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CORRECTIVE EFFECT OF MILK PHOSPHOLIPIDS IN PATHOLOGICAL CONDITIONS

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The monograph reflects the results of recent studies on the corrective action of milk phospholipids in the form of the liposomal form of biologically active additives “FLP-MD” for the effects of ecopathogenic environmental factors (ionizing radiation, heavy metals (cadmium), chemicals) and the development of entero- and hepatopathology, which It is recommended for implementation as an element of endoecological technology for the treatment, prophylactic and rehabilitation measures in veterinary medicine.

For specialists in the field of clinical biochemistry, clinical diagnostics, physiology and pathophysiology, therapy, morphology and pharmacology, practicing doctors of veterinary medicine, as well as for undergraduates, postgraduate and doctoral students, scientific research institutes, scientific and pedagogical workers of higher educational institutions of veterinary and biological profile.

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Preface

An urgent issue of applied veterinary medicine and other related industries is the determination of the adaptive potential of the body, which determines its tolerance to the negative effects of extreme environmental factors. The body's resistance is due to physiological and biochemical processes that underlie the development of specific reactions, which are closely interconnected with the structural organization of their cells and, above all, membrane systems. In mammals, in the course of evolution, a complex of structural and metabolic adaptation mechanisms has been formed both at the cellular and at other levels of organization, as a whole ensures their adaptation to changing conditions of existence (temperature fluctuations, oxygen concentration in the environment, radiation background, contamination of drinking water and feed xenobiotics [1, 2].

Now, the key role of structural and functional destabilization of cell membranes due to phospholipid deficiency, lipid peroxidation and their hydrolysis in the pathogenesis of inflammatory, dystrophic and degenerative processes has been scientifically and experimentally proven [3–5]. This indicates the feasibility of introducing reparative therapies in veterinary medicine and medicine, which include phospholipid-containing drugs, in particular, the most famous in the world - Essentiale® Fort N. Numerous studies in experiments on laboratory animals and when used in clinical practice have confirmed the positive effect of essential phospholipids on the structural organization of cell membranes, intracellular metabolism, the functional ability of mitochondria, antioxidant processes Sys, explains the active use of phospholipid-containing drugs in hepatopathology and enteropathology, cardiovascular diseases, intoxication of the body, etc. [7-9].

The structural similarities with phospholipids of mammalian tissues are raw materials of animal origin, in particular, obtained from milk, as well as a by-product of its processing - butterdish, which is a cheap and safe source of biologically active substances. Based on them, we created a biologically active additive (BAA) “FLP-MD”, which is characterized by membrane-active properties and reparative effect of action. Therefore, it is advisable to use reparative therapy in applied veterinary medicine, focused on the peculiarities of pathogenetic disorders, taking into account the natural potential and physiological ability of cells to self-restore lost functions, especially damaged intracellular structures – membrane systems. This approach, along with traditional therapy in the medical field, helps to reduce the economic damage from animal death, the occurrence of relapses and complications of relevant pathologies, as well as the solution of environmental, food and biological safety problems.

Respectfully,
team of authors

CHAPTER 1
INFLUENCE OF MILK PHOSPHOLIPIDES ON THE FUNCTIONAL CONDITION OF BODIES AND ORGANISM SYSTEMS

1.1 The use of dietary supplements in veterinary medicine

Preventive medicine that studies the problems of common non-communicable diseases, based on the identification of risk factors for the disease. An urgent task is to assess the risk of developing a chronic process, the formation of which correlates with the duration of the action of risk factors and their intensity. A chronic pathological process is formed under the influence of risk factors for a long period. In this regard, to suspend it or achieve regression of the disease is possible only as a result of prolonged exposure to corrective factors while eliminating or attenuating the effects of negative factors. Inhibition of the progression of the pathological process and, especially, its reverse development is an extremely difficult task that requires the use of rehabilitation measures.

New rehabilitation technologies for rehabilitation treatment include endoecological rehabilitation aimed at improving the natural cavities and spaces formed by the serous and mucous membranes, endothelial structures, as well as the restoration of the extracellular and cellular structures [1, 2]. Currently, there are four levels of endoecological rehabilitation. Particularly noteworthy is the fourth level, which is aimed at stimulating cell regeneration and structural and functional restoration of cell formations: external and intracellular membranes, protoplasm, membrane receptors, nuclear structures. The task of the fourth level of rehabilitation is solved by correcting the diet of animals with biologically active substances of membranotropic action, local action of membranotropic drugs [3].

The criterion for assessing the effectiveness of endoecological rehabilitation of the fourth level can be methods that characterize the processes that occur in cell (biological) membranes. The membrane responds to external signals and induces the production of the corresponding metabolites by the cell; it is able to effectively rebuild in response to various factors. The physical state of the membrane is known to be determined by phospholipids, primarily phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, which contain the bulk of the polyene fatty acids of the ω-6 and ω-3 structure [4, 5].

In the process of rehabilitation therapy at the fourth level of endoecological interaction, there is a directed regulation of cell lipid metabolism, which is carried out through the enzymatic block of desaturation of fatty acids, as well as due to the stabilization of vasodilator function in the synthesis of eicosanoids [6]. Moreover, the intensity of lipid modification of cell membranes depends on the depth and nature of the initial metabolic disorders. The fourth level will be especially effective in rehabilitation for years, but the effect can be seen on several generations of the rehabilitated.

Most drugs from the arsenal of humane and veterinary medicine are artificially synthesized. Along with the quick effect of their use, the development of severe complications is possible, it is extremely dangerous in case of complex clinical situations [7, 8]. The likelihood of side effects requires deliberate and careful prescription of such drugs in each case of the disease. Often the risk of negative effects of synthetic drugs on a living organism limits the possibility of their appointment due to existing contraindications. The vast majority of such drugs has cumulative properties and, if the recommended therapeutic and preventive doses are not followed, produces a toxic effect on the body.

The unqualified prescription of drugs, especially synthetic ones, often causes the development of a "drug" disease. According to statistical materials, the “drug” disease is the cause of death of about 100,000 people and the cause of the development of serious diseases in 2,200,000 people a year, unfortunately, no such studies have been conducted on the situation with animals. In many cases of "drug" disease, life-threatening side effects are noted: cardiac arrhythmia, renal failure, internal bleeding, and a critical decrease in blood pressure.

Along with this, the source of chemicals in the human body are food, including animal origin. The use of various chemicals in the livestock industry is carried out in order to increase the productivity of farm animals, prevent diseases and maintain feed quality. These are antibacterial substances (antibiotics, sulfonamides, nitrofurans), hormones, tranquilizers, antioxidants and the like. The systematic use of food contaminated with these substances impairs their quality, makes it difficult to conduct a sanitary and veterinary examination, and leads to the emergence of resistant forms of microorganisms. Therefore, it is extremely important to ensure the necessary control of the residual amounts of these contaminants in food products using fast and reliable methods.

The use of natural, environmentally friendly raw materials with low cost to create biologically active additives (BAA) of therapeutic and prophylactic action should help reduce the negative impact of enteropathology on the health status of newborn animals and the formation of their productive qualities [9].

So, modern pharmaceutical production focuses on the search for new, efficient, environmentally friendly and at the same time more cost-effective technologies; as a whole, it must solve an extremely important task - preserving human health and the environment.

The components of dietary supplements can be various organic and mineral substances of natural origin, which, along with other properties of membranotropic action. These are phospholipids, essential fatty acids, amino acids, vitamins, etc. [10, 11].

The structural and functional role of phospholipids. Cell membranes are 60% phospholipids [12]. Homeostasis of transport processes through membranes mainly determines their phospholipid composition. Phospholipids determine the intensity of the current repair of membranes and affect the functional condition of cell membranes [13]. Especially high in phospholipids are nerve and liver cells. Phospholipids also exhibit antioxidant properties, blocking the negative effects of free radicals on the body, which also cause age-related pathologies. Phospholipids are the main factor in the longevity of the body, because it is they that remove cholesterol from soft atherosclerotic plaques. Phospholipids even exhibit an anti-cancer effect, increasing the life expectancy of patients for cancer by more than 2.5 times.

Neutral phospholipids, such as sphingomyelin and phosphatidylcholine, are predominantly localized on the outside of the membrane in combination with a small amount of phosphatidylethanolamine. The inner (cytosolic) part of it consists of a large amount of phosphatidylethanolamine, as well as phosphatidylserine and phosphatidylinositol. Sphingomyelin and phosphatidylcholine mainly control the laminar configuration of the surface of membranes, which ensures their stability [14].

With the aging of the body, the content of phospholipids decreases both in the blood and in various tissues. A decrease in the content of phospholipids is associated with a decrease in the rate of their synthesis and self-renewal [12].

The main mechanism of cell damage is oxidative stress and impaired membrane integrity, as well as the development of phospholipid deficiency [15].

Exogenous phospholipids are material for the restoration of cell membranes in exchange for chemically converted lipids. In experiments on rats, it was proved that the introduction of essential phospholipids significantly reduces the percentage of cells that are subject to apoptosis under the influence of alcohol [16]. It has been demonstrated that with prolonged alcohol exposure, the administration of essential phospholipids reduces the activity of cytochrome P450 2E1 [17]. Exogenous phosphatidylcholine under experimental conditions helps to reduce the level of inflammatory activation of Kupffer cells and their production of proinflammatory cytokines IL 1b and TNFa, which means an important additional therapeutic effect [18, 19].

The antifibrotic effect of essential phospholipids in vitro and in vivo (with alcoholic liver disease) has already been proven [20]. This effect is explained by their effect on stellate (collagen-producing) liver cells. The use of phosphatidylcholine reduces the level of activation of stellate cells and the production of procollagen.

Damage to the surface membrane of cells is accompanied by an increase in its permeability, disorganization of receptor structures, disruption of carrier molecules and a decrease in transmembrane potential [21–24]. As a result of the violation of the integrity of the lipid bilayer of mitochondrial membranes and the loss of the transmembrane potential, accelerated release of reactive oxygen species, as well as cytochrome C (under physiological conditions isolated inside the mitochondria) into the cytoplasm is observed. The interaction of cytochrome C with the cytoplasmic protein of caspase 9 is a key step in the activation of the cascade of reactions, resulting in DNA degradation and cell death. So programmed cell death, apoptosis, can occur. Under light microscopy, histological signs of necrosis are cytoplasm edema, neutrophilic infiltration [25]. The development of apoptosis is indicated by karyopicnosis, karyorexis, and the formation of apoptotic bodies [26]. In such situations, the positive effect of the use of essential phospholipids, which have antioxidant potential, has been proven. Thus, when they enter the body as components of drugs, free radicals bind.

Under experimental conditions, phosphatidylcholine helps to reduce the level of inflammatory activation of Kupffer cells and their production of pro-inflammatory cytokines IL-1β and TNF-α, which has an important additional therapeutic effect [27].

Deficiency of phospholipids in the diet of animals leads to the accumulation of fat in the liver, its fatty degeneration, and then to cirrhosis [28].

Biological properties of fatty acids. Fatty acids are an important component of lipid homeostasis [29]. Now more than 200 fatty acids are known. In humans and animals, about 70 representatives of this class of compounds are found. The main biological functions of fatty acids: energy, structural, the creation of fat depots in the body, the formation of lipid regulators, participation in the formation of secondary messengers [30] and the regulation of the expression of a number of genes, the regulation of free radical oxidation, protein acylation and the formation of a lipid "anchor", necessary for the interaction of lipid and protein molecules.

Fatty acids are divided into saturated (SFA), monoenoic (MUFA) and polyenoic (PUFA) [31]. All mammals are able to synthesize de novo fatty acids from acetyl-CoA. The final product of this synthesis is palmitic acid ($C_{16:0}$), which is capable of converting into a stearic fatty acid ($C_{18:0}$) by attaching a carbon atom (elongation). However, in order to maintain microviscosity, structure and function of cell membranes, unsaturated fatty acids (UFA) are necessary for the formation of lipid mediators.

Essential fatty acids are linoleic, arachidonic, linolenic and α-linolenic acids. They are formed from oleic acid in plants, but not in animal cells [6].

Now the main focus is on the study of the unique biological properties of PUFA, which are essential fatty acids and are not synthesized in the human body. However, SFA and MUFA are the energy reserve of the body, they are involved in the acylation of cellular proteins and the conduct of signals in the cytoplasm. They create a "background" on which the more subtle action of PUFA is manifested. Monoenic fatty acids have antioxidant properties.

Fatty acids perform different functions in cells. They participate in the formation of microviscosity of the lipid bilayer, modify the conformational rearrangements of channel-forming proteins, receptors and enzymes, is a substrate for the synthesis of lipid mediators of free radical products, and regulate cellular metabolism and expression of certain genes [6].

The main functions of fatty acids in the body:

1. Creation of a system of cell membranes, regulation of microviscosity of a lipid bilayer.

2. The formation of biologically active lipid mediators - eicosanoids. 3. Ensuring the energy needs of the cell.

3. Participation in free-radical processes of oxidation, the formation of secondary and final products of lipid peroxidation modifiers of the state of lipids, proteins and nucleic acids.

4. Participation in the transmission of intracellular signals, the regulation of the activity of protein kinase and other enzymes.

5. Acylation of cellular proteins and providing lipid-protein and protein-protein interactions.

6. Prevention of proteins from denaturation (serum albumin) and glycosylation, reducing the content of proteins of the "acute phase".

7. Antioxidant effect of MUFA.

8. Regulation of endothelial cell migration.

9. Participation in postnatal formation (morphogenesis) of bone tissue in animals.

The stabilizing effect of ω-3 PUFA and palmitate by plasma proteins, including serum albumin, has been established. This protein is a physiological carrier of fatty acids, bile acids and biologically active factors in the blood. Fatty acids are not only “passengers” of this protein, but also change its conformation, increase resistance to hydrolytic and denaturing effects. Reducing the content of ω-3 PUFA on the surface of serum albumin determines the "aging" of the protein, contributes to its elimination from the bloodstream. Polyene fatty acids ω-3 are more powerful stabilizers of plasma proteins than chemical compounds. It is believed that the ability to suppress biochemical changes in serum proteins determines a decrease in the formation of autoantigens and the protective effect of ω-3 PUFAs in chronic autoimmune diseases [14].

The oxidation of SFA and MUFA in lymphocytes involving carnitine-palmitoyltransferase and the formation of CO_2 is an important point for the energy supply of proliferation. With mitogen-induced proliferation, the oxidation of fatty acids in lymphocytes increases to 35–40% [6, 12].

Changes in the phospholipid composition of cell membranes under the influence of PUFA affect the migration activity of cells. It is known that the migration and proliferation of endothelial cells is a key stage in the regeneration and restoration of blood circulation in ischemic tissues. Polyene fatty acids - eicosapentaenoic ($C_{20:5}$), docosapentaenoic ($C_{22:5}$) and docosahexaenoic ($C_{22:6}$) dose-dependently stimulate the migration and proliferation of endothelial cells in culture. This effect is more pronounced in docosapentaenoic fatty acid, which stimulates the migration of endotheliocytes at a concentration of 0.5 mg/ml. This allowed us to recommend these PUFAs for the prevention of atherogenesis [6, 12].

It has been proven that with PUFA accumulation in the membranes of splenocytes, the expression of HSp 25 and HSp 27 increases, and the sensitivity of these cells to apoptosis decreases [6].

Thus, taking into account the important biological functions of fatty acids –

structural, regulatory, and others, one should use the capabilities of feed rations and lipid additives not only to normalize lipid metabolism, but also to correct the state of cell membranes, immune function and prevent pathological processes – obtaining an anticoagulant, vasodilating, anti-inflammatory, immunomodulatory effects in the body.

The antioxidant and membrane-stabilizing effect of fat-soluble vitamins A and E. The problem of providing various types, primarily in the application of industrial technologies for the production of livestock products, biologically active substances, including vitamins and vitamin-like compounds, remains one of the most difficult and pressing issues in animal husbandry and veterinary medicine. Of considerable interest are applied developments to create new highly effective new-generation drugs and feed additives based on natural sources of vitamins and other biologically active compounds [32, 33].

Today, quite quickly you can create a new generation of highly effective medical preparations based on active derivatives and metabolites of vitamins, coenzymes and other biologically active compounds.

Fat-soluble vitamins are necessary for the normal functioning of all organs and systems of the body [34]. In the liver of animals 75–90% of the total supply of vitamin A in the body is deposited. The fundamental function of vitamin A (retinol) in the body is its participation in the processes of proliferation and differentiation, which underlies the growth and development of animals. This vitamin plays a leading role in the differentiation of epithelial cells. Therefore, with A-hypovitaminosis, a decrease in the synthesis of growth factor is observed, DNA synthesis slows down, the number of mucosynthetic goblet-like cells and enterocytes decreases, xerophthalmia (dryness of the cornea of the eye) develops, which ends with keratomalacia or panophthalmitis. Recent experimental data indicate that retinol is a minor component of cell membranes, that is, a deficiency or excess of it leads to a violation of their structure and functions. Vitamin A deficiency reduces the regulatory mechanisms of natural resistance of animals, disrupts the synthesis of collagen in bone tissue (the growth of the tubular bones of the extremities is delayed). Directly or indirectly, it affects protein synthesis, the formation of glycosaminoglycans, the ultrastructures of goblet-like cat cells, and lipid synthesis [35]. Its deficiency leads to an increase in the likelihood of infectious diseases, a decrease in night vision and the occurrence of conjunctivitis.

Vitamin E (tocopherol) lowers insulin levels, normalizes blood circulation, participates in restoring vascular elasticity and strength, prevents hemorrhages, improves oxygen transport to the eye and brain cells, increases the resistance of cell membranes to oxidation, and has a protective effect on vascular and heart cells. As an antioxidant, vitamin E prevents cardiovascular disease. It plays a significant role in eliminating cataracts and glaucoma along with carotenoids and selenium, which is necessary for tissue repair. It is necessary for the normal functioning of the nervous and muscle systems, strengthening the walls of the capillaries, improving immune function; purification of blood from fats [36]. Vitamin E, along with other antioxidants, reduces the damaging effect of free radicals [37].

According to scientists, dietary supplements with antioxidant properties can prevent oxidative stress [38]. Fat-soluble antioxidants (alpha-tocopherol and carotenoids) play a major role in protecting the basic structural components of biomembranes, such as phospholipids and proteins immersed in the lipid layer [39]. Vitamin E is a fat-soluble antioxidant that is located in the cell membrane. According to scientists, only 20-40% of alimentary vitamin E is absorbed by the body, so it is recommended to use it as part of dietary supplements. Vitamin E contains a phenolic ring with a double bond system, so it easily gives away the electron to free radicals, restoring them to stable products. The phenoxyl radical, which is formed in this case, is itself quite stable and is not involved in chain extension [40].

For the absorption of exogenous origin of retinol and tocopherol in the intestine, a necessary condition is the sufficient entry of bile acids into the duodenal lumen [41].

Proteinsynthetic and phospholipid-forming functions of methionine. One of the important micronutrients in animal feeding is protein [42]. High-protein diets contribute to the restoration of the disturbed structure of internal organs and stimulate the synthesis of enzymes necessary for the decontamination of toxins. Characteristic of liver diseases is an increase in protein catabolism, causing excretion, and loss of muscle mass. Protein deficiency in feed is very unfavorable for viral liver damage, as it violates the adequate response of the immune system. The development of the immune response is preceded by the development of proliferation and differentiation of immune cells, increases energy costs and the need for adequate protein supply.

It was established that the initial stage of the immunological response is accompanied by a 7–15-fold increase in the transmembrane flow of amino acids, nucleotides, and other plasma substances [43]. The inclusion of amino acids is accompanied by a high intensity of protein synthesis in the cell. It is known that antigen-dependent activation of lymphocytes is characterized by their high metabolic rate, increased activity and requires continuous energy and substrate support at a higher level.

In diseases of internal organs, it is important not so much the absolute amount of protein in the diet as its quality. Sources of protein should contain essential amino acids, primarily methionine, N-acetylcysteine, glutathione, arginine, lysine, etc. Methionine is important to reduce toxic effects on the liver, because it converts to glutathione due to cysteine and thereby participates in the neutralization of toxins. In addition, without methionine, synthesis of choline, an important component of cell membrane phospholipids, is impossible; methionine reduces fatty liver, acts as an energy source for the immune system. Arginine has the ability to bind ammonia, stimulates the ornithine cycle, which is important for detoxification, promotes liver regeneration, improves the function of T-lymphocytes, and has a beneficial effect on the thymus gland and the muscular system. Cysteine is a precursor to glutathione, which has a detoxifying effect. Amino acids have a positive effect on the restoration of liver cells, improve the function of all organs and tissues, including the immune system.

SH-amino acids (cysteine, cystine, methionine) possess certain antioxidant activity. Moreover, SH groups act as competitors with other substrates — objects of oxidation that do not produce free radicals and actually terminate the chain reaction of free radical oxidation [44]. SH compounds are capable of prolonging the lifespan of the NO molecule. However, the therapeutic use of compounds containing SH groups is limited due to their low permeability through cytoplasmic membranes, they can be protectors from intracellular oxidative stress, and also because of the ability to stimulate peroxide reactions in the intercellular medium [45].

1.2 Biological properties of milk phospholipids

The toxicity of the biologically active phospholipid-dietary supplement "FLP-MD" was determined in laboratory conditions on CBA mice by oral administration. The duration of the observation period is 16 days.

These tests are widely used by toxicologists as a relatively quick, uncomplicated, low-cost and statistically reliable method for assessing the negative effects of drugs on objects such as laboratory animals.

The studies were conducted on laboratory mice of the CBA line obtained from healthy individuals of the vivarium of the Institute of Oncology of the Academy of Medical Sciences of Ukraine. Used experimental mice that reached puberty at the beginning of the experiments. Healthy individuals were selected, which had approximately the same size and weight, had never before been used in other laboratory studies and manipulations. The weight of the mice was monitored throughout the experiment. So, at the beginning of the experiment, males weighed 19–20 g, females – 18–19 g.

To study the biological properties of the phospholipid-containing dietary supplement "FLP-MD" groups of experimental animals were composed of ten mice (five of each sex), which were placed in cells. Mice were kept in five individuals in a separate cage.

The drug was fed to mice every day for 16 days. After administration of the test substance, the mice had free access to food and water. Food intake was monitored by both test and control mice.

We observed changes in the general condition of the animals, the presence of symptoms of intoxication. Every 24 hours of the experiment, the mice were weighed and the amount of feed consumed was monitored. After the experiment, the mice were decapitated under ether anesthesia, blood samples were taken for hematological and biochemical studies, stomach and intestine samples for histological studies. Histological samples were stained with hematosinyl-eosin, blood smears according to Romanovsky.

Throughout the experiment, the behavior of animals in the experimental group did not differ from that in mice of the control group. When assessing the appearance (condition of the skin, coat, mucous membranes) in mice of the experimental group, no differences were revealed compared with animals of the control group. After administration of the dietary supplement “FLP-MD”, no symptoms of intoxication (tremors, seizures, paresis and paralysis) were observed in animals of the experimental group.

During visual observation in experimental animals, the frequency of urine output and defecation, as well as the color of urine and feces did not differ from those in the control group.

The dynamics of changes in body weight of CBA mice of various groups are given in Table. 1.1. According to the table, when feeding a phospholipid-containing

Table 1.1. The average change in body weight of mice (g) over the period of the experiment (M ± m, n = 5 in each group)

Group of animals		Body weight (g)				
		2 day	5 day	8 day	10 day	16 day
Experimental Group	females	17.7±0.3	18.4±0.1	18.6±0.3	18.8±0.3*	19.0±0.3*
	males	19.5±0.4	20.5±0.6	21.1±0.6	22.1±0.4*	22.85±0.3*
Control Group	females	18.1±0.2	18.6±0.3	18.7±0.2	18.9±0.2	19.7±0.6
	males	21.0±0.3	22.0±0.6	20.9±0.7	20.9±1.0	21.1±1.3

Note: * - $p < 0.05$, compared with the initial data on the second day of the experiment in the context of the corresponding groups.

dietary supplement “FLP-MD” to mice for 16 days, a significant increase in the body weight of mice was observed after 10 days of experiment in both males and females.

The results of the study of hematological parameters of the peripheral blood of mice treated with phospholipid-positive dietary supplement "FLP-MD" for 16 days are presented in table. 1.2.

Table 1.2. Hematological parameters of experimental mice

(M ± m, n = 5 in each group)

Indicator		Males		Females	
		experimental group	control group	experimental group	control group
Erythrocytes, x 10^{12}/l		6.01± 0.25*	7.22± 0.25	6.97± 0.51	7.60± 0.66
Hemoglobin, g/l		112.0± 1.7	116.8± 2.7	116.3± 3.03	115.3± 1.8
Hematocrit, conv. units		0.46± 0.03	0.45± 0.01	0.47± 0.01	0.43± 0.02
Platelets, x 10^9/l		548.67± 145.60	515.20± 41.20	611.25± 26.56	607.25± 39.02
Leukocytes, x 10^9/l		6.78± 0.56	6.80± 0.44	5.97± 0.35	5.40± 0.77
Stab leukocytes	10^9/l	0.021± 0.014	0.040± 0.023	0.051± 0.021	0.056± 0.021
	%	0.30± 0.20	0.20± 0.12	0.83± 0.33	1.00± 0.36
Segmental leukocytes	10^9/l	1.08± 0.06	1.06± 0.28	1.07± 0.25	1.14± 0.19
	%	16.60± 1.96	15.00± 3.38	17.67± 3.77	21.29± 2.93
Eosinophiles	10^9/l	0.17± 0.05	0.22± 0.08	0.15± 0.03	0.13± 0.02
	%	2.50± 0.63	3.30± 1.35	2.58± 0.45	2.71± 0.56
Monocytes	10^9/l	0.22± 0.05	0.39± 0.14	0.28± 0.03	0.33± 0.09
	%	3.20± 0.66	5.50± 1.75	4.75± 0.64	6.21± 1.60
Lymphocytes	10^9/l	5.30± 0.56	5.13± 0.20	4.44± 0.38	3.73± 0.68

	%	77.60± 2.14	76.00± 2.76	74.50± 4.61	68.36± 4.58
ESR, mm / hour.		0.80± 0.10*	1.60± 0.14	1.33± 0.17	1.51± 0.29

Note: * - p <0.05, the difference is significant compared with the control.

According to the results presented in mice of the experimental group, the amount of erythrocytes and the ESR in the blood decrease under the influence of phospholipids of the dietary supplement, which is better expressed in females (correspondingly from (7.22±0.25) x 10^{12}/l to (6.04±0.25) x 10^{12}/l s (1.6±0.24) mm/h to (0.8±0.2) mm/h, $p<0.05$). All other indicators of animals treated with dietary supplement "FLP-MD" do not significantly change.

As a result of biochemical studies, which are presented in Table. 1.3, when mice were fed phospholipids of milk in the form of liposomal dietary supplement “FLP-MD” for 16 days, no significant changes in blood plasma parameters were found, with the exception of alanine transaminase (ALT, EC 2.6.1.2) in females.

Table 1.3. Blood biochemical parameters of mice of different groups

(M ± m, n = 5 in each group)

Indicator	Males		Females	
	experimental group	control group	experimenta l group	control group
Alanine transaminase, IU	126.3± 6.1	139.3± 1.9	97.0± 1.6*	83.3± 1.1
Aspartate transaminase, IU	565.6± 30.7	612.3± 57.9	537.0± 50.1	527.6± 71.6
Alkaline phosphatase, IU	864.0± 16.2*	755.0± 31.3	792.3± 45.3	969.0± 73.9
Lactate dehydrogenase, IU	2496.3± 203.2	2996.3± 79.9	10199.0± 102.7	10802.0± 488.1
Glucose, mM	20.7± 1.2	19.4± 0.8	17.8± 0.6	18.21± 1.5
Total protein, g/l	39.3± 1.9	37.3± 0.6	31.0± 0.4	31.0± 1.2

The above allows us to conclude that there is no toxic effect of the components of the dietary supplement on the functional condition of the body.

The results of histological studies of the stomach and intestines. On the preparation of the cardial esophagus of the females of the experimental group, the transition of the stratified squamous epithelium of the esophagus into the golden epithelium of the cardial part of the stomach and the absence of cytological or structural changes in these organs are noted. In the esophagus, strata of the stratified epithelium are surrounded by keratin masses with foci of mineralization (dystrophic liming). Dystrophic liming indicates the antiquity of the mineralization process and is not a pathology. No structural changes were detected in the epithelial cells of the esophagus glands and in the cardiac glands of the stomach. The muscle membrane with an active response of smooth muscle cells, the serous membrane is thickened with an active reaction of fibroblasts. At the intersection of the esophagus into the stomach, there are massive round-cell infiltrates (inflammatory), stasis (plethora) of blood vessels. The mineralization of keratin masses in the esophagus and at the same time an active reaction of the basal cells of the esophagus are noted.

The mucous membrane of the stomach rejected into the lumen with stick necrosis fragments of the golden epithelium and the storage of individual Brunner glands, which are represented by a cylindrical epithelium. The rejection of the gastric mucosa extends to the limit of its own plate. The last thinned, homogeneous, poor cells with signs of mineralization. The muscular membrane is thinned with signs of intermuscular edema; smooth muscle cell response and lymphocyte infiltration. Some drugs have no structural changes. All layers of the structure are clearly recognizable: kerkring lobes, fossa of the mucosa and own glands. The submucous membrane of the membrane is thinned, the muscle and serous membranes are thickened.

The small intestine corresponds to the structure of the duodenum. The structure of the mucous membrane is preserved, despite the rejection of the mucous layers in the intestinal lumen and necrosis of part of the villi. The submucosa and serous membrane are absent. The muscular membrane, they are separated by a thinned one.

In the stroma of the intestinal villi, lymphocytic infiltration is expressed. The structure of the small intestine is not broken in all layers. The villi of the mucous membrane are large with a moderate endothelial reaction. The cavernous expansion of the blood vessels of the submucosal layer is determined. Hypertrophied muscle plate (middle section). The serous membrane is absent.

In males, in the study of the drug (transition of the esophagus to the cardial part of the stomach), hyperkeratization between the cells of the stratified squamous epithelium and the subepithelial layer with foci of mineralization (dystrophic liming) is noted. Massive foci of hemorrhage (intramuscular). The active reaction of nuclear forms of fat cells is the upper section. Fragmentation and decay of connective tissue fibers, which borders the squamous epithelium.

Transitional zone of the esophagus into the stomach: on the verge of a multilayer squamous epithelium of the esophagus and glandular epithelium of the cardiac section of the stomach, round-cell infiltrates were found. Dystrophic liming of the intermediate tissue of the esophagus. The structure of the stomach corresponds to the structure of the normal mucous membrane with pyloric glands and muscle membrane.

Small intestine: pockets (liberknon) crypts are clearly distinguished, villi are flattened, sent by a single-layer cylindrical epithelium, without visible pathological changes, mainly edged in the absence of other cells that make up the intestinal villus (goblet-like mucus-producing and endocrine-hormone-producing). Edema of the intermediate tissue of the Brunner glands with microcenters of inflammatory round-cell infiltrates was revealed.

When studying the transition of the esophagus to the cardial stomach of males and females of the control group, the pattern of changes corresponds to the characteristic changes for the research group described above, with the difference that a more pronounced inflammatory reaction is noted in the underlying connective tissue stroma of the esophagus. It turns dystrophic liming subepithelial tissue of the esophagus. The cardiac glands of the esophagus (Brunner) are not structurally altered; there is an intra-plasmic retardation of mucus with infiltration of the submucosal layer of lymphocyte.

In its plate of the mucous membrane of the esophagus, the structure is not broken, it contains all types of cells with a predominance of mucus-producing. In structure, it does not differ from the mucous membrane, characteristic of normal. In the small intestine, high villi were studied with a pronounced reaction of lymphocytes and plasma cells in their own plate of the mucous membrane.

CONCLUSION

During the feeding to mice of the CBA line of the liposomal form of dietary supplement “FLP-MD” based on milk phospholipids (within 16 days), a significant increase in body weight was observed after the tenth day of the experiment in both males and females. The amount of feed consumed during the entire period of the experiment in the control and experimental groups do not differ.

The obtained biochemical blood parameters indicate the absence of toxic effects of the liposomal form of dietary supplement “FLP-MD” based on milk phospholipids on the functional condition of the body.

The hematological parameters of the peripheral blood of experimental animals receiving the liposomal form of dietary supplement “FLP-MD” do not change significantly, with the exception of a decrease in the number of red blood cells and the ESR in animals of the experimental group.

According to the results of morphological studies, there was no negative effect of the liposomal form of dietary supplement “FLP-MD” based on milk phospholipids on the histostructures of the mucous membrane of the organs of the gastrointestinal tract. The normal structure of this section of the digestive tract is distinguished only by pronounced inflammatory infiltration (squamous) in the submucosal layer. Hyperplasia of the lymphoid elements of Peyer's plaques is noted. The epithelial cells of the Brunner glands are filled with mucus (delayed withdrawal), like the glands of the cardiac section of the stomach.

CHAPTER 2
CORRECTING EFFECTIVENESS OF MILK PHOSPHOLIPIDES BY CALVES ENTEROPATHOLOGY

An important issue in the current state of the livestock industry is the preservation of the stock of young animals and their productive qualities, the most intensive formation of which occurs in the early postnatal period of life. The applied value of these studies is confirmed by the high percentage of cases of young animals of different species on enteropathology. It is known that 60–90% of newborns are sick, of which 15 to 50% die [1].

Numerous studies by employees of the National University of Life and Environmental sciences of Ukraine (NULES of Ukraine) prove that the metabolic status of the body of newborn animals is distinguished by a significant lability of biochemical parameters, which is associated with the normalization of the acid-base state, the genetically determined process of replacing the fetal type of blood proteins with an adult, the formation of a unique natural phenomena - colostral immunity with endocytotic-pinocytotic mechanism of assimilation colostrum immune proteins in the gastrointestinal tract and with a number of features of the morphological and functional condition of the structural elements of cells and, above all, their membranes [2–5].

The development of enteropathology in this “critical” period significantly violates the formation of adaptive changes in the digestive tract and other organs and systems of newborns [5, 6]. In these animals, significant metabolic disturbances were noted during their clinical recovery [5, 7]. This indicates deep metabolic disorders at the cellular level, which correlates with the intensity of restoration of the structural and functional condition of damaged cells of the digestive system.

A significant and diverse arsenal of modern therapeutic and prophylactic agents is not always effective in treating patients with enteropathology of newborn animals, which is explained by the need for timely diagnosis of the development of acute digestive disorders and monitoring the functional condition of this system. At the same time, the restoration of metabolic processes and the structural and functional condition of epithelial cells of the intestinal mucosa, hepatocytes does not end three weeks after the clinical recovery of newborn calves [8–11].

This further creates the problem of repeated relapses of enteropathology, the development of complications from other organs and systems (hepatodystrophy, bronchopneumonia, nephritis, etc.), a decrease in the body's resistance state and productive qualities in animals of older age groups [10].

In the monograph, the authors present the results of a study of the characteristics of metabolic changes associated with the disorganization of the structural and functional condition of entero- and hepatocytes, which experience significant damage during the development of functional enteropathology in newborn calves; studies of the intensity of recovery processes during the period of their clinical recovery and the use of a phospholipid-containing additive in liposome form for corrective therapy with this pathology is proposed.

2.1 Morphofunctional changes in the digestive system with enteropathology of calves

The epithelial layer of the mucous membrane of the gastrointestinal tract undergoes significant structural changes during the neonatal period, which is a genetically determined process associated with a change in the type of feeding [12, 13]. This gives reason to consider the neonatal period as critical.

The occurrence of gastrointestinal pathology in newborn animals disrupts the functioning of adaptive mechanisms from the digestive tract. This provokes the development of complications and repeated relapses of acute digestive disorders in animals, including those who have been ill [14, 15].

We have investigated that in such calves the restoration of the structural and functional condition of the digestive tract does not end even at the age of 30 days [15, 16]. Slow normalization of impaired functions of enterocytes and, above all, the system of active transport of food nutrients through the apical and basolateral membranes inhibits the restoration of metabolic processes in the tissues of calves that have had enteropathology.

Enteropathology is more often observed in newborn animals born of cows in violation of the animal health conditions. Histological and morphological studies show that such animals have signs of tissue underdevelopment. This primarily affects the digestive and protective functions of the stomach and intestines. Therefore, even with minor changes in the chemical composition and physical properties of the diet feed, as well as against the background of the action of conditionally pathogenic microflora of the digestive organs, functional enteropathology develops [3, 4, 17].

It is known that parietal digestion is characteristic of newborn animals and the vast majority of colostrum (milk) nutrients are digested with the participation of membrane-bound enzymes of the bordering enterocytes of the small intestine mucosa. In violation of the intrauterine development of calves, the activity of these enzymes decreases by 8–10 times [18]. Morphological and functional immaturity of the digestive organs cause insufficient digestion of the nutrients of colostrum (milk). As a result of this, the physical properties and composition of the chyme change. The remnants of undigested casein excite intestinal mechanoreceptors, and the altered chyme pH, products of incomplete cleavage of colostrum (milk) and rot, as well as microbial toxins irritate chemoreceptors. Under these conditions, increased antigenic load leads to a "breakdown of tolerance" with the formation of autoimmune and allergic reactions, triggers a local inflammatory reaction of the mucous-submucosal layer and causes local damage. In combination with a deterioration in the trophism of the intestinal wall, this leads to the formation of mucosal defects. In patients, lysis of microvilli of enterocytes occurs, which are the structural basis of membrane digestion, enzyme synthesis is reduced, the hydrolysis of proteins, carbohydrates, lipids is completed, and their progress on the surface of intestinal epithelial cell membranes is disrupted. Irritation of the mucous membrane of the stomach and intestines promotes the release of histamine, which in turn enhances their peristalsis [19].

During the development of enteropathology in newborn animals, there is a violation of the structural and functional condition of cell membranes due to changes in the prooxidant-antioxidant balance, accompanied by regular metabolic disorders and inflammatory processes, primarily in the tissues of the intestines, liver and kidneys [20, 21].

The determining factor in the pathogenesis of metabolic disorders in patients with enteropathology of newborn animals, in addition to inflammation, is the development of tissue hypoxia. The mechanism of the negative effect of hypoxia on the body of newborns is due to a violation in the functioning of the electron transport chains of cell membrane complexes, which leads to the formation of reactive oxygen species. This leads to hypoxic cell necrobiosis in the affected organs and tissues [21].

Therefore, the development of pathological processes in enteropathology is quite complex, and in the pathogenesis of the disease there are four groups of basic interconnected pathogenetic mechanisms [23]:

1. Violation of the motor and secretory absorption function of the digestive canal;

2. Violation of water-electrolyte metabolism, which causes dehydration, toxicosis, decompensated acidosis, blood thickening, complications of the heart and its blockade due to excess sodium ions;

3. Disorders caused by a deficiency of nutrients in the body and a significant level of catabolic processes;

4. Dysbacteriosis and the possibility of endogenous intoxication in the presence of associations of highly virulent microorganisms.

If the feeding conditions (regime, quantity and quality of feed, technology) are not observed in newborn animals, the processes of digestion and assimilation of colostrum change. In violation of the conditions of detention of newborn animals (hypothermia or overheating of the body), changes in intestinal motility occur. This causes the movement of non-pathogenic microorganisms characteristic of newborn animals from the large intestine to the thin, where conditions are created under which they acquire pathogenicity (spore-forming bacilli, anaerobes, coca, microorganisms, etc.). Thus, in the intestinal microflora, the ratios of gram-negative and gram-positive microorganisms change. The waste products of the intestinal microflora, which accumulate in significant quantities, change the environment and digestive conditions. This negatively affects the mucous membrane of the digestive system, and once in the bloodstream it affects the whole organism. The toxicosis that arises further disturbs the metabolism and negatively affects the nervous, endocrine, cardiovascular and respiratory systems, and liver and kidney functions [24, 25].

With disorders of the digestive and absorption processes in the intestines of sick animals, the absorption of mineral elements (iron, copper, zinc, cobalt, manganese) is inhibited, which negatively affects the state of the central organs of the blood, especially the red bone marrow, which leads to impaired hemocytopoiesis, the development of sideropenia, iron deficiency anemia with hypochromia, which is compensated by erythrocytosis [26, 27].

With neonatal enteropathology of newborns, the epithelial layer of the mucous membrane of the abomasum (stomach) and intestines is desquamated with the formation of erosions and ulcers. This leads to a sharp decrease in the barrier functions of the digestive system and causes the penetration of toxic substances into the circulatory and lymphatic systems, which, after entering the portal vein, and subsequently into the liver, suppress the still unstable neutralizing ability of hepatocytes. In turn, disorders of the neutralizing function of the liver contribute to the disruption of the metabolism of proteins and nitrogen-containing substances, the processes of deamination and transamination of amino acids, and cause protein and fat degeneration of hepatocytes, and therefore a violation of their functions [28].

In toxic enteropathology, symptoms of encephalopathy are observed that arise as a result of the toxic effect on the central nervous system of the insufficiently neutralized by the liver rot products that accumulate in the abomasum and intestines (amines, phenols, indole, skatol, bacterial toxins) [29].

Along with the disturbance of the digestion processes in sick animals, an increase in intoxication of the body occurs, which leads to a change in the cardiovascular system, which are manifested by symptoms of acute dystrophy of the heart muscle [25].

With the onset of the symptom complex of diarrhea, the animal's body loses a large amount of water, which leads to its dehydration. Due to dehydration, blood thickening occurs, characterized by an increase in its specific gravity, a relative increase in the hemoglobin content and the number of blood cells. With obstructed blood circulation, oxygen transport is disrupted, which leads to oxygen starvation of tissues and, asa result, to tissue hypoxia [30].

The secretion of water and electrolytes in the first stages is aimed at removing toxic products from the intestine. However, prolonged secretion causes a violation of osmotic phenomena in the mucous membrane, the formation of prostaglandins and the development of local inflammatory processes [31].

It was found that in gastrointestinal digestive disorders in newborn calves, epithelial cells with a striped border (bordering enterocytes) of the small intestine mucosa increase the content of cGMP and the activity of guanylate cyclase and decrease the corresponding values of cAMP and adenylate cyclase, increase the content of PG F2 and PG F1α. As the author notes [32], the emergence and development of the gastrointestinal pathology of non-infectious etiology in these animals occurs with a GMP-dependent mechanism. Pathological factors, when interacting with glycoprotein receptors of the apical membranes of edging enterocytes, modify G-proteins, which increase the activity of membrane-bound guanylate cyclase and phospholipase C. Cyclic guanosine monophosphate, through the activation of cGMP-dependent protein kinases that phosphorylate the protein substrates of the apical membranes, enhances the secretion of Na^+, Cl^- i HCO_3^- Phospholipase C hydrolyzes phosphatidylinositol, which leads to the formation of IP3, which promotes the release of Ca^{2+} deposited in cells. Calcium ions stimulate the activity of phospholipase A_2 and calmodulin. Phospholipase A_2 interacts with phospholipids, accompanied by the growth of their lysoform in the membranes and the release of arachidonic acid into the cytoplasm. Oxidized forms of phospholipids reduce the density of membranes than cause an increase in their passive permeability. With arachidonic acid, prostaglandins E_2, groups F, etc. are formed, which stimulate the secretion of HCO_3^- and Cl^-. Ca-calmodulin exhibits a stimulating effect on protein kinase. Ca^{2+} ions, diacylglycerols, LYSO-phosphatidylcholine stimulate the activity of protein kinase C, which inhibits the functioning of ion pumps, in particular the activity of Na^+, K^+-ATPase. The intake of Ca^{2+} from colostrum (milk) to the epithelium of the intestinal mucosa of Suteev reinforces the described effects, as a result of which diarrhea, as a compensatory, adaptive, protective reaction of the patient's organism, is transformed into an uncontrolled stage of secretion of Na^+, H_2O, Cl^-, HCO_3^-, K^+ and things like that.

2.2 Milk phospholipids and enteropathology of newborn calves

The development of enteropathology in experimental calves was noted from the second day of life. The course of the disease in these animals was characterized by: diarrhea, dehydration, weakness, general depression, lack or loss of appetite, exhaustion, often cachexia.

In calves with the traditional treatment regimen for enteropathology, the duration of the disease course averaged 6–8 days (up to 10 days). In this case, fatal cases of the course of the disease were recorded. During the recovery period, relapses in 45% of calves were noted. Such animals were significantly behind in their development and productivity from the calves of the control group.

In contrast to the described group of animals, in seriously ill calves according to a comprehensive treatment regimen (traditional therapy + dietary supplement “FLP-MD”), the pathology duration on average was 4–6 days. At the same time, feces were characterized by a uniform consistency, natural color and lack of putrid odor. The reaction of these animals to external stimuli, motor activity and appetite were better expressed, exhaustion was rarely observed. No cases of death and relapse during enteropathology were noted among the experimental calves of this research group. This proves the effective effect of the phospholipid-containing dietary supplement "FLP-MD" of reparative action on the clinical status of calves during symptoms of enteropathology and during rehabilitation.

The results of a laboratory study of biochemical parameters characterizing various types of metabolism and the structural and functional condition of the affected organs and tissues during the development of enteropathology, significantly supplemented the described clinical picture. So, the level of total protein in the blood plasma of calves on the 7–8th day of life was characterized by a tendency to increase in animals with a traditional treatment regimen and likely increase in experimental calves with complex treatment with dietary supplement “FLP-MD”, respectively, by 39 and 30% staying high on the 30th day of their life. Hyperproteinemia has been established and has a relative character and is the result of dehydration, and in calves, according to a comprehensive treatment regimen, it is also probably a consequence of activation of protein-synthesizing processes in tissues, including increased immunogenesis, since exogenous phospholipids of dietary supplements “FLP-MD” certainly have an immunomodulatory effect on the structural – the functional condition of the cell membranes of immunocompetent organs, tissues and cells. The assumption is made that one can explain given the basic theory of immunogenesis, according to which the structure that is responsible for the initial stages of the immune response is the membrane of immunocompetent cells. In this case, immune reactions are initiated at the level of intermembrane contacts of lymphocytes, phagocytes and stromal elements. The above is consistent with literature data on the participation of phospholipids in the process of lymphocyte activation, the formation of intracellular messengers, and the transmission of signals from the cell surface to the cytoplasm.

At the same time, there was no significant difference in the level of albumin in the blood serum of the calves of the experimental groups both in separate age periods and in the dynamics of time. Moreover, in animals of all experimental groups, the albumin content in the blood plasma was within normal limits. However, the high value of the hematocrit value in calves with traditional treatment on the 7–8th day of their life (55–58 l/l) and the average degree of increase (42–50 l/l) in calves with complex treatment indicate the probability of hidden changes in the albumin content in the blood plasma of calves after the disappearance of the clinical symptoms of the disease due to the development of dehydration.

After the disappearance of the clinical symptoms of the disease (on the 7–8th day of life), the activity of hepat-specific enzymes significantly increases in experimental calves: γ-glutamyl transpeptidase (GGT) 2.8 times (traditional treatment), alkaline phosphatase (AP) 1.8 times (traditional treatment) and 1.4 times (complex treatment in calves with traditional treatment at the age of 30 days). This confirms the presence of violations of the biliary function of the liver (cholestasis) and the inflammatory process in the endothelium of the bile ducts and liver parenchyma. At the same time, the enzymatic activity of aspartate transaminase (AST) and alanine transaminase (ALT) remains virtually normal in sick calves. Only in calves with traditional treatment after the disappearance of the clinical symptoms of the disease, AST activity was significantly higher (1.4 times) for the control level, which proves the occurrence of deep structural changes in hepatocytes (at the level of mitochondria) during the clinical manifestation of the disease. The absence of significant fluctuations in the activity of aminotransferases in calves during complex treatment may be the result of stabilization of the structural and functional condition of cell membranes and enhancement of their protective properties when exogenous phospholipids of dietary supplements “FLP-MD” are used. At the same time, on the 30th day of calf life, there was a significant decrease in the activity of most of these enzymes in experimental animals with complex treatment, except AST, γ-GTP and exercise therapy in calves with traditional treatment, compared with their activity by 7–8-th day of life.

Hyperbilirubinemia and significantly higher direct bilirubin (3 times) in calves suffering from enteropathology on the 7-8th day of life, compared with the control, confirms the fact of impaired biliary function of the liver during the disappearance of clinical symptoms of digestive disorders. At the same time, the physiological parameters of the total bilirubin content in the blood plasma of calves with complex treatment and probably its decrease on the 30th day of life, indicates the absence of bile secretion disorders in animals treated with the experimental phospholipid-positive dietary supplement “FLP-MD”.

Disorders of the functional condition of the liver, possibly, is a consequence of the anatomically close arrangement of nerve and humoral connections between the intestines and the liver. In gastrointestinal pathology, damage to the liver structure and its functional changes are often noted, since, in acute and chronic digestive disorders, hepatocyte dystrophic changes are determined in the liver tissue, and in severe cases, small, medium and large-droplet fatty degeneration.

At the same time, an increase in the concentration of urea (2.7 times) and creatinine (by 22%) in the blood plasma of experimental animals with traditional treatment on the 7-8th day of life, indicates some tension in nitrogen metabolism (azotemia) and possible disorders structural and functional condition of the kidneys in sick calves. The absence of significant changes and the physiological boundaries of these indicators in the blood plasma of calves during complex treatment characterizes the protective and reparative properties of the phospholipid-containing dietary supplement "FLP-MD" with respect to normalizing the functional ability of the kidneys. However, in calves who had had an enteropathology at 28–30 days of age with the traditional and complex treatment regimen, there were no significant changes in the blood plasma content of urea and creatinine compared with the corresponding control.

Pathology of the digestive tract negatively affects the exchange of hemoglobin. It was proved that in experimental calves treated according to the traditional regimen, a significant decrease in hemoglobin level in the blood was observed in all studied periods of life, which characterizes the state of anemia, which, according to the literature, is classified as iron deficient. This is especially true for the early postnatal period of animal life, which accounts for the development of natural hemolytic anemia resulting from the genetically determined replacement of the fetal type of hemoglobin (HbF) with an adult (HbA). Enterocyte dysfunction in these calves disrupts the transport and absorption of feed nutrients, including those that are factors of hematopoiesis (essential amino acids, iron, copper, cobalt, zinc, group B vitamins). It should be noted that Fe^{2+} are part of the iron-porphyrin compounds, the representatives of which are enzymes of the antioxidant defense system (catalase, peroxidase). Therefore, iron deficiency, which is noted for this pathology, contributes to membrane pathological changes due to the activation of lipid peroxidation, and causes a disorganization of metabolic processes occurring on cell membranes.

At the same time, in the blood of calves, additionally received a phospholipid-dependent dietary supplement "FLP-MD" for the period of the experiment, a decrease in hemoglobin content, and, accordingly, the absence of iron deficiency anemia, was less intensive than in animals with traditional treatment, indicates membrane-stabilizing and at the same time stimulating, the effect of dietary supplement phospholipids on the synthesis of hemoglobin molecules in specialized tissues.

Probably a high glucose level (2.1 times) in the blood plasma of calves with the traditional treatment regimen on the 7-8th day of life, may indicate adaptive-compensatory changes in the neuro-endocrine regulation of the processes of synthesis and utilization of glucose in the body of seriously ill animals and, possibly , increased processes of glycogenolysis and gluconeogenesis in their tissues. However, in experimental animals, for complex treatment, no significant changes in glucose concentration were detected on both the 7–8th and 28–30th days of life, which characterize the absence of deep disturbances in the carbohydrate metabolism in the body of these calves. In addition, the glucose concentration significantly increased in the blood plasma of calves of these groups at the end of the experiment, compared with 7–8 days of life.

So, in calves, the rehabilitation period according to the traditional treatment regimen for enteropathology established the development of hyperenzymemia (AST, ALT, GGT, exercise therapy), hyperbilirubinemia, iron deficiency anemia and azotemia, which is consistent with the results of a clinical examination of these animals and manifests itself in a long and harder course of the disease (up to 10 days) with repeated relapses (in 45% of animals). And, on the contrary, in animals, along with the traditional treatment, they also received a phospholipid-positive dietary supplement "FLP-MD", which exerts a reparative effect, there are no significant changes in the studied parameters, except for hyperproteinemia, which may be the result of increased protein synthesis processes in tissues, and a tendency to normalization of their values. Indicators of the clinical status of the body of calves who were ill on enteropathology, the significant tension of metabolic processes in the tissues and the slow nature of their recovery, along with the existing dysfunction of enterocytes, hepatocytes and other body cells, are studied, indicate the need for such animals to use reparative therapy, including during rehabilitation. This can significantly accelerate the restoration of damaged cell membranes and, in general, the functional condition of cells involved in the pathological process of organs and tissues. To solve this problem, an experimental phospholipid-containing dietary supplement "FLP-MD" can be recommended, which significantly improves the therapeutic and prophylactic effect of traditional dyspepsia therapy regimens when used in combination with the disease and during rehabilitation.

CHAPTER 3
CORRECTIVE EFFECTIVENESS OF MILK PHOSPHOLIPIDES IN TETRACYCLIN-INDUCED HEPATOSIS

Despite the progress in creating and introducing into clinical practice new antimicrobial agents with high activity and minimal side effects, traditional and affordable drugs, such as penicillin, ampicillin, tetracycline and others continue to be used in therapeutic regimens [1, 2]. Today, according to statistics, up to 7–8% of the pharmaceutical market belongs to tetracyclines and cephalosporins [3, 4]. The main side effect of these antibiotics during prolonged high-dose therapy is various disorders of the liver – from jaundice to hepatosis, followed by the development of cirrhosis and fibrosis [5, 6]. In some cases, hepatopathology goes into the stage of decompensation, which requires prolonged use of hepatoprotectors.

Tetracycline refers to drugs with a direct cytotoxic effect on the liver [4, 5]. One of the first manifestations of toxic liver damage is fatty degeneration of varying degrees, or fatty hepatosis – the accumulation of triacylglycerols in hepatocytes. One of the causes of this pathology is the imbalance between the formation and catabolism of lipids [7, 8]. The introduction of tetracycline causes a decrease in the activity of mitochondrial beta oxidation of fatty acids, an increase in the synthesis of endogenous fatty acids, and insufficient incorporation or export of triacylglycerols into low density lipoproteins [9].

An important effect of the toxic effect of tetracycline is the suppression of the synthesis of mitochondrial oxidative phosphorylation enzymes, regulation of which occurs at the level of transcription, of the enzyme complexes of the respiratory chain of mitochondria I and IV, resulting in an increase in the number of lipid peroxidation products in the liver and blood [5, 10, 11]. The latter can cause a violation of the structural organization of the membranes of hepatocytes and their organelles, which is manifested by the depolarization of mitochondrial membranes with their subsequent swelling due to increased permeability for ions of the inner membrane caused by free radicals [4].

The above correlates with the known facts of an increase in the level of calcium ions in hepatocyte microsomes, which confirms the membranotropic and prooxidant effect of tetracyclines, and also indicates the main role of mitochondrial dysfunction in the development of tetracycline-induced hepatosis.

Tetracycline liver damage in animals is widely used in pharmacological experiments to determine the therapeutic efficacy of hepatoprotective drugs [4, 12]. With tetracycline hepatosis, destruction of cell membranes with a violation of their phospholipid organization, stimulation of collagen formation with subsequent formation of fibrosis is noted [6, 8]. Therefore, in recent years, essential phospholipids have been used to restore the structure and functions of hepatocyte membranes, increase their "fluidity", activate membrane enzymes, normalize lipid metabolism and transport during fatty hepatosis [13]. This became the basis for testing the effectiveness of the influence of milk phospholipids, which in their fatty acid spectrum correspond to the lipid component of the cell membranes of the internal organs of mammals. The aim of the work was to determine the functional state of the liver and the reparative properties of milk phospholipids in an experimental model of hepatosis in rats, which is tetracycline-induced.

3.1 Morpho-functional changes in the liver with tetracycline-induced hepatosis

Clinical and morphological manifestations of toxic liver lesions are diverse [14]. There are three main types of damage: hepatocellular, cholestatic and mixed. The target of toxic effects may be hepatocytes (dystrophy, necrosis), bile ducts and tubules (cholestasis) or sinusoidal cells (endothelium). Drug-induced liver damage, in particular damage to the parenchyma in the form of functional disorders (induction of microsomal enzymes, hyperbilirubinemia) lead to necrosis or apoptosis. Other hepatotoxic effects of pharmacotherapy include the formation of steatosis in the form of acute fat changes, steatohepatitis, cholestasis, granulomatous changes and damage to the vascular system of the liver, etc. [6, 15].

A significant compensatory antitoxic reserve of the body eliminates the negative impact of toxic substances [16]. In the liver, which performs a detoxification function, they are inactivated and bound for further elimination. The implementation of this function during the first phase substantially depends on the activity of cytochrome P450. With the participation of the corresponding enzymatic systems, oxidation, reduction, hydrolysis, hydration and dehalogenation of toxins occur. In the next phase – conjugation, deactivation of toxic substances due to glucuronidation, acetylation, methylation, binding to amino acids and glutathione. However, the detoxification ability of an organ is significantly reduced if the influence of harmful factors develops against the background of hepatopathology. The severity and severity of symptoms is determined by the dose of xenobiotics [17].

Morphological heterogeneity of toxic liver lesions and predominant localization of necrosis leads to metabolic zoning. According to metabolic activity, hepatocytes are heterogeneous. Their zoning, with differences in metabolic functions, leads to the selective sensitivity of hepatocytes to various pathological factors. In particular, the cells of the I zone contain more mitochondria, oxidative processes, gluconeogenesis, and the synthesis of cholesterol, urea and bile acids are more intense. In hepatocytes of zone III, glycolysis, lithogenesis, cytochrome P450-dependent hydroxylation, and glucuronidation of xenobiotics are most pronounced. The use of tetracycline antibiotics leads to the development of hepatocyte necrosis mainly of the III zone [17].

Toxic lesions of the liver due to the use of drugs present a certain diagnostic complexity [1]. Sometimes, against the background of prolonged therapy, they occur without clinical manifestations, which indicates chronic intoxication. The above encourages a comprehensive study of the pathogenesis of the development of drug hepatopathology, primarily at the molecular level, the creation of sensitive test systems for early diagnosis, as well as means of prevention and treatment of such conditions. Therefore, it remains relevant to develop methods for the artificial reproduction of pathological conditions of the liver due to its drug damage. We have already developed a method for modeling drug hepatopathology by introducing a non-steroidal anti-inflammatory drug diclofenac sodium into the body of rats, which provokes the development of toxic hepatitis [18].

The goal of this work was to artificially reproduce in laboratory rats the acute form of toxic hepatodystrophy without drastic changes in the clinical condition, which, according to the complex of general clinical, biochemical, morphological and pathological changes, corresponds to the spontaneous course of this hepatopathology in animals but does not lead to death.

For carrying out experimental studies, white laboratory rats (males) of 3 months of age with a body weight of 200–220 g were used, from which the experimental and control groups of twenty individuals each were formed. Prior to the experiment, rats were quarantined with clinical examination for two weeks. The animals were kept on a balanced diet containing all the necessary biologically active and nutrients. They had free access to feed and drinking water. The changes in body weight and feed intake were monitored by research animals. The duration of the experiment was 7 days.

The acute form of fatty hepatosis in laboratory rats was induced by intragastric administration with a probe of 4% tetracycline hydrochloride solution included in the group of antibacterial agents for systemic use (bacteriostatic antibiotic of the tetracycline group of a wide spectrum of action) at a dose of 250 mg/kg body weight, once a day for 7 days. We observed changes in the general clinical condition of the animals. Intact animals, which were orally administered an equivalent volume of distilled water, were assigned to the control group.

Regular administration of tetracycline hydrochloride to rats for seven days was accompanied by the appearance of pronounced clinical symptoms of fatty hepatosis, which began to appear in animals already on the second or third day of its administration and was characterized by depression of the general condition, decreased appetite, increased or decreased thirst, stable decrease in average body weight for a group of 10–15 g, dull coat, decreased elasticity and dry skin, hair loss, enlargement and soreness of the abdomen, rarefaction feces. No deaths were noted.

The clinical manifestations of fatty hepatosis supplement the results of pathological studies of the liver: light yellow or have a mottled mosaic pattern: brown-red areas alternate with yellow, slightly enlarged, rounded edges, flabby consistency. Histomorphological examination shows decomposition of hepatocytes, diffuse placement of fat droplets of various sizes (small-, medium- and large-droplet) in the liver cells, focal histiolymphoid infiltration, vasodilation and blood overflow. Other dystrophic changes (except for obesity) are mild. In some places, small Kupffer cell proliferates are found. Changes in the portal tracts were expressed in sclerosis with a slight thickening and inflammatory reaction – the appearance of small, local histiocytic and lymphoid elements.

In the study of biochemical parameters of blood plasma in rat fatty hepatosis patients (Table 3.1), a significant increase in activity was observed relative to liver-specific enzymes: ALT – 3 times, AST – 81%, alkaline phosphatase – 60% and GGT 73% with a simultaneous decrease in the content of total protein – by 11%, an increase in the concentration of total bilirubin by 15 times due to the conjugated fraction, the level of which increased by 19 times compared to the control (intact animals), which indicates destructive changes in hepatocytes, a decrease in the intensity of the intensity of protein synthesizing processes, impaired pigment metabolism and the development of intrahepatic cholestasis.

Table. 3.1. Biochemical parameters of blood plasma of rats of experimental fatty hepatosis (M ± m, n = 20)

Indicator	Control group	Experimental group
Total protein g/l	68.0±0.2	60.9±0.1*
Total bilirubin, μM	3.0±0.2	45.5±3.0*
Conjugated bilirubin, μM	1.46±0.24	27.34±0.83*
Alanine-transaminase, IU	0.24±0.03	0.72±0.03*
Aspartate-transaminase, IU	0.70±0.01	1.27±0.05*
Alkaline phosphatase, IU	0.25±0.01	0.40±0.01*
γ-Glutamyl-transpeptidase, IU	12.33±0.69	21.33±1.11*

Note. * - p <0.05, the results are likely compared with the values in the control group of rats.

The method of modeling fatty hepatosis provides artificial reproduction in laboratory rats of an acute form of fatty hepatosis with pronounced clinical, pathological, anatomical and biochemical changes characteristic of the development of fatty degeneration of the liver parenchyma, which can be used in veterinary medicine to study the characteristics of ultrastructural and metabolic changes in the animal body with the development of hepatopathology, as well as for the clinical trial of new drugs and the introduction of effective effective treatment regimens for liver diseases.

3.2 Milk phospholipids and fatty liver

The experiment involved 60 white laboratory male rats of the Wistar strain with a body weight of 200–220 g, which were selected according to the analogue principle [19]. Rats were separately housed in cages. Prior to the experiment, they were quarantined with a clinical examination for two weeks. The animals were kept on a balanced diet containing all the necessary biologically active and nutrients. They had free access to feed and drinking water. During quarantine, changes in body weight and animal feed were monitored.

For research, three groups of rats were formed (control and two experimental), 20 animals each. In accordance with the existing criteria for standardizing and the quality of biological experiments and the principles of biomodelling [19], rats of the first and second research groups for modeling the acute form of fatty hepatosis using our modified method [20] for 7 days using a probe intragastrically introduced a suspension of tetracycline hydrochloride in 4% starch gel solution once a day at a dose of 250 mg/kg of animal body weight, which was determined by their daily weighing. This helped to maintain the toxic effect of tetracycline hydrochloride on rat liver during the experiment. Animals of the control group were injected with a similar volume of bidistilled water.

During the experiment, the animals of the first experimental group were left without treatment (self-rehabilitation), and the animals of the second experimental group were intragastrically injected with a 1% solution of phospholipids and dietary supplements "FLP-MD" in liposome form at a dose of 13.5 mg/kg body weight one hour before the use of tetracycline and in the next 2 days after completion of the seed. Bioadditive "FLP-MD" is the author's development [21] and contains a complex of various classes of milk phospholipids (nipples), which have a fatty acid spectrum natural for animal membranes, a mixture of unsaturated fatty acids (oleic, linoleic, linolenic) and antioxidants (vitamins A and E).

The duration of the experiment was 9 days. The selection of biological material in rats was performed on the 10th day of the experiment under ether anesthesia.

Blood for hematological studies was taken from the abdominal aorta of rats. For biochemical studies, heparin stabilized whole blood was used. Hemoglobin content was determined in heparin-stabilized blood using a Micro CC-20 Plus Auto HTI analyzer (USA) according to the instructions to the last. In plasma, the content of total protein, albumin, total and conjugated bilirubin, creatinine, urea, glucose was studied: enzymes activity: aspartate-transaminase (AST, EC 2.6.1.1), alanine-transaminase (ALT, EC 2.6.1.2), γ-glutamyl-transpeptidase (GGT, EC 2.3.2.2), α-amylase (α-AM, EC 3.2.1.1) electrolyte concentration (Potassium, Phosphorus, Calcium) using a GBG Stat Fax 1904 Plus semi-automatic biochemical analyzer (Awareness Technology, Inc., Florida, USA) open type using reagents DAC-SPECTROMED SRL (Moldova).

Modeling in rats of research groups of tetracycline-induced fatty hepatosis is accompanied by functional-biochemical changes at the level of the whole organism and the liver in particular. So, in sick animals of the first experimental group, a slight increase in the total protein content in the blood plasma was found (by 10.4%), while the albumin level remained at the control level (Table 3.2). This suggests that quantitative changes in the organism of rats of research groups involve proteins of globulin fractions, which perform homeostatic, protective, transport, regulatory, immune and other numerous functions [22]. Such changes indicate the intensive development of compensatory-adaptive processes in animals with experimental fatty hepatosis aimed at maintaining homeostasis of the internal environment of the body through the active participation of the liver in the intermediate exchange of proteins.

An increase in the blood plasma of rats of the first experimental group of the activity of AST and ALT by 4.0 and 1.7 times, respectively (Table 3.2) indicates an

Table 3.2. Biochemical parameters of native blood/plasma of rats during experimental hepatosis with the introduction of tetracycline and with the use of corrective therapy (M ± m, n = 20)

Indicator	Control group	The first experimental group (self-rehabilitation)	The second experimental group (dietary supplement therapy "FLP-MD")
Total protein, g/l	66.7±2.10	73.6±2.20*	71.9±3.01
Albumin, g/l	37.5±1.5	34.2±1.3	40.5±1.6
Alanine-transaminase, IU	97.6±6.9	162.3±10.3*	137.3±7.1*
Aspartate-transaminase, IU	90.3±3.6	361.9±14.8*	178.2±8.2*
AST/ALT ratio	0.93±0.01	2.23±0.08*	1.30±0.02*
Creatinine, μM	40.40±2.11	47.71±2.23*	42.30±2.04
Urea, мM	6.9±0.3	13.9±0.4*	6.8±0.2
Hemoglobin, g/l	184.5±12.4	156.0±6.1*	310.5±20.2*
Total bilirubin, μM	3.1±0.2	54.4±2.1*	7.3±0.6*
Conjugated bilirubin, μM	1.43±0.24	27.22±0.84*	3.02±0.23*
Glucose, mM	6.40±0.13	6.60±0.07	7.1±0.09*
γ-Glutamyl-transpeptidase, IU	6.9±0.4	7.1±0.2	7.0±0.4
α-Amylase, IU	609.6±22.4	649.7±18.2	446.5±16.3*
K^+, mM	3.54±0.18	6.30±0.21*	3.70±0.16
Ca^{2+}, mM	2.03±0.10	4.37±0.23*	2.58±0.06*
Pinorganic, mM	3.35±0.24	4.05±0.12*	1.85±0.10*

Note: * - p <0.05 compared with the values of the control group of rats.

increase in the intensity of intermediate amino acid metabolism at the liver level and an increase in the role of transamination and deamination in the development of fatty hepatosis. This partially compensates for the deficiency of energy substrates characteristic of this pathology in hepatocytes.

At the same time, a 2.4-fold increase in the AST/ALT ratio in the blood plasma of rats of the first experimental group in relation to the control indicates mitochondrial cytopathy and structurally functional disorders of the cells are interconnected with it. An increase in the blood concentration of the main end products of nitrogen metabolism, urea, by 2.0 times and creatinine by 18.1% (Table 3.2) in the blood of these sick animals, in accordance with changes in the activity of aminotransferases, confirms the tendency to intensify deamination processes in the tissues and, at the same time, may indicate the development of secondary nephropathy. As a rule, this leads to general intoxication of the organism of sick animals with the end products of nitrogen metabolism.

At the same time, studies of biochemical parameters of blood plasma in rats of the second experimental group indicate a positive effect of milk phospholipids in the form of liposomal dietary supplement “FLP-MD” on protein metabolism (Table 3.2). So, the content of total protein and albumin corresponded to the boundaries of control values (Table 3.2). A tendency towards a decrease in the activity of AST and ALT was established, although the indicators were still quite high compared with the control: 1.7 and 1.4 times, respectively. The value of the ratio of the ratio AST/ALT also decreased, however, relative to the control, it remained 1.4 times higher.

Despite the increased activity of AST, ALT and the magnitude of their ratio, the obtained data indicate a gradual normalization of the activity of aminotransferases in the blood plasma of sick rats using dietary supplements "FLP-MD" in liposome form. In addition, the normalization of urea and creatinine was recorded in the blood of rats of this group (Table 3.2).

The development of experimental hepatosis negatively affects the exchange of hemoglobin, which undergoes significant changes in animals of the first experimental group. As a result, hypochromia is noted (a 15.4% decrease in hemoglobin content compared with the control) and the development of anemia (Table 3.2). The manifestation of the latter may be the result of a decrease in the hematopoietic activity of the red bone marrow, an increase in hemolytic processes due to the negative effect of toxins on the structural organization of the erythrocyte membrane systems in the bloodstream, and the induction of the pathological process in the liver parenchyma, which is an optional organ of hematopoiesis. As a result, the development of hyperbilirubinemia in the blood plasma of animals of this group (total bilirubin grows 17.6 times compared with the control) against the background of an increase in the level of its conjugated and unconjugated forms indicates a violation of the pigment function of the liver.

In rats of the second experimental group, when applying the dietary supplement “FLP-MD” in liposomal form, the blood hemoglobin level even exceeds the control 1.7 times, which is probably the result of compensatory mobilization of red blood cells from the corresponding depot. Indicators of pigment metabolism also have elevated values, but they are already much closer to the level of control (Table 3.2).

An increase in the concentration of glucose in the blood plasma of rats of the second experimental blood was found to be 11% compared with the control, which indicates the mobilization of glucose by the body to maintain energy balance and accelerate recovery processes in the tissues.

Along with this, the absence of significant changes in the blood plasma of rats of the first experimental group of GGTP and α-AM activity excludes the development of intrahepatic cholestasis and pancreatic complications (Table 3.2). A decrease in the activity of α-AM by 26.8% in the blood plasma of rats of the second experimental group as compared with the control may indicate metabolic changes in the pancreas, which accordingly cause the above-described changes in the concentration of glucose in blood plasma.

The acute course of fatty hepatosis in rats of the first experimental group is characterized by an increase in plasma intracellular macronutrients, in particular potassium by 1.8 times, calcium by 2.2 times and phosphorus by 1.2 times, which may negatively affect intracellular metabolism (Table 3.2). At the same time, mobilization of calcium and phosphorus from the main place of their deposition – bone tissue can lead to the development of secondary osteopathy, which will obviously enhance the negative consequences of anemia, which was diagnosed in animals of the first experimental group for self-rehabilitation. Moreover, a certain deviation from the normal content of calcium and phosphorus (respectively, an increase of 27% and a decrease of 45%) in the blood plasma of rats of the second experimental group indicates a gradual nature of the restoration of the structural organization of bone tissue even with the use of corrective therapy.

CONCLUSION

When modeling tetracycline-induced fatty hepatosis, significant destructive changes in the cell membranes of hepatocytes, including mitochondrial ones, were ascertained, as evidenced by the increased activity of aminotransferases and their ratio, inhibition of the protein synthesizing function of the liver. At the same time, an increase in the content of creatinine, urea and total bilirubin was revealed in the blood plasma. The use of milk phospholipids in the form of a 1% solution of liposomal dietary supplement “FLP-MD” with a pronounced membrane-tropic and reparative effect on damaged liver cells stimulates the restoration of metabolic disorders, especially proteins. The use of this dietary supplement prevents the development of possible complications - anemia, nephro- and osteopathy, parenchymal jaundice and intoxication of the body. The obtained results allow us to consider the liposomal dietary supplement “FLP-MD” based on milk phospholipids as a means for the prevention and pharmacological correction of drug-induced fatty hepatosis.

CHAPTER 4
CORRECTIVE EFFICIENCY OF MILK PHOSPHOLIPIDES AT EXPERIMENTAL IMMUNO DEFICIENCY

Exogenous and endogenous substances, which are capable of influencing the development of the immune response at any stage, are immunotropic. Depending on the mode of action, they can stimulate the immune response, enhance or weaken its final course or intermediate stage, as well as completely or partially suppress the immune response [1–3].

Now more than 40 different biologically active substances are isolated from the thymus tissue and from blood serum, which are believed to be produced mainly by the gland epithelial cells. These substances most often refer to polypeptides, although some of them are glycoproteins or steroid compounds. Of all the substances obtained, only 4–8 polypeptides meet the criteria of hormones and they are regarded as potential thymic hormones. One of them is thymic serum factor (TSF) – a real hormone of the gland, is secreted by its epithelium, which Bach et al. (1973) discovered the ability to restore the sensitivity of splenocytes of thymusectomized mice to antilymphocytic serum or azathioprine. However, TSF only in the presence of Zn^{2+} becomes biologically active and in such a composition is known as thymulin, a hormone that circulates in the blood. This hormone regulates the maturation and differentiation of T-lymphocytes. It is known that injections of some immunomodulators (thymostimulin, levamisole, splenin) with thimectomized animals induce the appearance in the blood serum of substances with thymosin-like activity – RTPA [4–8].

Almost all nosological forms of diseases are accompanied by disorders of the immune system and the development of secondary immunodeficiencies [9–13]. It has already been shown earlier that created at the Department of Animal Biochemistry and Physiology Academician M. F. Gulyi of the National University of Life And Environmental Sciences of Ukraine based on milk phospholipids BAA “FLP-MD” is not inferior in its reparative properties to the well-known phospholipid-containing drug Essentiale-forte and at the same time helps to restore the immune system during experimental gastroenteropathology of mice [10]. Therefore, the study of the effect of dietary supplements "FLP-MD" on the endocrine function of the thymus and indicators characterizing the state of the body's immune system as a whole is very relevant for a more complete understanding of the mechanisms of the immunotropic effect of phospholipid-acceptable bioadditives. Based on this, the effect of dietary supplement “FLP-MD” on the endocrine function of the thymus and indicators characterizing the state of the immune and hematopoietic systems in intact animals and under conditions of experimental immunodeficiency were studied.

The studies were conducted on laboratory mice of the CBA line obtained from healthy individuals of the vivarium of the Institute of Oncology of the Academy of Medical Sciences of Ukraine. Used laboratory mice that have reached puberty at the beginning of the experiment. The experiment involved 120 healthy individuals (females) with a body weight of 18–20 g.

We studied the effect of the liposomal form of dietary supplement “FLP-MD” on the endocrine function of the thymus, immunological and hematological parameters in animals with experimental immunodeficiency.

To simulate experimental immunosuppression, CBA mice were intraperitoneally injected with cyclophosphamide (CF, Orion Corporation Farmos, Finland) at a dose of 200 mg/kg body weight.

For research, a therapeutic dose of a 0.6–1.0% solution of the liposomal form of dietary supplement “FLP-MD” was used, which was administered orally to animals at the rate of 1.0–1.5 ml/kg body weight (20–30 μl per animal), one once a day, daily, for 30 days.

For the study, animal research groups were completed from 12 female mice, which were placed in cages. Animals were attracted to the experiment on the 3rd, 8th, 15th and 30th days after cyclophosphamide injection. Mice were weighed, decapitated under ether anesthesia, blood was taken for examination and organs (thymus, spleen) were weighed. The absolute mass of lymphoid organs, thymic and splenic indices were determined: the ratio of organ mass (mg) to body weight (g); the number of leukocytes was counted and the peripheral blood leukogram was studied; determined the absolute and relative number of lymphoid cells in the thymus and spleen. The content of T-lymphocytes in the peripheral blood of animals was determined by the method of spontaneous rosette formation (E-ROCK), which is based on the presence of receptors on the surface of T-cells in mice to rabbit erythrocytes. In Romanovsky stained smears, the number of large granular lymphocytes (LGL), which are a morphological substrate of NK- and K- cells, was determined. The endocrine function of the thymus, which was evaluated by the TSF titer,was determined by the Bach et al. (1973).

The study of the level of spontaneous apoptosis and proliferation of lymphoid cells of the organs of the immune system was carried out using a flow cytometric method on a FACScan instrument (Becton Dickinson, USA) equipped with an argon laser with a wavelength of 488 nm, using the CellQuest program for Mac computers. The proliferative activity index (PAI) was determined by dividing the indicator of the relative number of cells of the proliferative pool in the case of spontaneous proliferation by the indicator of the relative number of cells in a state of apoptosis. To assess the fractional content of cells in the main phases of the mitotic cycle (G1 / 0, S, G2 + M), distribution histograms were processed using the specialized mathematical program Mod Fit LT 2.0 (BDIS, USA) for Mac computers.

For research, 4 groups of mice were formed: 1 group – intact (control group) 2 group – intact animals that received the liposomal form of dietary supplement “FLP-MD”; Group 3 – animals with experimental immunodeficiency; Group 4 – animals with experimental immunodeficiency receiving the liposomal form of dietary supplement “FLP-MD”.

The composition of dietary supplements "FLP-MD". The liposomal form of dietary supplement “FLP-MD” contains a complex of various classes of phospholipids isolated from milk (butterdish), which contain a natural fatty acid spectrum for animals, a complex of unsaturated fatty acids (oleic, linoleic, linolenic) and antioxidants (vitamins A and E).

The phospholipids of the liposomal form of dietary supplement "FLP-MD" in chemical structure and physico-chemical properties are as close as possible to the lipid component of the cell membranes of the animal organism, which contributes to their better absorption.

The results of a study of the effect of the liposomal form of dietary supplement “FLP-MD” on the general condition of the organs of the immune system of CBA mice with experimental immunodeficiency are presented in Table. 4.1. As can be seen from these data, the introduction of CF causes a significant decrease in the mass of the thymus and spleen in mice. So, on the 3rd day after the injection of CF, the thymus decreases by 3.6, and the spleen – by 1.3 times. In mice of the control group receiving only CF, a gradual restoration of these parameters occurs and after 30 days they no longer differ from those in intact animals. The change in the mass of lymphoid organs in this group of animals is also reflected in their indices, especially the thymic ones (Table 4.1). The administration of the liposomal form of dietary supplement “FLP-MD” to intact animals did not change the studied parameters. In animals treated with dietary supplements "FLP-MD" and CF, there is also a decrease in the mass of the thymus on the 3–8th day of observation, but after 15 days it does not differ from that in the control group. Unlike the thymus, the spleen mass of animals of this group does not change and remains constant throughout the experiment.

The introduction of CF also affected the number of cells in the lymphoid organs (Table 4.2). At the beginning of the experiment (day 3), a significant decrease in the relative and absolute numbers of both thymocytes and splenocytes is observed. A reduced number of lymphoid cells in the thymus was detected throughout the experiment, while in the spleen, their recovery occurs already on the 15th day of observation. The introduction of the liposomal form of dietary supplement "FLP-MD" does not significantly affect the thymus cellularity, but increases the number of splenocytes in animals of the experimental group on days 3–8, $p<0.05$.

Table 4.1. The effect of the use of the liposomal form of dietary supplement "FLP-MD" on the general condition of immunogenesis organs in CBA mice after the introduction of cyclophosphamide into the body

Observation time, days	Indicator				
	Weight			Index	
	Body, g	Thymus, mg	Spleen, mg	Thymus	Spleen
Intact animals					
	21.84±1.90	65.40±3.75	114.0±6.50	3,09±0,30	5,38±0,57
Animals that received dietary supplement «FLP-MD» (before introduction CF)					
	21.18±0.92	61.40±6.40	102.20±12.50	2.94±0.33	4.82±0.53
Animals that received CF					
3	21.95±2.06	18.00±4.81*^{,,0}	85.00±5.80*	0.93±0.34^{*,0}	3.78±0.23^{*,0}
8	23.00±0.49	38.01±5.96*^{,0}	115.00±7.40	1.05±0.25^{*,0}	5.06±0.77
15	22.90±1.05	45.20±6.22*	153.60±1.99	1.94±0.22^{*,0}	6.86±1.20
30	23.05±1.07	56.25±11.64	146.30±24.80	2.40±0,40	6.38±1.44
Animals that received CF + dietary supplement «FLP-MD»					
3	23.32±0.95	22.50±4.63^{*,0}	148.00±27.60^{1}	0.99±0.25^{*0}	6.31±0.12
8	23.40±1.29	34.20±3.22^{*,0}	148.00±17.76	1.49±0.19^{*,0}	6.32±0.27
15	22.50±0.75	55.20±2.95	154.40±28.40	2.46±0.14	6.90±1.38
30	23.15±1.30	56.75±7.33	135.0±27.9	1.54±0.23	6.10±1.62

Note: * - the difference is statistically probable in comparison with the group of intact animals, p <0.05;

º - the difference is statistically probable in comparison with the group of animals that received dietary supplement "FLP-MD", p <0.05;

¹ - the difference is statistically probable in comparison with the group of animals that received cyclophosphamide, p <0.05.

Table 4.2. The effect of the liposomal form of dietary supplement "FLP-MD" on the number of lymphoid cells in the thymus and spleen of CBA mice after cyclophosphamide administration

Group animals	Day observa-tions	The number of cells in			
		thymus		spleen	
		10^6	10^6/mg	10^6	10^6/mg
Intact		156.00±15.06	2.42±0.27	78.08±7.27	0.68±0.04
+ Dietary supplement «FLP-MD»		139.20±23,70	2.26±0.22	66.90±7.28	0.69±0.11
+CF	3	16.50±7.65^{xo}	0.77±0.32^{xo}	44.90±10.90^{x}	0.29±0.03^{xo}
	8	77.00±14.39^{xo}	2.10±0.35	31.68±12.66^{xo}	9.67±0.06
	15	38.60±5.68^{xo}	0.94±0.25^{xo}	72.40±5.56	0.48±0.05
	30	37.00±8.66^{xo}	0.94±0.09^{xo}	68.88±14.65	0.48±0.03^{x}
+CF + Dietary supplement «FLP-MD»	3	14.00±3.60^{xo}	0.69±0.20^{xo}	76.00±2.16 ^{1}	0.53±0.03^{x}
	8	104.80±13.76^{x}	2.19±0.56	60.26±5.79^{1}	0.54±0.08
	15	38.60±5.67^{xo}	0.94±0.25^{xo}	72.40±5.76	0.48±0.05^{x}
	30	40.00±4.55^{xo}	0.77±0.14^{xo}	61.22±13.89	0.63±0.10

Notes: * - the difference is statistically significant compared with the group of intact animals, $p < 0.05$;

^{o} - the difference is statistically significant compared with the group of animals treated with dietary supplement "FLP-MD", $p < 0.05$;

^{1} - the difference is statistically significant compared with the group of animals treated with CF, $p < 0.05$.

The introduction of the liposomal form of dietary supplement "FLP-MD" to intact animals does not cause changes in the studied parameters.

Thus, the oral administration of the liposomal form of dietary supplement “FLP-MD” at a therapeutic dose does not change the general condition of the studied organs of the immune system in intact mice, and in animals with experimental immunodeficiency, in which significant changes in these organs occurred under the influence of cytostatic, contributes to a faster restoration of thymus mass with an increase in the thymic index and preservation of the spleen mass at the level of intact animals during the entire observation period.

The data on the effect of the liposomal form of dietary supplement "FLP-MD" on the endocrine function of the thymus of mice treated with CF are presented in Table. 4.3.

It has been established that the titer of TSF in intact animals is 1:8–1:16, and the introduction of such animals the liposome form of dietary supplement "FLP-MD" enhances the endocrine function of the thymus by 2 times. CF causes inhibition of the endocrine function of the gland, as a result of which its hormone is not determined on the 3rd day after injection in the circulation.

Table 4.3. TSF titer * in animals of different groups

Group animals	Day after cyclophosphamide administration			
	3	8	15	30
	TSF, Log $_2$ titer			
+ CF	0	1:2	1:4	1:4
+ CF + Dietary supplement «FLP-MD»	1:8	1:16	1:32	1:32

Notes: * TSF was determined in a pool of sera from 5-6 animals; The TSF titer in intact animals is 1:16, in normal animals receiving the liposomal form of dietary supplement "FLP-MD", 1:64.

In the future, a gradual restoration of the endocrine function of the thymus occurs, but even after 30 days the level of its hormones remains 1.5–2.0 times lower than that of intact animals. The introduction of the liposomal form of "FLP-MD" does not significantly affect the thymus cellularity, but increases the number of splenocytes in animals of the experimental group on days 3–8, $p < 0.05$. The introduction of the liposomal form of dietary supplement "FLP-MD" to intact animals does not cause changes in the studied parameters.

Thus, the oral administration of the liposomal form of dietary supplement

"FLP-MD" at a therapeutic dose does not change the general condition of the studied organs of the immune system in intact mice, while in animals with experimental immunodeficiency it contributes to the preservation of the endocrine function of the thymus during the entire observation period at the level of intact mice.

Since the thymus hormones play an important role in the regulation of maturation and differentiation of T-lymphocytes, we studied the effect of the liposomal form of dietary supplements "FLP-MD" on their number in mice of the CBA line with immunodeficiency. The experimental data are presented in Table. 4.4.

Table 4.4. The effect of the liposome form of dietary supplement "FLP-MD" on the number of T-lymphocytes and LGL in the peripheral blood of CBA mice treated with cyclophosphamide

Group animals	Units	The term after the introduction of cyclophosphamide, day			
		3	8	15	30
T-lymphocytes (E-ROCK)					
Intact	%	9.20± 1.01	-	-	-
	x10^9/l	0.377± 0.084	-	-	-
+ Dietary supplement «FLP-MD»	%	8.20± 1.49	-	-	-
	x10^9/l	0.335± 0.013	-	-	-
+ CF	%	7.630± 0.076	9.75± 1.50	9.00± 0.90	9.88± 0.49
	x10^9/l	0.076± 0.019^{o}	0.213± 0.024^{xo}	0,213± 0,025^{xo}	0,390± 0,098
+ CF + Dietary supplement «FLP-MD»	%	6.63± 0.69	10.50± 1.50	8.40± 0.60	7.75± 1.18
	x10^9/l	0.093± 0.003^{xo}	0.383± 0.084	0.348± 0.055	0.363± 0.063
LGL					
Intact	%	1.38± 0.38	-	-	-
	x10^9/l	0.039± 0.090	± -	-	-
+ Dietary supplement	%	2.50± 0.87	-	-	-

«FLP-MD»	x10⁹/l	0.057± 0.018	-	-	-
+ CF	%	1.38± 0.38	1.63± 0.24	2.30± 0.77	1.13± 0.31
	x10⁹/l	0.039± 0.003	0.112± 0.030	0.092± 0.019	0.075± 0.028
+ CF + Dietary supplement «FLP-MD»	%	2.50± 0.87	2.40± 0.19[1]	3.10± 0.89	2.25± 0.48
	x10⁹/l	0.056± 0.018	0.175± 0.013	0.172± 0.032[1]	0165± 0.030

Notes: * - the difference is statistically significant compared with the group of intact animals, $p < 0.05$; º - the difference is statistically significant compared with the group of animals receiving the liposomal form of dietary supplement "FLP-MD", $p < 0.05$.

It was found that the introduction of CF leads to a decrease in the number of T-cells in the peripheral blood of animals. The absolute number of these cells significantly decreases during 3–15 days of observation, and only on the 30th day their number in intact animals increases. The restoration of the absolute amount of E-ROCK under the influence of the liposomal form of dietary supplement “FLP-MD” occurs much faster (already on the 8th day). The use of dietary supplements "FLP-MD" also contributes to the maintenance of the amount of LGL at a higher level in animals during the entire observation period. It was found that the introduction of CF leads to a decrease in the number of T cells in the peripheral blood of animals. The absolute number of these cells significantly decreases during 3–15 days of observation, and only on the 30th day their number in intact animals increases. The restoration of the absolute amount of E-ROCK under the influence of the liposomal form of dietary supplement “FLP-MD” occurs much faster (already on the 8th day). The use of dietary supplements "FLP-MD" also contributes to the maintenance of the amount of BGL at a higher level in animals during the entire observation period.

Thus, the introduction of dietary supplements “FLP-MD” enhances the endocrine function of the thymus in normal conditions and in conditions of immunodeficiency, contributes to a more rapid restoration of the number of T-lymphocytes and an increase in the number of BGL in mice with experimental immunodeficiency.

In addition, the effect of the liposomal form of dietary supplement “FLP-MD” on the hematological parameters of animals with experimental immunodeficiency was studied. It was found that the introduction of intact animals BAA "FLP-MD" does not cause quantitative changes in these indicators. Under experimental immunodeficiency, the number of red blood cells on the 3rd day after the introduction of CF is not significantly different from that in intact animals and those that received only the liposomal form of dietary supplement “FLP-MD”. In the group of animals that were administered CF and dietary supplements "FLP-MD", this indicator is characterized by a high level throughout the experiment.

In CBA mice, under the influence of CF, there is a decrease in the total number of leukocytes and a shift in the leukogram, which is characterized by an increase in the number of segmented granulocytes and monocytes due to a decrease in the number of lymphoid cells in the peripheral blood. Oral administration of the liposomal form of dietary supplement “FLP-MD” to such animals helps to restore the leukogram in mice with experimental immunodeficiency and does not cause its changes in intact animals.

The study of the effect of the liposomal form of dietary supplement "FLP-MD" on apoptosis and proliferative activity of lymphoid cells of the thymus and spleen is presented in Table. 4.5. Thus, the introduction of CF is accompanied by a slight increase in the proliferative activity of thymocytes on the 3rd day of observation and a decrease in the number of cells in the apoptosis stage on the 8th day after the administration of CF. The proliferative activity index was characterized by values ranging from 1.83±0.38 to 4.31±1.33. At the same time, in the group of animals with experimental immunodeficiency, the proportion of proliferating splenocytes

4.5. The influence of dietary supplements "FLP-Table MD" on the course of proliferation and apoptosis of lymphoid cells of the thymus and spleen in experimental mice

Group animals	Observation time, days	Thymocytes			Splenocytes		
		% of cells that are at the stage of			% of cells that are at the stage of		
		apoptosis	proliferation	PAI	apoptosis	proliferation	PAI
Intact		12.67±2.06	21.79±5.90	1.71±0.33	3.62±0.38	26.85±1.51	7.72±0.84
+dietary supplement «FLP MD»		14.45±2.29	19.31±1.64	1.60±0.32	4.42±0.24	26.56±0.49	6.34±0.39
+CF	3	8.52±1.93	29.37±3.51^{0}	4.31±1.33	11.89±1.40^{*0}	22.45±3.77	1.98±0.38^{*0}
	8	6.55±0.87^{0}	25.32±3.32	4.06±0.69^{0}	10.74±1.370	22.85±1.29	2.61±0.45^{*0}
	15	10.00±1.12	16.80±1.52	1.83±0.38	7.24±0.59*	21.90±0.62*	3.21±0.32^{*0}
	30	5.35±1.67^{*0}	28.04±1.24^{0}	4.92±1.94	3.76±0.50	29.16±1.99	8.03±0.14^{0}
+CF + dietary supplement «FLP MD»	3	4.07±0.65^{*0}	27.97±0.11^{0}	7.32±1.11^{0}	4.74±0.38^{1}	14.41±1.85^{*0}	3.02±0.22^{*01}
	8	7.19±0.51^{0}	23.73±1.51	3.33±0.34^{0}	9.56±1.12^{*0}	22.96±2.37	2.59±0.52^{*0}
	15	14.61±1.44	16.39±1.85	1.19±0.23	6.14±0.59^{*0}	24.87±1.17	4.08±0.39^{*0}
	30	6.42±1.10^{0}	22.64±2.32	4.36±1.63	8.44±3.19	30.86±1.24^{0}	4.85±1.06^{*1}

Note: * - the difference is statistically probable in comparison with the group of intact animals, $p < 0.05$;

^{o} - the difference is statistically probable in comparison with the group of animals that received dietary supplement "FLP-MD", $p < 0.05$;

^{1} - the difference is statistically probable in comparison with the group of animals that received cyclophosphamide, $p < 0.05$.

decreases and acquires minimal values on the 15th day of observation. At the same time, the level of apoptosis increases by 2–3 times compared with intact animals that received or did not receive the liposomal form of dietary supplement “FLP-MD”. The proliferative activity index is significantly reduced, $p<0.05$. The introduction of the liposomal form of dietary supplements to these animals only affects the number of splenocytes undergoing apoptosis and significantly reduces the value of this indicator to the level in intact animals, as a result of which IPA also decreases (to 3.02±0.22) compared to intact animals. However, its value remains higher in the group of animals with CF on the 3rd day of observation. Thus, the introduction into the animal organism of the liposomal form of dietary supplement “FLP-MD” does not change the course of proliferation and apoptosis in the lymphoid cells of the organs of the immune system of intact animals.

The use of the liposomal form of dietary supplement “FLP-MD” in mice with experimental immunodeficiency inhibits the development of apoptotic processes in the lymphoid cells of the spleen of these animals.

As a result of the studies, it was found that immunodeficiency modeled by the administration of 200 mg/kg CF to mice of the CBA line is accompanied by a decrease in the mass of the thymus and spleen, the number of lymphoid cells in these organs of the immune system, a decrease in the endocrine function of the thymus and the absolute number of T-lymphocytes in the peripheral blood of animals, as well as a change in hematological parameters. Oral administration of the liposomal form of dietary supplement “FLP-MD” to animals with experimental immunodeficiency accelerates the restoration of the mass of the thymus and thymic index and helps to maintain spleen mass at the level of intact animals throughout the observation period, suppresses the level of apoptosis of splenocytes, improves the condition of the T-link of the immune system (for by preserving the endocrine function of the thymus and faster restoration of the number of T-lymphocytes), increases the level of natural killers (the number of LGL) and ensures the normalization of mathematical indicators.

CONCLUSION

One of the reasons for the development of immunodeficiency, especially secondary, is an increase in the permeability of damaged cell membranes, the entry of immunosuppressive substances into the vascular bed, and the release of immune response mediators by immunocompetent cells under their influence. Effective correction of immunological functions and the dependent immune processes can be achieved by introducing compounds into the body of sick animals that can affect the structural and functional condition of cell membranes. From this point of view, the liposomal form of dietary supplement “FLP-MD” was created on the basis of milk phospholipids, its effectiveness was tested when used in animals under experimental immunodeficiency.

It was found that immunodeficiency modeled by the administration of 200 mg / kg of cyclophosphamide to CBA mice is accompanied by a decrease in the mass of the thymus and spleen, the number of lymphoid cells in these organs of the immune system, a decrease in the endocrine function of the thymus and the absolute number of T-lymphocytes in the peripheral blood of animals, as well as a change hematological indicators. Oral administration of the liposomal form of dietary supplement "FLP-MD" based on milk phospholipids to animals with experimental immunodeficiency promotes faster restoration of the thymus and thymic mass in them and preserves the spleen mass at the level of intact mice throughout the observation period, suppresses the level of splenocyte apoptosis, improves the condition T-chains of the immune system (due to the preservation of the endocrine function of the thymus and faster restoration of the number of T-lymphocytes), increases the level of natural killers and stabilizes hematological parameters.

Thus, the complex use of reparative and immunomodulatory agents by means of sick animals prevents the development of immunodeficiency of their body, that is, exhibits a systemic immunomodulatory effect, which accelerates their recovery and shortens the duration of the rehabilitation period in severe diseases of any etiology.

CHAPTER 5
CORRECTING EFFECTIVENESS OF MILK PHOSPHOLIPIDES UNDER ACTION ON ANIMAL ORGANISM OF IONIZING RADIATION

Violation of the body under the influence of various negative factors, including ionizing radiation, one way or another, is associated with a change in the functional state of the cells. Moreover, an increase in the volume and severity of cell damage under the influence of a pathological factor reduces the likelihood of their self-healing [1–4].

The action of many exogenous factors on the body is gradually enhanced as a result of the formation of metabolites that interact with the cell in many ways, from covalent binding to molecules to stimulate peroxidation and decrease the lipid content in the membrane structures of cells [4].

The lipid bilayer of cell membranes performs two main functions – barrier and matrix (structural) [4]. Damage to the cell barrier as a result of a violation of the density of hydrophobic hydrocarbon chains and an increase in the volume of structural defects of the membranes leads to an increase in their permeability to ions and metabolites and, as a result, to disrupt regulation of intracellular processes with significant changes in numerous cell functions.

To date, it has been proven that the use of phospholipid-containing dietary supplement "FLP-MD" in animals under conditions of radiation exposure to the body has a high prophylactic effect. This is due to the membrane-stabilizing properties of its components, as well as the stimulation of antioxidant processes in organs and tissues and the prevention of metabolic status of their body [2, 3].

5.1 Radiation-induced modification of the structural and functional state of cell membranes

The prospect of an irradiated cell – death or preservation of viability is determined not only by the severity of the initial damage, which depends on the radiation dose or its power, but also on the physical condition of the cell, the functional activity of its enzyme systems, the presence of radioprotectors and other factors that determine the development of biological amplification processes or reparation of radiation injuries [5, 8].

It is generally recognized that damage to biomembranes is the initial stage in the development of radiobiological effects. Morphological and functional disorders of cell membranes due to ionizing radiation appear almost immediately after irradiation in both high and relatively low doses [4, 7].

Various reversible and irreversible structural and functional changes in cells and tissues are based on a certain sequence of radiation-induced membrane response, the indicators of which can act as bioindicators of the action of ionizing radiation [8, 10]. The radiation-physical and radiation-chemical processes that occur in different cell membranes are similar in nature - the transition of molecules to an excited state (formation of free radicals), breaking of macromolecules, chemical modification of molecules, etc. The final manifestation of membrane reactions to the action of ionizing radiation depends from the structural features of the membranes and the environment of their environment [11].

It is known that the effect of negative factors on tissues is largely based on the interaction of radicals that are formed as a result of radiolysis of water with biologically important molecules. The processes accompanying the development of oxidative stress can cause damage to a significant number of cellular components. But most often, the main damage to the cells is called lipid peroxidation (LPO) of the membranes and the associated violation of barrier properties and inactivation of membrane-bound enzymes. Membrane damage and subsequent activation of the POL course, an increase in the concentration of lipoperoxides and free UFA, which are inhibitors and disconnectors of oxidation and phosphorylation processes, determine the indirect effects of the action of primary-induced short-lived radicals. It is these effects that play a major role in the development of damaging cell damage, and its long-term effects. In the intracellular medium and cell organelles, the accumulation of lipid peroxidation products – diene conjugates and TBA-active products – takes place [12, 13].

It is believed that in the cooperative system of the protein-lipid matrix of membranes, radiation-induced changes in the functional activity of intracellular organelles are caused by changes in the structure of membrane proteins and the physical condition of the lipid bilayer [13].

The radiosensitivity of cells is associated with the features of their metabolism and is directly proportional to the speed of metabolic reactions. Cells with a high level of metabolic processes and oxidative phosphorylation are more sensitive to radiation than cells that are at rest – in the stationary phase [3].

To understand the radiation effects of various doses, there is an ever more convincing need to take into account the systemic response of cells to radiation: along with the intensification of destructive processes in the cell, the presence of systems in it that control and ensure the restoration of damaged structures. The basic principles of the systemic response of cells to radiation damage were formulated by Yu. B. Kudryashov (2001) and are as follows [1, 3]:

- irradiation of the cell (as well as any "complex biological systems" in general) activates the functions of autoregulation of homeostasis. Their significance lies in the mobilization of compensatory mechanisms designed to prevent damage or activate the restoration of damaged structures and excited dynamic equilibrium of the irradiated system;

- control over the excess accumulation of lipid peroxidation products in the biological membrane is performed by a complex system of cellular protective resources (water and fat-soluble products), which includes enzymatic and non-enzymatic "antiradical", "hypoxic" and "antioxidant" mechanisms, the activity of which depends on the dose and time after irradiation. Changes in the ratio of the content of anti- and prooxidants in the cell characterize the level of the energy-generating background of radioresistance, as well as the degree of cell damage.

The successes of quantitative radiobiology, radiation biochemistry and molecular biology, the analysis of the sequence of physicochemical processes in an irradiated cell - all this can serve as the basis of a general theory that would reveal the mechanisms of action of ionizing radiation on a living cell, which allows a scientifically sound approach to the prevention and treatment of damage. Such an approach should take into account the complex nature of the manifestation of radiobiological effects, the possibility and nature of the development of initial molecular damage to visible final biological effects, and requires a phased study of the mechanisms of development of reparative processes at the cell level with the use of prophylactic and therapeutic agents.

For clinical trials, outbred male rats weighing 180–200 g were used, kept on a standard diet of vivarium. Animals were divided into groups of ten animals each. Group I – control (intact animals) Group II - the animals were orally administered the liposomal form of dietary supplement “FLP-MD”; III and V groups – animals were totally once irradiated with x-rays at a dose of 2.0 Gy; IV and V and groups – animals were given dietary supplements "FLP-MD" for 5 days, and then subjected to x-ray irradiation at a dose of 2.0 Gy. Rats were decapitated after 1 day (groups III and IV of animals) and after 2 days (groups V and VI of animals).

Irradiation was carried out on a RUM-17 installation with a tube under the following conditions: dose rate 0.17 Gy/min, filters 0.5 mm Cu and 1 mm Al, current 10 mA, voltage 200 kV, skin focal length 50 cm. Dose 2 Gy selected taking into account the fact that this dose is sublethal, because $LD_{100/30}$ for rats is 7.78 Gy.

5.2 Phospholipids of milk and ionizing radiation of animals

High biological activity is inherent in ionizing radiation: it can cause excitation and ionization of atoms and molecules of any biological structures, the formation of active radicals and thereby induce long-term reactions in the tissues of living organisms. Therefore, the result of the biological effect of radiation is, as a rule, a violation of biochemical processes with the following morphological and functional changes in the cells and tissues of the body [15–17].

As a result of the studies, it was found that the content in the blood serum of animals of all research groups: total protein and albumin does not significantly change. The concentration of urea and creatinine in the blood serum of experimental animals significantly increases after irradiation at a dose of 2 Gy. So, 2 days after irradiation, the indicated increase is 50 and 27%, respectively. The results obtained may indicate a violation of the excretory function of the kidneys under irradiation conditions. In addition, an increase in the activity of alkaline phosphatase (by 18%) and GGT (by 22%) in the blood serum of these animals on the 2nd day after irradiation was revealed. An increase in the activity of intracellular enzymes in rat blood serum under the action of irradiation may indicate the development of destructive processes in the cells of internal organs.

Preventive administration to animals of the liposomal form of dietary supplement “FLP-MD” prevents significant changes in the parameters of the studied biochemical parameters in the blood serum of irradiated animals. It should be noted that the introduction of the liposomal form of dietary supplement “FLP-MD” to control (healthy) animals does not lead to changes in the biochemical parameters of blood serum, with the exception of a possible increase in the content of total protein, indicates an intensification of protein synthesizing processes in tissues [18, 19].

The main lipid components of the blood are cholesterol (Ch), PL and triacylglycerols (TAG). Serum lipids are mainly found in lipoproteins (LPs), the main function of which is the interorgan transport of lipids and the regulation of lipid metabolism. They provide lipid transport of both exogenous and endogenous origin. High density lipoproteins (HDL) transport cholesterol from cells of peripheral organs to the liver, where it is transformed into bile acids. Low density lipoproteins (LDL) are the particles that are richest in cholesterol, the content of which can reach 45-50%. LDL is the main transport form of cholesterol for the needs of cells of the vascular wall, and under certain pathological conditions it is a source of accumulation in the walls of blood vessels. Very low density lipoproteins (VLDL) are formed in the liver and are the main transport form of endogenous TAG. With this in mind, it is advisable to determine the content of lipids and LP in the blood serum, used for laboratory diagnosis of lipid metabolism disorders.

The results obtained indicate that the content of cholesterol and TAG in the blood serum of experimental rats after irradiation at a dose of 2 Gy does not change significantly: after 1 day, the content of TAG increases on average by 17%, and cholesterol - by 11%; through 2 days, their content even decreases slightly. The introduction of the liposomal form of dietary supplement "FLP-MD" before irradiation reveals a stabilizing effect in relation to these indicators.

An analysis of the results indicates a redistribution of the drug content of various densities under irradiation conditions. First of all, it should be noted that HDL content increases, especially 1 day after exposure to radiation (by 30%). Under the same conditions, the content of VLDL is increased (by 17%), along with an increase in the level of TAG in serum. At the same time, under irradiation conditions there is a decrease in the level of LDL that are involved in the transport of cholesterol (on average by 15-30%). In rats, LP, the lipid transfer function (modification of LP) is low, therefore, they are resistant to the development of atherosclerosis, since it is modified LPs that can provoke the development of atherosclerosis. Since modified LDL is removed from the bloodstream even faster than native LPs, it is possible that this leads to a decrease in LDL. Preventive administration to animals of dietary supplement “FLP-MD” does not lead to a significant difference between these indicators and control values characterizing the formation of lipid homeostasis in their body.

Ionizing radiation is one of the external factors that enhances LP. Under its action, the equilibrium in the system of "free radical processes-antioxidant activity" is disturbed. The chronic effect of ionizing radiation contributes to an increase in the concentration of free radicals and lipid peroxidation products, which causes depletion of the content of endogenous antioxidants and a decrease in the effectiveness of the antioxidant (AO) protection system [1, 20–23].

In experiments on rats, LPO activation was evaluated by the rate of accumulation of TBA-active products in blood serum and liver and intestinal tissues. It was found that 1 day after irradiation of animals at a dose of 2 Gy, the level of TBA-active products in the cells of the small intestine mucosa increases by 68%. 2 days after irradiation of animals at this dose, the accumulation rate of TBA-active products in liver cells increases by 45%, in the cells of the small intestine mucosa by 42% and in blood serum by 71%. An increase in the content of TBA-active products in blood serum is possible both due to the oxidation of blood lipids and as a result of receipt from other tissues. The introduction of dietary supplement “FLP-MD” for 5 days with subsequent irradiation is not accompanied by an increase in the content of TBA-active products in the studied biological material.

The results of determining the activity of superoxide dismutase (SOD) and catalase (C_{at}) indicate that in the period after irradiation, the predominant changes in their activity are observed after 1 day. SOD activity decreases slightly. The most pronounced changes in the activity of SOD are observed in the cells of the mucous membrane of the small intestine (decreased by 18%). At the same time, C_{at} activity under irradiation conditions increases, especially 1 day after irradiation: in liver cells – by 69%, in cells of the small intestine mucosa – by 43% and blood serum – by 200%. 2 days after irradiation, C_{at} activity remains increased only in the cells of the small intestine mucosa – by 43%. The introduction of animal dietary supplements "FLP-MD" before irradiation leads to less pronounced changes in the activity of SOD and C_{at} in the studied biological material.

In addition, the activity of enzymes of the glutathione-dependent AO-system of protection of various organs of rats was investigated. For the liver, a decrease in the content of reduced glutathione (RGL) was found to be 25% after irradiation at a dose of 2 Gy. The activity of glutathione peroxidase (GP) is growing – by 12%. At the same time, the activity of glutathione transferase (GT) decreases slightly under these conditions. In the cells of the mucous membrane of the small intestine, a decrease in the content of RGL is observed (on average by 20% after irradiation), the activity of GP increases by 50%, and the activity of GT almost does not change. Serum RGL levels are reduced by an average of 15% after irradiation. The activity of GP under these conditions is growing – by 24%, the activity of GT is reduced – by 22%. Introduction of dietary supplements “FLP-MD before irradiation prevents changes in the activity of GT and GP in tissues, however, the content of RGL in the studied objects is reduced compared to the control. In addition, under these conditions, the activity of GT and GP of blood serum increases, which may indicate an intensification of the processes of AO-protection.

Analyzing the results obtained, it should be noted that there are violations in the functioning of the prooxidant-antioxidant defense system of the body under irradiation conditions. An increase in the accumulation of TBA-active products in the cells of the mucous membrane of the small intestine, liver and blood serum was established. However, the functioning of the AO-protection system varies in different ways. During the action of ionizing radiation on the body, there is the formation of reactive oxygen compounds, in particular hydrogen peroxide and superoxide radical. However, the activity of SOD, an enzyme that neutralizes superoxide radicals, decreases slightly under these conditions, possibly as a result of damage to its molecule as a result of irradiation. At the same time, Cat activity in all studied objects increases, possibly as a result of the accumulation of hydrogen peroxide molecules after irradiation. The activity of another enzyme involved in the disposal of highly toxic hydroperoxides – GP also increases after irradiation, especially in the cells of the small intestine mucosa and blood serum. The content of reduced GL – compounds, refers to the non-enzymatic AO-protection system decreases after irradiation.

When using the liposomal form of dietary supplement “FLP-MD” before irradiation, there are no significant changes in the indicators characterizing the state of the prooxidant-antioxidant equilibrium: the content of TBA-active products is almost the same as the control values, the activity of Cat and GP increases in the blood and cells of the thin mucous membrane gut, relative to control values, which indicates activation of the AO-defense system. At the same time, a reduced content of RGL, relative to control values, may indicate its mobilization to protect cell structures from exposure to radiation.

Thus, the obtained results allow us to recommend the specified liposomal form of dietary supplements "FLP-MD" as a means of medical protection of animals under conditions of ionizing radiation.

CONCLUSION

The study of the radioprotective properties of the liposomal form of dietary supplement "FLP-MD" was carried out in in vivo experiments under the action of ionizing radiation.

Studies of biochemical parameters of blood serum, epithelial cells of the liver and small intestine of rats indicate the damaging effect (the occurrence of destructive processes in cells) of ionizing radiation on the cellular structures of the animal organism under experimental conditions. Preventive administration to animals of the liposomal form of dietary supplement "FLP-MD" based on milk phospholipids is effective because it leads to less significant deviations in the values of the studied biochemical parameters of blood serum, liver and small intestine relative to control values.

The intensification of the processes of free radical oxidation and the disruption of the functioning of the antioxidant defense system of animals for the effects of ionizing radiation are established. It was shown that the liposomal form of dietary supplement “FLP-MD” prevents the spread and intensification of oxidative processes in rats under the action of ionizing radiation. First of all, this is due to the composition of the dietary supplement “FLP-MD”, since its main component is phospholipids, which, on the one hand, undergo oxidation due to the action of oxygen radicals and, on the other hand, stabilize cell membranes. The liposomal form of dietary supplement “FLP-MD” also includes vitamins with membrane-active and antioxidant properties.

The results of the studies indicate the effectiveness of the use of the liposomal form of dietary supplement "FLP-MD" to stabilize the lipid fraction of blood serum, restore the intensity of oxidative processes and improve the functional activity of membranes under irradiation conditions.

Thus, application to animals within five days before the start of their x-ray irradiation of the liposomal form of dietary supplements "FLP-MD" with the original combination of lipids, mainly phospholipids, which correspond to membrane in their fatty acid spectrum, obtained from natural and cheap raw materials (oiler) animal cell lipids, a mixture of mono- and polyunsaturated fatty acids derived from linseed oil, and fat-soluble vitamins – α-tocopherol and retinol acetate, stimulates the development of reparative processes in damaged tissues and restores the metabolic status of the organism of animals exposed to radiation, and also has a stabilizing effect on lipid metabolism and pro-antioxidant balance.

CHAPTER 6
CORRECTING EFFICIENCY OF MILK PHOSPHOLIPIDES UNDER CONDITIONS OF ANIMAL POISONING BY CADMIUM

Today, due to the intensive development of industry, the environment has significantly increased the content of heavy metals. Among toxic metals - cadmium, mercury, arsenic, lead, included in the group of highly dangerous ecotoxicants. The wide distribution of cadmium in minerals, along with its use in industrial production, determines the gradual increase in the content of this element in the environment (air, soil, water). Anthropogenic cadmium load can induce a complex of factors that can cause disturbances at almost all levels of the ecosystem and directly in the human body, as its integral part. Heavy metals affect the body, which leads to the emergence of various pathological conditions, not only due to acute intoxication, but also in conditions of entry into the body at relatively low concentrations [1–3].

The toxic effect of cadmium is associated with its effect on the structural state of cell membranes. Biological membranes are that cellular structure, changes and damages of which cause disturbances in the structure and functioning of the cell as a whole. The effects that develop as a result of the action of cadmium can be either specific or non-specific (the absence of a strict dependence on the structure of the substance). Due to the nonspecific action of cadmium ions, the structural integrity of the membranes can be violated, which leads to deformation, lysis of the cell and its death. The selective effect of cadmium on membrane-bound enzymes, transport systems, and receptor complexes is mediated mainly through its interaction with protein SH-groups [3, 4].

Today, we have proved that the use of a liposome form of a phospholipid-containing dietary supplement “FLP-MD” in animals under conditions of toxic effects on the cadmium organism is characterized by high prophylactic and therapeutic effects, which is explained by the membrane-stabilizing properties of its components, stimulation of antioxidant processes in organs and tissues and prevention of metabolic disorders the status of their body [5–7].

6.1 Cytotoxicity of cadmium and its manifestation

There are three main ways in which cadmium enters the body: through the gastrointestinal tract with food (feed) and water, through the lungs with air, and through the skin. Cadmium that enters through the lungs is more easily absorbed by the body – 10–30%. When entering through the gastrointestinal tract, the absorption rate is only 4–7% (0.2–5 μg Cd per day). The average daily rate of cadmium withdrawal from the body is insignificant, approximately 0.001% of its total content. Slow excretion of cadmium, mainly in urine, is explained by the absence of a specific biochemical mechanism of metal removal from the body associated with reabsorption processes in the proximal tubules, which ensures the formation of nephrotoxicity mechanisms without its additional supply [8–11].

When cadmium enters the body through the gastrointestinal tract, its absorption mainly occurs in the small intestine. The process of absorption of cadmium ions in the intestine is characterized by its rapid accumulation in the mucous membrane with subsequent slow entry into the circulation system. When cadmium enters the cavity of the small intestine, its digestive functions may be impaired. As a result of absorption, the metal is absorbed in the intestine, and then enters the liver with portal blood flow and is intensively absorbed by hepatocytes [11, 14].

Cadmium metabolism in the body is characterized by the following features:

1) The lack of an effective mechanism for homeostatic control;

2) Retention (cumulation) in the body with a long half-life (average 25 years);

3) The predominant accumulation in the kidneys and liver;

4) intense interaction with other divalent metals both in the process of absorption and accumulation.

It was found that cadmium combines with negatively charged groups of membranes and thus modifies the charge of their surface. At the same time, a significant increase in the microviscosity of the lipid phase of the membrane is recorded without changing its polarity. This situation is associated with the fact that the metal "cross-links" the negatively charged groups of phospholipids located nearby and, thus, limits their mobilit [14].

Due to the presence of UFA, phospholipids of cell membranes are most susceptible to the oxidation reaction initiated by free radicals that form in the cell. Activation of the process of formation of free radicals leads to an increase in the intensity of production of highly reactive secondary radicals, easily diffuse over considerable distances, and activation of lipid peroxidation [3, 11].

An important mechanism of damage to biological membranes is the hydrolysis of phospholipids due to activation of phospholipases (especially phospholipases A_2). The result of the action of phospholipase A_2 on lipids of biological membranes is the release of arachidonic acid. The latter, in turn, is a substrate of cyclooxygenase. The conversion of arachidonic acid with the participation of cyclooxygenase leads to the formation of eicosanoids (prostaglandins, thromboxane, prostacyclins) - substances that activate the development of inflammatory processes in tissues. Under the influence of another enzyme (5-lipoxygenase), arachidonic acid is converted into leukotrienes and eicosatetraenoic acids. They are neutrophil chemoattractants - substances that regulate vascular permeability [4, 8].

Biological effects of heavy metals on membranes:

1) As a result of the growth of membrane permeability, the rate of entry of ions and substrates into the cell and the exit of metabolic products from it changes. This leads to metabolic disorders in the cell, electrical properties of the membranes;

2) The structural organization and functional activity of the cell are disrupted, cell death is possible;

3) The formation of a number of active substances that are involved in the pathogenesis of the toxic process.

One of the main mechanisms by which most heavy metals realize their toxic effect is the activation of free radical oxidation, which is accompanied by damage to macromolecules and supramolecular complexes, including biological membranes. A variety of biologically important molecules can be drawn into the processes of free radical oxygen transformations. The formation of oxygen reduction products in living systems creates the possibility of their interaction between themselves and other molecules or ions, the appearance of toxic products and the launch of pathological processes such as LPO [8].

Lipoperoxidation processes play an important role in the mechanisms of toxic action of cadmium ions [1, 2]. This element, unlike other heavy metals, does not directly generate free radicals in cells. However, numerous data indicate the generation of superoxide radical, hydroxyl radical and NO - radicals in the cells as a result of the indirect action of cadmium. The generation of cadmium hydrogen peroxide is shown, which becomes a significant source of free radicals as a result of the Fenton reaction. Cadmium can replace ferum and copper in a number of proteins like ferritin, which, in turn, leads to an increase in the concentration of Fe^{2+} and Cu^{2+}.

The study of the prooxidant effect of cadmium on liver cells showed that the mechanism of induction of lipid peroxidation by metal is associated with the displacement of ions that initiate the oxidation process from cellular structures [11]. In addition, it was found that animal intoxication with cadmium promotes the production of oxygen radicals in the mitochondria and microsomes of hepatocytes, and also leads to a decrease in the content of reduced glutathione (RGL) in the liver. The obtained similar results also indicate that under the influence of cadmium, oxygen radicals are produced in the cells. The introduction of cadmium chloride to animals leads to a significant activation of lipid peroxidation in the cerebral cortex and myocardium, however, there is no change in the activity of antioxidant enzymes in these organs.

Studies of the activity of a number of antioxidant protection enzymes (AO) - protection (catalase (C_{at}), superoxide dismutase (Cu, Zn-SOD, Mn-SOD) of the liver and kidneys after chronic intake of cadmium with drinking water indicate their sensitivity to the action of this element. In vitro experiments have shown that a decrease in the content of TBA-active products as a result of vitamin E supplementation does not restore the activity of AO-defense system enzymes under the action of cadmium. It is believed that cadmium binds to the imidazole group His-74 protein. This is caused by the disruption of the decomposition of hydrogen peroxide. Cadmium acts as an inhibitor of the activity of the Mn-dependent SOD of the mitochondria of the liver, probably due to the replacement of Mn (II) by cadmium ions [3].

It was shown that acute intoxication of animals with cadmium leads to an increase in the activity of enzymes of the AO-defense system: Cu, Zn-SOD, C_{at}, glutathione peroxidase (GP), glutathione reductase (GR) and glutathione transferase (GT). In particular, the influx of metal into the animal organism causes a significant increase in the LPO intensity (the intracellular level of TBA-active products increases) and inhibition of the activity of antioxidant enzymes (SOD, C_{at}, GP, GR). Activation of free radical oxidation due to the action of heavy metals is associated with the depletion of the reserve of natural antioxidants (ascorbic acid and tocopherol) in cells along with a change in the activity of antioxidant enzymes (C_{at} and SOD) [3].

The administration of large doses of cadmium or arsenic salts to animals leads to overproduction of superoxide radicals and the accumulation of metabolites of the oxidative reaction. A previous incubation of cells with methionine and cysteine, protects them from oxidative stress caused by the action of mercury, cadmium, and copper, which indicates the leading role of inactivation of SH-groups of proteins in the induction of free radical oxidation by metals [4, 8].

It is known that active radicals can cause oxidative modification of not only lipids, but also proteins. Tryptophan, tyrosine, histidine and cysteine are actively oxidized. In addition, the hydroxyl radical (·OH), as a rule, causes aggregation of proteins, and in combination with O_2 and O_2 – fragmentation. Fragmentation of proteins can also cause lipid radicals. Attack of ·OH aromatic and sulfur-containing amino acid residues is accompanied by their irreversible changes. Oxidative modification of enzymes of the AO-defense system leads to changes in their activity: O2· inhibits the activity of C_{at} and GP; H_2O_2 causes inactivation of SOD and cytochrome P-450. Thus, free aggressive radicals affect various target structures of cells: membrane lipids, free amino acids, polysaccharides, receptor molecular complexes, transport proteins, etc. [11, 14].

The result of this is a change in the functional condition of the cell, a mutation of the genetic code, at the macroorganism level, leads to mutagenesis and neoplasms in the remote periods after the action of the toxicant, necrosis, cell lysis and accelerated apoptosis. That is why drugs with membrane-active properties acquire significant relevance in cadmium intoxication of animals, stimulate the development of reparative processes in damaged cell structures [4, 8].

Again, the liposomal form of dietary supplement “FLP-MD” contains a mixture of various classes of phospholipids isolated from milk (butterdish), which have a natural fatty acid spectrum for animals, a mixture of unsaturated fatty acids (oleic, linoleic, linolenic) and antioxidants (vitamins A and E) [10, 13, 14].

For clinical trials, outbred male rats, weighing 180–200 g, contained in a standard vivarium diet were used. Animals were divided into 4 groups of ten animals each. The duration of the experiment was 20 days. Group I included control animals (intact); Group II – healthy animals were orally administered dietary supplement “FLP-MD” (13.5 mg/kg body weight) for 14 days; Group III - for animals, cadmium chloride was administered orally at a dose of 1.0 mg per 1 kg of body weight for 14 days (once a day), corresponds to 1/50 LD_{50} (single dose); Group IV – animals were given dietary supplement " FLP-MD ”(13.5 mg/kg body weight) for 6 to and 14 days after daily administration of cadmium chloride at a dose of 1.0 mg / kg body weight.

During slaughter, by decapitation, in laboratory animals, for the study of biochemical parameters, samples of the liver and small intestine were taken to obtain homogeneous preparations, as well as blood, from which serum was further obtained.

6.2 Corrective properties of liposomes based on milk phospholipids for cadmium poisoning of animals

Cadmium, which is recognized as one of the significant pollutants of the biosphere, upon entry into the body of animals causes a number of toxic effects, affecting various organs and systems. The main target organs for cadmium intoxication are the gastrointestinal tract, liver and kidneys. It is known that one of the main mechanisms by which most heavy metals realize their toxic effect is the activation of free radical oxidation, accompanied by damage to all major classes of biological macromolecules and supramolecular complexes, including biological membranes. There is evidence that disruption of lipid peroxidation processes also plays an important role in the mechanism of toxic effects of cadmium ions on the body. It is believed that cadmium causes a significant increase in lipid peroxidation and a decrease in the activity of antioxidant enzymes: GP, SOD, Cat [1–4].

The determination of the content of lipid peroxidation products for the action of harmful factors of various origin is highly informative, determines its use in order to diagnose the effectiveness of treatment and prevention of diseases of various etiologies.

Constant monitoring of the content of reactive oxygen species, free radicals, etc. clearly regulates the reaction of lipid peroxidation and is aimed at maintaining homeostasis in the body. Failure of such a control, which may occur as a result of exogenous or endogenous exposure, leads to intensification of lipid peroxidation, an increase in the concentration and accumulation in the body of lipid peroxidation products, which are characterized by a high reactivity with respect to systemic damaging effects on cells. Indicators that determine the level of lipid peroxidation and the state of the antioxidant system that characterize the functioning of the body's protective and adaptive mechanisms, and therefore, can be sensitive tests for the effects of various exogenous factors.

So, under the conditions of administration of cadmium chloride to blood rats, a significant increase in the concentration of creatinine (on average by 21%) and glucose (by 30%) was established. It is likely that the regular intake of cadmium chloride in the body of animals within 14 days causes metabolic disturbances in the renal parenchyma, glomerular filtration and tubular reabsorption. In addition, under the conditions of the introduction of cadmium into the body, a pancreatic function may be impaired, which leads to a weakening of the glycogen-forming and glycogen-fixing functions of the liver and is manifested by an increase in the concentration of glucose in the blood. At the same time, this condition is accompanied by a characteristic increase in enzyme activity: AST - by an average of 22% and GGT – by 40%, which indicates the hepatotoxic effect of cadmium. Oral administration to animals of the liposomal form of dietary supplement “FLP-MD”, against the background of cadmium intoxication, leads to partial normalization of GGT activity and serum glucose concentration, and AST activity and creatinine content do not differ from control values. It should be noted that the introduction of the liposomal form of dietary supplement “FLP-MD” to control animals does not lead to changes in the biochemical parameters of blood serum.

The activation of LPO intensity was evaluated by the accumulation of the final product, TAC-active products. It was found that the introduction of cadmium chloride into the animal organism leads to the accumulation of TBA-active products in the epithelial cells of the small intestine (on average by 300%), in liver cells (by 50%) and blood serum (by 36%). Under the conditions of the introduction of the liposomal form of dietary supplement “FLP-MD”, along with the intake of cadmium chloride, the level of accumulation of TBA-active products remains increased (by 203% compared to control values) only in blood serum.

The results of determining the activity of SOD and C_{at} indicate multidirectional changesin their activity under experimental conditions.

Under the conditions of cadmium administration, the activity of SOD in liver cells decreases, on average, by 50%, and in epithelial cells of the small intestine – by 37%. At the same time, the activity of C_{at} in the studied organs under these conditions does not change. However, in the blood serum, C_{at} activity decreases – by 77%, while the activity of SOD does not change. The introduction of the liposomal form of dietary supplement “FLP-MD”, along with the intake of cadmium chloride, indicates the normalization of SOD activity indicators in the studied objects. At the same time, Cat activity in liver and blood serum preparations even exceeds the control values by 39 and 25%, respectively. Since Cat is involved in oxidative phosphorylation and oxygen transfer to intracellular structures, an increase in the activity of this enzyme may indicate the mobilization of the body's defenses.

The content of RGL under the conditions of cadmium administration decreases, on average, by 26%, 12 and 18% in preparations of the liver, mucous membrane of the small intestine and blood. Under these conditions, the activity of the studied enzymes – HT and GP – decreases. The greatest changes are observed for blood, respectively, at 61 and 37%.

Under the conditions of the introduction of cadmium chloride against the background of the liposomal form of dietary supplement "FLP-MD", less pronounced changes in the studied parameters are observed. Although the content of RGL in blood serum and liver remains reduced, respectively, by 18 and 27% relative to the control, as in the conditions of cadmium administration, the activity of the enzymes HT and GP decreases to a lesser extent. Thus, the activity of HT and GP changes in blood serum by 43 and 19%, respectively, and in the liver by 9 and 17% relative to control values.

The obtained results indicate the intensification of lipid peroxidation against the background of a decrease in the potential of enzymatic and non-enzymatic AO-defense units in the blood, liver and mucous membrane of the small intestine under the conditions of 14-day intake of cadmium chloride in animals at a dose of 1.0 mg/kg body weight. The accumulation of the final product of LPO – TBA-active products is observed in all the studied objects. Oral administration of cadmium chloride to rats leads to the activation of lipid peroxidation in the cells of internal organs. Intoxication of animals with cadmium chloride causes an increase in the content of oxygen radicals in cells, including mitochondria and hepatocyte microsomes. It is known that cadmium ions have a pro-oxidant effect: active radicals can cause oxidative modification of not only lipids, but also proteins and nucleic acids.

When LPO is activated, the state of the AO-system of the body becomes important.

The activity of C_{at} and SOD, which are involved in the neutralization of active oxygen compounds, varies in different ways in the internal organs when cadmium is introduced into the body of rats. SOD activity, especially in the liver, decreases, which can be explained by damage to the enzyme molecule or an increase in the concentration of hydrogen peroxide as its inhibitor. Confirmation is the results that indicate that in the liver and small intestine, according to the studied conditions, the activity of C_{at} almost does not change, and the activity of the GP, enzyme, also participates in the neutralization of hydrogen peroxide, decreases. The glutathione peroxidase system is one of the universal systems for the decomposition and neutralization of peroxides, which prevents the initiation of secondary lipid oxidation reactions and is involved in the inactivation of xenobiotic oxidative metabolism products.

Glutathione transferase plays a leading role in the biotransformation and detoxification of xenobiotics of various origins by catalysis of their conjugation reactions with restored GL [1, 2]. A decrease in the activity of this enzyme, along with a decrease in the content of RGL, as well as a decrease in the activity of GP, C_{at}, and SOD, testifies to the inhibition of the functioning of the OA-defense systems of the body under the conditions of cadmium chloride administration.

Simultaneously with cadmium, the liposomal form of dietary supplements “FLP-MD” enters the body of animals, leading to an improvement in the state of pro-antioxidant balance. A less pronounced accumulation of TBA-active products in the studied biological objects is observed. The activity of SOD almost does not differ from the control values, and the activity of C_{at} even exceeds their value, which may indicate the mobilization of the body's defenses.

Thus, the obtained results allow us to recommend the specified liposomal form of dietary supplement “FLP-MD” as a means of drug protection of the animal’s body during cadmium poisoning.

CONCLUSION

The cytotoxic effects of heavy metals, including cadmium, are based on various mechanisms, which include the following: impaired energy metabolism and intracellular calcium homeostasis activation of free radical processes in cells; damage to cell membranes. The use of membrane stabilizing drugs can be used in the complex treatment of cadmium intoxication.

The study of the correcting properties of milk phospholipids in the form of liposomal dietary supplement "FLP-MD" was carried out in vivo experiments under the action of cadmium chloride.

Studies of the biochemical parameters of blood serum, epithelial cells of the liver and small intestine of rats indicate a damaging effect (the presence of destructive processes in the cells) of cadmium on the cellular structures of the animal organism under experimental conditions. Preventive administration to animals of the liposomal form of dietary supplement “FLP-MD” based on milk phospholipids is effective because it leads to less pronounced changes in the values of the studied biochemical parameters of blood serum, liver and small intestine relative to control values.

According to the toxic effect of cadmium on the body, the main factor loads are introduced by indicators characterizing: the functioning of the glutathione system in the tissues of the liver, small intestine and blood serum, the functioning of the body systems (indicators of blood serum), as well as the processes of pro-antioxidant balance of the body. The state of the object of research under the conditions of the action of cadmium for using the liposomal form of dietary supplement "FLP-MD" is approaching the control group. That is, the use of poisoned animals liposomal form of dietary supplement "FLP-MD" exhibits a pronounced corrective effect. First of all, this is due to the composition of the dietary supplement “FLP-MD”, since its main component is phospholipids, which, on the one hand, undergo oxidation due to the action of oxygen radicals and, on the other hand, stabilize cell membranes. The liposomal form of dietary supplement “FLP-MD” also includes vitamins with membrane-active and antioxidant properties.

Thus, simultaneously with the introduction into the body of rats of cadmium chloride for fourteen days, the use of the liposomal form of dietary supplement "FLP-MD" with the original combination of lipids, mainly phospholipids, which are fatty acid in their composition, obtained from natural and cheap raw materials (oilers) correspond to membrane lipids of animal cells, a mixture of mono- and polyunsaturated fatty acids obtained from linseed oil, and fat-soluble vitamins – α-tocopherol and retinol acetate, stimulates the development of e reparative processes in damaged tissues and restores the metabolic status of the organism of animals exposed to cadmium ions, and also has a stabilizing effect on the pro-antioxidant balance.

LIST OF CONVENTIONAL SYMBOLS AND ABBREVIATIONS

BAA «FLP-MD»	- biologically active additives «FLP-MD»;
GGTP	- γ-glutamyl-transferase (EC 2.3.2.2);
LP	- lipoprotein;
UFA	- unsaturated fatty acids;
PUFA	- polyenoic unsaturated fatty acids;
MUFA	- monoenoic unsaturated fatty acids;
SFA	- saturated fatty acids;
NULES	- National University of Life and Environmental Sciences of Ukraine;
POL	- peroxide oxidation of lipids;
SOD	- superoxide dismutase (EC 1.15.1.1);
GR	- glutathione reductase (EC 1.6.4.2);
TAG	- triacylglycerol(s);
TBA-active products	- thiobarbituric acid active products;
PL	- phospholipid(s);
Ch	- cholesterol;
AO	- antioxidant;
RGL	- reconstituted glutathione;
Gy	- radiation level unit;
LPL	- low density lipoproteins;
VLDL	- very low density lipoproteins;
HDL	- high density lipoproteins;
LD	- lethal dose;
GP	- glutathione peroxidase (EC 1.11.1.9);
GT	- glutathione transferase (EC 2.5.1.18);
C_{at}	- catalase (EC 1.11.1.6);

TSF	- thymic serum factor;
LGL	- large granular lymphocytes;
PAI	- proliferative activity index;
CF	- cyclophosphamide;
Hb	- hemoglobin;
AP	- alkaline phosphatase (EC 3.1.3.1);
AST	- aspartate-transaminase (EC 2.6.1.1);
ALT	- alanine-transaminase (EC 2.6.1.2);
LPO	- lipid peroxidation;

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