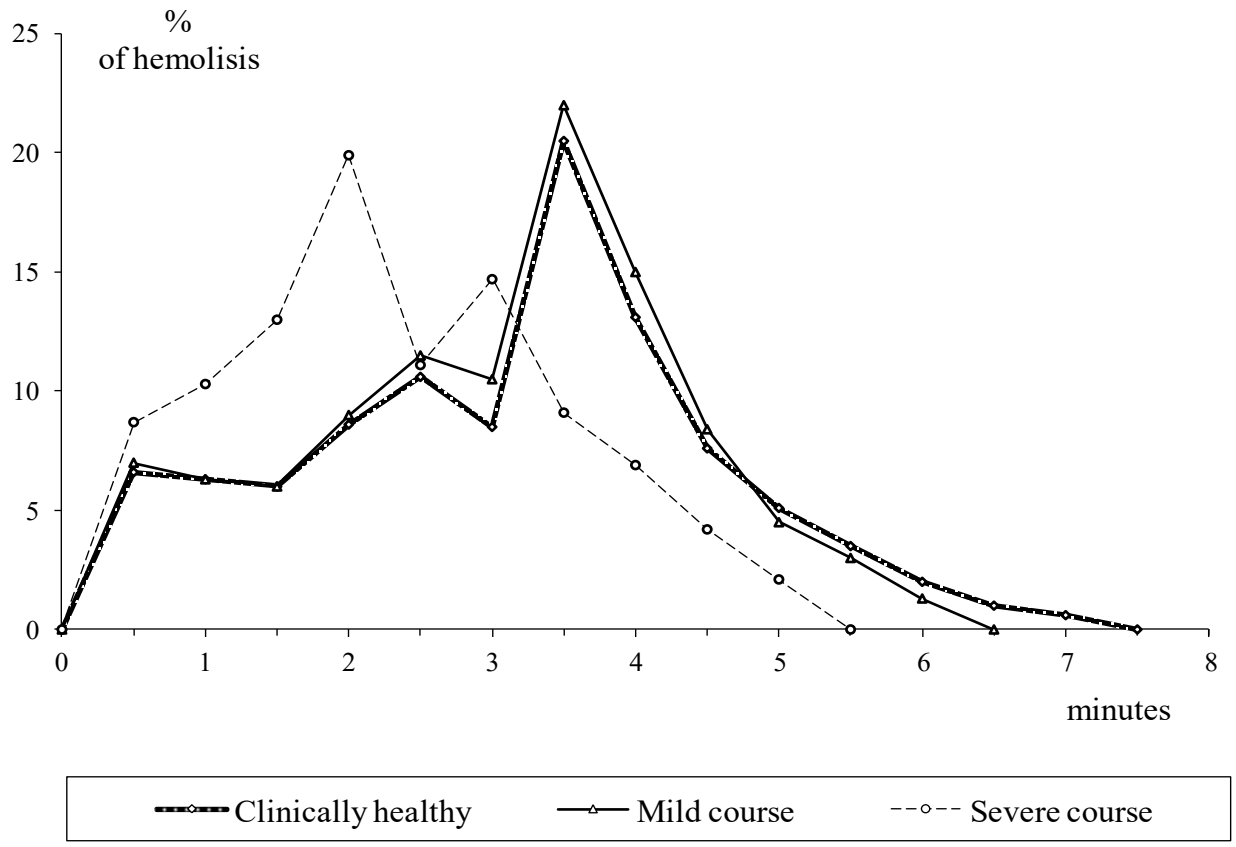


Рис. 4.1 – Acid Resistance of Red Blood Cells in Venous Blood from Clinically Healthy Calves and Calves with Bronchopneumonia

The time it takes for red blood cells to hemolyze depends on the time required for the hemolytic agent to overcome the barrier of membrane impermeability, the rate of destruction of intracellular structures, and the time during which the mechanical strength of the membrane resists the increasing osmotic pressure inside the cell. Thus, the stability of red blood cells depends on their age and the optimal content of lipid

components in their membranes [197].

The onset of the main peak on erythrograms of calves with mild bronchopneumonia, as in clinically healthy animals, began at 3 minutes and reached its maximum at 3.5 minutes. In calves with a mild course of the disease, the height of the main peak was 1.5% higher compared to clinically healthy animals. However, the height of the first peak and the structure of the erythrograms in these calves were virtually identical.

In our opinion, no profound changes in the structure of erythrocyte membranes had yet occurred during this time, and those that did occur were of a more functional nature, as evidenced by the nearly identical hemolytic resistance of erythrocytes in clinically healthy calves and those with a mild course of bronchopneumonia.

The acid resistance of erythrocytes in calves with severe bronchopneumonia differed significantly in their graphical representation. The main peak on their erythrograms was bifurcated and originated from the zero mark. Its maximum occurred at the second minute and amounted to 20%, which is 0.5% less than in clinically healthy animals and 2% less than in calves with mild bronchopneumonia. These changes indicate a significant structural reorganization in erythrocyte membranes due to changes in the ratio of lipid components and the population composition of red blood cells (Table 4.3).

The erythrogram parameters of arterial blood from calves with bronchopneumonia differed from those of venous blood in that they showed slightly higher peaks and a reduction in hemolysis time by 20–50 s. Since the acid erythrogram primarily reveals the relationship between the stability and age distribution of red blood cells, the characterization of red blood cell populations provides a better understanding of the processes occurring in the blood of animals with bronchopneumonia.

The significant decrease in the number of “old” red blood cells ($p < 0.001$) and the tendency toward an increase in “young” red blood cell forms in sick calves with mild bronchopneumonia (in both venous and arterial blood) represent a compensatory phenomenon. In the event of oxygen deprivation, the bone marrow is stimulated, and

a large number of "young," possibly even immature, red blood cells are released into the bloodstream in an effort to eliminate the oxygen deficit and excess carbon dioxide in the body. The worsening of the pathological condition in animals with bronchopneumonia leads to the depletion of the body's defensive and compensatory mechanisms.

Table 4.3

Peripheral blood eritrocytes population composition in clinically healthy calves and calves with bronchopneumonia, %

Group of animals			Eritrocytes population		
			"Old"	"Adult"	"Young"
Clinically healthy		Venous	11,9±0,8	36,1±1,1	52,0±1,7
		Arterial	14,0±0,2	38,8±0,6	47,2±1,0
		p<	0,05	0,05	0,02
Sick animals	Mild course	Venous	8,0±0,5	36,0±1,5	56,0±1,2
		p'<	0,001	0,1	0,1
		Arterial	10,0±0,3	37,8±1,0	53,2±1,1
		p*<	0,001	0,1	0,01
		p<	0,01	0,1	0,1
	Severe course	Venous	16,5±1,0	34,5±2,0	49,0±1,0
		p'<	0,001	0,1	0,1
		p"<	0,001	0,1	0,001
		Arterial	22,3±0,6	35,5±0,9	42,2±1,1
		p*<	0,001	0,001	0,01
		p**<	0,001	0,1	0,001
		p<	0,001	0,1	0,001

Note. p< – comparison of venous and arterial blood parameters in the respective groups; p'< – comparison of venous blood parameters in sick and clinically healthy calves; p"< – comparison of venous blood parameters in sick calves with different disease courses; p*< – comparison of arterial blood parameters in sick and clinically healthy calves; p**< – comparison of arterial blood parameters in sick calves with different disease courses

This is confirmed by the indicators of erythrocyte population composition in calves with severe disease progression. There is a statistically significant increase in the number of "old" erythrocytes (pv < 0.001; pa < 0.001) with a tendency toward a decrease in the number of "young" red blood cells in venous blood and a statistically significant decrease in arterial blood of these animals indicates a reduction or

complete cessation of the release of “young” red blood cells into the bloodstream. The result of this is a retention of “old” red blood cell forms in the blood of sick animals. These changes cause a very rapid rise in the main peak on the erythrogram, its shift to the left, and rapid hemolysis of red blood cells. When comparing the ratio of erythrocyte populations in venous and arterial blood, we observed a decrease in this ratio in “old” cells (clinically healthy – 0.85; mild course – 0.80; severe course – 0.74). These changes indicate a greater rate of “aging” of red blood cells in arterial blood compared to venous blood. A slight increase in this ratio in “mature” cells, depending on the course of the disease (clinically healthy – 0.93; mild course – 0.95; severe course – 0.97), indicates a tendency toward faster maturation of “young” red blood cells in the venous blood of calves with bronchopneumonia.

4.4. Lipid content in the membranes of red blood cells from calves with bronchopneumonia

We attribute the reduced hemolytic resistance and the different population composition of red blood cells in calves with bronchopneumonia to changes in the lipid composition of red blood cell membranes (Table 4.4). The increase in cholesterol and phospholipid content in the erythrocyte membranes of venous and arterial blood from calves with bronchopneumonia depends on the severity of the disease. Thus, in animals with a mild course of the disease, the cholesterol content increased 1.6-fold ($p<0.001$) in venous blood and 2.2-fold ($p<0.001$) in arterial blood, while the phospholipid content increased 1.5-fold ($pv<0.001$; $pa<0.02$) compared to the same indicators in clinically healthy calves. In severe cases of the disease, the cholesterol content in the erythrocyte membranes of venous and arterial blood was 3.6 ($p < 0.001$) and 3.1 ($p < 0.001$) times higher, respectively, compared with clinically healthy calves. This indicates an intense incorporation of the sterol component into the erythrocyte membranes of affected animals. The phospholipid content in calves with severe disease increased 1.7-fold ($p < 0.01$) in venous blood and 1.3-fold ($p < 0.02$) in arterial blood compared to clinically healthy calves.

Table 4.4

Changes in the Lipid Components of Erythrocyte Membranes in Calves with Bronchopneumonia

Group of animals		Total lipids, g per g of erythrocyte membrane protein	Cholesterol	Phospholipids	Cholesterol / Phospholipids
			mmol/g erythrocyte membrane suspension protein		
Clinically healthy	Venous	2,10±0,11	0,50±0,03	0,92±0,04	0,54±0,01
	Arterial	3,04±0,07	0,73±0,04	1,32±0,06	0,55±0,02
	p<	0,001	0,001	0,001	0,1
Mild course	Venous	1,50±0,09	0,82±0,05	1,20±0,07	0,68±0,03
	p'<	0,001	0,001	0,001	0,001
	Arterial	2,60±0,08	1,62±0,09	1,66±0,10	0,98±0,04
	p*<	0,001	0,001	0,02	0,001
	p<	0,001	0,001	0,01	0,001
Severe course	Venous	1,08±0,10	1,80±0,15	1,60±0,10	1,12±0,06
	p'<	0,001	0,001	0,001	0,001
	p"<	0,01	0,001	0,01	0,001
	Arterial	1,64±0,06	2,26±0,11	1,73±0,12	1,31±0,05
	p*<	0,001	0,001	0,01	0,001
	p**<	0,001	0,001	0,1	0,001
	p<	0,001	0,05	0,1	0,05

Note. p< – comparison of venous and arterial blood parameters in the respective groups; p'< – comparison of venous blood parameters in sick and clinically healthy calves; p"< – comparison of venous blood parameters in sick calves with different disease courses; p*< – comparison of arterial blood parameters in sick and clinically healthy calves; p**< – comparison of arterial blood parameters in sick calves with different disease courses

The level of total lipids in the erythrocyte membranes of sick calves decreased significantly depending on the severity of bronchopneumonia. A mild course of the disease was characterized by a 1.4-fold ($p < 0.001$) decrease in this parameter in

venous blood and a 1.2-fold ($p < 0.001$) decrease in arterial blood; in the erythrocyte membranes of animals with a severe course of the disease, the total lipid content was reduced by a factor of 1.9 ($p < 0.001$) in venous blood and by a factor of 1.8 ($p < 0.001$) in arterial blood, compared with the values in clinically healthy calves. An increase in cholesterol and phospholipid levels against a background of decreased total lipid content leads to membrane thickening and a reduction in their permeability. This is confirmed by an increase in the cholesterol-phospholipid ratio in the red blood cells of calves with bronchopneumonia compared to clinically healthy calves. Moreover, in arterial blood, this ratio is significantly higher in animals with both mild ($p < 0.01$) and severe ($p < 0.001$) disease progression. This indicates not only a shift in the balance of lipid components in the blood of affected animals but also a significantly greater incorporation of cholesterol into the erythrocyte membranes of these animals in various organs, particularly the lungs. Changes in the bilayer structure of the erythrocyte membrane lead to impaired permeability, which reduces the stability and functional properties of erythrocytes.

4.5. Total and inorganic phosphorus and 2,3-diphosphoglycerate (2,3-DPG) levels in the red blood cells of calves with bronchopneumonia

The total phosphorus content in red blood cells increased significantly in accordance with the severity of the disease (Table 4.5). The more severe the course of the disease, the higher the total phosphorus content. In mild cases, its level in venous blood increased 1.4-fold ($p < 0.001$) and in arterial blood 1.5-fold ($p < 0.001$); and in severe cases, by 1.8-fold ($p < 0.001$) and 1.9-fold ($p < 0.001$), respectively, compared with the same indicators in clinically healthy calves. Changes in the total phosphorus content in the erythrocytes of calves with bronchopneumonia can be explained by changes in membrane permeability to this element and its intensive use for the synthesis of organic compounds.

The content of inorganic phosphorus in the cytoplasm of erythrocytes in the venous blood of affected animals increases ($p < 0.001$), but it remains stable as the

disease progresses ($p < 0.1$), while in arterial blood, changes in inorganic phosphorus depend on the severity of the disease: in mild cases, its concentration increases 2.4-fold ($p < 0.001$), and in severe cases, 3.7-fold ($p < 0.001$), compared to clinically healthy calves. A decrease in the ratio of this parameter in venous and arterial blood (1.27 in clinically healthy calves; 0.91 in calves with a mild course of the disease; 0.78 in those with a severe course) indicates a more intensive incorporation of this parameter into the cytoplasm of arterial red blood cells in affected animals.

Table 4.5

Phosphorus (P) and 2,3-DPG levels in the red blood cells of calves with bronchopneumonia

Group of animals		Common Phosphorus	Non-organic Phosphorus	2,3-DPG	2,3-DPG•100% P common
		мμmol/mL of eritrocyte suspension			
Clinically healthy	Venous	3,30±0,02	1,30±0,04	2,00±0,05	60,6±1,6
	Arterial	4,22±0,11	1,02±0,02	3,20±0,07	75,8±2,5
	p<	0,001	0,001	0,001	0,001
Mild course	Venous	4,60±0,12	2,20±0,09	2,40±0,08	52,2±1,1
	p'<	0,001	0,001	0,001	0,001
	Arterial	6,14±0,13	2,42±0,08	3,72±0,09	60,6±1,4
	p*<	0,001	0,001	0,001	0,001
	p<	0,001	0,1	0,001	0,001
Severe course	Venous	5,90±0,14	2,20±0,07	3,70±0,10	62,7±1,8
	p'<	0,001	0,001	0,001	0,1
	p"<	0,001	0,1	0,001	0,001
	Arterial	7,83±0,16	3,57±0,12	4,26±0,11	54,4±1,2
	p*<	0,001	0,001	0,001	0,01
	p**<	0,001	0,001	0,01	0,1
	p<	0,001	0,001	0,01	0,01

Note: p< – comparison of venous and arterial blood parameters in the respective groups; p'< – comparison of venous blood parameters in sick and clinically healthy calves; p"< – compares venous blood parameters in sick calves with different disease courses; p*< – compares arterial blood parameters in sick and clinically healthy calves; p**< – compares arterial blood parameters in sick calves with different disease courses

Hypoxia in the body and higher cholesterol content in erythrocyte membranes lead to increased anaerobic glucose breakdown. Since 2,3-diphosphoglycerate, rather than lactate, is the end product of this process in erythrocytes [200], the level of 2,3-

DPG in the erythrocyte cytoplasm increases depending on the severity of bronchopneumonia.

In calves with a mild course of the disease, this indicator increased 1.2-fold in venous and arterial blood ($p < 0.001$); in those with a severe course, it increased 1.8-fold ($p < 0.001$) and 1.3-fold ($p < 0.001$), respectively, compared with clinically healthy animals.

The increase in 2,3-DPG content in the red blood cells of affected animals is evidently an adaptive response of the organism to the development of oxygen deprivation and serves as a compensatory factor that facilitates tissue respiration. The thickening of erythrocyte membranes in animals with bronchopneumonia reduces their permeability and leads to a more prolonged gas exchange. Therefore, the organism of affected animals responds to changes in the lipid composition of the membranes by increasing the level of 2,3-DFG in erythrocytes of both venous and arterial blood.

Despite the higher content of this phosphoric acid ester in the red blood cells of arterial blood compared to venous blood, the more intensive utilization of phosphorus for 2,3-DPG occurs specifically in the red blood cells of venous blood in affected animals. This is evidenced by an increase in the percentage ratio of 2,3-diphosphoglycerate to total phosphorus in venous and arterial blood. In clinically healthy calves, this ratio was 0.80; in those with mild bronchopneumonia, it was 0.86, and in those with severe bronchopneumonia, it was 1.15. This indicates a decrease in the utilization of phosphorus from the cytoplasm of arterial blood erythrocytes for the synthesis of 2,3-DPG. In calves with bronchopneumonia, a state of hypoxia and hypoxemia develops, resulting in increased anaerobic breakdown of glucose with the formation of 2,3-DPG. An increase in the level of this enzyme leads to faster binding and release of gases (O2, CO2). More active gas exchange increases the rate of lipid oxidation in erythrocyte membranes, since it is the lipid components that provide the transport capacity of the erythrocytes. Increased oxidative activity in the membranes, in turn, leads to premature “aging” of red blood cells in sick calves with severe bronchopneumonia.

UNIT 5

CLINICAL STATUS, CHANGES IN THE STRUCTURE AND FUNCTIONAL PROPERTIES OF RED BLOOD CELLS DURING THE TREATMENT OF CALVES WITH BRONCHOPNEUMONIA

5.1 Structure and Functional Properties of Red Blood Cells in the Treatment of Sick Calves with Mild Bronchopneumonia Using 15% Amoxicillin Trihydrate

The use of the same antibacterial drugs on farms to treat bronchopneumonia leads to the development of antibiotic-resistant microflora. As a result, treatment effectiveness decreases, the disease takes a severe course, and often proves fatal. Therefore, in addition to the standard treatment regimen used on farms, we decided to evaluate a new, extended-release, broad-spectrum antibiotic – 15% amoxicillin trihydrate.

Based on the results of a clinical study and a bronchopulmonary test, four groups of calves were formed. The first three groups consisted of sick calves with mild bronchopneumonia, while the fourth – the control group – consisted of clinically healthy animals.

Bacteriological examinations of bronchial mucus from the sick calves (microscopy, plating on MPA and Endo medium) identified a culture of Streptococcus pneumoniae.

Treatment regimens: the first group – Bicillin, Trivitamin, 0.5% sodium bicarbonate solution, “Prana”; second group – 15% amoxicillin trihydrate, trivitamin, 0.5% sodium bicarbonate solution; third group – 15% amoxicillin trihydrate, trivitamin, 0.5% sodium bicarbonate solution, “Prana” (see Section 2).

We observed clinical recovery in the experimental calves at different stages of treatment. In the first group, recovery occurred on the eighth day; however, only 40% of the animals recovered, 20% died, and in 40% of cases, the disease took a severe course. The bronchopulmonary test index in the recovered calves was

1.68 ± 0.04 mL, indicating the restoration of protein metabolism in the body.

In the animals of the second group, the disappearance of bronchopneumonia symptoms was observed on the fifth day, and in the third group – on the fourth day. Although the bronchopulmonary test value increased, it did not return to the level observed in clinically healthy calves (second group – 1.47 ± 0.07; third group – 1.59 ± 0.09 mL). Since we did not observe symptoms of bronchopneumonia in these animals, the low test result can be explained by the toxic effect of amoxicillin trihydrate on the liver, which leads to a disruption of its protein-synthesizing function. In addition, pallor of the visible mucous membranes was observed in calves from the second and third groups after treatment. We reported the hematological parameters of animals in the first group after treatment only for those in which complete clinical recovery was observed.

5.1.1. The changes in blood count results

An analysis of blood counts following treatment shows that the blood parameters of calves in the first group improved the most (Table 5.1). However, considering the duration of their recovery (8 days), the death of 20 percent of the animals, and the progression of the disease to a chronic form in 40 percent of the calves, it can be concluded that the selected treatment regimen had low therapeutic efficacy. The mean packed cell volume in these animals never returned to normal; at the end of treatment, it was 5.3 μm3 lower than in clinically healthy calves ($p < 0.05$). In calves from the second and third groups, we observed a decrease in blood hemoglobin concentration after treatment compared to both the sick calves (by 14.2% in both groups; $p < 0.001$) and to clinically healthy animals (by 9.1%; $p < 0.02$ for the second group; $p < 0.01$ for the third group). However, the hemoglobin levels in the blood of these calves increased significantly: in the second group – by 3.2 times ($p < 0.001$), and in the third – by 2.6 times ($p < 0.001$), compared to the start of the experiment. At the end of treatment, this indicator was higher than in the animals of the control group by a factor of 5.7 ($p < 0.001$) and 4.6 ($p < 0.001$), respectively.

The red blood cell count in calves from the second and third experimental groups did not decrease after treatment compared to baseline values; in fact, it even increased by 13.8% in the second group ($p < 0.01$). At the end of treatment, this value was significantly higher than in clinically healthy animals, by 26.4% and 12.5%, respectively.

Table 5.1

Blood count results for calves' venous blood after treatment

Group of animals	Eritrocytes, T/l	Hemoglobin, g/l	Hemoglobin, %	Hematocrit, %	MCH, pg	MCV, μm³
Clinically healthy	7,2±0,2	107,8±2,1	2,9±0,2	36,0±0,8	15,0±0,4	50,0±1,1
Sick animals	8,0±0,2	114,2±2,2	5,1±0,2	32,0±0,7	14,3±0,3	40,0±1,2
p<	0,01	0,05	0,001	0,001	0,1	0,001
1st group	7,6±0,4	104,0±0,6	3,0±0,2	34,0±0,4	13,7±0,7	44,7±1,2
p<	0,1	0,1	0,1	0,1	01	0,05
p*<	0,1	0,01	0,001	0,1	0,1	0,05
2nd group	9,1±0,2	98,0±2,0	16,4±0,4	30,5±0,4	10,5±0,2	33,5±0,9
p<	0,001	0,02	0,001	0,001	0,001	0,001
p*<	0,01	0,001	0,001	0,1	0,001	0,01
3rd group	8,1±0,3	98,0±1,5	13,5±0,4	28,0±1,0	11,0±0,2	34,6±0,8
p<	0,05	0,01	0,001	0,001	0,001	0,001
p*<	0,1	0,001	0,001	0,01	0,001	0,01

Note. p< – compared to clinically healthy individuals; p*< – compared to patients

The increase in red blood cell count and the decrease in hemoglobin concentration in the blood of calves in the second and third groups resulted in a decrease in the average hemoglobin content in red blood cells. After treatment, this value decreased by a factor of 1.3 ($p < 0.01$) and 1.2 ($p < 0.01$), respectively,

compared to the start of the experiment, and was 1.5 ($p < 0.001$) and 1.4 ($p < 0.001$) times lower than in animals in the control group.

A significant decrease in the mean corpuscular volume in the blood of animals in these groups following treatment led to a reduction in hematocrit levels. In the second group, this value decreased by 1.5% compared to the start of the experiment ($p < 0.1$) and was 5.5% lower than in clinically healthy animals ($p < 0.001$), in the third group – by 4.0% ($p < 0.01$) and 8.0% ($p < 0.001$), respectively. Analysis of blood count parameters in the treated calves showed that microcytosis developed in all experimental groups, as the mean red blood cell volume in these animals was significantly lower compared to clinically healthy calves. In addition, we observed pronounced hypochromic erythrocytosis in calves from the second and third groups. Animals treated with amoxicillin exhibited pronounced hyperhemoglobinemia. In our opinion, this can be explained by the toxic effect of the antibiotic on red blood cells, leading to the formation of Ehrlich–Heinz bodies in the cytoplasm of the latter, which caused a significant increase in hemoglobin concentration in the blood of animals in the second and third groups during treatment.

5.1.2. Changes in the Population Composition and Acid Resistance of Red Blood Cells

The erythrocyte population composition at the end of treatment in the various animal groups was quite diverse (Table 5.2).

The blood of calves in the first group exhibited the most optimal ratio among the different erythrocyte populations. In these calves, the number of “old” red blood cells increased 1.3-fold compared to that in sick animals ($p < 0.05$). The difference between the red blood cell population composition indicators of calves in the first group and the control group was not statistically significant ($p < 0.1$ for all age groups). In calves of the second group, following treatment, the number of “old” red blood cell populations increased sharply (2.2-fold; $p < 0.001$), and the number of “mature” red blood cell populations decreased significantly (1.2-fold; $p < 0.05$). The

number of “young” cells decreased by a factor of 1.1 ($p < 0.05$) compared to the sick calves and became comparable to that in clinically healthy calves ($p < 0.1$).

Table 5.2

Population composition of eritrocites in the venous blood of calves after treatment

Group of animals	Eritrocites population, %		
	“Old”	“Adult”	“Young”
Clinically healthy	11,9±0,8	36,1±1,3	52,0±1,7
Sick (mild course)	8,0±0,5	36,0±1,5	56,0±1,2
p<	0,001	0,1	0,1
First group	10,4±0,8	36,6±1,2	53,0±1,0
p<	0,1	0,1	0,1
p*<	0,05	0,1	0,1
Second group	18,0±1,0	31,0±1,0	51,0±1,8
p<	0,001	0,05	0,1
p*<	0,001	0,05	0,05
Third group	15,0±1,2	53,0±1,7	32,0±0,9
p<	0,05	0,001	0,001
p*<	0,001	0,001	0,001

Note: p< – comparing the experimental and control groups at the end of the experiment; p*< – comparing the beginning and end of the experiment

The age distribution of red blood cells in the third group of animals was characterized by a significantly higher proportion of “old” (1.9 times; $p < 0.001$) and “mature” (1.5 times; $p < 0.001$) populations and a lower proportion of “young” cells (1.8 times; $p < 0.001$) compared to sick calves.

After treatment, the percentage of “old” red blood cells in calves from the second and third groups was higher than in clinically healthy animals by 1.5 ($p < 0.001$) and 1.3 times ($p < 0.05$), respectively. The “older” a red blood cell is, the smaller its volume. This is why, despite the large number of red blood cells in the blood of animals in these groups, their hematocrit values were significantly lower compared to clinically healthy calves.

Changes in the age distribution of red blood cells are also reflected in their acid resistance (Fig. 5.1). The restoration of the age distribution of red blood cells in

animals of the first group is accompanied by a restoration of these cells' acid resistance following treatment.

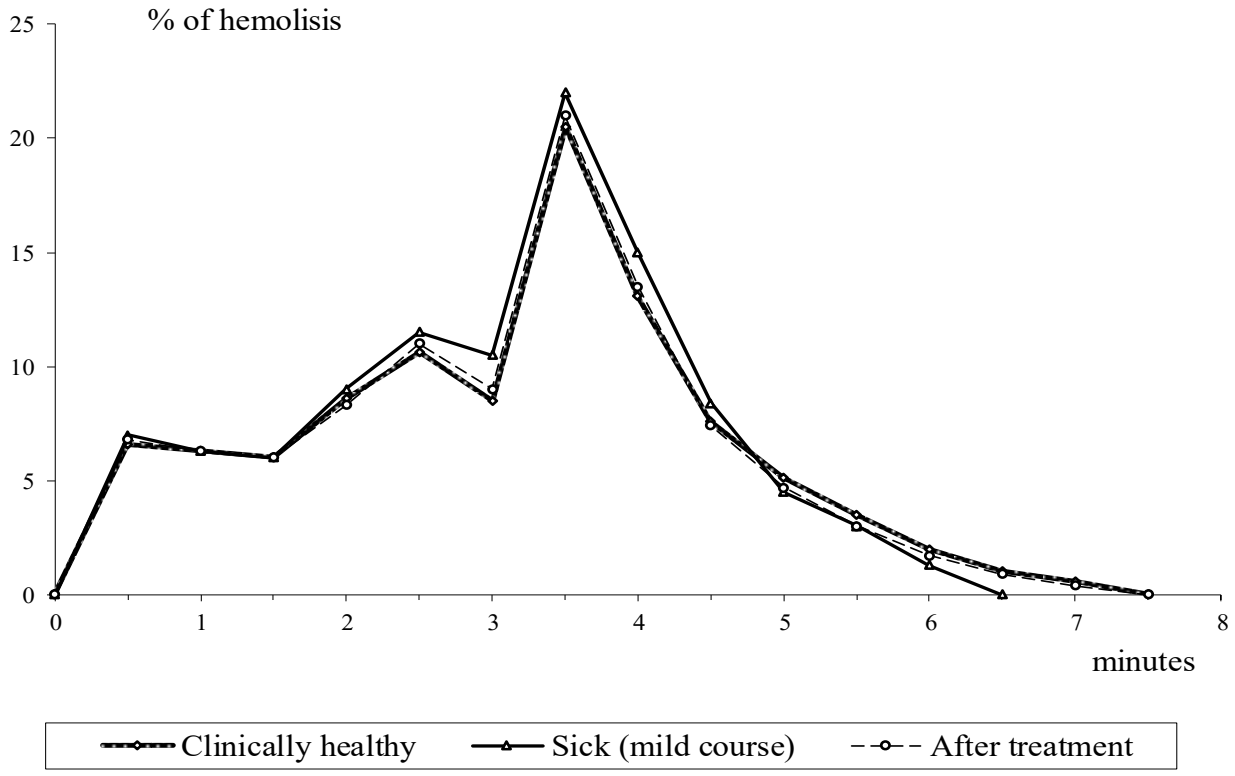


Fig. 5.1. Acid resistance of red blood cells in clinically healthy calves and animals in the first experimental group before and after treatment

The erythrogram of the calves in the second group showed a curve that was completely atypical for clinically healthy animals (Fig. 5.2). A large number of "old" red blood cells caused the main peak to begin in the first second; it reached its maximum as early as the 30th second and amounted to 17%. The second peak, formed by these same cells, reached 12%. The split peak in the middle of the graph is characteristic of "mature" populations, as confirmed by their lower concentration compared to that in the control group. Since the number of "young" red blood cells in the animals of the second group recovered after treatment and did not differ from that in clinically healthy calves, the right-hand sides of the graphs for both groups were

nearly identical by the end of the experiment. Hemolysis also concluded at the same time – at 7.5 minutes. In animals of the second group, the maturation of "young" red blood cells does not occur, as a result of which they are unable to function properly. This leads to a retention of "old" red blood cells in the bloodstream.

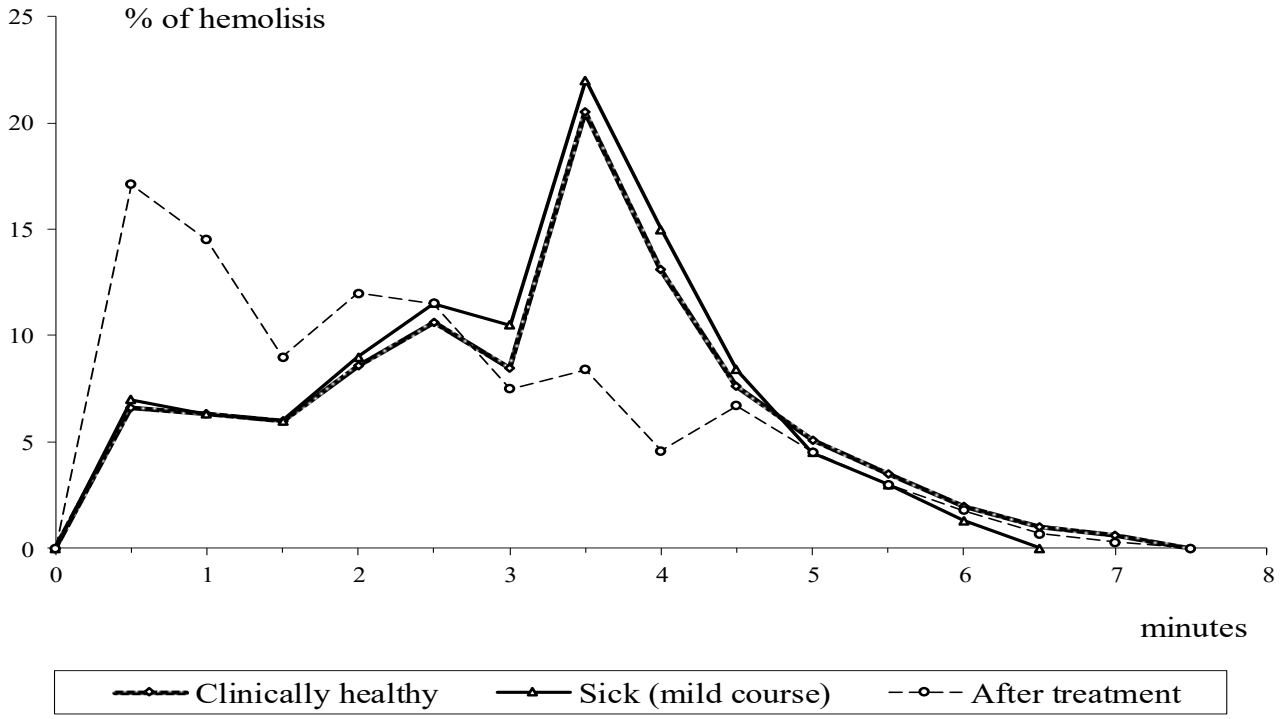


Fig. 5.2. Acid resistance of red blood cells in clinically healthy calves and animals in the second experimental group before and after treatment

In calves of the third group, the structure of the left side of the erythrogram – in terms of the timing of the peaks and their duration – was similar to that of clinically healthy animals. The difference lay in the greater height of the first and second additional peaks. This is explained by the greater number of "old" and "mature" red blood cell populations (Fig. 5.3). The main peak of the graph differed from that of calves in the control group by 1.5% in height. The right side of the graph was abrupt and ended at the 6th minute, which was 0.5 minutes earlier compared to the start of

treatment and 1.5 minutes earlier compared to the erythrograms of clinically healthy animals. We attribute the decrease in the number of "young" red blood cells and the increase in the populations of "mature" and "old" red blood cells in the animals of the third group to the inhibitory effect of 15% amoxicillin trihydrate on hematopoiesis.

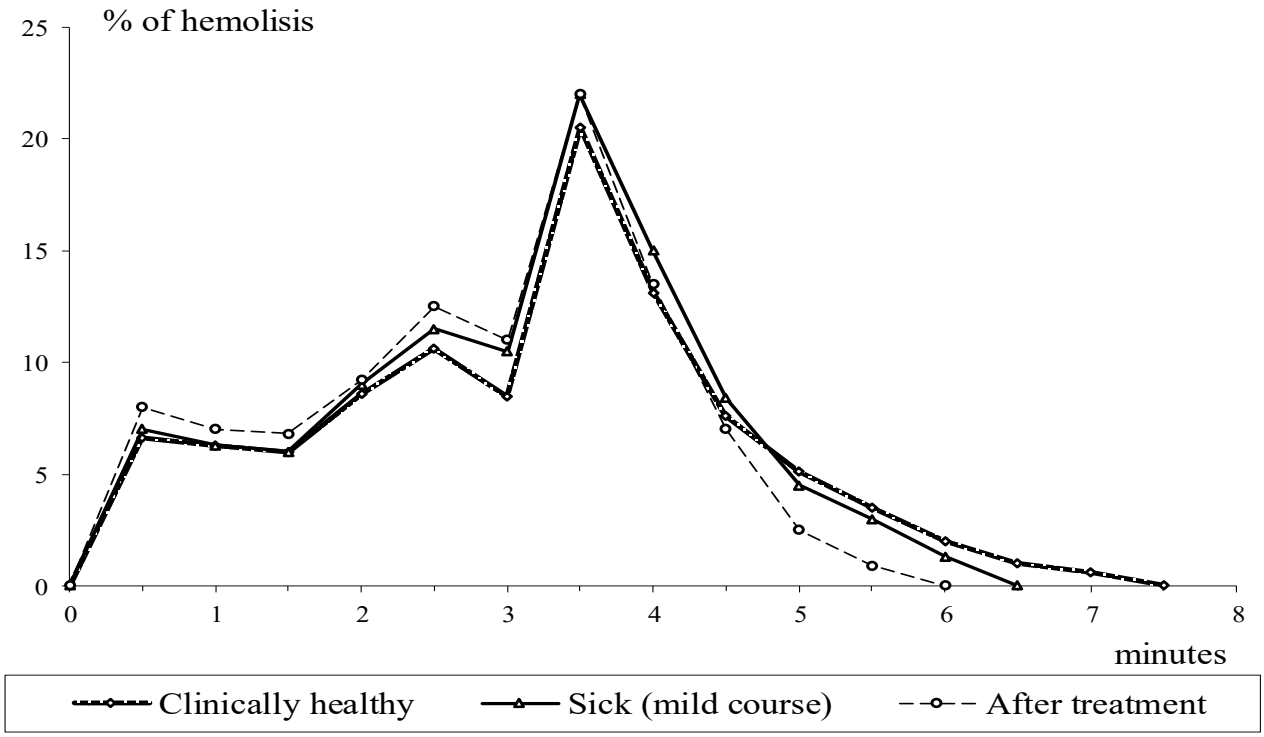


Fig. 5.3. Acid resistance of red blood cells in clinically healthy calves and animals in the third experimental group before and after treatment

The maturation of red blood cells in the bloodstream of calves in the third group proceeds normally, unlike in animals in the second group. We attribute this phenomenon to the effect of the homeopathic remedy "Prana," which increases overall resistance and indirectly promotes the maturation of red blood cells.

5.1.3. Changes in the lipid composition of red blood cell membranes

In the animals of the first group, total lipid content increased by a factor of 1.3 ($p < 0.02$) compared with the pre-treatment level (Table 5.3). Cholesterol and phospholipid levels in these calves decreased compared to the start of the experiment by 1.5-fold ($p < 0.001$) and 1.3-fold ($p < 0.01$), respectively. Although all lipid profile parameters did not show a statistically significant difference from those in the control group ($p < 0.1$ for all), the ratio between them did not return to normal. In calves of the first experimental group, the cholesterol-phospholipid ratio decreased significantly compared to the start of treatment, by a factor of 1.2 ($p < 0.05$). However, after treatment, this indicator was 9.3% higher than in clinically healthy animals ($p < 0.05$).

Table 5.3

Changes in the lipid composition of erythrocyte membranes in the venous blood of calves following treatment

Group of animals	Total lipids, g per g of erythrocyte membrane protein	Cholesterol	Phospholipids	Cholesterol / Phospholipids
		mmol/g erythrocyte membrane suspension protein		
Clinically healthy	2,10±0,11	0,50±003	0,92±0,04	0,54±0,01
Sick animals (mild course)	1,50±0,09	082±0,05	1,20±0,07	0,68±0,03
p<	0,001	0,001	0,001	0,001
First group	1,92±0,12	0,55±0,02	0,93±0,03	0,59±0,02
p<	0,1	0,1	0,1	0,05
p*<	0,02	0,001	0,01	0,05
Second group	1,72±0,10	0,50±0,01	0,86±0,04	0,58±0,02
p<	0,05	0,1	0,1	0,1
p*<	0,1	0,001	0,01	0,02
Third group	1,80±0,07	0,52±0,02	0,86±0,02	0,59±0,02
p<	0,05	0,1	0,1	0,05
p*<	0,1	0,001	0,01	0,05

Note: p< – comparison between the experimental and control groups at the end of the experiment; p*< – comparison between the beginning and end of the experiment

The total lipid content in the erythrocyte membranes of the second and third experimental groups showed only a tendency to increase after treatment ($p < 0.1$). Their levels remained quite low compared to those in the control group ($p < 0.05$). The cholesterol and phospholipid content in the erythrocyte membranes of calves in these groups decreased significantly compared to the start of treatment ($p < 0.001$ for cholesterol; $p < 0.01$ for phospholipids in both groups). The cholesterol-phospholipid ratio in the cell membranes of animals in the second and third groups decreased significantly by a factor of 1.2 after treatment ($p < 0.02$; $p < 0.05$). Changes in lipid content and the ratio between them in erythrocyte membranes lead to disturbances in cellular metabolic processes. As a result, the structure and functions of the membranes change, as evidenced by differences in the buoyancy and stability of erythrocytes.

5.1.4. Changes in the levels of phosphorus and 2,3-DPG in red blood cells

Phosphorus and 2,3-DPG levels are important indicators for assessing the body's state of hypoxia and the permeability of erythrocyte membranes.

The data presented above in this section show an improvement in nearly all of the parameters studied in animals from the first experimental group following treatment. Phosphorus and 2,3-DPG levels in red blood cells were no exception. The levels of these parameters decreased significantly compared to the start of the experiment and were indistinguishable from those in clinically healthy calves ($p < 0.1$ for all parameters; Table 5.4).

In animals from the second and third groups, the total phosphorus content at the end of the experiment did not change compared to the beginning ($p < 0.1$). Although the level of inorganic phosphorus decreased by a factor of 1.3 ($p < 0.001$) in the second group and by a factor of 1.2 ($p < 0.01$) in the third group, its concentration remained quite high compared to that in calves of the control group: 1.3 ($p < 0.001$) and 1.4 ($p < 0.001$) times, respectively. The functional immaturity of the red blood cells that entered the animals' bloodstream following administration of

15% amoxicillin trihydrate led to a worsening of hypoxia in the affected calves. This resulted in increased anaerobic glucose breakdown and subsequent activation of the 2,3-diphosphoglycerate (2,3-DPG) shunt of glycolysis in erythrocytes. Consequently, inorganic phosphorus was utilized more intensively in the 2,3-DPG shunt. All these processes led to an increase in the concentration of 2,3-DPG in the cytoplasm of the cells, compared both to sick animals ($p < 0.01$ for the second group; $p < 0.02$ for the third group) and to clinically healthy animals ($p < 0.001$ for both groups).

Table 5.4

Changes in phosphorus (P) and 2,3-DPG levels in venous blood erythrocytes of calves after treatment

Group of animals	P common	P non-organic	2,3-DPG	$\frac{\text{2,3-DPG} \bullet 100\%}{\text{P common}}$
	μmol/g erythrocyte suspension			
Clinically healthy	3,30±0,02	1,30±0,04	2,00±0,05	60,6±1,6
Sick animals (mild course)	4,6±0,12	2,20±0,09	2,40±0,08	52,2±1,1
p<	0,001	0,001	0,001	0,001
First group	3,40±0,03	1,30±0,03	2,10±0,06	61,8±1,3
p<	0,1	0,1	0,1	0,1
p*<	0,001	0,001	0,05	0,01
Second group	4,60±0,10	1,70±0,03	2,90±0,08	63,0±1,8
p<	0,001	0,001	0,001	0,1
p*<	0,1	0,001	0,01	0,001
Third group	4,60±0,10	1,80±0,04	2,80±0,10	60,9±1,6
p<	0,001	0,001	0,001	0,1
p*<	0,1	0,01	0,02	0,01

Note: p< – comparison between the experimental and control groups at the end of the experiment; p*< – comparison between the beginning and end of the experiment

The ratio of 2,3-DPG to total phosphorus at the end of treatment increased 1.2-

fold in all experimental groups ($p < 0.01$ for the first group; $p < 0.001$ for the second group; and $p < 0.01$ for the third group). At the end of the experiment, the percentage of 2,3-DPG relative to total phosphorus did not differ from that in the control calves ($p < 0.1$ for all experimental groups). Clinical status indicators demonstrate a rapid therapeutic effect of the treatment regimens using 15% amoxicillin trihydrate. We attribute the rapid decrease in temperature and the resolution of bronchopneumonia symptoms in the calves to the drug's high antibacterial activity and the attenuation of the inflammatory response in the body. However, a detailed examination of red blood cells following the administration of this antibiotic reveals profound structural changes in the cells, causing them to lose their functional properties. This, in turn, leads to the development of various types of anemia and prolonged oxygen deprivation in the animals' bodies.

5.2. Changes in the Structure and Functional Properties of Red Blood Cells During the Treatment of Calves with Mild Bronchopneumonia Using 5% Enroxil

Treatment regimen: 5% Enroxil, Trivitamin, a 0.5% sodium bicarbonate solution, and "Prana."

We observed clinical recovery in only 20% of the treated animals. In the remaining 80%, only the body temperature decreased to 38.3 ± 0.15 among the symptoms of bronchopneumonia. Other signs of the disease worsened. The bronchopulmonary test index after treatment (7 days) decreased to 1.11 ± 0.07 mL.

After using this drug to treat calves with bronchopneumonia, we observed interesting changes in the quantitative and qualitative composition of red blood cells. The number of red blood cells in the blood of the affected animals did not decrease after treatment but remained significantly higher compared to clinically healthy calves ($p < 0.05$; Table 5.5). The total hemoglobin concentration showed a tendency to decrease and, by the end of the experiment, occupied an intermediate position between the values of the sick animals and those of the control group. The

hemoglobin content in the blood increased by 10.7% compared to the baseline value ($p < 0.05$), and by the end of the experiment was 2.0 times higher than that of the control group ($p < 0.001$). The hematocrit level remained virtually unchanged ($p < 0.1$), which can be explained by the identical mean red blood cell volume in the calves' blood both before and after treatment ($p < 0.1$). Although the hemoglobin content in the red blood cells of the venous blood of these calves tended to decrease, we did not observe a statistically significant difference in this parameter in the affected animals ($p < 0.1$). At the end of treatment, the VGE in the experimental animals was 9.3% lower than in clinically healthy calves ($p < 0.02$).

Table 5.5

Hematogram indicators in calves after administration of 5% enroxil

Group of animals	Erytrocytes, T/l	Hemoglobin, g/l	Hemoglobin, %	Hematocrit, %	MCH, pg	MCV, μm^3
Clinically healthy	7,2±0,2	107,8±2,1	2,90±0,18	36,0±0,8	15,0±0,4	50,0±1,1
Sick animals	8,0±0,2	114,2±2,2	5,14±0,17	32,0±0,7	14,3±0,3	40,0±1,2
p<	0,01	0,05	0,001	0,001	0,1	0,001
After treatment	8,1±03	110,5±2,0	5,69±016	32,8±0,7	13,6±0,2	40,5±09
p<	0,05	0,1	0,001	0,02	0,02	0,001
p*<	0,1	0,1	0,05	0,1	0,1	0,1

Note: p< – compared to clinically healthy individuals; p*< – compared to patients

An analysis of the blood count results suggests that the condition of animals with bronchopneumonia did not improve after treatment; on the contrary, it worsened. This is confirmed by the distribution of red blood cell populations and their acid resistance.

The number of "old" cells increased significantly by 5.3% compared to the

start of the experiment ($p < 0.001$) and was 1.4% higher than in the control group ($p < 0.1$; Table 5.6). The proportion of "mature" red blood cells did not change after treatment of the sick calves ($p < 0.1$), but the proportion of "young" red blood cells decreased significantly. The percentage of the latter was 6.3% lower compared to the start of the experiment ($p < 0.02$) and 2.3% lower compared to this indicator in clinically healthy animals ($p < 0.1$).

Table 5.6

Population of erytrocytes in the Venous Blood of Calves
after treatment with 5% Enroxil

Group of animals	Erytrocyte population, %		
	"Old"	"Adult"	"Young"
Clinically healthy	11,9±0,8	36,1±1,3	52,0±1,7
Sick animals (mild course)	8,0±0,5	36,0±1,5	56,0±1,2
p<	0,001	0,1	0,1
After treatment	13,3±0,9	37,0±1,3	49,7±1,6
p<	0,1	0,1	0,1
p*<	0,001	0,1	0,02

Note: p< – compared to clinically healthy animals; p*< – compared to sick animals

The acid resistance of red blood cells in calves did not recover after treatment, indicating low cellular resilience compared to clinically healthy animals. A relatively high peak on the left side of the erythrogram indicates an increase in the number of "old" populations. Their hemolysis peaked at the second minute, which is characteristic of severe bronchopneumonia (Fig. 5.4).

The main peak of erythrocyte hemolysis in venous blood from calves (after treatment) did not differ from that in animals of the control group in terms of onset time, duration, or amplitude. The total time of cell resistance to the hemolytic agent did not increase, which is explained by the low percentage of "young" populations.

The lipid composition of the erythrocyte membranes in the venous blood of calves treated with enroxil showed the opposite trend compared to that of diseased animals. After treatment, the total lipid content in the membranes increased, while the cholesterol and phospholipid contents decreased (Table 5.7).

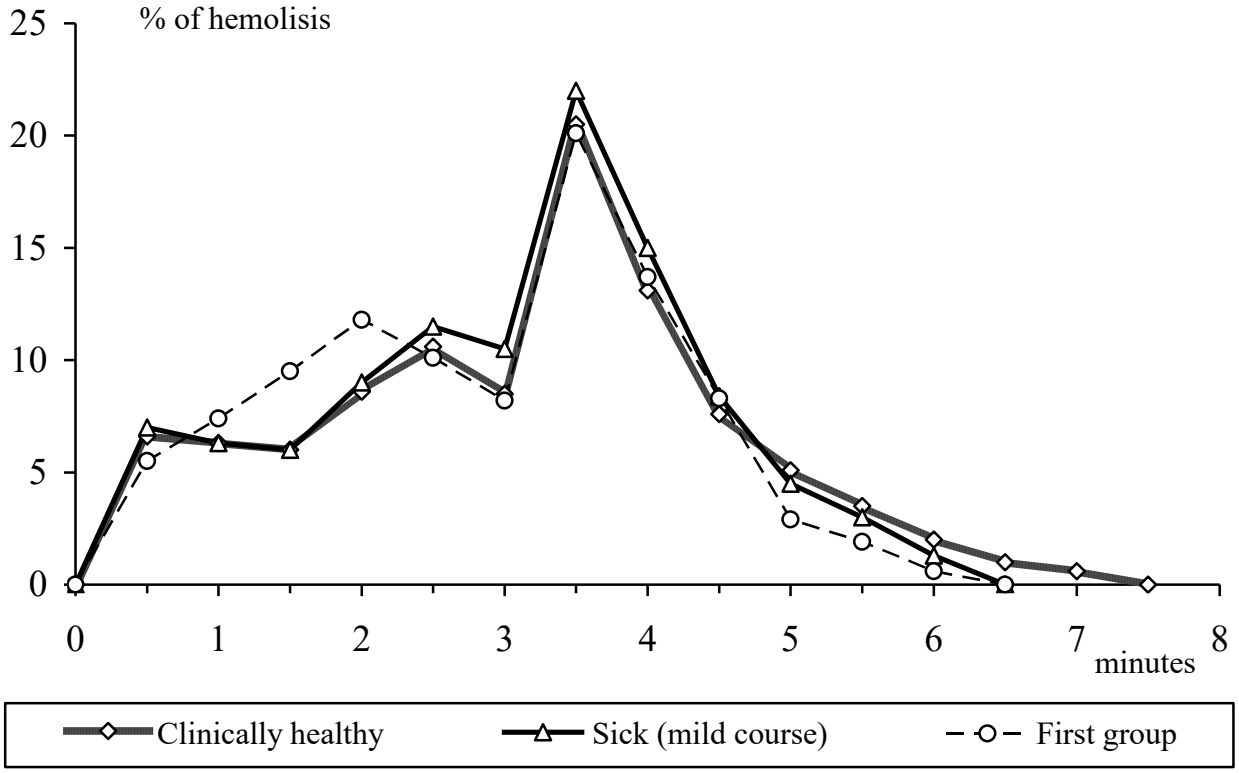


Fig. 5.4. Acid resistance of red blood cells in clinically healthy calves and animals after treatment

The lipid composition of erythrocyte membranes in venous blood from calves treated with enroxil showed the opposite trend compared to that in diseased animals. After treatment, the total lipid content in the membranes increased, while the cholesterol and phospholipid contents decreased (Table 6.7). Only phospholipids reached an optimal level, with their values not differing from those of the control group ($p < 0.1$). The total lipid content increased 2.1-fold compared to the start of treatment ($p < 0.001$) and was 1.5 times higher than in clinically healthy calves ($p <$

0.001). A significant 2.6-fold decrease in cholesterol in the erythrocyte membranes of venous blood from the experimental animals after treatment ($p < 0.001$) resulted in a statistically significantly lower concentration of this sterol compared to the control group (1.6-fold; $p < 0.001$). Changes in the content of lipid components indicate significant structural rearrangements in erythrocyte membranes. This is evidenced by a significantly lower cholesterol-phospholipid ratio in the erythrocytes of venous blood from treated animals. This ratio decreased by a factor of 2.1 compared to the start of the experiment ($p < 0.001$) and was 1.7 times lower than that observed in clinically healthy calves ($p < 0.001$).

Table 5.7

Lipid composition of erythrocyte membranes in the venous blood of calves after administration of 5% enroxil

Group of animals	Total lipids, g per g of erythrocyte membrane protein	Cholesterol	Phospholipids	Cholosterol / Phospholipids
		mmol/g erythrocyte membrane suspension protein		
Clinically healthy	2,10±0,11	0,50±003	0,92±0,04	0,54±0,01
Sick animals (mild course)	1,50±0,09	0,82±0,05	1,20±0,07	0,68±0,03
p<	0,001	0,001	0,001	0,001
After treatment	3,10±0,14	0,31±0,01	0,96±0,03	0,32±0,02
p<	0,001	0,001	0,1	0,001
p*<	0001	0,001	0,02	0,001

Note: p< – compared to clinically healthy animals; p*< – compared to sick animals

The structural changes that occurred in red blood cells could not help but affect their functional properties. Since the primary function of these cells is to transport oxygen and carbon dioxide, changes in the structural and functional properties of red blood cells should primarily be reflected in the energy metabolism of the cells themselves. One characteristic indicator of this is the content of phosphorus and its

derivatives in the cytoplasm of red blood cells. Analysis of these parameters in the blood of animals after treatment indicated an increase in the activity of the 2,3-diphosphoglycerate shunt of glycolysis in the cells. This is a sign of enhanced anaerobic glucose breakdown, which in turn indicates a worsening of oxygen deprivation in the body (Table 5.8). The total phosphorus content in erythrocytes increased significantly, both by 10.0% compared to the start of the experiment ($p < 0.02$) and by 53.3% compared to the control group ($p < 0.001$). Although the level of inorganic phosphorus in the cytoplasm of the cells decreased by 10.9% after treatment ($p < 0.05$), it remained 50.8% higher than in clinically healthy calves ($p < 0.001$). Given the changes in total and inorganic phosphorus, it was to be expected that the 2,3-DPG content in the red blood cells of the treated calves would increase.

Table 5.8

Phosphorus (P) and 2,3-DPG levels in the red blood cells of calves' venous blood following administration of a 5% enroxil solution

Group of animals	P common	P non-organic	2,3-DPG	$\frac{\text{2,3-DPG} \bullet 100\%}{\text{P common}}$
	µmol/g erythrocyte suspension			
Clinically healthy	3,30±0,02	1,30±0,04	2,00±0,05	60,6±1,6
Sick animals	4,60±0,12	2,20±0,09	2,40±0,08	52,2±1,1
p<	0,001	0,001	0,001	0,001
After treatment	5,06±011	1,96±0,05	3,10±0,07	61,3±1,7
p<	0,001	0,001	0,001	0,1
p*<	0,02	0,05	0,001	0,01

Note: p< – compared to clinically healthy animals; p*< – compared to sick animals

At the end of treatment, its level increased by 25.8% ($p < 0.001$) compared to that in the diseased animals and was 51.0% higher than in the calves of the control group. The increase in the amount of 2,3-diphosphoglycerate in red blood cells led to

an increase in its percentage relative to total phosphorus. This value was 17.4% higher than at the start of the experiment ($p < 0.01$) and did not differ significantly from that in clinically healthy calves ($p < 0.1$). All of the changes in erythrocyte parameters described above indicate a worsening of the pathological condition in the experimental animals. Changes in the population composition and acid resistance of the studied cells indicate structural rearrangements in their membranes, which is confirmed by changes in lipid components, and the increase in 2,3-DPG levels in the erythrocyte cytoplasm indicates a worsening of oxygen deficiency in the body due to a decrease in the functional activity of red blood cells.

5.3. Structure and Functional Properties of Red Blood Cells in the Treatment of Calves with Severe Bronchopneumonia

Pathological changes in the bodies of calves with bronchopneumonia depend on the course of the disease. The more severe the course of bronchopneumonia, the more profound the changes that have occurred in the body's tissues and cells. Therefore, it is much more difficult to treat animals with a severe course of the disease than those with a mild one. This section describes two series of experiments. In the first, we used two treatment regimens that had already been applied to calves with mild bronchopneumonia. In the second, we modified them slightly by adding or replacing certain drugs, hoping for a greater therapeutic effect from the improved regimens.

5.3.1. Treatment of calves with 15% amoxicillin trihydrate and 5% enroxil

We divided the animals with bronchopneumonia into two groups. For one group, we used 15% amoxicillin trihydrate as the antibacterial agent in the treatment regimen; for the other, we used 5% enroxil.

Treatment regimens: the first group received 15% amoxicillin trihydrate, trivitamin, a 0.5% sodium bicarbonate solution, and "Prana"; the second group received 5% enroxil, trivitamin, a 0.5% sodium bicarbonate solution, and "Prana."

We observed clinical recovery in the calves of the first group on the 5th–6th

day of treatment. However, as in previous experiments (using 15% amoxicillin trihydrate), the biochemical indicator for predicting the course of bronchopneumonia did not return to normal in the animals and remained at 1.08 ± 0.06 mL after treatment. Signs of anemia were also present in these calves. The general condition of the animals in the second group improved after treatment; however, the presence of disease symptoms and a low bronchopulmonary test index (1.04 ± 0.04 mL) indicate the ineffectiveness of the chosen treatment regimen for calves with severe bronchopneumonia. Analysis of blood count parameters after treatment showed a decrease in the number of red blood cells in the animals of the first experimental group ($p < 0.01$) to a level comparable to that of clinically healthy calves ($p < 0.1$; Table 5.9).

Table 5.9

Hematogram indicators in calves after treatment

Group of animals	Erytrocytes, T/l	Hemoglobin, g/l	Hemoglobin, %	Hematocrit, %	MCH, pg	MCV, μm3
Clinically healthy	7,2±0,2	107,8±2,1	2,90±0,18	36,0±0,8	15,0±0,4	50,0±1,1
Sick animals	8,0±0,3	102,0±2,0	6,77±028	32,0±06	12,7±03	40,0±0,8
p<	0,05	0,05	0,001	0,001	0,02	0,001
1st group	6,8±0,2	89,0±1,6	18,12±0,51	28,4±0,7	13,1±0,2	41,8±0,7
p<	0,1	0,001	0,001	0,001	0,01	0,001
p*<	0,01	0,001	0,001	0,01	0,1	0,1
2nd group	14,4±0,5	140,0±2,3	8,83±0,18	39,0±1,0	9,7±0,1	27,1±06
p<	0,001	0,001	0,001	0,05	0,001	0,001
p*<	0,001	0,001	0,001	0,001	0,001	0,001

Note: p< – compared to clinically healthy animals; p*< – compared to sick animals

In the second experimental group, by contrast, the red blood cell count increased 1.8-fold ($p < 0.001$) compared to the start of the study, and at the end of

treatment was 2.0 times higher than in the animals of the control group ($p < 0.001$).

We observed a similar pattern with hemoglobin. In the first group, hemoglobin levels decreased significantly after treatment ($p < 0.001$) and were 1.2 times lower than in clinically healthy calves ($p < 0.001$), while in the second group, it increased significantly ($p < 0.001$) and was 1.3 times higher than in the control group ($p < 0.001$). The hemoglobin level in calves in the first experimental group increased 2.7-fold ($p < 0.001$) compared to baseline values, while in animals in the second group, it increased 1.3-fold ($p < 0.001$). The hemoglobin content was 6.2 times ($p < 0.001$) and 3.0 times ($p < 0.001$) higher, respectively, than in calves in the control group.

The mean hemoglobin concentration per red blood cell in the blood of calves in the first group remained virtually unchanged after treatment ($p < 0.1$) and was 12.7% lower than in the control group ($p < 0.01$). A significant increase in the number of red blood cells in the blood of animals in the second group, despite the increase in hemoglobin concentration, led to a decrease in mean corpuscular hemoglobin (MCH) in the blood of these calves compared to the baseline value ($p < 0.001$). The average hemoglobin content per red blood cell at the end of treatment in animals in the second group was 1.6 times lower than in clinically healthy animals ($p < 0.001$).

The decrease in the number of red blood cells in the blood of calves in the first experimental group after treatment led to a 3.6% reduction in hematocrit ($p < 0.01$), and it was 7.6% lower compared to the control group ($p < 0.001$). In animals of the second group, this value was 7.0% higher than at the start of the experiment ($p < 0.001$) and 3.0% higher compared to clinically healthy calves ($p < 0.05$).

The increase in erythropoiesis in the animals of the second group was reflected not only in the hematocrit value but also in the mean volume of the red blood cells themselves, which decreased by a factor of 1.5 after treatment ($p < 0.001$). Compared to the control group, this value was 1.8 times lower ($p < 0.001$). In animals from the first group, we observed no changes in the mean red blood cell volume. At the end of treatment, it remained 1.2 times lower than in clinically healthy calves ($p < 0.001$).

Changes in blood count parameters at the end of the experiment indicate the development of hypochromic microcytic anemia in the first group and hypochromic

microcytic erythrocytosis in the second group of animals. The increase in hemoglobin concentration in the blood of treated calves indicates increased systemic intoxication. The population composition of erythrocytes in the blood of calves in the first experimental group after treatment differed from that of clinically healthy animals in that it contained a greater number of "mature" cells (by 12.9%; $p < 0.01$) and a lower proportion of "young" cells (by 14.2%; $p < 0.001$). The proportion of "old" cells decreased by 3.3% compared to the sick calves ($p < 0.05$) and became similar to that in calves of the control group ($p < 0.1$; Table 5.10).

Table 5.10

Population composition of red blood cells in calves after treatment

Group of animals	Population of erytrocytes, %		
	"Old"	"Adult"	"Young"
Clinically healthy	11,9±0,8	36,1±1,3	52,0±1,7
Sick animals (severe course)	16,5±1,0	34,5±2,0	49,0±1,0
p<	0,01	0,1	0,1
First group	13,2±1,0	49,0±1,7	37,8±1,5
p<	0,1	0,001	0,001
p*<	0,05	0,001	0,001
Second group	9,6±0,7	50,1±2,1	40,3±1,6
p<	0,1	0,001	0,001
p*<	0,001	0,001	0,001

Note: p< – compared to clinically healthy animals; p*< – compared to sick animals

In the second group (at the end of treatment), we observed a similar trend: a decrease in the number of "old" and "young" red blood cells and an increase in "mature" red blood cells. The concentration of "old" cells decreased by 5.9%

compared to the start of the experiment ($p<0.001$) and was not significantly different from that in the control group ($p<0.1$). The number of "mature" cells increased by 15.6% ($p < 0.001$) and was 14% higher than in the blood of clinically healthy calves ($p < 0.001$). The percentage of "immature" cells after treatment was 8.7% lower than at the start of the experiment ($p < 0.001$) and 11.7% lower compared to clinically healthy calves ($p < 0.001$). The high number of "mature" and low number of "young" red blood cells in calves from the experimental groups is also reflected in the cells' acid resistance (Figs. 5.5; 5.6).

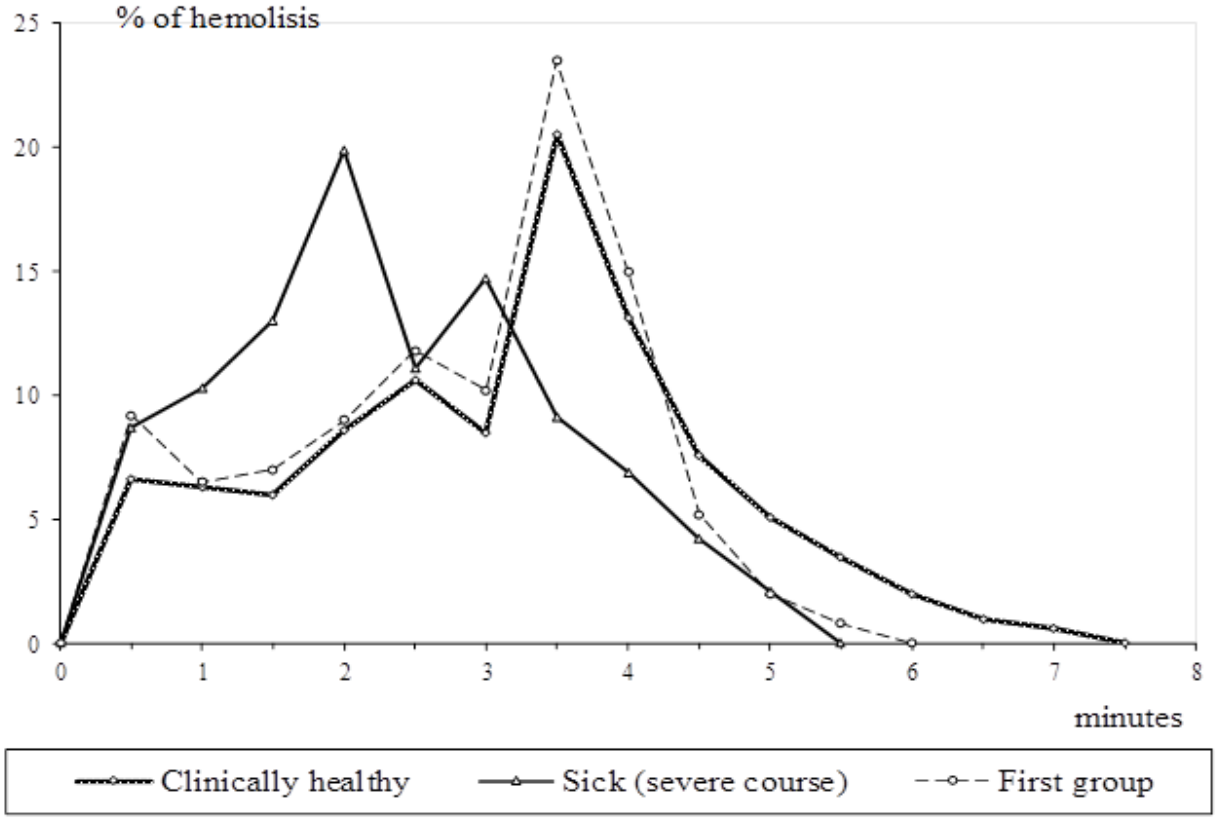


Fig. 5.5. Acid resistance of red blood cells in clinically healthy calves and animals in First Group before and after treatment

The changes in the erythrogram of the calves in the first group (after treatment) were revealing. The main peak shifted by 1.5 min. It corresponded to the onset time and duration in the same way as in the control calves. However, the negative effect of 15% amoxicillin trihydrate on erythropoiesis was still evident.

The additional peaks on the left side of the graph in these animals were higher and differed in duration compared to those in clinically healthy calves. Acid hemolysis of erythrocytes in the venous blood of animals in the first group ended 1.5 minutes earlier than in clinically healthy animals. The right side of the curve was abrupt, which is characteristic of a low concentration of "young" cell populations.

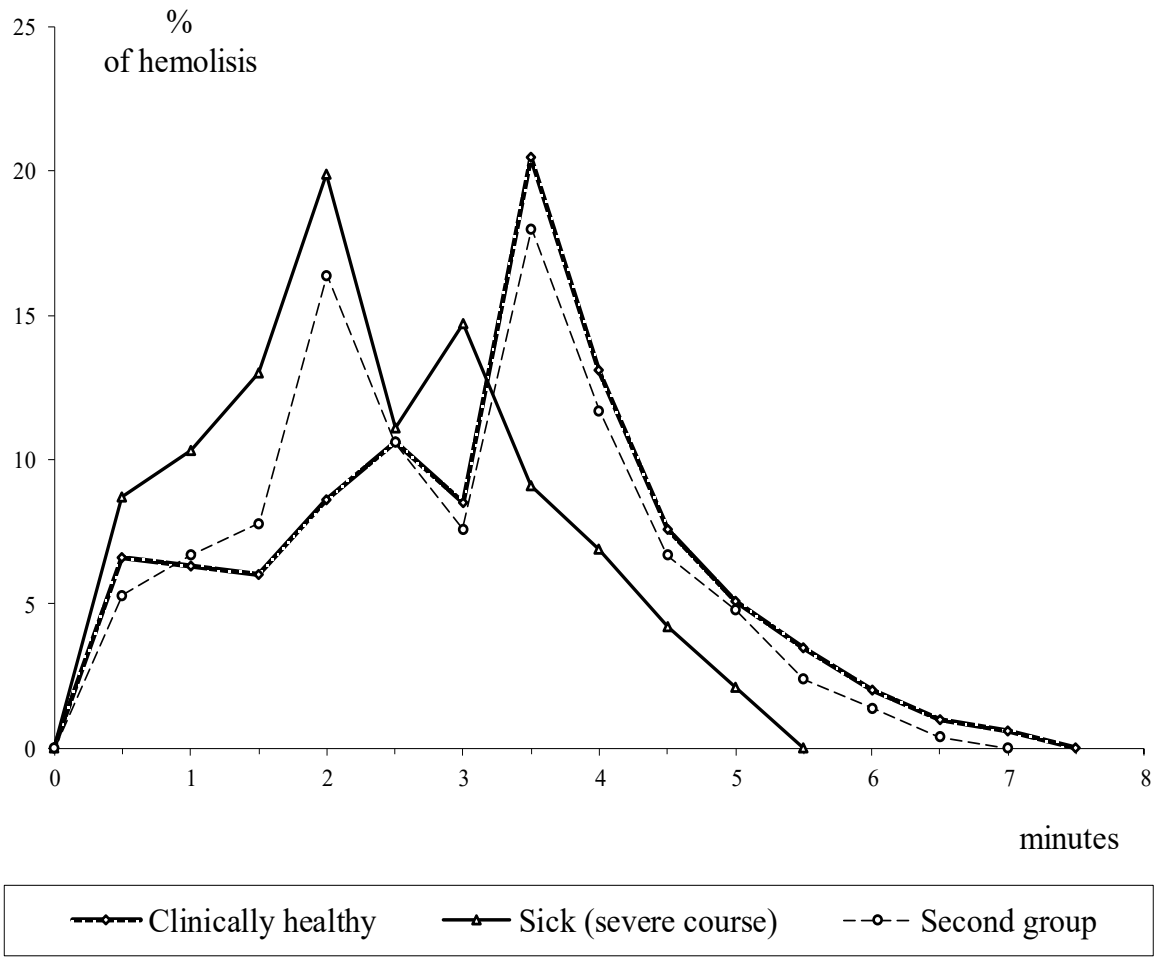


Fig. 5.6. Acid resistance of red blood cells in clinically healthy calves and animals in Second Group before and after treatment

The graph depicting the acid resistance of erythrocytes in calves from the second group (after treatment) had changed compared to the initial state, but was still not at all characteristic of clinically healthy animals. The first peak of the erythrogram differed from that observed before the start of the experiment in both

height (it was 3.6% lower) and duration (it was 0.5 minutes longer). The slope of this peak and the time to reach its maximum remained unchanged.

The main peak began to emerge at the 3-minute mark and reached its peak at 3.5 minutes, just as in the control group, but its amplitude was 2.5% lower. The total duration of hemolysis in the experimental calves of the second group increased by 1.5 minutes, but it was still 0.5 minutes shorter than in the control group. We attribute the high peaks in the central part of the graph to the large number of “mature” red blood cells in the blood of these animals.

Analysis of the lipid composition of erythrocyte membranes in the venous blood of calves treated with different regimens revealed varying levels of lipid components. In the erythrocyte membranes of the first group of animals (after treatment), we observed a trend toward an increase in total lipids and a decrease in cholesterol. However, only the content of phospholipids was fully restored in the cell membranes. Their level had decreased 1.8-fold by the end of the experiment ($p < 0.001$) and did not differ from that in clinically healthy calves ($p < 0.1$; Table 5.11).

The total lipid content in the erythrocyte membranes of venous blood from animals in the first group after treatment was 1.5 times lower ($p < 0.01$), while the cholesterol content was 2.9 times higher ($p < 0.001$) than in animals in the control group. These changes led to a 1.5-fold increase in the cholesterol-phospholipid ratio in cell membranes compared to the start of the experiment ($p < 0.001$), and a 3.0-fold increase compared to the control group ($p < 0.001$).

As for these parameters in the second group of calves (after treatment), the total lipid content of venous blood erythrocyte membranes increased 3.3-fold ($p < 0.001$) and was 1.6 times higher compared to the control group ($p < 0.001$). The cholesterol level in cell membranes decreased significantly by a factor of 5.8 ($p < 0.001$) and, at the end of treatment, was 1.6 times lower than in the control group ($p < 0.001$). The phospholipid content decreased by a factor of 1.6 ($p < 0.001$) and showed no difference from this value in clinically healthy animals ($p < 0.1$). The significant reduction in cholesterol levels was reflected in the cholesterol-to-phospholipid ratio. A 3.7-fold decrease in this ratio (after treatment; $p < 0.001$) resulted in a significantly

lower value compared to clinically healthy calves (1.8-fold; $p < 0.001$). Analysis of phosphorus and 2,3-diphosphoglycerate in the cytoplasm of red blood cells from the venous blood of treated animals showed an increase in these parameters in calves of the first group and only a tendency toward normalization in the second experimental group (Table 5.12).

Table 5.11

Lipid composition of calf red blood cell membranes before and after treatment

Group of animals	Common lipids, g/g erytrocytes suspensial protein	Cholesterol	Phospholipis	$\frac{Cholesterol}{Phospholipids}$
		mmol/g erytrocytes suspensial protein		
Clinically healthy	2,10±0,11	0,50±003	0,92±0,04	0,54±0,01
Sick animals (severe course)	1,08±0,10	1,80±0,15	1,60±0,10	1,12±0,06
p<	0,001	0,001	0,001	0,001
First group	1,46±0,13	1,44±0,09	0,88±0,03	1,64±0,07
p<	0,01	0,001	0,1	0,001
p*<	0,1	0,1	0,001	0,001
Second group	3,47±0,14	0,31±0,02	1,02±0,04	0,30±0,03
p<	0,001	0,001	0,1	0,001
p*<	0,001	0,001	0,001	0,001

Note: p< – compared to clinically healthy animals; p*< – compared to sick animals

The proportional increase in total and inorganic phosphorus in the red blood cells of calves in the first group following treatment [by 1.1 ($p<0.05$) and 1.2 ($p<0.001$) times, respectively] did not significantly affect the 2,3-DFG content ($p<0.1$) or the ratio of 2,3-DPG to total phosphorus ($p<0.1$) compared to the start of the experiment. All these indicators (except the last one) were approximately twice as

high as in clinically healthy animals ($p < 0.001$ for all). In the second group at the end of the experiment, despite a 1.2-fold decrease in total phosphorus ($p < 0.01$), a 1.1-fold decrease in inorganic phosphorus ($p < 0.02$), and a 1.2-fold decrease in 2,3-DPG ($p < 0.02$), their levels were significantly higher than in the red blood cells of calves in the control group ($p < 0.001$ for all).

The decrease in the studied parameters did not affect the ratio of 2,3-DPG to total phosphorus, expressed as a percentage ($p < 0.1$ – for both baseline and control values).

Table 5.12

Phosphorus (P) and 2,3-DPG levels in the red blood cells of calves' venous blood after treatment

Group of animals	P common	P non-organic	2,3-DPG	2,3-DPG•100% / P common
	μmol/ml of erytrocite emulsion			
Clinically healthy	3,30±0,02	1,30±0,04	2,00±0,05	60,6±1,6
Sick animals (severe course)	5,90±0,14	2,20±0,07	3,70±0,10	62,7±1,8
p<	0,001	0,001	0,001	0,1
First group	6,40±0,12	2,61±0,03	3,79±0,12	59,2±1,4
p<	0,001	0,001	0,001	0,1
p*<	0,05	0,001	0,1	0,1
Second group	5,06±0,13	1,94±0,06	3,12±0,18	61,7±1,5
p<	0,01	0,001	0,001	0,1
p*<	0,01	0,02	0,02	0,1

Note. p< – compared to clinically healthy animals; p*< – compared to sick animals

Studies related to the treatment of calves with severe bronchopneumonia confirm the mixed results of the chemotherapeutic agents we used. Thanks to its high antibacterial activity, 15% amoxicillin trihydrate rapidly neutralizes the microorganisms that caused inflammation of the bronchi and lungs. The homeopathic remedy “Prana” also has antimicrobial and anti-inflammatory effects. By enhancing

the drainage function of the mucous membranes and the body's overall resistance, this remedy, when used in combination with an antibiotic, rapidly eliminates both the cause and symptoms of bronchopneumonia. However, "Prana" is unable to completely neutralize the negative effects of amoxicillin on the animals' bodies. As a result, hypochromic microcytic anemia develops, accompanied by structural abnormalities in red blood cell membranes. Consequently, the cells are unable to function properly, which in turn can lead to irreversible pathology in the body. Enroxil 5% has a pronounced antibacterial effect, but like amoxicillin, it has side effects that negatively impact erythropoiesis and the animal's overall health. The development of hypochromic microcytic erythrocytosis in the blood of animals treated with Enroxil was accompanied by significant changes in the lipid composition of cell membranes. This prevents the restoration of the membranes' structure and function, resulting in the persistence of hypoxia and hypoxemia in the animals' bodies. Because it contains a piperazinyl radical in its structure, the drug exhibits immunosuppressive effects. Therefore, when using chemotherapeutic agents, it is necessary to consider not only their antibacterial properties but also their side effects on the animals' bodies. If amoxicillin and enroxil are used in a treatment regimen, it is necessary to administer detoxifying, hematopoietic, and general stimulant drugs concurrently.

5.3.2. Treatment of calves with 15% amoxicillin trihydrate in combination with liver hydrolysate (Syrepar) and 5% enroxil with Catosal

Based on the findings of previous studies, we decided to refine our treatment regimens. Hoping to enhance the therapeutic effect and striving to prevent the side effects of antibacterial drugs on the erythropoietic system and the calves' bodies, we supplemented the regimen with 15% amoxicillin trihydrate, trivitamin, a 0.5% sodium bicarbonate solution, and "Prana" – a liver hydrolysate (Syrepar) – and treated the animals in the first experimental group; while in the regimen consisting of 5% Enroxil, trivitamin, a 0.5% sodium bicarbonate solution, and "Prana" – the latter

of which was replaced with Katosal – we treated the sick calves in the second group.

Clinical recovery of the calves occurred in both the first and second groups on the 5th–6th day. After treatment, we did not observe any symptoms of bronchopneumonia or other diseases. The bronchopulmonary test result in these animals was 1.93 ± 0.14 mL in the first group and 1.88 ± 0.10 mL in the second group. A clinical examination of the animals after treatment confirmed their complete recovery, with no side effects or adverse effects from the administered drugs.

5.3.2.1 Blood test results for calves after treatment

In the venous blood of animals in the first group (after treatment), we observed a decrease in the number of red blood cells ($p < 0.02$); in the second group, this parameter remained virtually unchanged ($p < 0.1$). We observed a similar situation in arterial blood. The red blood cell count in the calves of the first group decreased by a factor of 1.2 ($p < 0.05$). We did not observe a statistically significant decrease in these cells in the animals of the second experimental group by the end of the study ($p < 0.1$; Table 5.13). After treatment, the red blood cell count in the calves of both groups did not differ from that of clinically healthy animals.

Hemoglobin levels also returned to those of the control group ($p < 0.1$). The hemoglobin concentration in the blood of calves in the experimental groups had significantly decreased by the end of treatment compared to the start of the study: by a factor of 2.2 ($p < 0.001$) in venous blood and by more than a factor of 1.6 in arterial blood ($p < 0.001$). The difference between this indicator in treated animals from both groups and clinically healthy calves was not statistically significant ($p < 0.1$).

Since the red blood cell count in the venous blood of calves in the second group increased compared to the control group, against the backdrop of a recovery in hemoglobin content, the mean hemoglobin concentration at the end of treatment did

Table 5.13

Erythropoiesis Parameters in Calves with Bronchopneumonia

Group of animals		Erytrocytes, T/л	Hemoglobin, g/l	Hemoglobin, %	Hematocrite, %	MCH, pg	MCV, μm³
Clinically healthy	Venous	7,2±0,2	107,8±2,1	2,90±0,18	36,0±0,8	15,0±0,4	50,0±1,1
	Arterial	6,2±0,2	99,8±1,3	3,70±0,18	32,3±0,6	16,1±0,3	52,1±0,8
	p<	0,01	0,01	0,01	0,02	0,05	0,1
Sick animals (severe course)	Venous	8,0±0,3	102,0±2,0	6,77±0,28	32,0±0,6	12,7±0,3	40,0±0,8
	p'<	0,05	0,05	0,001	0,001	0,02	0,001
	Arterial	7,2±0,2	90,3±1,4	6,62±0,18	28,0±0,7	12,5±0,2	38,8±1,2
	p*<	0,01	0,001	0,001	0,001	0,001	0,001
	p<	0,05	0,001	0,1	0,001	0,1	0,1
First group	Venous	7,3±0,2	107,8±1,8	3,10±0,07	36,5±0,7	14,8±0,2	50,0±1,3
	p'<	0,1	0,1	0,1	0,1	0,1	0,1
	p"<	0,02	0,1	0,001	0,001	0,001	0,001
	Arterial	6,5±0,2	100,6±1,5	3,94±0,11	33,0±0,5	15,7±0,3	51,6±1,1
	p*<	0,1	0,1	0,1	0,1	0,1	0,1
	p*<	0,05	0,001	0,001	0,001	0,001	0,001
	p<	0,02	0,05	0,01	0,01	0,1	0,1
Second group	Venous	7,8±0,2	105,3±1,9	3,23±0,19	36,4±0,5	13,5±0,3	46,7±0,9
	p'<	0,1	0,1	0,1	0,1	0,05	0,1
	p"<	0,1	0,1	0,001	0,01	0,1	0,01
	Arterial	7,3±0,3	103,2±1,6	4,13±0,15	31,6±0,9	14,1±0,4	49,4±1,0
	p*<	0,05	0,1	0,1	0,1	0,1	0,1
	p**<	0,1	0,01	0,01	0,05	0,02	0,01
	p<	0,1	0,1	0,02	0,01	0,1	0,1

Note: p< – comparison of venous and arterial blood parameters in the respective groups; p'< – comparison of venous blood parameters, p*< – comparison of arterial blood parameters in sick and clinically healthy calves; p"< – comparison of venous blood parameters, p**< – comparison of arterial blood parameters in animals before and after treatment

not change significantly ($p < 0.1$) and was 1.5 pg lower than in animals in the control group ($p < 0.05$). In the arterial blood of calves in this group, the mean hemoglobin content per red blood cell increased by 1.6 pg ($p < 0.02$) and showed no statistically significant difference from the control value ($p < 0.1$). In the first group (after treatment), the mean hemoglobin concentration increased by 2.1 pg in venous blood ($p < 0.001$) and by 3.2 pg in arterial blood ($p < 0.001$). At the end of the experiment, this value did not differ from that in clinically healthy animals. The mean corpuscular volume in the blood of animals in both experimental groups increased: in the first group – by 1.2 times ($p < 0.001$) in venous blood and by 1.3 times ($p < 0.001$) in arterial blood; in calves of the second group, respectively, by 1.2 times ($p < 0.01$) and 1.3 times ($p < 0.01$). This increase led to a statistically significant rise in hematocrit (first group: $pv < 0.001$; $pa < 0.001$; second group: $pv < 0.01$; $pa < 0.05$).

At the end of the experiment, the mean red blood cell volume and hematocrit levels in both venous and arterial blood had returned to normal and showed no statistically significant difference compared to clinically healthy calves.

The ratios of blood count parameters in venous and arterial blood in animals of the first group after treatment were identical to those in the control group; only the red blood cell count was slightly lower (1.12 vs. 1.16). In calves of the second experimental group, these parameters returned to levels seen in clinically healthy animals – for hemoglobin, HCT, and mean corpuscular volume. The erythrocyte ratio in these calves was 6% lower than in the control group, and the hemoglobin ratio was 5.6% lower, indicating a more intensive influx of normochromic cells into the bloodstream.

5.3.2.2. Age distribution and acid resistance of calf red blood cells after treatment

The age distribution of red blood cells was fully restored at the end of treatment only in calves from the second group (Table 5.14). A statistically significant decrease in “old” cells [by a factor of 1.3 ($p < 0.05$) in venous blood and by a factor of 1.4 ($p < 0.001$) in arterial blood] and a trend toward an increase in the

populations of “mature” and “young” red blood cells contributed to the complete restoration of the age composition of the cells to the levels observed in the control group ($p < 0.1$ for all populations compared with the control group).

Table 5.14

Population composition of erytrocytes in calves after treatment

Group of animals		Population of erytrocytes, %		
		“Old”	“Adult”	“Young”
Clinically healthy	Venous	11,9±0,8	36,1±1,1	52,0±1,7
	Arterial	14,0±0,2	38,8±0,6	47,2±1,0
	p<	0,05	0,05	0,02
Sick animals (severe course)	Venous	16,5±1,0	34,5±2,0	49,0±1,0
	p'<	0,001	0,1	0,1
	Arterial	22,3±0,6	35,5±0,9	42,2±1,1
	p*<	0,001	0,001	0,01
	p<	0,001	0,1	0,001
First group	Venous	5,4±0,4	40,3±1,2	54,0±2,8
	p'<	0,001	0,05	0,1
	p"<	0,001	0,05	0,1
	Arterial	7,6±0,5	43,9±1,0	48,5±1,6
	p*<	0,001	0,01	0,1
	p**<	0,001	0,001	0,01
	p<	0,002	0,1	0,1
Second group	Venous	13,1±0,6	35,8±1,3	51,1±1,9
	p'<	0,1	0,1	0,1
	p"<	0,05	0,1	0,1
	Arterial	15,6±0,7	38,1±1,0	46,0±1,7
	p*<	0,1	0,1	0,1
	p**<	0,001	0,1	0,1
	p<	0,05	0,1	0,1

Note: p< – comparison of venous and arterial blood parameters in the respective groups; p'< – comparison of venous blood parameters, p*< – comparison of arterial blood parameters in sick and clinically healthy calves; p"< – comparison of venous blood parameters, p**< – comparison of arterial blood parameters in animals before and after treatment.

The optimal ratio of erythrocyte populations of different ages is also confirmed by the venous-arterial coefficients, which returned to normal by the end of treatment and showed no difference from this indicator in clinically healthy calves. In the venous and arterial blood of animals in the first group, we observed fewer “old” and more “mature” cell populations. The number of “young” red blood cells in the blood of these calves did not differ significantly from that in clinically healthy calves. A 4.0% decrease in the venous-to-arterial ratio of “old” populations compared to the start of the experiment – and a value 16.5% lower than that in calves of the control group – indicates a higher intensity of antioxidant processes in the red blood cells of calves in the first group. The values of this indicator in “mature” and “young” cells did not differ significantly from those of clinically healthy animals.

The complete or partial restoration of the erythrocyte population composition was also reflected in their acid resistance. The erythrograms of calves in both groups changed significantly after treatment compared to the start of the experiment (Figs. 5.7; 5.8).

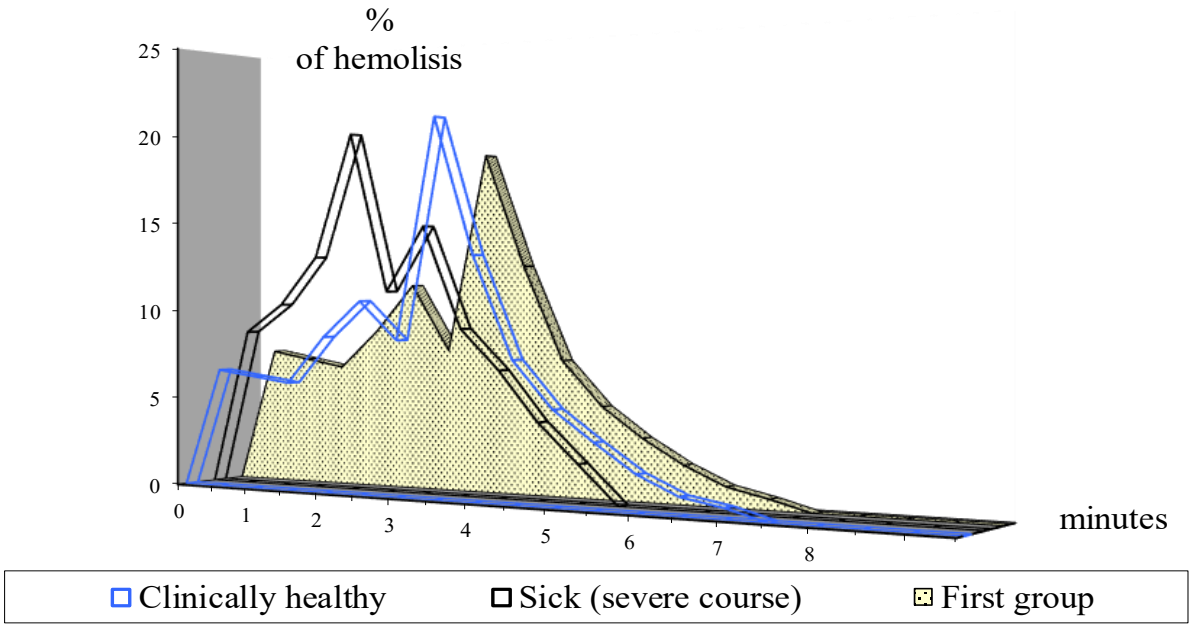


Fig. 5.7. Changes in the Acid Resistance of Red Blood Cells Following Treatment of Calves with Bronchopneumonia

In the first experimental group, we observed slightly lower peaks on the left side of the graph and a higher main peak. In the second group, these were almost identical to those in the control group. The time of peak appearance, their duration, and the overall duration of erythrocyte hemolysis in these animals were completely consistent with the erythrograms of clinically healthy.

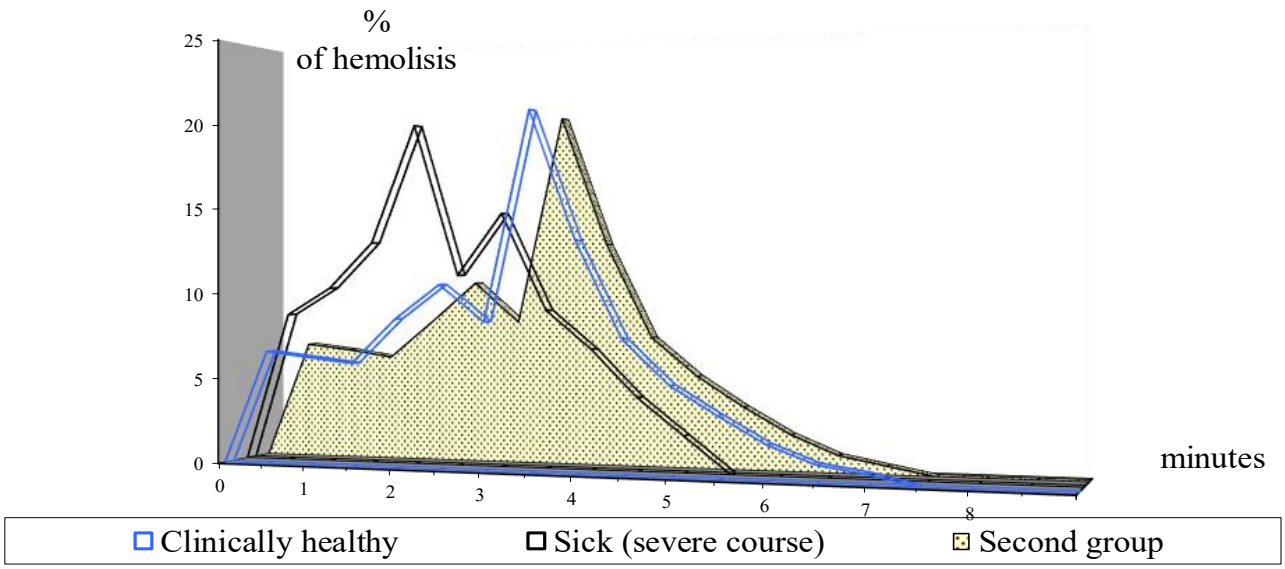


Fig. 5.8. Changes in the Acid Resistance of Red Blood Cells Following Treatment of Calves with Bronchopneumonia

5.3.2.3. The lipid composition of membranes and the phosphorus and 2,3-DFG content in the cytoplasm of calf red blood cells after treatment

The restoration of the buoyancy and stability of red blood cells can be explained by the optimization of the content of lipid components and the ratio between them in cell membranes. The increase in total lipid content and the decrease in cholesterol and phospholipid levels in the erythrocyte membranes of animals in both groups, in our opinion, indicated the restoration of the structural and functional properties of the cell membranes. This is confirmed by the decrease in the cholesterol-phospholipid ratio in the erythrocyte membranes of both venous and

arterial blood in calves from the experimental groups after treatment (Table 5.15).

Table 5.15

Lipid composition of calf red blood cell membranes before and after treatment

Group of animals		Common lipids, g/g erytrocytes suspensial protein	Cholesterol	Phospholipids	*Cholesterol / Phospholipids*
			mmol/g erytrocytes suspensial protein		
Clinically healthy	Venous	2,10±0,11	0,50±0,03	0,92±0,04	0,54±0,01
	Arterial	3,04±0,07	0,73±0,04	1,32±0,06	0,55±0,02
	p<	0,001	0,001	0,001	0,1
Sick animals (severe course)	Venous	1,08±0,10	1,80±0,15	1,60±0,10	1,12±0,06
	p'<	0,001	0,001	0,001	0,001
	Arterial	1,64±0,06	2,26±0,11	1,73±0,12	1,31±0,05
	p*<	0,001	0,001	0,01	0,001
	p<	0,001	0,05	0,1	0,05
First group	Venous	2,04±0,10	0,54±0,02	0,98±0,04	0,55±0,02
	p'<	0,1	0,1	0,1	0,1
	p"<	0,001	0,001	0,001	0,001
	Arterial	3,00±0,08	0,79±0,05	1,39±0,09	0,57±0,03
	p*<	0,1	0,1	0,1	0,1
	p**<	0,001	0,001	0,05	0,001
	p<	0,001	0,001	0,01	0,1
Second group	Venous	2,31±0,12	0,52±0,04	0,94±0,07	0,55±0,04
	p'<	0,1	0,1	0,1	0,1
	p"<	0,001	0,001	0,01	0,001
	Arterial	3,20±0,13	0,69±0,07	1,30±0,10	0,53±0,06
	p*<	0,1	0,1	0,1	0,1
	p**<	0,001	0,001	0,05	0,001
	p<	0,01	0,1	0,05	0,1

Note: p< – comparison of venous and arterial blood parameters in the respective groups; p'< – comparison of venous blood parameters, p*< – comparison of arterial blood parameters in sick and clinically healthy calves; p"< – comparison of venous blood parameters, p**< – comparison of arterial blood parameters in animals before and after treatment

At the end of the experiment, all indicators of membrane lipid composition showed no statistically significant difference from those in clinically healthy animals ($p < 0.1$ for both experimental groups).

The optimization of lipid component levels was accompanied by a restoration of the venous-arterial ratio of these parameters (Fig. 5.9). While in diseased animals we observed a decrease in this ratio for total lipids and an increase for cholesterol and phospholipids, after treatment, we noted the opposite trend. At the end of the experiment, the venous-arterial ratio for total lipids increased, while that for cholesterol and phospholipids decreased compared to the beginning of the experiment. The value of the venous-arterial ratio for the cholesterol-phospholipid ratio also returned to normal after treatment.

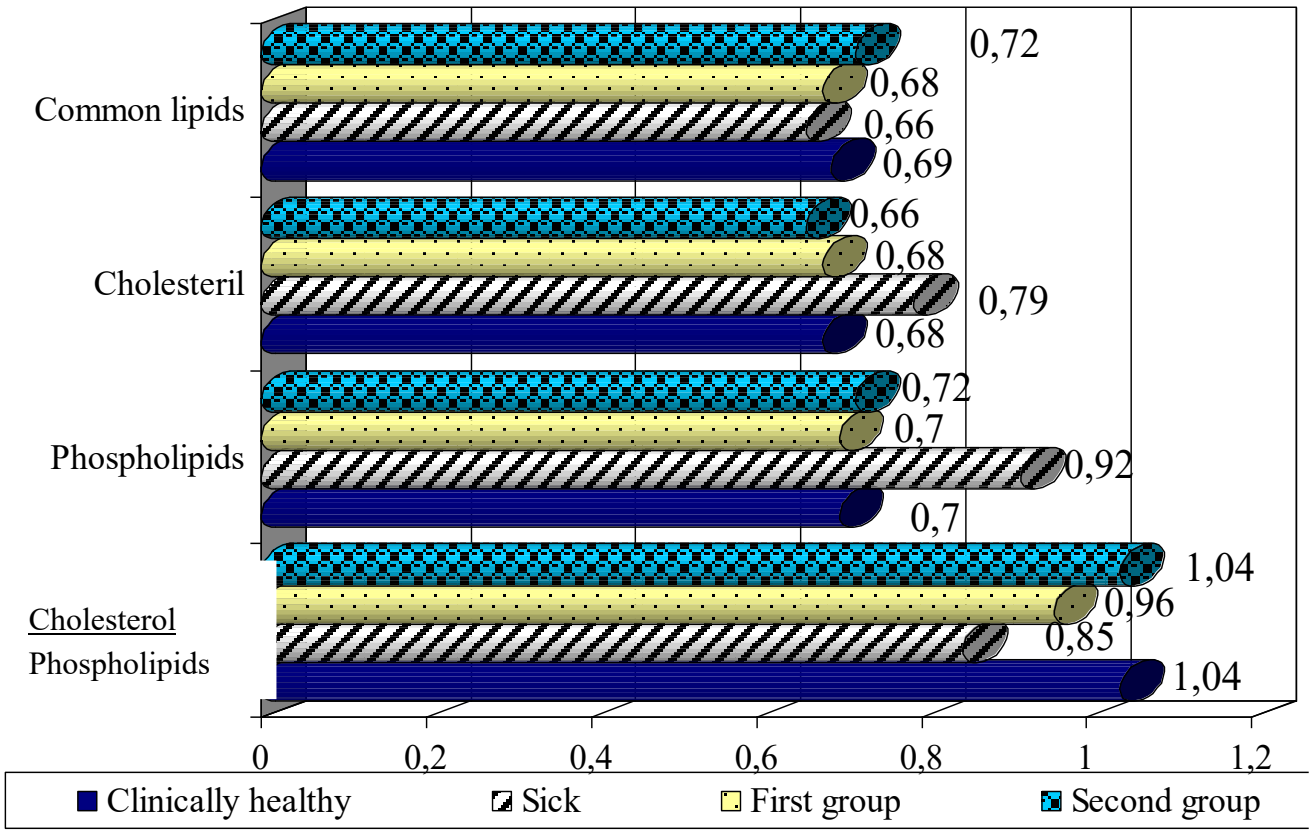


Fig. 5.9. Venous-Arterial Ratio of Lipid Parameters in Red Blood Cell Membranes

The formation of lipid components in erythrocyte membranes contributed to the normalization of metabolic processes in the erythrocytes. As a result, the functional properties of the cells were restored. This is confirmed by the levels of phosphorus and 2,3-diphosphoglycerate in the erythrocyte cytoplasm (Table 5.16).

Table 5.16

Changes in phosphorus (P) and 2,3-DPG levels in venous blood erythrocytes of calves after treatment

Group of animals		P common	P non-organic	2,3-DPG	2,3-DPG•100% / P common
		μmol/g erythrocyte suspension			
Clinically healthy	Venous	3,30±0,02	1,30±0,04	2,00±0,05	60,6±1,6
	Arterial	4,22±0,11	1,02±0,02	3,20±0,07	75,8±2,5
	p<	0,001	0,001	0,001	0,001
Sick animals (severe course)	Venous	5,90±0,14	2,20±0,07	3,70±0,10	62,7±1,8
	p'<	0,001	0,001	0,001	0,1
	Arterial	7,83±0,16	3,57±0,12	4,26±0,11	54,4±1,2
	p*<	0,001	0,001	0,001	0,01
	p<	0,001	0,001	0,01	0,01
First group	Venous	3,48±0,11	1,32±0,08	2,16±0,06	62,1±2,1
	p'<	0,1	0,1	0,1	0,1
	p"<	0,001	0,001	0,001	0,1
	Arterial	4,25±0,16	1,07±0,04	3,18±0,08	74,8±2,2
	p*<	0,1	0,1	0,1	0,1
	p**<	0,01	0,001	0,001	0,001
	p<	0,01	0,02	0,001	0,01
Second group	Venous	3,51±0,14	1,43±0,07	2,08±0,07	59,3±1,9
	p'<	0,1	0,1	0,1	0,1
	p"<	0,001	0,001	0,001	0,1
	Arterial	4,33±0,15	1,12±0,05	3,21±0,10	74,1±2,3
	p*<	0,1	0,1	0,1	0,1
	p**<	0,001	0,001	0,001	0,001
	p<	0,01	0,01	0,001	0,001

Note: p< – comparison of venous and arterial blood parameters in the respective groups; p'< – comparison of venous blood parameters, p*< – comparison of arterial blood parameters in sick and clinically healthy calves; p"< – comparison of venous blood parameters, p**< – comparison of arterial blood parameters in animals before and after treatment

The decrease in total and inorganic phosphorus and 2,3-DPG in the cytoplasm indicates the restoration of energy metabolism in red blood cells. The statistically insignificant difference in these parameters compared to clinically healthy calves indicates the resolution of hypoxia and hypoxemia in the treated animals. The percentage of 2,3-DPG relative to total phosphorus in the red blood cells of arterial blood from the experimental groups also returned to control levels after treatment ($p_a < 0.1$ for both groups). Consequently, the ratio of 2,3-DPG·100% to total phosphorus between venous and arterial blood decreased by a factor of 1.4 compared to the start of treatment and was 0.83 in the first group, and 0.80 in the second group (in clinically healthy calves, the venous-arterial ratio of this parameter is 0.80).

The studies in this section indicate that the drugs have an ambiguous effect on calves. The use of 15% amoxicillin trihydrate to treat animals with bronchopneumonia – whether mild or severe – leads to their rapid clinical recovery. However, following treatment, the animals develop anemia, which is a consequence of the drug's toxicity. The use of "Prana" in combination with this antibiotic slightly reduces its toxic effects and promotes a more rapid recovery in animals with bronchopneumonia. We observed the elimination of amoxicillin's side effects after administering it together with "Prana" and liver hydrolysate – Syrepar.

Enroxil 5% is ineffective for treating animals, whether used with or without "Prana." In addition, it causes the development of hypochromic microcytic erythrocytosis; however, these cells are not functionally mature. The high content of total lipids and low cholesterol in the erythrocyte membranes of treated calves indicates significant structural changes in the bilayer. This results in changes in the functional properties of the cells. We were able to counteract the negative effects of enroxil using 10% catosal. The use of vitamin and expectorant preparations is a mandatory requirement during the treatment of calves with bronchopneumonia. That is why, through comprehensive treatment that takes into account the mechanism of action and the effects of the drugs used on both the micro- and macro-levels, it is possible to achieve high treatment efficacy and full recovery in calves with both mild and severe forms of the disease.

CONCLUSIONS

1. This monograph presents a theoretical synthesis of changes in the biochemical structure and functional properties of red blood cells in calves with catarrhal bronchopneumonia, as well as new approaches to their treatment. an intense increase in the population of “old” red blood cells, changes in the lipid composition of cell membranes, and an increase in the activity of the 2,3-diphosphoglycerate shunt of erythrocyte glycolysis were observed in calves with catarrhal bronchopneumonia. the effectiveness of various treatment regimens for calves was investigated. the adverse effects of 15% amoxicillin trihydrate and 5% enroxil on erythropoiesis in affected animals were identified. comprehensive treatment regimens for calves with catarrhal bronchopneumonia were developed and experimentally validated, involving the use of antibacterial, expectorant, immunostimulatory, and hematopoietic stimulants.

2. A difference was found in erythron levels between venous and arterial blood in clinically healthy calves. blood from the jugular vein, compared to blood from arterial vessels, has a significantly higher hemoglobin content and a greater number of red blood cells at the same mean cell volume, as well as a lower content of hemoglobin (methemoglobin).

3. The erythrocyte membranes of arterial blood from clinically healthy calves, compared to red blood cells from the jugular vein, contain a significantly higher amount of total lipids (by 44.8%), phospholipids (43.5%), and cholesterol (46.0%), which significantly affects their properties.

4. There is a higher proportion of “old” ($p<0.05$) and “mature” ($p<0.05$) populations and a lower proportion of “young” ($p < 0.02$) populations of arterial blood erythrocytes, as well as higher activity of the 2,3-diphosphoglycerate shunt in these erythrocytes compared to those from the jugular vein, indicate an increase in the functional load on erythrocytes after they pass through the pulmonary circulation.

5. As a result of oxygen deprivation, populations of “young,” functionally immature red blood cells are intensively released into the blood of calves with

bronchopneumonia, leading to the development of hypochromic erythrocytosis; however, the depletion of the body's compensatory mechanisms during a protracted course of the disease leads to the rapid "aging" of both "young" and "mature" red blood cells.

6. In calves with bronchopneumonia, the biochemical structure of the erythrocyte membrane bilayer is disrupted: the total lipid content decreases significantly (by 48.6% in venous blood and 53.9% in arterial blood); cholesterol levels increase (by 260.0% and 209.6%) and phospholipid levels increase (by 73.9% and 31.0%, respectively), which leads to a reduction in the acid hemolysis time of red blood cells compared to that in healthy calves. as a result of changes in the conformational structure of erythrocyte membranes in calves with bronchopneumonia, the 2,3-diphosphoglycerate shunt is activated in the cells, which serves as a compensatory mechanism during hypoxia.

7. Amoxicillin trihydrate 15% has high antimicrobial activity and, together with trivitamin and sodium bicarbonate, promotes rapid clinical recovery in calves with catarrhal bronchopneumonia. However, under the influence of this antibiotic, intense "aging" of red blood cells occurs in the calves' blood, leading to the development of hypochromic microcytosis and hyperhemoglobinemia (methemoglobin levels increase 3.2-fold), hypoxia worsens, the structure of hepatocytes is disrupted, and the filtration function of the renal glomeruli is reduced.

8. Treatment of calves with bronchopneumonia using the following regimen – 15% amoxicillin trihydrate, syrepar, "prana," trivitamin, and sodium bicarbonate solution – proved to be the most effective. A 100% clinical recovery rate was observed within six days, along with normalization of the broncho-pulmonary test (1.93 ± 0.14 ml; in healthy calves: 1.89 ± 0.12; $p < 0.1$), erythropoiesis parameters, the lipid composition of erythrocyte membranes, and their acid resistance; signs of hypoxia decreased (the 2,3-dpg level decreased by 41.6% in venous blood and 25.4% in arterial blood).

9. A 5% solution of enroxil has a pronounced antibacterial effect but causes the development of hypochromic microcytic erythrocytosis, which is accompanied by

changes in the lipid composition of the membranes (total lipid content after treatment of sick calves with severe bronchopneumonia was 15.2% higher than in clinically healthy calves, while the cholesterol-phospholipid ratio was 44.4% lower). As a result, persistent oxygen deprivation was observed even seven days after treatment with this drug (the 2,3-DPG content was 56.0% higher compared to healthy calves).

10. As a result of treating calves with bronchopneumonia using 5% enroxil, 10% katosal, trivitamin, and sodium bicarbonate, clinical recovery occurred within five to six days. indicators of erythropoiesis, acid resistance, and the functional properties of erythrocytes, as well as the lipid composition of their membranes (the amount of lipid components and the cholesterol-phospholipid ratio after treatment did not differ from those in clinically healthy calves), were restored; the state of hypoxia resolved (the level of 2,3 -DPG levels after treatment were 2.08 ± 0.07 μmol/l in venous blood and 3.21 ± 0.10 μmol/l in erythrocyte suspension in arterial blood, compared with 2.00 ± 0.05 and 3.20 ± 0.07, respectively, in clinically healthy calves).

PROPOSITIONS

1. The treatment of calves with catarrhal bronchopneumonia, use the following: 15% amoxicillin trihydrate, intramuscularly, 150 mg (1 mL) per 10 kg of body weight, twice daily; Syrepar, intramuscularly, 2.5 mL, once daily; “Pranu,” intramuscularly, 4 mL, three times daily; Trivitamin, intramuscularly, 3 mL, once every three days; a 0.5% sodium bicarbonate solution, orally, 1 L, twice daily.

2. The treatment of calves with catarrhal bronchopneumonia, the following may be administered: 5% Enroxil, intramuscularly, 1 mL per 20 kg of body weight, for three consecutive days, in combination with Katosal, intramuscularly, 8 mL, once daily; Trivitamin and sodium bicarbonate.

3. While using 15% amoxicillin trihydrate, liver hydrolysate (Sirepar) must be included in the treatment regimen for calves, in accordance with the “Recommendations for the Use of 15% Amoxicillin Trihydrate for the Treatment of Calves,” approved by the Chair of Technical Committee No. 132 “Animal Health Products, Feed, and Feed Additives,” the Director of the State Scientific and Research Institute for Veterinary Preparations and Feed Additives, and Corresponding Member of the Ukrainian Academy of Agrarian Sciences M.V. Kosenko (Minutes No. 4 dated June 26, 2002).

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