
Liliia Kalachniuk
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Kalachniuk Liliia

INTELLEKTUELLES KAPITAL - DIE GRUNDLAGE FÜR INNOVATIVE ENTWICKLUNG

EINIGE MITTEL DER REGULIERUNG IM ORGANISMUS DER TIERE BEI DEN WIRKUNGEN DER FAKTOREN VERSCHIEDENER NATUR

INTELLECTUAL CAPITAL IS THE FOUNDATION OF INNOVATIVE DEVELOPMENT

SOME MEANS OF REGULATION IN AN ORGANISM OF ANIMALS AT ACTIONS OF FACTORS OF DIFFERENT NATURE

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Einleitung / Introduction

All living organisms have the ability to adapt to the influence of exogenous factors within certain limits of their adaptability and potency of these factors [1-6]. If in natural conditions such ability of an organism is lost, then possibility of its functioning and life disappears. Exogenous exposure changes the metabolism, generating endogenous levers of further changes in metabolic processes.

Important in the aspect of life of biological objects under the influence of toxic substances and other negative effects of exogenous factors is the release of biological products with immunomodulatory properties, which would help stabilize the functioning of the body as a whole, increasing its resistance.

The leading niche in the structure of preclinical and clinical research in the medical-biological and veterinary fields is occupied by determining the mechanisms of influence of biologically active substances of natural origin and elucidating their effectiveness in stabilizing the functioning of cells, organs and the body as a whole. Cells and organs of the immune system are key targets for testing various substances. Changes in immunological parameters may be a manifestation of the body's normal response to physiological or pathological factors, reflect excessive activation / depletion of the immune system, characterize a congenital or acquired defect of certain parts of the immune system.

The purpose of the study is to develop methods and means of regulating metabolism in the animal's body under the action of various factors and the correction of its disorders. Comprehensive research was aimed at studying metabolic processes in animals by determining the biochemical parameters of metabolism, elucidating sensitive to changes in biochemical processes under the influence of harmful exogenous factors and the correction of their biological products with immunomodulatory properties.

To achieve the goal of the tasks you need to solve the following tasks:

- improvement / development of methods for obtaining the drug Biofosfomag (based on macronutrients and some components of milk) and biological products of

natural origin OVA and OVA+ for further testing and evaluation of their immunomodulatory properties;

- approbation of the developed methods of obtaining biological products with immunomodulatory properties and their evaluation according to the following criteria / indicators: stimulating / inhibiting activity of biological products (OVA, OVA+, Biophosphomag and others) against T- and B-lymphocyte cultures; determination of proliferative parameters under the influence of the studied components using modern methods of colorimetric test with the inclusion of specific dyes - substrates of mitochondrial dehydrogenases; assessment of glucose metabolism by cells and activity of tricarboxylic acid cycle enzymes; determination of phagocytic activity OVA, OVA+ and Biophosphomag in the conditions of spontaneous and induced stimulation; determination of the mechanisms of oxidative explosion by cells of the phagocytic system, comparison of the studied components with the classical activator of phagocytosis - phorbol-12-meristat-13-acetate.

The above studies are promising, as in the future may reveal some elements of the regulatory units of metabolic processes for the correction of the obtained biologicals with immunomodulatory properties.

KAPITEL 1 / *CHAPTER 1*
SOME METABOLIC PROCESSES AND THEIR REGULATION IN CELLS

1.1. Intracellular metabolism under the influence of factors of different nature

Disturbances in lipoprotein metabolism may be the result of abnormal synthesis, processing or catabolism of lipoprotein particles in blood plasma. Impaired LDL synthesis in the liver can lead to hyper- or hypo-secretion of these triacylglycerol-enriched lipoproteins in humans and other mammals, including farm animals.

LDL hypersecretion is associated with an increased risk of cardiovascular disease in primates [7]. These metabolic abnormalities are mainly seen in people with hormonal (non-insulin-dependent diabetes) or digestive (obesity) disorders. However, cases of VLDL hypersecretion have not yet been definitively identified [8]. Non-insulin dependent forms of diabetes. Overproduction of very low-density lipoprotein particles in the liver is common in people with non-insulin-dependent diabetes mellitus, which is characterized by tissue resistance to insulin. This metabolic abnormality is not correlated with apoB hypersynthesis, as VLDL particles are mostly disturbed in size and altered chemical composition. Several metabolic abnormalities are associated with non-insulin-dependent diabetes, such as hyperglycemia, high plasma esterified fatty acid levels, and hypoglucagonemia, each associated with increased secretion of very low-density lipoproteins [9, 10].

Adiposity. Obesity is also associated with hepatic hypersecretion of very low-density lipoproteins. In this metabolic disease, the secretion of apoB and triacylglycerols increases so that it leads to higher production of VLDL particles that have a constant size and chemical composition [11]. Their hypersecretion by the liver (which is observed in "smooth" patients) can be partly explained by the more intensive work of this organ, its utilization of non-esterified fatty acids of very low-density lipoproteins and its insulin resistance, often found in obesity [12]. Hyposecretion of LDL in the liver is the result of impaired synthesis of lipoprotein particles. Epidemiological studies have shown that patients with this disease have, on average, a lower risk of atherosclerotic cardiovascular disease, but higher - for

various types of cancer, lung and gastrointestinal diseases [13]. Steatohepatosis occurs when there is an imbalance between the formation of triacylglycerols in this organ and the synthesis and / or secretion of VLDL. This instability arises as a result of changes in metabolic mechanisms (Table 1.1), which are mainly caused by genetic, hormonal or digestive factors.

Genetic diseases. VLDL-hyposecretion can occur with a variety of genetic abnormalities that lead to hypobetalipoproteinemia [13]. In several cases of abetalipoproteinemia in humans [13, 14], mutations in the apolipoprotein B gene have been identified, which inhibit the synthesis of this protein or the assembly of several dysfunctional truncated molecules, and mutations in the large subunit gene of microsomal transfer protein [15] from the norm.

Insulin-dependent forms of diabetes reduce the yield of VLDL by stimulating insulin degradation of newly synthesized AROB [16]. Other digestive factors that also reduce VLDL secretion include fish oil (rich in n-3 polyunsaturated fatty acids), which increases peroxisomal oxidation of carboxylic acids in hepatocytes, altering the ability of long-chain fatty acids to synthesize and secrete triacylglycerols [17].

Alcoholic steatosis of the liver. Excessive ethanol consumption is often accompanied by malnutrition. This diet limits the absorption of fatty acids, but prefers their higher mobilization from adipose tissue with subsequent entry into the liver. Excess ethanol impairs the mitochondrial oxidation of long-chain fatty acids, leading to more of them and the ability to esterify into triacylglycerols. Hepatic steatosis may occur when the selection of exogenous long-chain fatty acids by this organ exceeds its ability to collect and / or secrete LDL particles [18]. Excessive consumption of ethanol also causes numerous damage to hepatocytes, which can disrupt protein synthesis in them, and therefore block the formation of apolipoprotein B. At the subcellular level, ethanol can affect intracellular transport, packaging or secretion in one or more stages. VLDL particles. Blockade of ethanol microtubular formation leads to impaired mobility of very low density lipoproteins and prevents their exit from the Golgi apparatus [18]. Alcoholic liver disease can be stopped with the use of appropriate nutrients [19-25]. Studies in rats have shown that long-term

alcohol consumption of high levels of unsaturated fats and low levels of energy in the diet contributed to the development of steatohepatosis, while higher energy yields reduced lipid infiltration [26].

Table 1.1 - Possible mechanisms that contribute to the development of steatohepatosis

#	Deviation	Reasons for deviation
1	Increased supply of long-chain fatty acids (LCFA) to the liver	Higher LCFA mobilization from adipose tissue; High levels of LCFA in the liver; Lower oxidation of LCFA in the liver.
2	Delayed incorporation of triacylglycerols into VLDL	Inhibition of apoB or microsomal transfer protein synthesis; Inadequate supply of apolipoprotein B or microsomal transfer protein molecules; Competition with cholesterol.
3	Defects in one or more stages of LDL transport and secretion	Changes in VLDL transport from the endoplasmic reticulum to the Golgi complex; Violation of the final glycosylation in the Golgi apparatus; Reducing the rate of formation of secretory vesicles; Disorders of migration of secretory vesicles from the Golgi apparatus to the cell membrane.

Parenteral nutrition and steatosis. Hepatic steatosis is a typical disorder for patients with general parenteral nutrition [27]. The development of this syndrome occurs depending on the amount of glucose ingested. Plasma glucose infusion results in hyperinsulinemia, which inhibits LDL secretion. Among the possible explanations are that the accumulation of triacylglycerols in hepatocytes may result from higher peripheral lipolysis, less oxidation of long-chain fatty acids, their higher de novo synthesis and their esterification to triacylglycerols, and higher degradation of aromatic production. Total parenteral nutrition can provide excess calories from glucose, which is associated with inadequate supply of protein or amino acids.

The addition of lipid emulsion to parenteral nutrition reduces portal vein

insulinemia and the molar ratio of insulin to glucagon in it, leading to a decrease in lipid infiltration in rat liver [28]. Hepatic steatosis in patients on long-term parenteral nutrition has been shown to be induced by plasma choline deficiency and altered by lecithin supplementation [29].

Hepatic steatosis in birds. Hepatic steatosis in poultry, particularly in laying hens, is associated with hemorrhagic syndrome, which is induced by permanent damage to the integrity of the liver reticulin [30]. During egg laying, high levels of estrogen in the blood plasma induce increased feed intake and a state of positive energy balance in chickens. Such metabolic "states" promote the synthesis of long-chain fatty acids, triacylglycerols and apolipoproteins in the liver [31]. However, most newly synthesized triacylglycerols accumulate in the cytoplasmic pool and appear to re-hydrolyze and esterify to triacylglycerols before incorporation into VLDL particles [32]. Such hydrolysis of triacylglycerols is stimulated by the need of birds for food/ *feed.*

Typical swallowing behavior of chickens is a result close to constant feeding. This constant feeding state may limit the transfer of triacylglycerol from the cytoplasmic depot to the secretory microsomal pool and their subsequent incorporation into VLDL particles. This constant positive energy balance observed in laying hens maintains relatively high insulinemia, which contributes to the inhibition of VLDL secretion [33]. Geese kept on a carbohydrate-enriched diet and forcibly fed more than normal for 2 weeks developed severe hepatic steatosis, leading to a large (10-fold) increase in liver weight. Induction of steatohepatosis is partly under genetic control, but the mechanisms of steatosis remain unclear [33]. This process of overfeeding promotes lipogenesis of the liver in geese and produces higher levels of VLDL in blood plasma. These particles are particularly enriched in cholesterol esters, indicating either a defect in the combination of triacylglycerols in immature LDL particles in the liver, or competition between hydrophobic lipids in LDL packaging [34]. Changes in the secretion of triacylglycerols in the liver, apparently, thus stimulate steatosis of this organ.

Hepatic steatosis, in general, appears to be a metabolic disorder in the period

before and after calving of highly productive dairy cows, which occurs in one third of the total number of such animals [35]. Maximum triacylglycerol infiltration occurs between 2 and 4 weeks after calving (80-100 mg / g fresh liver compared to the same 2 mg / g in non-lactating cows) and is considered to be the result of excessive fatty acid mobilization when energy content becomes incomplete to maintain milk content and production. This disappears after 12 weeks, when the positive energy balance is restored [36].

Another major factor in the development of such liver disease may be the slow chronic entry of triacylglycerols into the liver, which form part of the VLDL particles [35, 37]. In the early stage of lactation, a decrease in the concentration of apoB in blood plasma correlates with an increase in the content of triacylglycerols in the liver [38, 39]. A similar induction of steatohepatosis was observed when cows were given ethionine (an inhibitor of protein synthesis), suggesting that apolipoprotein synthesis is a limiting step for VLDL secretion [40]. Simultaneous liver levels of apolipoprotein B, its mRNA, and triacylglycerols clearly showed a negative correlation between apoB gene expression and the development of hepatic steatosis in the first week of lactation [41]. In other cases, disruption of the signal transduction pathway is mediated by the emergence of protein kinase (protein kinase C), which is also involved in the pathogenesis of this liver disease in cows [42]. Various studies suggest a lack of amino acid and phospholipid precursors in high-yielding dairy cows in early lactation. Nutritional feeding, such as intravenous L-methionine and L-lysine [38] or choline [43-45] as precursors of phosphatidylcholine synthesis in early lactating cows, promotes VLDL secretion and reduces lipid infiltration. Extensive hydrogenation of dietary polyunsaturated fatty acids by cicatricial bacteria appears to be a positive factor in limiting the spread of hepatic steatosis in dairy cows. Hence, the replacement of soybean oil (enriched in n-6 polyunsaturated fatty acid) with animal fat, which is rich in saturated and monounsaturated fatty acids in milk substitutes for "before ruminant" calves (functional monogastric animals), promotes the secretion of LDL particles in the liver. on cholesterol and the loss of triacylglycerols accumulated in this body [46-48], as in the case of forced feeding

above the norm of geese [34].

1.2. Metabolism and transport systems of lipid compounds

It is known that the predominant amount of lipids in their chemical structure are esters of high molecular weight fatty acids (FA) and alcohols (glycerol, sphingosine, cholesterol and others). Many of them also contain residues of phosphoric acid, nitrogen-containing bases (colamine, choline) and carbohydrates, etc. [49].

However, the most important feature of these compounds is FA, which largely determines the physical, chemical, biological and other properties of lipids. In particular, it is the length of the carbon chain and the degree of unsaturation of FA in molecules of natural lipid compounds (neutral fats, phospholipids, swingolipids, etc.) determine their consistency, availability for intracellular use and ability to bind to proteins and other substances, including , bilayers, transporting lipoprotein forms, lipid matrix of biomembranes, etc.

In animals and humans, FA with an even number of carbon atoms (C12 - C24) were found, mostly C16 - C20 [50], contained either in the free (non-esterified) state or in the lipid components of blood plasma and triacylglycerols (TAG) of adipose tissue. They are used as a mobile reserve, primarily carbon and hydrogen, to maintain energy and metabolic levels [49, 50]. Of the saturated fatty acids, palmitic acid ($C_{15}H_{31}COOH$) predominates, and of the unsaturated ones, oleic acid ($C_{17}H_{33}COOH$) predominates, accounting for almost 60% of the total triacylglycerols in adipose tissue. The presence in lipids of a significant amount of $C_{17}H_{33}COOH$ with a low melting point (10 - 15 °C) provides a liquid state of fats. For example, the melting point of saturated FAs such as myristic (C14:0), palmitic (C16:0) and stearic (C18:0) is +54.4, +62.8 and +69.6 ° C (content in triacylglycerols) human adipose tissue - 3, 20 and 5%, respectively). The melting point of mono- and polyene unsaturated FAs, such as palmitoleic (C16:1), oleic (C18:1), linoleic (C18: 2) and arachidonic (C20:4) is +1.0, +13.0, -11.0 and –49.5 ° C (content in triacylglycerols - 5.55-60, 10 and

0.2%, respectively) [1, 50].

1. *Volatile FA and ketone bodies.* Volatile fatty acids and ketone bodies are water-soluble molecules whose permeability exceeds that of free FA and triacylglycerols. Their concentrations in the blood of adult ruminants are much higher than in organisms with unicameral stomachs (e.g., rats [51] and humans [52]). This is caused by the formation of many volatile FA and ketone bodies due to microbial fermentation of feed in the rumen. The main representative of ketone bodies in the blood of ruminants is β-hydroxybutyrate, and in rats - acetoacetate [51]. Their content in all species of animals increases during starvation and lactation. In the absence of lactation, dairy cows tolerate food shortages more easily than rats because the concentration of ketone bodies in them increases much less. However, during lactation, the content of these compounds in the blood of cows is significantly higher than in rats [51].

In sheep fed traditional foods, almost 70% of ketone bodies enter the bloodstream from the intestines and only 30% from the liver. They are mainly used by muscles (60%) and to a lesser extent by liver (20%) and kidneys (20%). However, the relative fluxes of ketone bodies change significantly during starvation, pregnancy or lactation [52, 53].

Long-chain FA and triacylglycerols. The content of non-esterified FA in the blood of ruminants during feeding is less than 5% of the total mass of lipids, while triacylglycerols about 10%, which are mainly contained in the lightest lipoproteins (chylomicrons, VLDL). The flows of non-esterified FA and triacylglycerols for 24 hours in sheep fed on roughage were studied [54]. Experimental data show that 40% of them are mobilized from adipose tissue, and the source of 60% is the hydrolysis of unbound triacylglycerols by lipoprotein lipase. This indicates a key role of the enzyme in the regulation of all lipid metabolism, as the intestine produces 65% of triacylglycerols from the amount circulating in the blood [54], while the liver - only 35%. These FA streams, however, depend on the species of animals, the type of diet and the kinetics of their absorption of nutrients after a meal. However, it is believed that in dairy cows the source of triacylglycerols is mostly the intestines, not the liver

[55].

The structure and properties of lipoproteins were studied by different analytical methods under different physiological states of animals and their feeding conditions [37, 49]. It has been found that most of these compounds in blood plasma (over 80%) belong to high-density lipoproteins (HDL). Human blood contains mainly LDL (over 55% of the total).

Both in humans and in bulls, the main lipoproteins in the intestinal contents are chylomicrons and VLDL. A unique isoform of apolipoprotein B (apoB), which is similar to human apoB-48, has been found in the intestines of ruminants [56, 57]. In blood plasma VLDL, it is present in two isoforms: apoB-48 and apoB-100, whose genetic information is localized in the same gene [58]. The truncated isoform of apoB 48 is formed during post-translational RNA modification. In bulls, 95% of the information RNA (mRNA or mRNA) of apolipoprotein B is almost completely corrected in the intestine, while in sheep and horses - 40% and 73%, respectively [59]. That is why VLDLs available in the intestinal contents of cattle contain only apoB-48. Information RNA of the latter was detected in the liver of the fetus at an early stage of pregnancy of cows (after 90 days) [60].

Using FA. Absorbed acetate and butyrate are used primarily as a source of energy. In cell mitochondria, they are oxidized in the tricarboxylic acid cycle [61]. In addition, acetate is also the main substrate for lipogenesis in all fat depots, except intramuscular adipose tissue. In the liver, propionate is mainly used by hepatocytes for gluconeogenesis, and is therefore considered the main source of carbon for glucose biosynthesis in ruminants with a cicatricial type of digestion [53, 62].

FA in the composition of triacylglycerols are deposited in the liver or adipose tissue. They are either oxidized in hepatocytes, cardiomyocytes, and mycites, or re-metabolized by the liver, which secretes lipids and their metabolites in various forms, including acetate, ketone bodies, and lipoproteins. For example, in sheep fed on roughage, 34% of FA in the non-esterified state is deposited, 40% is oxidized, and 26% is included in the repeated cycle of transformations in the liver during the day [54].

The intensity of synthesis and secretion of VLDL by cattle liver is much lower compared to primates and rodents [63]. This is the case with high-FA feeds, as the liver, especially in high-fat calves, pregnant and lactating calves, ineffectively metabolizes free FA in the re-cycle. Animals often develop steatohepatosis. Thus, an in-depth understanding of the intracellular metabolism of LC in the liver and other tissues is especially important if there is a need to manipulate biological mechanisms to enhance the growth and development of ruminants, restore their health and increase production of high quality dairy or meat products [50, 64].

FA with a short carbon chain. Low molecular weight FA (including volatile forms and ketone bodies) enter the blood either from the intestine through the portal vein or from hepatocytes due to the catabolism of long-chain FA [53, 66, 67]. Then they are catabolized and included in the re-cycle of transformations for the synthesis of other metabolites or in the case of re-secretion are re-included in the overall metabolism.

It is known that 85-90% of propionate comes from the gastrointestinal tract through the portal vein to the liver and only a small amount reaches other tissues. In the liver, most propionate is converted to glucose. Therefore, in ruminants the degree of gluconeogenesis increases according to the amount of food consumed, ie quite differently than in animals with a single chamber stomach. Under such conditions, propionate inhibits the use of other gluconeogenic substrates, in particular, lactate [66], oxidation of short carbon chains [67] and ketogenesis. Several mechanisms for such inhibition are described in [35, 68]. Propionate also inhibits de novo lipogenesis, in particular the incorporation of acetate into the FA in rat liver [69 - 71].

More than 80% of the absorbed butyrate is immediately removed from the liver. The end products of its metabolism in hepatocytes are acetyl-CoA, long-chain FA and ketone bodies. However, more ketone bodies are formed in liver cells if long-chain FA are used as a substrate than when butyrate is the substrate [66, 71]. A smaller part of acetate is absorbed by the liver, and a larger part provides a significant supply of this metabolite to other tissues. However, the total output of acetate from propionate is masked by the corresponding products of his liver. In hepatocytes,

acetate is mainly used not for the production of ATP, but in anabolic processes and, above all, in the lipogenesis of long-chain FA de novo [71, 72].

Different directions of conversion of volatile FA may be due to differences in the ability of the bull's liver to activate them, as it contains acyl-CoA synthetases necessary for the release and use of propionate and butyrate. At the same time, low activity of acetyl-CoA synthetase was detected in this organ [70].

Metabolism of long-chain FA. Non-esterified blood FA are a source of long-chain FA that are deposited in the liver. The liver secretes 7 - 25% of FA received by it in a non-esterified state. Their transport to the liver depends on blood flow and concentration, which increases due to the mobilization of lipids from adipose tissue [56, 66, 73]. Hydrolysis of free triacylglycerols by lipase is also a source of long-chain FA in hepatocytes [64, 74, 75]. Liver lipase activity is much lower in cattle than in rodents. It can function on endothelial tissues and regulate plasma lipid levels. This enzyme is involved in the lipolysis of LDL and low-density lipoprotein (LDL), as well as in the hydrolysis of phospholipids and triacylglycerols in the process of HDL metabolism. It is possible that this lipase enters into a lipoprotein-lipase bond with the endothelium of the liver capillaries [64, 72].

In hepatocytes, long-chain FA are contained in an esterified or oxidized state. The distribution between these two pathways of FA conversion is regulated by at least two binding proteins associated with FA and acyl-CoA. As a result of their functioning, two cytoplasmic depots of FA are formed - inactivated and activated, which are further sent to the places of their esterification or oxidation in cells. These acyl-CoA binding proteins isolated from bull liver were sequenced and their primary structure determined. The expression of FA-binding protein in the liver of rodents increases under the influence of long-chain FA, but not short-chain [77]. Such a mechanism may be important for calves (which are not yet ruminant) for keeping them on a high-fat diet.

Metabolic pathways of long-chain FA are associated on the one hand with their esterification and synthesis of triacylglycerols, as a result of which they accumulate in the cytosol or are transported to microsomes, where they are secreted as part of

VLDL particles [8]. On the other hand, FA can be oxidized in peroxisomes and mitochondria with the release of free energy. Transport of acyl-CoA through membranes is carried out with the participation of carnitine-dependent system. Activated acid is transferred to various organelles of liver cells (microsomes, peroxisomes, mitochondria) and removed from them by the above system, which includes carnitine palmitoyltransferase and carnitinoctanyl transferase (CPT and COT, respectively). These enzymes are products of individual genes, and the regulation of their activity, especially in the transfer of malonyl-CoA, is considered one of the key reactions in the entire metabolism of liver lipids [53, 78].

2. The composition and nature of the diet (ratio of fats, proteins and carbohydrates, fatty acid spectrum, their concentration, physicochemical properties, etc.), as well as hormones regulate the distribution of fats in hepatocytes between different metabolic pathways. For example, insulin stimulates de novo lipogenesis and esterification of FA [79, 80], but inhibits their oxidation [81, 82]. In fed cows, 76% of palmitate is esterified and 24% is oxidized by sections of the liver, while in the state of starvation only 33% of it is esterified and 66% is oxidized [83].

Deviation of the process of regulation of the distribution of FA between the above metabolic pathways in hepatocytes can lead to excessive accumulation of triacylglycerols in the liver (to the formation of steatohepatosis) or ketosis. Steatohepatosis occurs in 20-60% of dairy cows during calving. This is accompanied by low functioning, decreased appetite in cows, reduced resistance to disease and slow recovery. This condition can be brought closer to normal by intravenous administration to animals of L-methionine and L-lysine or choline, which stimulate the secretion of VLDL in hepatocytes. As a result, the deposition of triacylglycerols in the liver is reduced. Normalize the state of steatohepatosis can also be reduced by feeding FA. This is observed, in particular, after 12 weeks of lactation of cows, when their mobilization from adipose tissue is significantly reduced [8, 55, 72]. Occurrence of ketosis and stimulation of FA oxidation are generally the main ways to remove excess fat from the liver. Ketone bodies inhibit lipolysis of adipose tissue and thus reduce the flow of FA to the liver and their accumulation in it [84, 85]. Fatty

hepatosis also occurs in calves (meat breeds), in which growth and development are inhibited, health deteriorates [86], metabolic processes are disrupted [72, 80, 87]. Low-fat diets or sorbitol are added to milk replacer to remove excess lipids from hepatocytes of beef calves [88].

Long-chain FA-CoA esters are transported to mitochondria by a complex carnitine-dependent process that involves the coordinated actions of two carnitine palmitoyltransferases (CPT I and CPT II) and translocase [78]. The level of carnitine in the liver of ruminants increases under different conditions of metabolic stress (starvation, diabetes, toxicosis during pregnancy, accumulation of triacylglycerols in the liver, etc.) [89, 90]. Experimental results published in [83, 91, 92] indicate that it stimulates the oxidation of palmitate in the liver of ruminants, which gives reason to believe that it is possible to regulate the oxidation of FA. However, it is known that the activity of CPT I can limit the rate of translocation of long-chain FA to mitochondria, which in turn regulates their oxidation and, consequently, ketogenesis. The activity of CPT I is inhibited by malonyl-CoA, the synthesis of which from acetate is stimulated by insulin. Malonyl-CoA, acetate, and insulin inhibit palmitate oxidation in liver sections [91-93]. CPT I from bull liver is much more sensitive (8-10 times) to inhibition of malonyl-CoA [92] by the same rat transferase [94]. If insulin levels are high, the effect of changes in the absolute concentration of malonyl-CoA may be increased by concomitant changes in the kinetic properties of CPT I or a decrease in gene expression of the enzyme [82]. On the other hand, low insulin levels in early lactation may lead to low concentrations of malonyl-CoA and, consequently, to increased ketosis. CPT I also inhibits methylmalonyl-CoA formed from propionate. With low propionate content in hepatocytes, the level of ketosis in the liver of ruminants may be high [95]. Other studies in rabbits have shown that incubation of cultured fetal hepatocytes with long-chain LCs induces the expression of a gene encoding CPT I [96]. If such a mechanism were to work in ruminants in vivo, very significant changes in CPT I gene expression should be expected during weaning. However, it is known that volatile and medium-chain FA and ketone bodies easily penetrate into the mitochondria of hepatocytes, regardless of the involvement

of carnitine and CPT I.

Fatty acids are broken down by β-oxidation in mitochondria to form acetyl-CoA, which is converted in the tricarboxylic acid cycle to other compounds with the release of H_2O, CO_2 and energy in the form of ATP [97]. Depending on the feeding conditions of calves in their liver, 3-4% of free acetate and 10-55% of β-hydroxybutyrate are formed due to oxidation of long-chain FA [75]. Starvation of animals causes ketogenesis due to increased mobilization of fat from adipocytes and a decrease in the insulin / glucagon ratio, which stimulates the transfer of FA to mitochondria. Pregnancy and, in part, lactation of cows increase the level of fat mobilization, which leads to hyperketonemia. At each stage of β-oxidation, several enzymes are involved, which differ in their specificity to the length of the carbon chain of the FA [98]. Two mitochondrial 3-hydroxyacyl-CoA dehydrogenases were detected in ruminant hepatocytes, while only one was detected in liver cells of single-chambered animals [99]. In hepatocytes, bull propionyl-CoA inhibits acyl-CoA dehydrogenase [74], which indicates a decrease in β-oxidation of FA propionate formed in the rumen. High NADH / NAD ratios also inhibit β-oxidation of FA. In the cytosol and mitochondria, this ratio is regulated by specific dehydrogenases, including β-hydroxybutyrate dehydrogenase. However, the activity of β-hydroxybutyrate dehydrogenase in the mitochondria of ruminant liver cells is 50-100 times lower than that of rats and humans [100]. All this is probably due to a special mechanism by which the ratio of NADH / NAD in the liver of ruminants is increased and thus limits the oxidation of FA and ketogenesis [75]. In addition, 3-hydroxy-3-methylglutaryl-CoA synthase inhibits the conversion of propionate to propionyl-CoA or succinyl-CoA, inhibiting ketogenesis [55, 101].

From studies on other animal species, it is known that FA molecules in peroxisomes are shorter than mitochondrial and therefore more active than saturated and unsaturated long-chain FA [97, 102]. The degree of β-oxidation of the laureate in the peroxisomes of rats [102] and calves [53] is higher than that of palmitate. FA to peroxisomes are also transported across membranes by a carnitine-dependent system that is sensitive to malonyl-CoA [438]. Soluble CPT, which is probably peroxisomal

in origin, has been identified in bull liver homogenates. The activity of this enzyme against long-chain FA is higher in calves than in rats and mice [103].

Free energy production. The last stage of ATP formation during FA catabolism is expressed in the process by which protons accumulate between two mitochondrial membranes in the electron transport airway. The mechanism of this process is the same in different tissues (heart, liver and muscles). However, cytochrome oxidase, which catalyzes the main regulatory stage of the respiratory chain, is different in kinetics and structural features in the liver and heart of cattle [104]. Research data confirm the presence of species-specific and tissue-specific regulation of energy supply: after all, tissue-specific expression of genes encoding subunits VIa and VIII cytochrome oxidase is different in the heart of bulls, rats and humans [105]. Generation of ATP with the participation of the proton gradient and ATP synthase is the next stage of metabolism. However, a certain amount of protons is lost due to penetration through the inner mitochondrial membrane, and therefore the production of ATP under such conditions is less effective compared to ATP production during catabolism of the FA. The level of proton leakage in hepatocytes was the same for many studied animal species [106]. This process probably [107] plays a key role in the regulation of energy processes in the liver.

Synthesis and deposition of triacylglycerols. The degree of de novo lipogenesis and triacylglycerol secretion by ruminant hepatocytes is lower than in rats, rabbits and chickens [63]. Because of this [8, 49, 72], considerable attention is paid to the study of both metabolism and secretion of VLDL in ruminants. Such data will be important to elucidate the biological mechanisms of steatohepatosis and, therefore, to prevent its occurrence.

There is no doubt that the distribution of FA between oxidation and esterification is crucial for the metabolism of long-chain FA in calf liver. Glucose and propionate probably increase their esterification [92, 93] as they are converted to glycerol. This may be evidence of inhibition of palmitate oxidation in hepatocytes.

Different stages of triacylglycerol formation from long-chain FA and triacylglycerol secretion are described in [8, 43, 108]. Three esterification reactions

and one hydrolytic reaction are required to bind three long-chain FA molecules into one glycerol-3-phosphate molecule [50]. Studies in sheep, rats [8, 64, 74] and calves [8, 50, 85] have shown that in hepatocytes, triacylglycerols combine into a large cytoplasmic depot and a small microsomal secretory pool. Microsomal transfer protein catalyzes their transfer from the microsomal membrane to the site of VLDL. Hepatocyte-synthesized triacylglycerols from unbound long-chain LCs released by the liver are apparently primarily transferred to the cytosolic depot. If they are used for the formation of VLDL, they are probably hydrolyzed with the release of long-chain FA before re-esterification in microsomes. However, it is suggested that the secretory triacylglycerols of VLDL particles mainly contain free FA, which are synthesized de novo in the liver. However, the degree of lipogenesis in hepatocytes of cattle is low and, therefore, the microsomal secretory pool of triacylglycerols can not be significant. This probably limits the secretion of VLDL, which causes steatohepatosis in animals. On the other hand, the reason for the low level of secretion of VLDL particles in cattle may be a decrease in the rate of synthesis of apoB and its inclusion in VLDL particles; catabolism of newly synthesized aroB in the liver and low activity of microsomal transport protein [8, 64, 74].

The nature of FA feeds, in particular polyunsaturated, is considered an important factor in regulating the metabolism of triacylglycerols in hepatocytes. These factors play a particularly important role in dairy calves and much less in animals weaned from the mother. This is due to the fact that the inflow of FA with milk in dairy calves is greater than in weaned animals, as well as significant hydrogenation of food long-chain FA in the rumen. Diets that are fortified with polyunsaturated FA (in soybean oil; C18: 2n-6) lead to the infiltration of triacylglycerols in the liver of calves and to a significant increase in its secretion of VLDL by this organ. Accumulation of triacylglycerols may occur due to the dominance of the rate of their biosynthesis over the secretion of VLDL; low oxidation of FA or, more likely, increased secretion of cholesterol esters compared to triacylglycerols. Indeed, diets enriched with polyunsaturated FA stimulate the secretion of HDL by the liver, leading to hypercholesterolemia [48, 109].

The effects of insulin on hepatic triacylglycerol secretion are still poorly understood. It is known that it stimulates esterification and lipogenesis, but reduces the ability of apoB to secrete due to the increased degree of its destruction. The balance between these effects depends on the conditions of the in vivo and in vitro experiments, as well as interactions with other regulatory mechanisms, in particular, the variability of cell volume and oxygen supply [82].

It should be noted that in general, the study of molecular mechanisms associated with esterification and catabolism of FA and secretion of triacylglycerols by the liver in lactating cows and calves of different ages has attracted the attention of many researchers. However, the possibility of correcting the distribution of FA between oxidation, deposition and secretion of triacylglycerols in VLDL in order to prevent (or treat) manifestations of steatohepatosis is still poorly understood [110-112].

Thus, sections 1 and 2 analyze the molecular aspects of the regulation of metabolism in pro- and eukaryotic cells, features of biochemical processes in living organisms and the influence of factors of different nature on them, which form the theoretical basis for further experimental research.

1.3. Improvement / development of methods for obtaining biologicals with immunomodulatory properties, their testing and evaluation

To improve / develop methods for obtaining biologicals with immunomodulatory properties, I series of studies were conducted, and for their testing and evaluation - II.

According to the first series of studies conducted on the basis of the interdepartmental educational and scientific laboratory of veterinary diagnostic research of the Department of Biochemistry and Physiology of Animals named after Academician M.F. Gulyi, 2 experimental batches of the medication Biophosphomag of phosphorylated magnesium chelate were made [113, 114]. The medication is a white amorphous powder that does not dissolve in water, but is highly dispersed

(turbid solution). When determining the molecular weight in SDS PAGE electrophoresis, the following characteristics are given (Fig. 1.1).

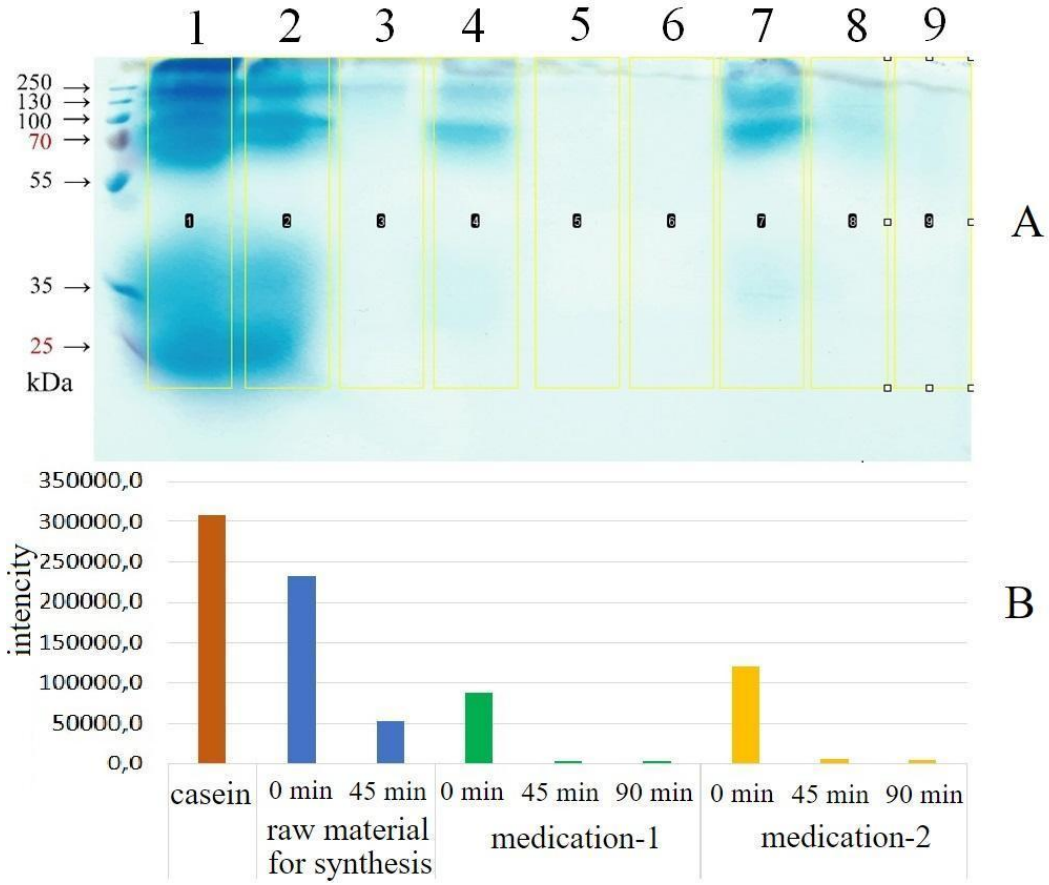


Figure 1.1 - Characteristics of the drug Biophosphomag by electrophoresis in SDS page, *where*

A is the electrophoregram of the products of magnesium hydrolyzate and phosphorus of the combined drug based on casein (1 - casein, 2 - source material for synthesis, 3 - source material after trypsin treatment after 45 min, 4 - medication-1 before trypsin treatment, 5 - medication-1 after trypsin treatment 45 min, 6 - medication-1 after trypsin treatment 90 min, 7 - medication-2 before trypsin treatment, 8 - medication-2 after trypsin treatment 45 min min, 9 - medication-2 after trypsin treatment after 90 min).

B - the results of densitometric analysis of the electropherogram A. The intensity of the color of the tracks in conventional units

This series of studies improved the method of creating the drug "Biophosphomag", which contains organic phosphorus and additionally contains

casein and magnesium, in the following weight ratio: casein - 75%, magnesium - 10%, phosphorus - 15%, and the synthesis occurs in two stages: direct phosphorylation of casein and chelation of magnesium with casein, resulting in a homogeneous powder preparation for oral administration to animals. This medication can be used in agriculture, in particular in the field of veterinary medicine, namely: for ways to correct the functional state and increase animal productivity.

The preparation is based on artificially phosphorylated casein of cow's milk as a ligand and magnesium ions as a complexing agent. The drug is a homogeneous powder, which can be easily tableted for oral use. To enhance and supplement the therapeutic and prophylactic effect, it is possible to add water- or fat-soluble vitamins in crystalline form to the mixture.

Biophosphomag is an effective treatment-and-prophylactic and adaptogenic agent for wide use in veterinary medicine and animal husbandry. Its effectiveness is associated with the stimulation and correction of metabolic processes a significant impact by its nature, the components of the drug have a protein, lipid and carbohydrate metabolism. Biophosphomag is promising for further study and use because it is easily modified and can be used as a raw material for further development of more complex drugs. Along with this, according to the original methods, biological products OVA, OVA^{+} and others were distinguished. The novelty of the research was confirmed by the patents of Ukraine for the utility model № 150210 [115-117].

The second series of studies was conducted in the laboratory of cultured cells of the ESC Institute of Biology and Medicine of Taras Shevchenko National University of Kyiv. To determine the effectiveness of the effects of compounds of natural origin obtained by extraction from plant raw materials: OVA and OVA^{+}, Biofosfomag and the corresponding controls used a number of biochemical and cell-biological methods, recording results using the following equipment: laminar (LS, laminar systems), CO2 incubator (Medcenter Einrichtungen GmbH MMM-Group), centrifuge (OPN-3), multiwell spectrophotometer (Labsystems Multiscan MS). In vivo observation, assessment of morphological parameters and visualization of cell

populations were performed using an inverted microscope AxioVert (Carl Zeiss), equipped with AxioVision software. Cellular preparations were photographed using a Digital Still Camera with a Carl Zeiss Vario-Sonar lens.

Cell lines. MT-4 - culture of T-cell leukemia; cells were obtained by co-culturing lymphocytes from the blood of the heart and peripheral blood of patients with T-cell leukemia.

Namalva - B-cell line derived from Burkitt's lymphoma - a human cell line containing hypodiploid chromosomes. The number of modal chromosomes 44, found in 30% of cells. The rate of cells with higher ploids is 2.8%. Twelve to four marker chromosomes were distributed to most cells. Among them were (q11q29), del (3) (p12 / 13), der (6) t (3; 6) (p21; p25), t (15q21q). Normal N3 was absent and N7 had three copies per cell. The X chromosome was single, the Y chromosome was missing.

Cultivation of cells. The cells of the studied lines, stored under cryopreservation conditions in liquid nitrogen, were rapidly thawed at 37 ° C in a water bath. 1-2 ml of cell suspension was taken and added to 25 ml of culture medium, stirring gently. Cells were pelleted by centrifugation at 800 g for 2–3 min, and the supernatant was removed. Carefully resuspended in complete medium (RPMI-1640, Sigma, USA) containing 10% FBS (Sigma, USA) and count the total number and ratio of living and dead cells after staining the latter with trypan blue. Cells in which the number of dead did not exceed 15% were used for cultivation.

Evaluation of cell survival using the vital dye trypan blue. Trypan blue staining (0.4% solution prepared on 0.1M phosphate-buffered saline, PBS pH 7.2) was used to quantify the ratio of living to dead cells. To do this, 2 samples were taken from each well of the plate, stained with trypan blue, for which an equal volume of 0.4% trypan blue solution was added to the cell suspension, and cells in the Goryaev chamber were counted. The results were averaged considering cell growth and incubation volume.

The proliferative parameters of MT-4 (T-lymphocyte cell line) and Namalva (B-lymphocyte cell line) cells were studied by Biophosphomag, OVA and OVA+ using

the MTT colorimetric test. According to this method, the assessment of proliferative activity is carried out by the intensity of metabolic processes in mitochondrial dehydrogenases [118]. MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) is a yellow monotherazolium salt, the reduction of which is most often used to measure cell proliferation and cytotoxicity [119].

The regenerative activity of dehydrogenases depends largely on the concentration of intracellular NADH and NADPH, the content of which is associated with the presence of extracellular glucose [120]. Mitochondrial succinate dehydrogenase and cytochrome C are mainly involved in the recovery of MTT [121]. Thus, compounds that affect the intensity of MTT reduction reflect the activity of mitochondrial respiration and are adapted to determine proliferative processes.

Lymphocytes isolated from the peripheral blood of rats were used to determine the effect of these compounds on primary T- and B-lymphocyte cultures. The method is based [122] on the reaction of "rosette formation" of T-lymphocytes.

Spontaneous and stimulated HCT test was used to determine oxygen-dependent biocidality of peritoneal macrophages [123]. Spontaneous activity of peritoneal macrophages was expressed in conventional units [124].

Statistical processing of the results was performed by the conventional method of variation statistics with the determination of arithmetic mean (M), deviations of each measured value from M (a), quadratic deviations for each group (σ), mean error (m), Student's coefficient t and probability between p values, the results for which $p \geq 0.05$ were considered reliable.

Determining the immunomodulatory effects of different classes of substances, especially of biotechnological and natural origin, occupies a leading niche in the structure of preclinical studies. That is why the newly created plant extracts OVA, OVA^+ and Biophosphomag were primarily studied on target cells of the immune system, namely the lines and primary cultures of T- and B-lymphocytes and phagocytic cells.

Determination of cell survival by OVA, OVA^+ and Biophosphomag. According to the count of living cells using trypan blue dye and MTT test, activation of MT-4

cell proliferation was detected under the influence of OVA^+ (Table 1.2, Fig. 1.2).

Along with the increase in the total concentration of cells under the action of OVA^+ a decrease in the percentage of dead cells was found. Also, a decrease in the percentage of dead cells was characteristic of OVA, but a significant increase in the total concentration of cells under the influence of OVA was not recorded.

Table 1.2 - The content of cells of the MT-4 line under the action of OVA, OVA^+ and Biophosphomag under the conditions of culturing cells for 2 days with substances under standard conditions

Sample	Control	OVA	OVA+	Biophosphomag
Concentration of living cells	$34.4x10^3 \pm 1.2\ x10^3$	$37.2\ x10^3 \pm 2.7x10^3$	$43.8\ x10^3 \pm 3.1x10^{3*}$	$37.4\ x10^3 \pm 5.6x10^3$
Concentration of dead cells	$2{,}6x10^3 \pm 0.7\ x10^3$	$0.9x10^3 \pm 0.2\ x10^3$	$1.4x10^3 \pm 1.1\ x10^3$	$3.8x10^3 \pm 2.2\ x10^3$
% of dead cells	7.02	2,36	3.09	9.22

*- $p < 0.05$ against control

A similar effect was found in the MTT colorimetric test - an increase in the content of living cells (function of reduction of yellow salt to formazan crystals, visualization of which was performed after dissolution in DMSO and determination of color intensity by optical absorption) (A540, λ = 540 nm). Fig. 1.2 and 1.3, OP_540 for the action of OVA^+ exceeds this indicator in the control by almost 1.3 times.

Because the MT-4 cell line has a malignant phenotype but also has markers of T-lymphocyte differentiation, it was important to test proliferative parameters in primary lymphocyte cultures derived from rat peripheral blood.

There was a slight inhibitory effect on the survival of cells Biofosfomag and activation under the influence of OVA^+, and for OVA, the indicators did not differ from the control (Fig. 1.4)

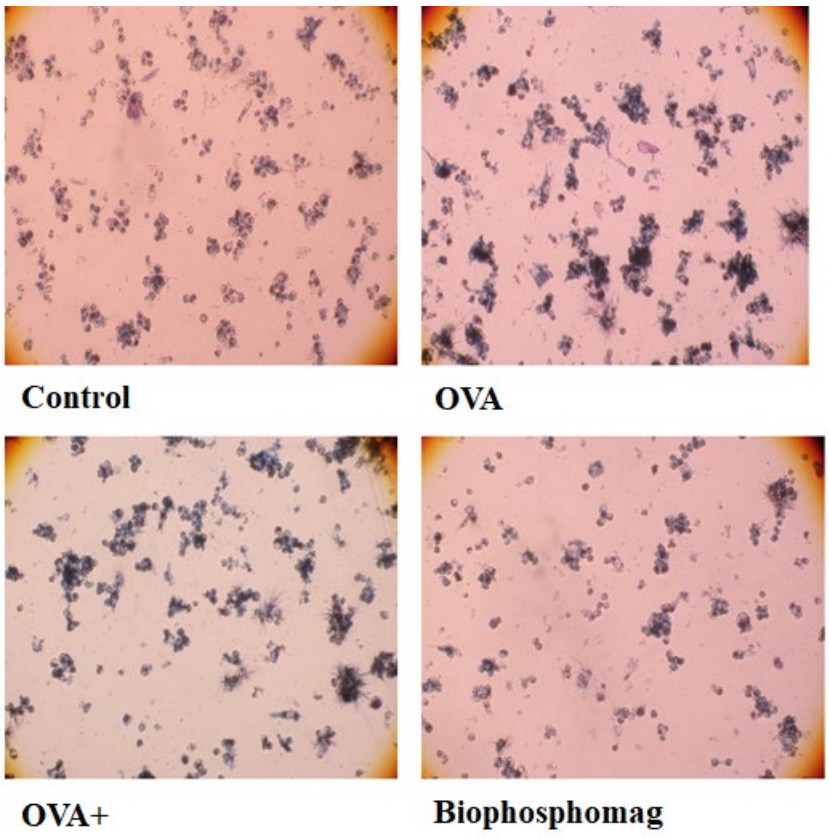


Figure 1.2 - Reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide by cells of T-lymphocyte origin of the MT-4 line under the action of OVA, OVA$^+$ and Biophosphomag.

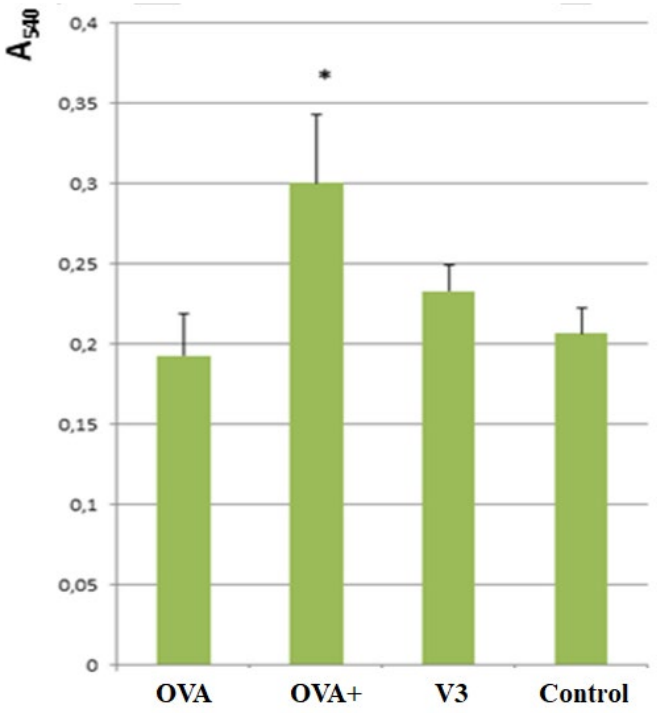


Figure 1.3 - Histograms of restoration of proliferative activity of T-lymphocytes under the action of Biophosphomag (V3), OVA and OVA$^+$; * - p <0.05 against control.

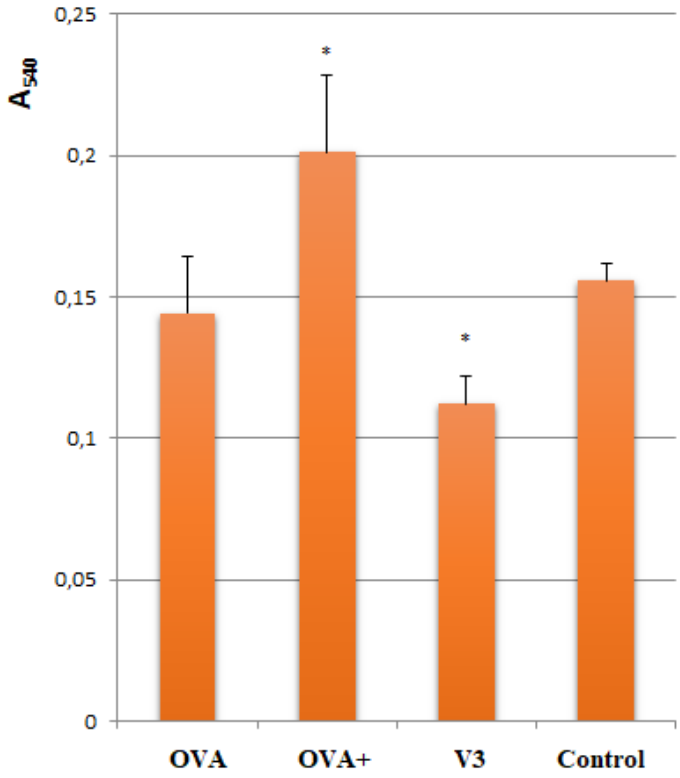


Figure 1.4 - Histograms of the effect of Biophosphomag (V3), *OVA and OVA$^+$ on the culture of primary T-lymphocytes of rats. Cultivation for 3 days under standard conditions of 5% CO2, 37o C and 100% humidity in RPMI 1640 (Sigma) and 10% ETS, * - p <0.05 against control.*

Regarding B-lymphocytes, changes in cell survival under the action of Biophosphomag, OVA and OVA$^+$ were not recorded (Figs. 1.5, 1.6). There was a slight inhibition of proliferation by the action of OVA, while other compounds did not show different from the control effect. As no changes in B-cell survival under the influence of OVA, OVA$^+$ and Biophosphomag were recorded, the primary culture of B-lymphocytes was not used in the test.

Determination of oxygen-dependent biocidality of peritoneal macrophages by the action of OVA, OVA$^+$ and Biophosphomag. The next step in determining the bioactive parameters under the action of Biophosphomag, OVA and OVA$^+$ was the study of oxygen-dependent bioactivity of peritoneal macrophages in rats in the HCT test with Nitro-Blue tetrazolium (Fig. 1.7).

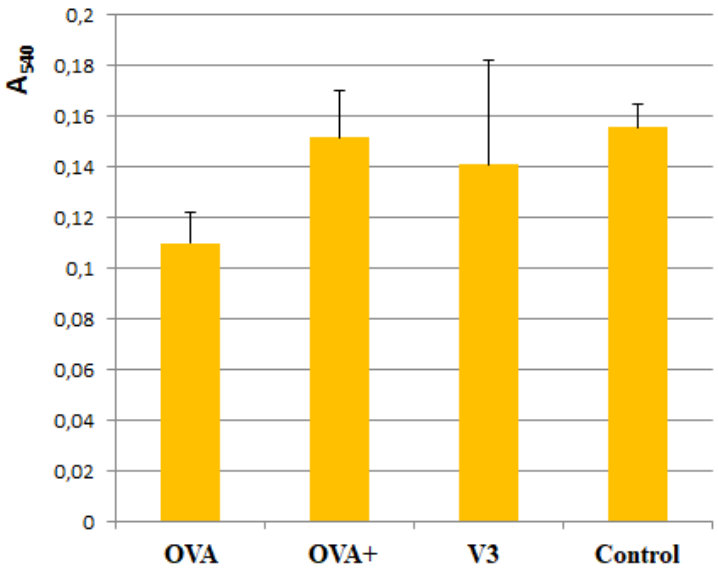
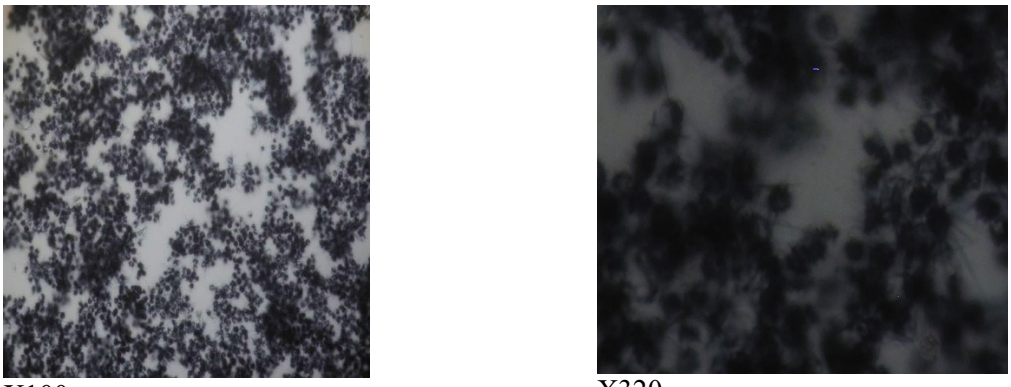


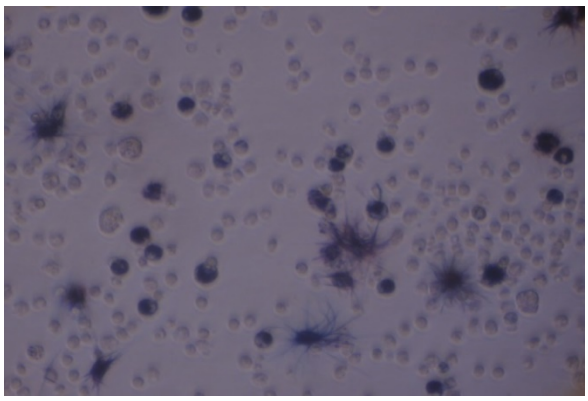
Figure 1.5 - Histograms of the effect of Biophosphomag (V3), *OVA and OVA$^+$ on the culture of B-lymphocytes of Namalva cells. Cultivation for 2 days under standard conditions of 5% CO2, 37˚ C and 100% humidity in RPMI 1640 (Sigma) and 10% ETC.*



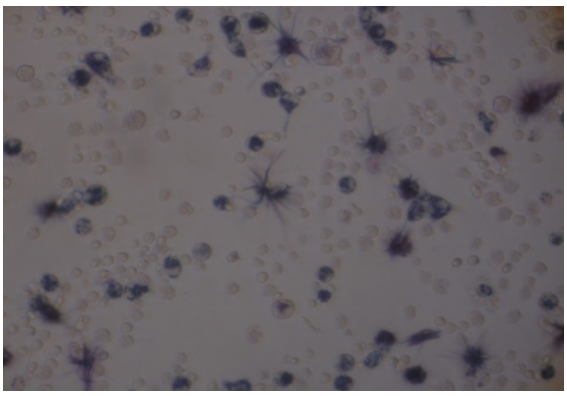
X100 X320

Figure 1.6 - Accumulation of formazan crystals by Namalva B-cell cells

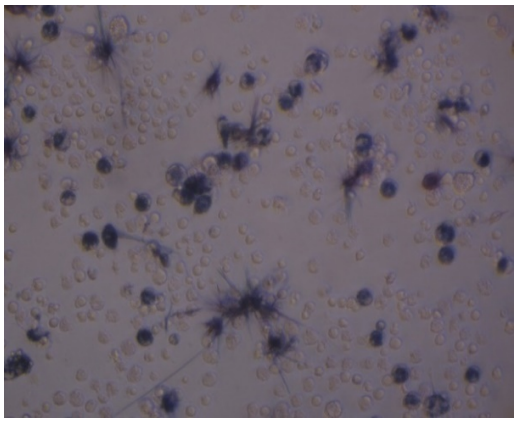
Obtaining peritoneal macrophages was performed according to the classical procedure [123, 124]. Cultivation with test compounds and nitrosin tetrazolium was performed according to the procedure for 2 hours. According to the data of optical absorption shown in Fig. 1.8 shows that in comparison with the control (increase) and phorbol 12-meristat-13-acetate (almost identical) means OVA$^+$ stimulates oxygen-dependent biocidality.



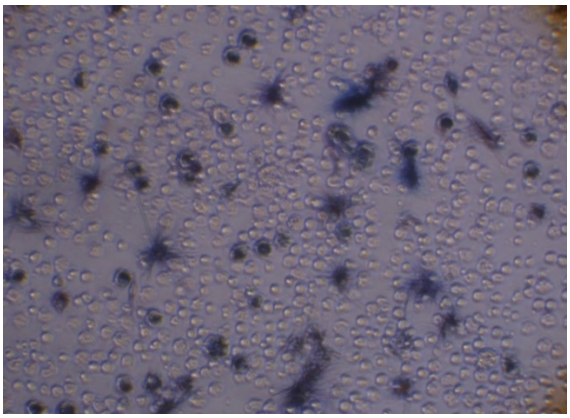
Control



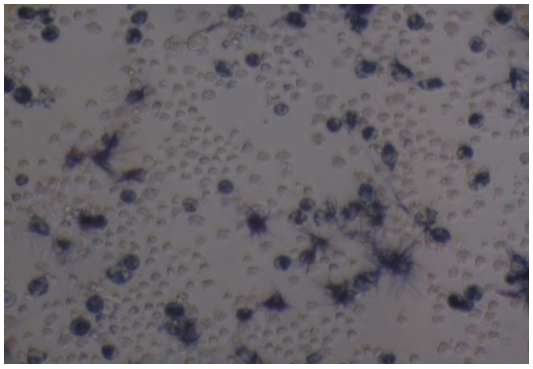
OVA



OVA^+



Biophosphomag



PMA

Figure 1.7 - Oxygen-dependent activity (conversion of nitrosine tetrazolium to crystals by active phagocytes) of peritoneal macrophages of rats under the action of various compounds.

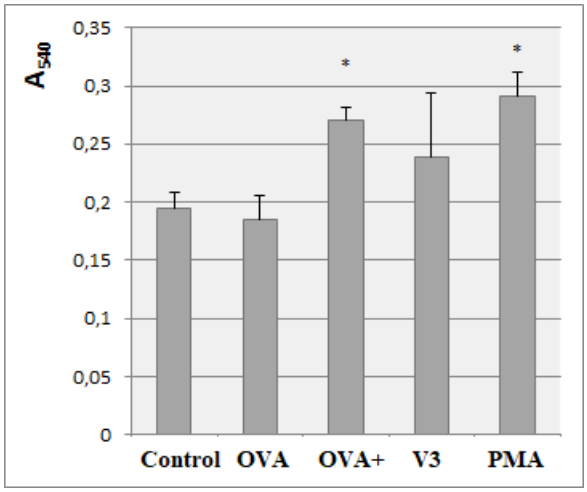


Figure 1.8 - Oxygen-dependent biocidality of peritoneal macrophages under the action of Biophosphomag (V3), *OVA and OVA^+, determined in the HCT test. * - $p < 0.05$ against control*

According to the obtained data, the functional reserve of peritoneal macrophages relative to spontaneous activity in the control was determined (Table 1.3).

Table 1.3 - Percentage of functional reserve of peritoneal macrophages under the action of Biophosphomag, OVA and OVA^+

Samples	OVA	OVA^+	Biophosphomag	*PMA (phorbol 12-meristat-13-acetate)*
% activation	4.96±0.71	38.86±3.8	22.4±14.7	49.82±4.69*

Thus, the stimulating activity of OVA^+ against oxygen-dependent activity of rat peritoneal macrophages was found to be almost 40% compared to controls and only 10% lower than the classical stimulant phorbol 12-meristat-13-acetate (PMA).

Thus, in the first series of studies was synthesized Biophosphomag, which is an effective therapeutic and prophylactic and adaptogenic agent for widespread use in veterinary medicine and animal husbandry, the effectiveness of which is associated with the stimulation and correction of metabolic processes. In addition, biological products of natural origin OVA and OVA^+ were identified for further testing and evaluation of their immunomodulatory properties.

The second series of studies revealed a multidirectional effect of the studied biological products: activation of T-lymphocyte proliferation by OVA^+ by 23% relative to control and no changes in the proliferative parameters of T-lymphocytes in the presence of OVA; slight toxicity to B-lymphocytes under the influence of OVA (approximately 13%) relative to control, while OVA^+ and Biophosphomag had no toxic effect; an increase in oxygen-dependent biocidality of rat peritoneal macrophages by 40% relative to the control of the action of OVA^+, which reached almost the same level of activity as stimulation with the classic activator - phorbol-12-meristat-13-acetate.

KAPITEL 2 / *CHAPTER 2*
INFLUENCES OF BIOLOGICALS ON CELL BIOCHEMICAL PROCESSES

Introduction

Metabolism in the cell in pathological conditions is considered an important therapeutic target. Precursors of metabolic drugs can be both synthetic (biophosphomag) and natural (betaine, OVA, OVA^{+}). Recently, betaine has been used as a bioactive substance [1-3]. This year's research was aimed at developing a scheme (schemes) to study the effects of bioactive substances along with their development to use them more effectively to normalize pathological conditions of the body. Betaine is trimethylglycine, which is widely used in animals, plants, and microorganisms. It plays the role of osmoprotector, donor of methyl groups and anti-inflammatory factor in many diseases. Betaine is involved as an antioxidant in the metabolism of S-containing amino acids, while inhibiting the activity of nuclear factor-kB and activation of the inflammatory process NLRP3, regulates energy metabolism, relieves endoplasmic reticulum stress and apoptosis [1, 4]. on the one hand, it is an important donor of methyl groups in the transmethylation process catalyzed by betaine homocysteine methyltransferase. During the reaction, homocysteine is converted to methionine directly in the liver and kidneys. On the other hand, betaine is an important osmoprotector, mainly in the kidneys, liver, and brain [5], and its large amount can accumulate in cells without disrupting their function, i.e., an important factor is that this role is protected by cellular proteins and enzymes. osmotic stress.

In mammalian cells, betaine, like osmolite, is responsible for reducing hypertensive stress, which leads to the preservation of their functioning and increased survival. It alleviates many types of cellular stressors, namely: hyperthermia, hypertension, acidity, hyperkalemia, and oxidative stress [6].

In addition, betaine inhibits the production of IL-1β by canonical mechanisms. (1) MAPK (JNK, p38 and ERK1 / 2) are responsible for the expression of proinflammatory cytokines; and NIK / IKK relieves IκB inhibition, leading to NF-κB activation to promote increased regulation of IL-1β. Betaine inhibits NF-κB activity

and IL-1β expression by inhibiting MAPK and NIK / IKK. (2) Betaine inhibits the expression of mMNA and HMGB1 protein, which regulates the activation of TLR4, which are involved in the activation of NF-κB. (3) Betaine inhibits HDAC3, which binds to IκBα to activate NF-κB. (4) NLR-controlled involvement of ASC stimulates the activation of pro-caspase-1, which leads to the cleavage of pro-caspase-1 and the maturation of caspase-1, subsequently caspase-1 cleaves pro-IL-1β to produce bioactive IL-1β. Betaine enhances the phosphorylation of IRS-1 to activate PKB / Akt, leading to inactivation of FOXO1, resulting in inhibition of FOXO1 TXNIP, which functions as an endogenous inhibitor of ROS-absorbing protein, thereby inhibiting NLP activation. (5) In addition, activation of inflammatory NLRP3 is associated with K+ leakage caused by ATP-mediated activation of P2X7R. Betaine inhibits NLRP3 activation by maintaining normal K + levels in the cytosol [4]. Betaine also inhibits IL-1β production by non-canonical mechanisms. (A) Caspase-8 plays a vital role in promoting NF-κB signaling and also modulates the cleavage of pro-IL-1β at exactly the same site as caspase-1. In fact, FADD and caspase-8 control the transcriptional priming of NLRP3-inflamasoma by modulating the expression of NLRP3 and pro-IL-1β. Betaine inhibits the production of IL-1β by preventing the induction of caspase-8 activity / activation. (B) Initiation of dectin-1 promotes Syk-dependent formation of the CARD9-Bcl-10-MALT1 complex, which induces NF-κB activation and IL-1β transcription; and the formation and activation of the complex MALT1-caspase-8-ASC, which mediates the processing of pro-IL-1β. Betaine reduces the production of IL-1β by inhibiting the formation and activation of the MALT1-caspase-8-ASC complex. (B) Caspase-11 is also important for the activation of NLRP3-inflamasoma, which ends with the production of IL-1β. Mechanically activated LPS caspase-11 induces cleavage of the plasma membrane channel of pannexin-1, which leads to the leakage of K^+ and the release of ATP that interacts with P2X7R, promoting the activation of NLRP3. Interestingly, betaine reduces IL-1β production by increasing hemoxygenase-1 expression to activate the cGMP-protein kinase G signaling pathway, which improves the activity of the pannexin-1 channel [1-4]. Thus, all of this emphasizes the anti-inflammatory properties of

betaine by inhibiting the production and secretion of IL-1β and points to the potential use of betaine supplements as adjunctive therapy in various inflammatory diseases associated with IL-1β secretion.

Given the current challenges of COVID and related pathological abnormalities, endothelial cell culture was selected for its versatility and broad functions. These functions include regulation of vascular tone, barrier formation, leukocyte transmigration, blood clotting, and angiogenesis. Endothelial function and dysfunction are associated with inflammatory processes (pneumonia due to COVID-19 virus), the development of cardiovascular disease (one of the key threats to COVID-19) [7-9].

In view of this, it can be concluded that an important target of betaine as a modulator of metabolic dysfunction may be endothelial cells, assessing the impact on which this bioactive substance.

The purpose of the study is to develop methods and means of regulating metabolism in the animal's body under the action of various factors and the correction of its disorders. Comprehensive studies were aimed at studying metabolic processes in animals by determining the biochemical parameters of metabolism, elucidation of sensitive to changes in biochemical processes in pathological abnormalities of organisms and the correction of their biological products with immunomodulatory properties.

To achieve the goal of the tasks you need to solve the following tasks:

- research and analysis of the effect of bioprotectors on the composition of some amino acids under oxidative stress caused by alcohol;

- improvement / development of the method of obtaining "Biophosphomag" (based on macronutrients and some components of milk) for further testing and evaluation of immunomodulatory properties;

- development of a scheme (schemes) to study the effects of bioactive substances of natural origin (betaine) to normalize pathological conditions of the body (oxidative stress, lipid metabolism, inflammation, destabilization of the endothelial-epithelial barrier as a powerful regulator of metabolic processes in cells

and tissues), using the MTT test to determine the activity of mitochondrial enzymes and cell survival and to assess the level of glucose uptake and morphological properties of endothelial cells and a similar assessment of the action of other biological products in further studies.

The above studies are promising, as in the future may reveal some elements of the regulatory units of metabolic processes for the correction of the resulting biological products with immunomodulatory properties.

2.1. Betaine, its participation, and role in metabolism

Bioactive substances of natural and artificial origin (betaine, biophosphomag, OVA, OVA^{+}) have a multifaceted effect on biochemical transformations in cells that can normalize abnormal metabolic processes. In our experimental studies of bioactive substances, betaine was used as a control substance as a hepatoprotector and antioxidant factor, while studying its properties, which are actively discussed in the scientific literature. Distributed in animal, plant, and microbiota betaine (trimethylglycine, Fig. 2.1) acts as an osmoprotector, donor of methyl groups and anti-inflammatory factor.

In addition, it is actively involved in the metabolism of sulfur-containing amino acids as a kind of antioxidant, while inhibiting the activity of nuclear factor-kV and activation of NLRP3 inflammatory, regulates energy metabolism and relieves stress of the endoplasmic reticulum and apoptosis [1-6].

Betaine is a stable and non-toxic natural substance with the quaternary Quaternary ammonium form [$(CH_3)_3N^{+}CH_2COO^{-}$] (Fig. 2.1). It was first found in the plant Beta avulgaris, and then in wheat bran, spinach, microorganisms, and invertebrates in water. The main natural sources of betaine include bran and wheat germ, spinach, beets, sunflower seeds, krill, pasta, some spices - paprika, curry, ginger, turmeric, oregano. It is also found in some herbs and berries, such as Echinacea purpurea, burdock root and goji berries. In the human body, some betaine

is formed naturally by the oxidation of choline (vitamin B4) in the liver and kidneys. The highest content of betaine and its plasma transporter is determined in the liver. In drugs, the substance is most often used in the form of betaine hydrochloride or citrate, it is classified as a metabolite (hypolipidemic drugs), and hepatoprotectors, and a group of digestive enzymes. Betaine is involved in many biochemical processes in the body. The main function of betaine is to reduce the level of potentially toxic homocysteine agent.

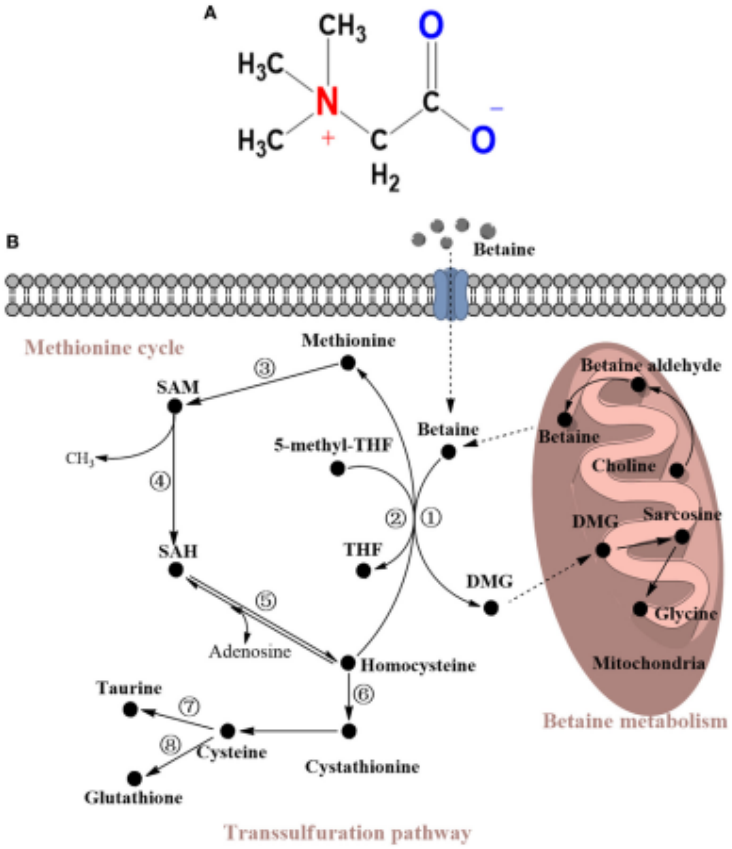


Figure 2.1 - Betaine (A) and its participation in the metabolism of sulfur-containing amino acids (B) [1].

It is also known about its normalizing effect on the digestive system, activation of lipid metabolism in the liver, increase bile production and improve its outflow; Betaine hydrochloride salt can be used for achlorhydria, increase appetite and improve the absorption of iron, calcium and vitamins. Betaine acts as an activator in the synthesis of phospholipids of cell membranes and a donor of methyl groups in the conversion of methionine to homocysteine and by involving these two reactions

affects the intermediate metabolism. Dietary use of betaine plays a crucial role in the body's betaine content. In addition, it can be synthesized from choline in the body. Studies show that its high concentrations are present in infants and newborns and indicate the effectiveness of this synthetic mechanism.

Betaine has been found in mouse ovaries, where it increases after fertilization. It may act as an osmolyte, and preimplantation embryos accumulate betaine through a Na-dependent amino acid transporter. Detection of betaine in high concentrations indicates its importance in methylation reactions at developmental stages. This is confirmed by observations that inhibition of these processes during embryogenesis leads to developmental defects [10]. Betaine is an important donor of methyl groups in transmethylation, a process catalyzed by betaine homocysteine methyltransferase (BHMT) in the reaction to convert homocysteine to methionine directly in the liver and kidneys. In addition, betaine is an important osmoprotectant, mainly found in the kidneys, liver and brain, and a large amount can accumulate in cells without disrupting their function: an important factor is that this role is protected by cells, proteins and enzymes under osmotic stress (Fig. 2.1). Betaine is a substrate of choline and is converted to dimethylglycine (DMG) by dimethylation to eventually become glycine. Most of these reactions occur in the mitochondria. After the dimethylation reaction, homocysteine is converted to methionine, which is converted to 5-methyl-THF, which catalyzes methylation to form THF. Methionine is then sequentially converted to SAM and finally to homocysteine, forming a methionine ring. Homocysteine can also undergo transulfurization to form cystathionine, cysteine, taurine or glutathione. The enzymes listed above in the description (Fig. 2.1) are represented and labeled in the cycle by individual numbers: 1 - betaine-homocysteine methyltransferase (BHMT); 2 - methionine synthase (MS); 3 - methionine adenosyltransferase (MAT); 4 - SAM-dependent methyltransferase; 5 - S-adenosylhomocysteine hydrolase; 6 - cystathionine β-synthase (CBS); 7 - cysteine dioxygenase (CDO); 8 - γ-glutamylcysteine synthetase (GCS) and THF - tetrahydrofolate; SAM - S-adenosyl-L-methionine; SAH - S-adenosyl-L-homocysteine; DMG - N, N-dimethylglycine. Aqueous extracts of Lycium chinensis

are known to contain high concentrations of betaine and are used in traditional medicine in Southeast Asia to treat liver disease [11]. Studies of betaine, its natural compounds as anti-inflammatory agents for diseases such as diabetes, as well as non-alcoholic and alcoholic fatty liver disease (NAFLD / AFLD) are still relevant [12].

Cells found in all living organisms, from bacteria to vertebrates, use betaine as an osmoprotector; it is well absorbed through the duodenum of the small intestine in animals [32, 33]. Betaine can be freely filtered by the kidneys and reabsorbed into the circulation, so it is excreted mostly in the body, not in the urine. Betaine accumulation depends on transporters and is distributed mainly in the kidneys, liver and brain. Although betaine is used in a variety of tissues as an osmoprotectant, its major role is the donation of methyl groups in hepatic metabolism.

Betaine as an osmoprotector. Unlike inorganic salts, osmoprotectors are well-soluble small organic compounds that accumulate in large quantities in cells without disrupting their functions and protecting them from osmotic stress. Hyperosmosis can cause water outflow and a gradual decrease in cell volume, so these effects can threaten the survival of body cells. Thus, to balance hyperosmosis and protect cells from drying out, they accumulate various types of osmoprotectors, including betaine, sorbitol, and taurine, which are mandatory.

Unlike other osmolites and inorganic salts (urea and Na^+), betaine reduces the ability of water molecules to dissolve proteins, stabilizing native protein structures. It can increase the cytoplasmic volume and free water content of cells to prevent compaction under hyperosmotic conditions and to inhibit the diversity of proteins associated with hyperosmotic apoptosis. Due to these benefits, extra betaine can be used to counteract the pressure when the tissues are hypertensive. For example, in the kidneys, hypertension increases the level of betaine - γ-aminobutyric acid (GABA) of the transport system (GAT4 / BGT1) in the basolateral plasma membrane to obtain more betaine. However, under normal physiological conditions, BGT1 levels are quite low, and this transporter is present mainly in the cytoplasm of Madina-Darby cells of canine kidneys (MDCK) [13].

Betaine as a donor of methyl groups. Betaine is a metabolite of choline and a

donor of methyl groups involved in methylation. Processes such as DNA or protein methylation are important biochemical processes in animals. In turn, the availability of methyl group donors affects methylation. Betaine, methionine, and choline are the most important donors of methyl groups present in food [14]. The main role of methionine is that it is a substrate for protein synthesis, and choline is mainly involved in the formation of cell membranes and neurotransmitters. The betaine transmethylation reaction is part of a single-carbon metabolism through the methionine cycle in the mitochondria of liver and kidney cells. In this reaction, VNMT catalyzes the addition of a methyl group from betaine to homocysteine to form methionine, which is eventually converted to dimethylglycine (DMG).

Dimethylglycine has two available methyl groups and may degrade to sarcosine and be converted to glycine. Similarly, vitamin B12-dependent enzyme methionine synthase (MS) can also catalyze the formation of methionine from homocysteine with a methyl group donor from N5-methyltetrahydrofolate. These reactions are important for animals because they retain methionine, detoxify homocysteine, which is the cause of cardiovascular disease, and form S-adenosylmethionine (SAM; a coenzyme involved in methyl transfer reactions). SAM is formed from methionine by methionine-adenosyltransferase (MAT), and SAM is the main methyl agent. After demylation, SAM is converted to S-adenosylhomocysteine (SAH). The SAM: SAH ratio affects a variety of SAM-dependent methyltransferases, including protein-L-isoaspartate methyltransferase (PIMT), protein arginine methyltransferase (PRMT), and isoprenylcysteinecarboxyl methyltransferase (ICMT). These enzymes are involved in the recovery of proteins, lipid metabolism, protein interactions and the activity of GTPase, which belongs to the class of hydrolases that synthesize and hydrolyze guanosine triphosphate (GTP).

One SAH molecule is hydrolyzed by SAH hydrolase to form homocysteine and adenosine. This reaction is reversible, and the direction of the reaction depends on whether these products are removed. All these reactions together form a methionine cycle. In addition, with cystathionine-β-synthase, a vitamin B6-dependent enzyme, homocysteine can be converted to cystathionine on the way to transulfurization.

Under these conditions, homocysteine catabolism leads to increased production of glutathione (GSH), taurine and other metabolites. Betaine supplementation has been shown to affect various S-containing amino acids (SAAs). For example, such an addition effectively increases the available methionine and SAM. Thus, betaine acts as a methyl donor and plays an important role in the metabolism of sulfur-containing amino acids (in detail, this metabolic pathway is shown in Fig. 1.1B).

2.2. Anti-inflammatory properties of betaine

Inflammation, as an immune response, is important and paramount in the body's defenses and in repairing and healing wounds. However, excessive, or prolonged inflammation can be a pathogenic condition of various diseases. Therefore, the use of natural compounds to treat diseases can be a means of controlling the intensity of the inflammatory response. For example, many studies show that GABA has anti-inflammatory effects [15]. Based on this, the following actions of betaine are described below.

Metabolism of S-containing amino acids in the presence of betaine under oxidative stress. Reactive oxygen species (ROS) are by-products of biological reactions that generate energy in mitochondria, where oxidative metabolism occurs. Under normal conditions, the body has two detoxification systems that can cleanse the body of ROS and free radicals: antioxidant enzymes and antioxidant agents. Catalase, superoxide dismutase (SOD), melatonin and GSH are examples of various detoxifying agents. Excessive levels of ROS pose a threat to cells because they alter the stability of nucleic acids, proteins and lipid components of the membrane; in addition, high levels of ROS cause pathological processes, including inflammation. S-containing amino acids (homocysteine, methionine, SAM, SAH, and cysteine) are involved in metabolic processes, including GSH synthesis, protein synthesis, and trasmethylation reactions. Although homocysteine promotes GSH synthesis, many different studies have shown that hyperhomocysteinemia ultimately causes oxidative

stress and apoptosis [16, 17]. Betaine treatment can directly affect homocysteine levels by stimulating the conversion of homocysteine to methionine to regulate SAA levels. For example, ROS and ethanol-induced free radicals can inhibit methionine synthase (MS) to inhibit remethylation and cause hyperhomocysteinemia.

To compensate for the decrease in MS activity, betaine was used as an alternative methyl donor, which improves BHMT activity to produce methionine and SAM, and as a result to remove homocysteine in the liver of Wistar rats fed ethanol specifically for the study.

In C57B6 mice, there was a decrease or no change in VNMT expression, rather than a compensatory increase. Hence, because betaine converts homocysteine to methionine, the concentration of methionine is closely related to the concentration of betaine. Methionine also plays an important role in antioxidants. It can reduce oxidative stress due to chelation and be used by hepatocytes to synthesize GSH. This reaction is necessary for the formation of SAM and removal of homocysteine. SAM is a direct antioxidant in living organisms and can regulate GSH metabolism. Moreover, based on the inverse reaction, as a result of which SAH is converted into homocysteine and adenosine, the concentration of homocysteine will continue to decrease. SAH is a potent inhibitor of SAM-dependent methyltransferases, which methylate nucleic acids, bproteins and other organic compounds.

Betaine can significantly increase the SAM: SAH ratio and MAT activity. It can prevent the development of nitric oxide synthase II (N2); expression of nitric oxide synthase II (NOS2) [18]. This process is initiated by inflammation, and the SAM: SAH ratio is increased to maintain methylation of the NOS2 promoter. Homocysteine can also be converted to cysteine by irreversible transulfurization. Next, cysteine is formed from either taurine through cysteine dioxygenase (CDO) or GSH through γ-glutamylcysteine synthetase. Betaine treatment inhibits CDO activity and lowers taurine levels, while increasing GSH production to neutralize oxidative stress in mice with subsequent NAFLD / AFLD diseases [19, 20]. Antioxidant enzymes SOD2 and glutathione S-transferase (GST) were modified after treatment with betaine [21, 22]. However, most results prove the absence of significant changes. Therefore, further

research is needed in the future to confirm whether these antioxidant enzymes have indeed changed after treatment with betaine.

Thus, the anticoxidant mechanism of action of betaine may be due to the acceleration of SAA metabolism. These changes after betaine treatment are associated with oxidation of the function of primary S-containing amino acids (Table 2.1).

Betaine inhibition of the NF-κB signaling pathway. The transcription pathway of nuclear factor-kV (NF-kV) controls a large number of genes involved in inflammation; genes contain proinflammatory cytokines: tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 23 (IL-23). Hence, most inflammatory diseases are associated with chronic NF-κB activation. Thus, the NF-κB pathway has become a very important candidate in the treatment of inflammation. It has been found that betaine can inhibit the activity of NF-κB, as well as structural genes [23].

In one of the first studies in old kidney cell cultures, betaine inhibited NF-κB activity and the expression of various related genes, including TNF-α, a vascular cell adhesion molecule - molecule-1 (VCAM-1), intracellular cellular adhesion molecules-1 (ICAM-1), induced nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). In addition, betaine inhibits NF-κB by inhibiting two important activators: mitogen-activated protein kinase (MAPKs) and kinase / IRB kinase, which induces nuclear factor (MIK / IKK). NIK / IKK may attenuate IQ inhibition and initiate transcriptional activation of NF-κB. MARKs consist of c-Jun NH2-terminal kinase (JNK), protein 38 (p38) and intracellular signal-regulated kinase (ERK1 / 2) and are involved in inflammation and responses to anti-inflammatory cytokine expression. The mechanism of action of betaine is based on the maintenance of thiol levels, including GSH, to inhibit ROS production and NF-κB activity. Betaine also inhibits some signaling molecules that cause NF-κB activation. Toll-like receptors (TLRs) are involved in an important signaling event that activates NF-κB. In an in vitro study, betaine affected lipopolysaccharide (LPS, a specific TLR-4 activator) that induces NF-κB activation in RAW 264.7 mouse macrophage cells.

Table 2.1 - The effect of oxidative functions of primary S-containing amino acids on biochemical processes under the action of betaine

Compound	Changes	Function
Methionine	Gain / increase	GSH synthesis; reduction of oxidative stress
S-adenosylmethionine	Gain / increase	Increased content in the GSH cell; antioxidant action
S-adenosylhomocysteine	Inhibition / reduction	Methyltransferase inhibitor; induces oxidative stress
Homocysteine	Inhibition / reduction	Induces oxidative stress; GSH synthesis
Cysteine	Gain / increase	GSH synthesis; reduces oxidative stress
GSH	Gain / increase	Antioxidant

Betaine reduces the potential for neurotrauma to the hypothalamus by inhibiting the TLR-4 / NF-κB signaling pathway to restore astrogylosis and fructose-induced inflammation. Betaine may inhibit the expression of histone deacylases 3, which may activate NF-κB due to IqBα binding. Another study showed that betaine treatment can reduce TLR-4 expression levels to limit inflammation [24]. Betaine can reduce the formation of endogenous molecular structure damage (DAMP) to inhibit the NF-κB pathway. Hence, betaine has an anti-inflammatory effect by inhibiting the NF-κB signaling pathway.

Inhibition of NLRP3 inflammation activation by betaine. The Leucine-rich family and NLRP3 inflammasome are a large cytosolic protein complex that contains a nucleotide-linked domain, a Leucine-rich content repeat (NLR) member of the NLRP3 family, an important adaptive ASC molecule, and mature caspase-1. When TLRs recognize DAMPs or pathogen-associated molecular patterns, NF-κB is activated, promoting mRNA expression of interleukin precursors containing pro-IL-18 and pro-IL-1β and NLRP3. Completely assembled inflammatory NLRP3 activates caspase-1 to mediate the production of mature IL-1β and IL-18, which are involved in initiating inflammation. It is important to reduce inflammatory responses in a timely manner by inhibiting the activity of inflammasome.

Betaine may directly increase the expression of hemoxygenase-1 in hepatocytes; this effect may inhibit NLRP3 inflammation to protect against LPS-induced and d-galactosamine-induced inflammation in the liver [25]. Betaine treatment can

significantly inhibit NLRP3-associated inflammatory proteins such as NLRP3 and mature caspase-1 and certain levels of anti-inflammatory cytokines, including IL-1β, in a dose-dependent manner in fructose-induced NAFLD models [26].

A similar situation was found in db / db mice treated with betaine. This demonstrates the mechanism involved in thymic transcription factor FOXO-1, which is inhibited by thyrodoxin-interacting protein (TXNIP), which can affect ROS production to trigger NLRP3 inflammatory components. The FOXO family contains six members, including: FOXO-1 and FOXO-6, which are found in mammals. The main role of these FOXO factors is in the regulation of cell growth, cell death, proliferation, differentiation, and oxidative stress response [27].

Activated FOXO-1 increases the activity of TXNIP, which is an endogenous inhibitor of the ROS-saving protein thioredoxin, which leads to the formation of large amounts of ROS. Activated PKB / Akt can phosphorylate the active form of FOXO-1 to cause it to exit the nucleus into the cytoplasm, causing inactivation of FOXO-1. The action of betaine increased the levels of PKB / Akt-mediated phosphorylation of FOXO-1. But betaine does not directly activate PKB / Akt, and its mechanism may be the result of enhanced insulin receptor substrate 1 (IRS-1) phosphorylation [28]. Hence, betaine may enhance IRS-1 activity to activate PKB / Akt; and subsequently, activated PKB / Akt inhibits FOXO-1 activation, which in turn limits TXNIP to inhibit NLRP3 components by inflammation to eliminate its anti-inflammatory effect. Betaine-mediated inhibition of inflammation-induced NLRP3 activation plays a more important role than NF-κB in response to renal inflammation. In general, the anti-inflammatory effects of betaine are very closely related to its inhibition of NLRP3 by activating the inflammasome.

Blockade of chronic inflammation by betaine regulation of energy metabolism. Impaired energy metabolism can lead to a variety of chronic diseases, including obesity and diabetes, which are usually accompanied by low-level systemic inflammation. Therefore, the restoration of normal metabolism is an important step that can reduce inflammation. Betaine has an effect on both lipid and glucose metabolism [29].

Regarding lipid metabolism: Excessive accumulation of fat, which occurs as a result of imbalance of lipid compounds during their synthesis and oxidation, is considered the cause of many diseases. Factors such as a high-fat diet, exposure to antibiotics and ethanol use can lead to obesity, antibiotic intoxication and ethanol poisoning.

The action of betaine can restore the imbalance between synthesis and oxidation, reducing the accumulation of fat. Increased activity of hepatic AMP-activated protein kinase (AMPK) can be mechanically involved in the process of reducing the accumulation of fat in the body [30]. AMRK serves as the main cellular energy sensor and as a vital regulator of metabolic hemostasis in the body. Many genes: sterol regulatory protein-binding element 1c (SREBP-1c), acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS), are involved in such regulation. Activated AMRK can inhibit fatty acid synthesis and promote fatty acid oxidation by regulating the expression of these genes. Betaine may increase the phosphorylation of AMPK and then inhibit ACC activity, as well as SREBP-1c and FAS expression. Similarly, AMRK can directly phosphorylate SREBP-1c and SREBP-2 on Ser372 to inhibit their activity to reduce lipogenesis and lipid accumulation in mice with dietary insulin resistance [31]. In addition, activated AMRK promotes glucose uptake by improving translocation of the glucose transporter type 4 (GLUT-4); therefore, these data suggest a beneficial effect on insulin resistance. Changing the ratio of AMP: ATP in cells under normal conditions promotes the activation of AMP. However, hepatic AMPK activation can occur regardless of the AMP: ATP ratio via adiponectin. Betaine can restore abnormal levels of adipokines in NAFLD, and it is betaine that increases adiponectin levels and decreases leptin and resistin levels in fat cells to reduce dysregulation of lipid metabolism. Similar effects of betaine are confirmed by other in vitro studies in human adipocytes [32]. Hence, increased adiponectin levels may contribute to the phosphorylation of AMPK. Because these adipokines play a role in inflammation, this normalizing process is anti-inflammatory. In addition to activating AMPK, betaine treatment may potentially affect other factors related to lipid metabolism.

Betaine may reduce the accumulation of triacylglycerols in apolipoprotein B (apoB)-deficient mice due to decreased methylation of peroxisome proliferator-activated receptor alpha (PPARα). In another study, betaine restricted the transcription of PPARγ by inhibiting the binding of FOXO-1 to the PPARγ promoter to reduce fat accumulation [33].

It has been shown that PPPAα and hepatic X-receptor α (LXRα) have elevated levels just when betaine restores inhibition of fatty acid oxidation. The mechanism of activation by betaine LXRα remains unclear. It may be associated with the enzyme PPMT-3, which is also associated with SAM, which may directly increase LXRα activity. In a cisplatin-induced nephrotoxicity study, betaine itself inhibited lipid peroxidation by inhibiting renal activation of TBA products, which is primarily triggered by the kidneys. Hence, the appearance of TB products is initiated by oxidative stress. In addition to changes in fat synthesis and oxidation, betaine may increase lipid transport. Betaine supports the CAM: SAH ratio in the liver to increase phosphatidylcholine synthesis and normalize the production of very low density lipoproteins (VLDL) due to the stimulation of PEMT activity [19, 20]. In addition, betaine stimulates the expression of the apoV gene to produce more VLDL [34].

Insulin resistance is known to be directly related to inflammation in glucose metabolism. Betaine supplements can directly affect the insulin pathway and facilitate the course of NAFLD [35]. A similar manifestation was observed in another study of type II diabetes, where the use of betaine led to a decrease in ser473-phosphorylated RKV / Akt and increased phosphorylation of IRS-1 and thr308-phosphorylated PKB / Akt. Thus, PBK / Akt regulates systemic and cellular metabolism, mainly the proliferation, differentiation and survival of cells required for insulin signaling. It should be noted that thr308-phosphorylated PKB / Akt may limit the activity of FOXO-1 and glycogen synthase kinase-3α. The first substance can reduce the expression of phosphoenolpyruvate carboxykinase to reduce gluconeogenesis in the liver, while the second can accelerate glycogen synthesis. In order to test and investigate whether betaine can initiate RCM / Akt, a RIZK inhibitor, wortmanin, was used and it was found that it is difficult to find activated

PKB / Akt. This suggests that betaine may directly enhance the phosphorylation of IRS-1 and not directly activate PKB / Akt. The mechanism by which betaine enhances the phosphorylation of IRS-1 to enhance insulin resistance remains unclear. However, it has been shown that PRMT-1 can methylate heterogeneous nuclear ribonucleoprotein (hnRNPQ) and may be involved in insulin signaling [36 - 38]. The mechanism is based on the fact that PRMT-1 can catalyze the addition of a methyl group from SAM to hnRNPQ; this process leads to the internalization of long-term activation of the insulin receptor. SAM concentrations have been shown to be related to betaine.

Therefore, betaine promotes the availability of SAM for the formation of large amounts of methylation of hnRNPQ through PRMT-1 and thus the activation of PKB / Akt. In addition to the IRS-PKB / Akt signaling pathway, betaine treatment has been shown to reduce levels of X-box-bound protein-1, a protein that is associated with endoplasmic reticulum stress. This is likely to increase p38-MAPK and mammalian rapamycin targets, which reduce hepatic gluconeogenesis and insulin resistance [39].

Hence, betaine has an anti-inflammatory effect due to the restoration of energy metabolism. These major metabolic pathways and key factors mediated by betaine treatment in chronic inflammation are listed in table. 2.2. and fig. 2.2.

Betaine reduction of endoplasmic reticulum stress and apoptosis. Endoplasmic reticulum (ER) stress is caused by an abnormal assembly of proteins in the form of irregular and deployed proteins in the lumen of the ER. Proteins such as C / EVR and homologous protein (CHOP), as well as glucose-regulating protein 78 (GRP 78), all participate in and are markers of EP stress. Massive EP stress is undesirable, causing cellular apoptosis. Apoptosis is one of the types of cell death and is involved in the pathogenesis of inflammatory diseases. Apoptosis has an external and internal pathway, and its final process ends with proteins of the caspase family, namely caspase 3. Betaine can directly affect homocysteine, and hyperhomocysteine can cause improper protein formation, which ultimately leads to EP stress. Betaine can stabilize homocysteine levels and inhibit GRP78 and CHOP levels, as well as prevent

cell death [40]. Similarly, in another experiment, betaine inhibited both GRP78 and CHOP, resulting in decreased activation of JNK, which can directly phosphorylate several portions of IRS-1, including serine-307. These modifications prevent the stimulating effect of insulin and tyrosine phosphorylation of IRS-1, and this leads to insulin resistance. However, in addition to stress EP, betaine also inhibits apoptosis. Studies of synovial fibroblasts in rheumatoid arthritis have shown that transcription factor-3 (ATF-3) is a molecule that is associated with apoptosis and is reduced by betaine [41].

Table 2.2 - Major metabolic pathways and genes / proteins affected by betaine in inflammatory diseases

Results	Main metabolic pathway	Gene / protein	Gene / protein function
Lipid metabolism ↑	AMPK path ↑	ACC ↑	Synthesis of fatty acids
		FAS ↑	Synthesis of fatty acids
		SREBP-1c ↑	Synthesis of fatty acids
	Others	PPARα ↑	Oxidation of fatty acids
		PPARγ ↑	Oxidation of fatty acids
		LXRα ↑	Oxidation of fatty acids
		TBARS ↓	Lipid peroxidation
		Apo B ↑	Cholesterol transport
Glucose metabolism ↑	IRS-1 / Act Way ↑	IRS-1 ↑	Insulin sensitivity
		FOXO-1 ↓	Gluconeogenesis
		GSK3α ↓	Inhibition of glycogen synthesis
	Others	XBP-1 ↓	Gluconeogenesis
		GLUT-4 ↑	Glucose transport

It is known that betaine can inhibit caspase family proteins. In vitro studies adding adenosine to hepatocytes, increasing liver SAH and caspase-3 activity, have shown that both factors are also inhibited by betaine. Inhibition of caspase-3 by betaine has also been observed in the induction of cispatin and the resulting nephrotoxicity. In addition, betaine significantly reduced the activity of caspase-8, caspase-9 and caspase 3/7 activity in human corneal epithelial cells and MDCK under conditions of reduced EP stress and apoptosis under the influence of betaine is important for its anti-inflammatory action.

From here it is necessary to allocate a logical number of processes under the influence of betaine, which can: 1) change the concentration of various sulfur-

containing amino acids (SAA), protecting metabolism with SAA from oxidative stress; 2) inhibit the activity of IKK, MAPKs, HDAC3 and Toll-like receptor-4 (TLR-4) to reduce the regulation of nuclear factor-kV (NF-kV) and transcription of anti-inflammatory genes; 3) reduce the expression of inflammatory components of

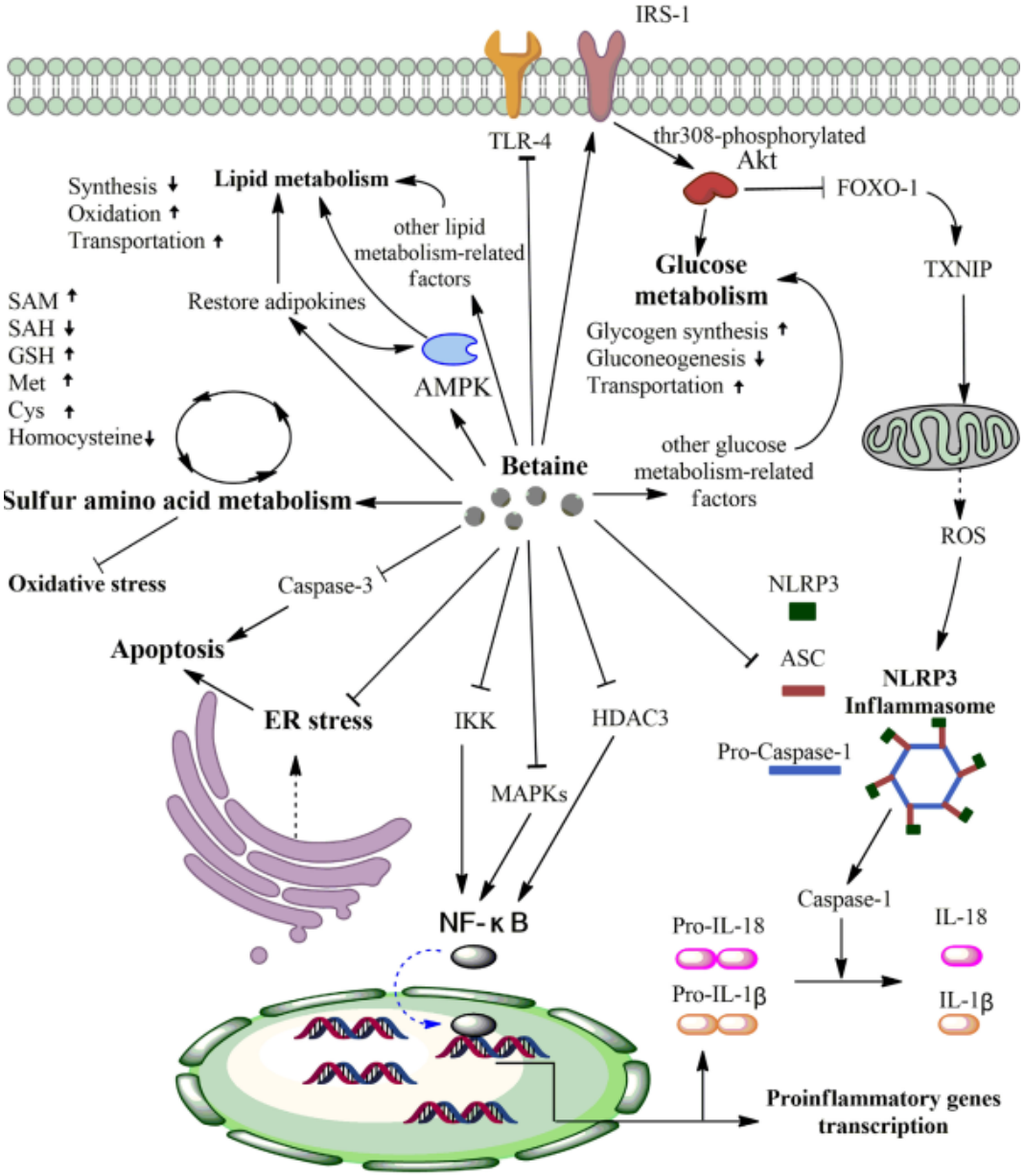


Figure 2.2 - The main anti-inflammatory mechanisms of betaine [1], *where Akt - protein kinase B; AMRK-AMP - activated protein kinase; FOXO-1 - thymic enzyme O1; TXNIP - thioredoxin-interacting protein; ROS - reactive oxygen species; IKK - nuclear factor-induced kinase / IkB kinase; MARKs - mitogen activating protein kinase; HDAC3 - histone deacetylase 3; SAM, S-adenosyl-L-methionine; SAH, S-adenosyl-L-homocysteine; GSH-glutathione; Met - methionine; Cys - cysteine.*

NLRP3 (pro-caspase-1, ASC and NLRP3) and inhibit FOXO-1-induced NLRP3 inflammation and by enhancing the IRS / Akt pathway; 4) significantly increase activated AMRK by restoring adipokines that can activate AMRK and other factors associated with lipid metabolism to regulate lipid metabolism; 5) on the one hand, increase the phosphorylated IRS, which phosphorylates Akt on threonine 308, to improve glucose metabolism, and on the other hand, to influence other factors associated with increased glucose metabolism; 6) inhibit caspase-3 to reduce apoptosis and restore endoplasmic reticulum (EP) stress.

Summarizing all the above, we have reason to believe that betaine is involved in many biochemical processes in the body. The main function of betaine is to reduce the level of homocysteine - a potentially toxic agent. In addition, it has a normalizing effect on the digestive system, activation of lipid metabolism in the liver, increase bile production and improve its outflow. Its hydrochloride salt can be used for achlorhydria, increase appetite and improve the absorption of iron, calcium and vitamins. Betaine plays the role of activator in the synthesis of phospholipids of cell membranes and the donor of methyl groups in the conversion of methionine from homocysteine. Hence, betaine affects the intermediate metabolism by involving it in the above two reactions.

Betaine can be used as a protective agent against hepatotoxic substances such as ethanol and carbon tetrachloride. Betaine administration was found to increase hepatic S-adenosylmethionine levels in control animals as well as in ethanol-treated animals and almost completely prevented ethanol-induced hepatosteatosis. By providing methyl groups for betaine-homocysteine-methyltransferase-mediated methionine and S-adenosylmethionine production under methionine synthetase inhibition, as well as protection against methylfolic acid traps, betaine may be a promising alternative for other disorders.

On the other hand, betaine can be used as a kind of control to assess the protective properties of biological products such as biophosphamide, OVA and OVA +.

Thus, the two previous sections analyze aspects of the theoretical basis for the

practical application of biologicals in the adjustment of relevant parts of biochemical processes, development of a scheme for assessing their protective properties and further experimental research and implementation.

2.3. The effect of bioprotectors on the composition of some amino acids under oxidative stress caused by alcohol

Two series of studies (III and IV) were conducted, which studied metabolic processes in animals through the determination of biochemical parameters of metabolism, clarified sensitive to changes in biochemical processes under the influence of harmful exogenous factors and the correction of their biological products with immunomodulatory properties.

The third series of research was carried out on the basis of the interdepartmental educational-scientific laboratory of veterinary-diagnostic research of the department of biochemistry and physiology of animals named after acad. M.F. Gulyi, Ukrainian Laboratory of Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine.

The experiments were performed on male rats weighing 180-220 g, which were divided into 7 groups (control and 1-6 experimental) of 7 animals each for 28 days. For 7 days of the adaptation period, control and experimental rats were kept in ad libitum drinking water and dry standard rodent feed. For another 3 weeks, the diet of rats remained unchanged, except for animals in experimental (1-6) groups (Table 2.3).

At the end of the experimental period, the animals were euthanized with deep chloroform anesthesia. The content of total protein, creatinine, urea and enzyme activity (alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), γ-glutamyltransferase (GGT, EC 2.3.2.2) were measured. Bioanalysis and biochemistry of the corresponding standard PLIVA-Lachema Diagnostika kits (Czech Republic) [42], as well as oxidoreductases such as lactate

dehydrogenase (LDH, EC 1.1.1.27), superoxide dismutase (SOD, EC 1.15.1.1) and catalase (EC 1.11.1.6), - according to the described methods [43-45] The content of TBA-active compounds (malonic dialdehyde, MDA) was determined by reaction with thiobarbituric acid [46].

Student's t-test was used in statistical analysis.

Table 2.3 - The composition of control and experimental rations

Groups of animals →	Control	Experimental					
Ration↓		1	2	3	4	5	6
Purina rodent chow	+	+	+	+	+	+	+
Additives (protein + Minerals)	-	-	-	-	+	-	+
Water	+	-	-	-	-	-	-
EtOH (30% v/v, 8 g/kg BW)	-	-	+	+	+	+	+
Carbohydrates' mix (in final solution of 35 %)	-	+	-	+	+	-	-
Sulfur-containing bio-protector betaine in the final concentration of 1%	-	-	-	-	-	+	-

Note: Animal groups: (C) control and experimental - (1) Carbohydrates' mix, 2) EtOH, (3) EtOH + Carbohydrates' mix, (4) EtOH + Carbohydrates' mix + Additives (Protein + Minerals), (5) EtOH + Betaine, (6) EtOH + Additives (Protein + Minerals). Additives (Protein + Minerals): proteins 22.5%, fats 5%, fiber 4.5%, ash 5.1%, moisture 10.2%, calcium 0.89%, phosphorus 0.67%, magnesium 0.24%, sodium 0.26%, potassium 1.07%; vit. A, D3, E, C, K3, B1, B2, B6, B12, nicotinamide, pantothenic acid, biotin, folate, choline, zinc, manganese, copper, iodine, iron.

Animal experiments were conducted in compliance with the Law of Ukraine "On Protection of Animals from Cruelty" (Article 230 of 2006), "General Ethical Principles of Animal Research", approved by the National Congress of Bioethics and the European Convention. on the protection of vertebrates used in experiments and other scientific purposes "(Strasbourg, 1986) [47].

III series of studies. Changes in body weight of rats during the experimental period confirm the depressing effect of alcohol on the dynamics of weight gain of animals during their growth and development, the positive protective effect of betaine and supplements (protein + minerals) (Fig. 2.3).

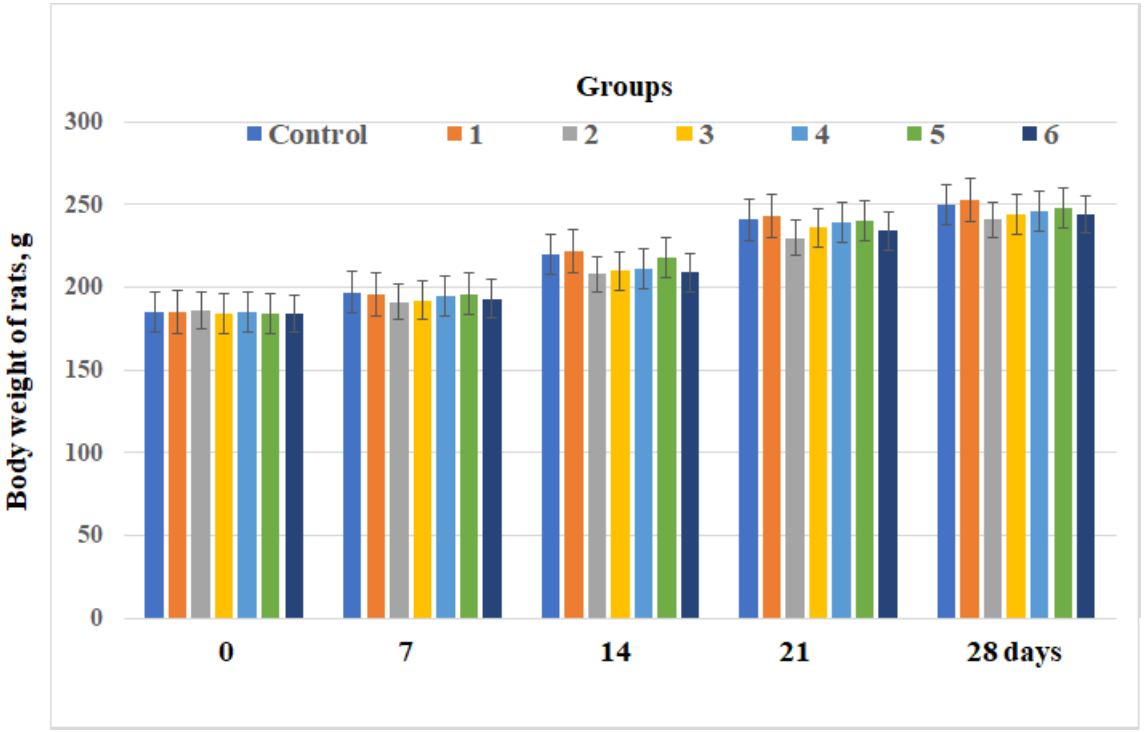


Figure 2.3 - Weight of rats in the control (control) and 1-6 experimental groups, g (M ± m, n = 7)

A significant increase in the activity of AST in the serum of rats of the 2nd experimental group indicates damage to liver cells and the release of this enzyme into the blood through damaged cell membranes. The use of protectors, apparently, restores the structural and functional state of cells, and therefore, probably a decrease in activity (experimental groups 4-6) and approach to the level of the control group (Table 2.4). Similar patterns of changes are observed when evaluating the results of determining the activity of ALT and GGT (Table 2.4).

The results (Table 2.5) show that in the serum of rats under the action of alcohol and alcohol-carbohydrate mixture, the total protein content is almost unchanged (there is a tendency to slightly increase), and creatinine and urea are significantly increased. Such changes in biochemical parameters indicate a possible pathological deviation of the kidney. Under the action of protectors (betaine and protein supplement with minerals) there was a significant decrease in creatinine and urea.

Table 2.4 - Transferase activities (AST, ALT and GGT) in the blood of rats, U / L (control and 1-6 experimental groups of animals; M ± m, n = 7)

Groups of animals →	Control	Experimental					
Enzyme activity↓		1	2	3	4	5	6
AST, U/L	98.1± 4.9	108.2± 3.8	197.6± 10.9**	206.3± 8.4**	199.8± 9.3**	110.1± 7.1##	170.2± 5.8**#
ALT, U/L	79.2± 4.7	83.8± 5.2	169.3± 8.8**	150.3± 6.9**	146.4± 7.4**	91.3± 6.9##	120.1± 7.2**##
GGT, U/L	6.9± 0.7	8.4± 0.9	19.7± 1.1**	21.3± 1.3**	20.7± 3.1**	9.2± 0.6*##	15.4± 1.1**#

Note: in Tables 2.4-2.6, the data are statistically significant (p< 0.05 and ** p< 0.001) compared to the control group and # p<0.05, and ## p< 0.001 compared to the 2nd experimental group, probably.*

Table 2.5 - Nitrogen-containing substances in the serum of rats in the control and 1 - 6 experimental groups (M ± m, n = 7)

Groups of animals →	Control	Experimental					
Biochemical indexes ↓		1	2	3	4	5	6
Total protein, g/L	64.5± 3.3	64.5± 1.9	64.4± 1.3	66.5± 1.4	64.5± 1.1	64.1± 0.8	64.6± 0.9
Creatinine, µmol/L	30.5± 2.9	34.7± 2.2	66.4± 3.1**##	68.4± 2.7**##	67.2± 1.7**	33.6± 2.3##	43.6± 2.4*#
Urea, mmol/L	3.4± 0.6	4.6± 0.7	8.2± 0.8**	9.1± 1.1**	8.3± 1.5*	5.1± 0.9#	6.1± 0.6*

Decreased activity of oxidoreductases (SOD and catalase) in the serum of the 2nd and 3rd groups of animals, increasing the concentration of malonic dialdehyde indicates the presence of alcoholic oxidative stress (Table 2.6). Under the conditions of use of protectors in 4-6 groups of rats opposite changes of value of biochemical indicators are observed.

Under the influence of alcohol and alcohol-carbohydrate mixture, the activity of LDH in the serum of rats increases almost 2 times (Table 2.6). This indicates a clear manifestation of alcoholic steatosis in the liver, the cells are unable to fully utilize lactic acid, which is formed from pyruvate in this organ or enters the blood through the plasma membranes of muscle cells, leading to general depletion of muscle cells. body. Betaine and protein supplements with minerals have an effect on the health of the body.

Table 2.6 - Activity of oxidoreductases (LDH, SOD and catalase) and the content of malonic dialdehyde (MDA) in the blood serum and liver tissue of rats (in control and 1 - 6 experimental groups of animals; M ± m, n = 7)

Groups of animals →	Control	Experimental					
Biochemical indexes ↓		1	2	3	4	5	6
LDH, U/L	489± 18.2	519± 26.7	990± 28.3**	998± 25.9**	734± 21.2**##	610± 19.1**##	680± 19.9**##
SOD, U/mg of protein/min	260 ± 21.2	220 ± 13.1	148.5± 15.3**	135.8± 13.3**	172.4± 17.1*	220.2± 14.2#	160.8± 12.3*
Catalase, U/mg of protein/min	239.8± 11.3	215± 7.8	139.3± 9.1**	128.7± 8.9**	154.3± 7.7**	179.7± 12.1*#	161.3± 6.7*
MDA, nmol/mg of protein	40.9± 2.3	47.7± 3.5*	56.3± 4.1*	66.1± 2.4**	50.7± 3.1*	42.1± 1.8#	45.4± 2.1#

A study of the levels of amino acids involved in the conversion of methionine (taurine, serine, a cystine dipeptide consisting of two cysteine residues and methionine) in rat serum shows a decrease in their content in animals of experimental groups 2 and 3. When using bioprotectors, the content of these amino acids in animals of groups 4-6 is close to the results of the control group (Fig. 2.4).

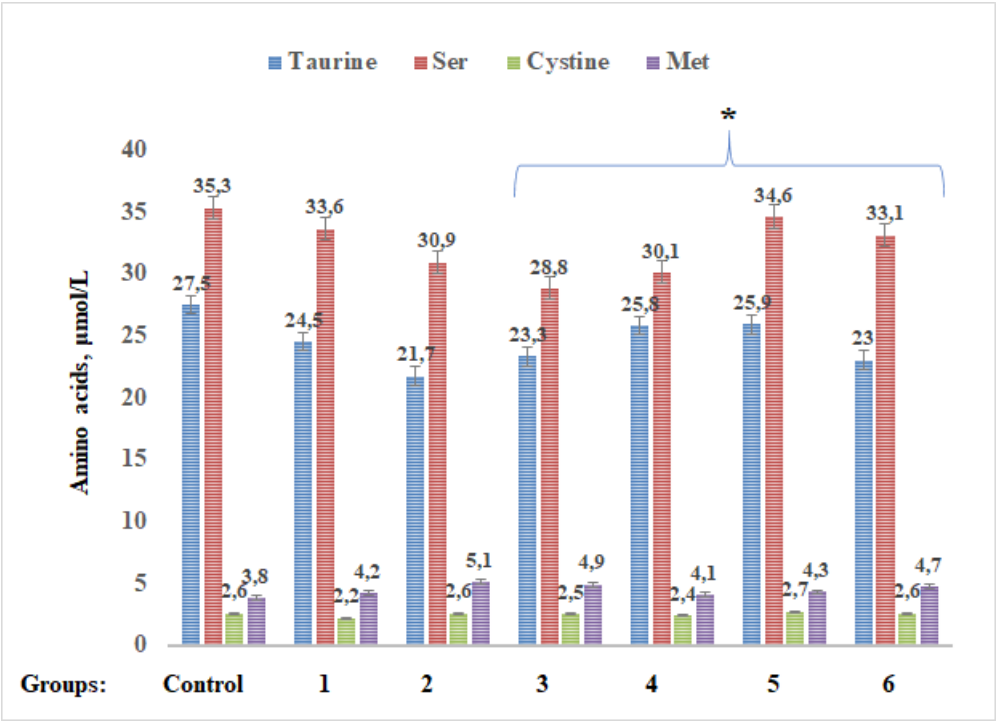


Figure 2.4 - The level of individual amino acids involved in the Met – transformation in the serum of rats, µmol / L (in the control and 1-6 experimental groups of animals; M ± m, n = 7; * p <0.05)

In the blood of rats, the activity of ALT is lower than that of AST (Table 2.4). The activity of transferases (ALT, AST, GGT) in the blood of animals of experimental groups (2 and 3) was higher than in control animals, indicating a deviation in the functional capacity of the liver. GGT is a diagnostic marker of liver function. GGT plays a key role in the gamma-glutamyl cycle, glutathione synthesis and degradation, and in the detoxification of drugs and xenobiotics. GGT also plays a prooxidant role by regulating effects at different levels in cellular signaling and cellular pathophysiology [12].

Transferases are involved in the conversion of alanine to pyruvate, from which glucose is synthesized by a number of reactions, and previous experimental data [53] also indicate an increase in glycogen amino acids in serum, indicating their use in many processes in the body, especially liver, in adaptation under the influence of toxic substances. In addition, indicators of transferase activity may indicate pathomorphological abnormalities in the tissues of the kidneys, as, for example, when working with microscopic examination of histopreparations of kidney kidneys of rats on an alcoholic carbohydrate diet [54].

Prophylactic use of protective substances (beta-containing and protein-rich supplements enriched with minerals in chelated form) restores the activity of the studied enzymes, normalizes redox processes and the intensity of transamination in animals under the influence of alcohol.

Alcohol intoxication increases the level of biosynthesis of triacylglycerols (TAG) and cholesterol in the liver, which, in turn, affects their content in the serum of rats [55]. Increased serum TAG in ethanol-consuming rats is most likely due to increased levels of lipoproteins such as LDL. The high content of TAG induces the synthesis of apolipoprotein B-100 (apo B) in liver cells. In turn, apoV in combination with TAG forms very low-density lipoproteins (VLDL), which are released into the blood. An increase in the content of TAG in the blood of rats with the development of alcoholic steatosis of the liver leads not only to an increase in LDL, but also to a decrease in HDL [56].

Such processes cause probable changes in the activity of oxidoreductases,

namely: its increase for LDH and decrease for SOD and catalase. Along with this, the concentration of MDA in liver tissues increases (Table 2.6).

Chronic hyperglycemia is observed in animals with excessive carbohydrate load in the diet (experimental groups), which can lead to the development of pre-diabetic conditions. This is confirmed by the results of histological studies, which indicate a certain correlation between the development of pathological conditions of liver tissue from blood glucose levels [57].

Betaine and protein supplement with minerals in chelated form normalize metabolic processes, especially the conversion of methionine and its intermediates, which are involved in the mechanisms of antioxidant protection (Fig. 2.4). It should be noted that in animals of group 2 the level of amino acids decreased unevenly. The amount of cystine and methionine decreased slightly compared to the control group, while the level of taurine decreased by almost 13% compared to the control group, and the level of serine decreased by 15%. It is likely that the decrease in serine levels is because it is a precursor of methionine (which is part of S-adenosylmethionine or SAM) and cysteine (which is part of glutathione - a tripeptide consisting of γ-glutamic acid residues). acid, cysteine, and glycine).

Decreased levels of taurine (synthesized from cysteine) - an amino acid with antioxidant properties, may indicate that this amino acid is an antioxidant system "first action". In this case, we can assume that serine was used for cysteine synthesis and taurine for synthesis. These results may also indicate the "secondary" nature of SAM as an antioxidant system compared to glutathione and the amino acid taurine, as it was not activated with a significant reduction in the number of amino acids - taurine and serine.

According to the results of a study [59-63], theoretically betaine is a more promising bioprotector than S-adenosylmethionine. It is planned to study the immunomodulatory properties of betaine, protein-containing supplements with minerals in chelated form, biophosphomag (synthesized on the basis of additionally phosphorylated casein with magnesium in chelated form) and other biological products in vitro.

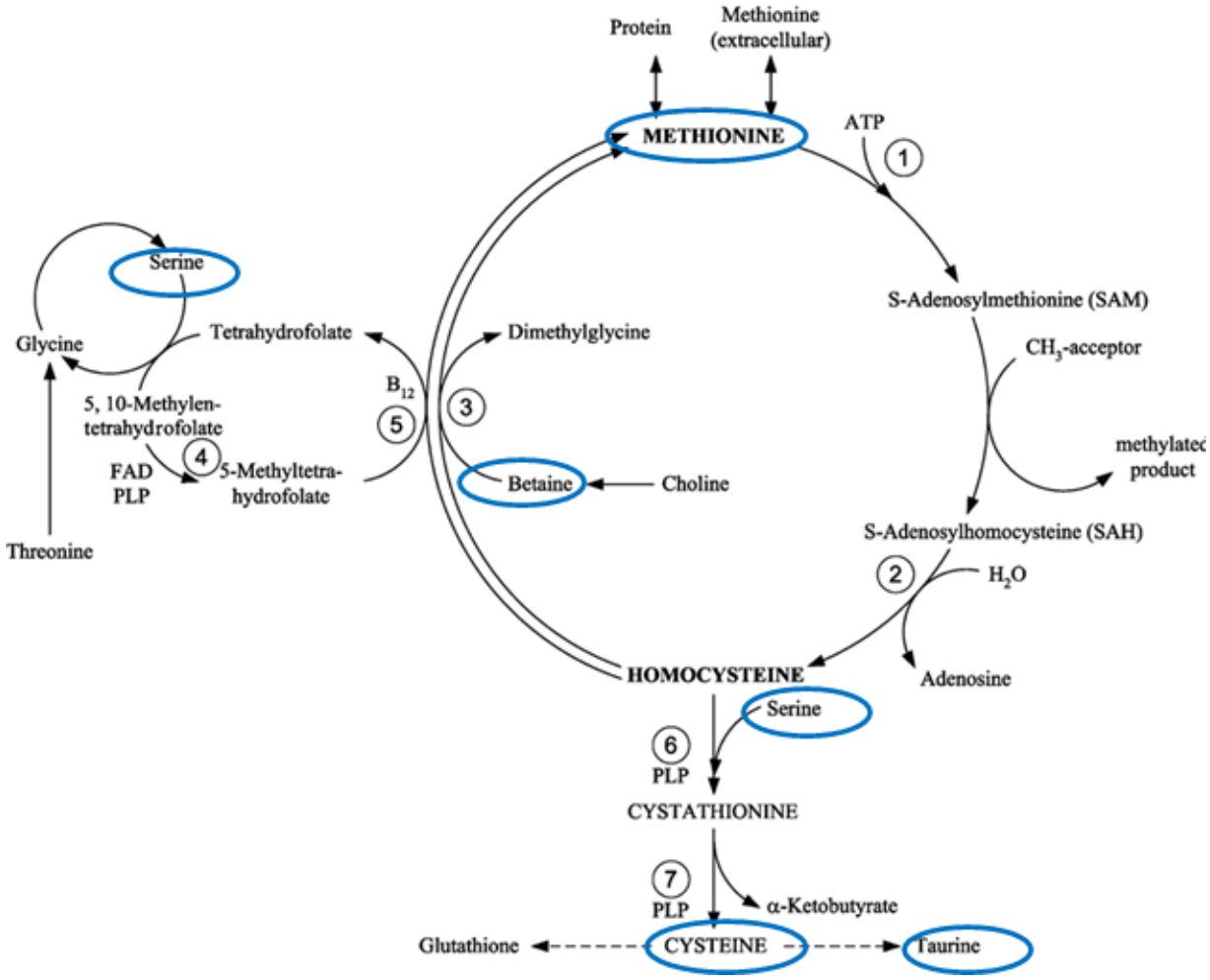


Figure 2.5 - Methionine cycle and intermediates (adapted [58])

2.4. The effect of betaine on endothelial cells and the development of a scheme for assessing the protective properties of biological products

The fourth series of studies was conducted in the laboratory of cultured cells of the ESC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv. The following equipment was used for the research: laminar flow cabinet (LS, laminar systems), CO2 incubator (Medcenter Einrichtungen GmbH MMM-Group), magnetic stirrer for isolation of primary colonocyte culture, thermostat with temperatures 370C and 560C, inverted microscope Ax Verio -40 with Axio Vision software (Carl Zeiss), light microscope (Carl Zeiss), shakers, electronic scales, and plastic utensils: vials, multi-well plates (Nunclon, Denmark). Visualization of the obtained results was performed on a spectrophotometric

multiwell scanner (Labsystems Multiscan MS) at a wavelength of 540-570 nm. Cell preparations were photographed using a Digital Still Camera with a Carl Zeiss Vario-Sonar lens (100-, 200-, 320-fold magnification).

Cell line. The study was performed on an RAE porcine aortic endothelial cell line. Cells were cultured in a DMEM medium (Sigma, USA) with the addition of 10% FBS (Sigma, USA), 2 mM L-glutamine, 40 μg / ml gentamicin at 37 ° C in a humid atmosphere (100%) with 5% CO2. Sterile 96-well plates, 6-well plates, 25 cm2 vials, and 10 and 6 cm Petri dishes (Nunclon, Denmark) were used for cell culture.

Isolation of cells from culture. The cell medium was collected from the vial for incubation in centrifuge tubes and centrifuged for 10 minutes at 1500 rpm. The precipitate was dissolved in 1 ml of saline. Cells were counted in a hemocytometer.

The supernatant (incubation medium) was collected in microtubes to further determine the level of glucose, TBA active products and catalase activity.

MTT colorimetric method for estimating the number of living cells by the activity of mitochondrial dehydrogenases of cells. The MTT test is an indirect method for determining the proliferative parameters of cultured cells associated with the activity of their mitochondrial dehydrogenases and cytotoxic / cytostatic effects on cells in vivo [48, 49].

Determination of adhesive properties of cultured cells by violet crystal color. When staining endothelial cells adhered to the plates, used vital dye violet crystal [50]. Evaluation of the rate of cell attachment to the substrate by modification of the test substances was performed on a highly adhesive substrate - 96-well plates (Falcon, USA).

Determination of glucose levels in the incubation environment of cells using glucose oxidase reaction, which is based on the oxidation of glucose by the enzyme glucose oxidase (using air oxygen) to gluconic acid and hydrogen peroxide as described [51].

Determination of total protein in cell culture using a set of reagents LLC "Philisit-Diagnostics". The content of TBA-active products (malonic dialdehyde) was

determined in endothelial cell culture. [52]. Catalase activity in cells was determined by MA Koroliuk et al. [45].

Evaluation of morphological characteristics. Determination of morphological characteristics under the action of ascorbic acid and lysozyme hydrochloride was performed on RAE cells (porcine aortic endothelial cells). To do this, different concentrations of betaine were added to cells in the 50-60% monolayer. After 24 hours of cultivation, the culture medium was collected, washed twice with phosphate-buffered saline and fixed with 95% ethanol for 30 minutes. Ethanol was then collected, the cells were dried and stained with purple crystalline dye for 20 minutes at 37 ° C. Morphological parameters were assessed and cell populations were visualized using an AxioVert inverted microscope (Carl Zeiss) equipped with AxioVision software. Cellular preparations were photographed using a Digital Still Camera with a Carl Zeiss Vario-Sonar lens.

Statistical processing of the received data. Statistical processing of the obtained results was performed using the program "STATISTICA 8.0". The probability of differences between the indicators of the control and research groups was determined by the criteria of Student and Fisher. The level of reliability was taken at $p < 0,05$.

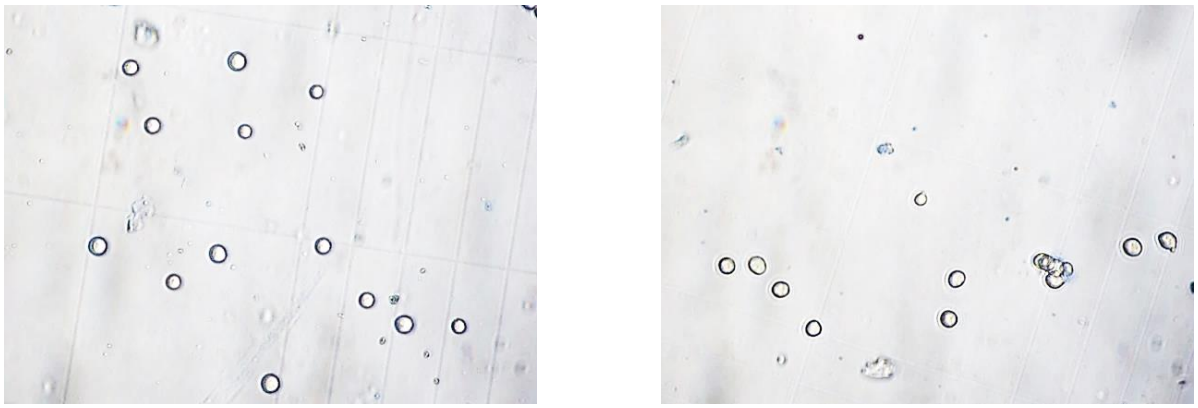
III series of studies. Determination of the effectiveness of betaine as a proangiogenic factor was performed on pig endothelial cells of the PAE line. These cells are immortalized, produce autocrine various biologically active factors, tolerate 6-8 passages when reconstituted with liquid nitrogen. The cell doubling period is 27 ± 4 hours. These cells are also used as a model to determine the mechanisms of differentiation under the influence of various natural and synthetic substances [64] and at the same time to develop a scheme for assessing the protective properties of biological products.

Endothelial function and dysfunction are associated with inflammatory processes, the development of cardiovascular disease, which include the regulation of vascular tone, barrier formation, leukocyte transmigration, blood clotting and angiogenesis in general [65 - 67]. Therefore, determining the effect of various drugs

on vascular cells determines their suitability and effectiveness as proangiogenic factors. Therefore, an important target of betaine as a modulator of metabolic dysfunction may be endothelial cells, the impact of which on this bioactive substance was chosen for the purpose of this study.

Determination of survival of endothelial cells when stained with trypan blue under the action of betaine. To determine betaine for endothelial cell survival, it was screened in the concentration range of 0.125-4 mg / ml. To do this, betaine was added to RAE (porcine aortic endothelial) cells adapted to standard culture conditions in RPMI-1640 medium with 10% FBS and cultured for 24 hours. After the deadline, the cells were detached from the substrate, centrifuged, the precipitate was dissolved in phosphate-buffered saline, 0.4% trypan blue solution was added, and the cell concentration and the ratio of living and dead cells were calculated (Fig. 2.6).

Toxic and antiproliferative effects under the influence of betaine for endothelial cells were not recorded (Table 2.6). When stained with trypan blue in the presence of 0.5 mg / ml and 1 mg / ml betaine, an increase in the concentration of endothelial cells was recorded in comparison with the control (Table 2.6). Similar data have been found in studies by other authors [68].



Control Betaine 4mg / ml

Figure 2.6 - Typical photos of endothelial cell count in the hemocytometer in the control and experimental (with the addition of betaine) samples (x100)

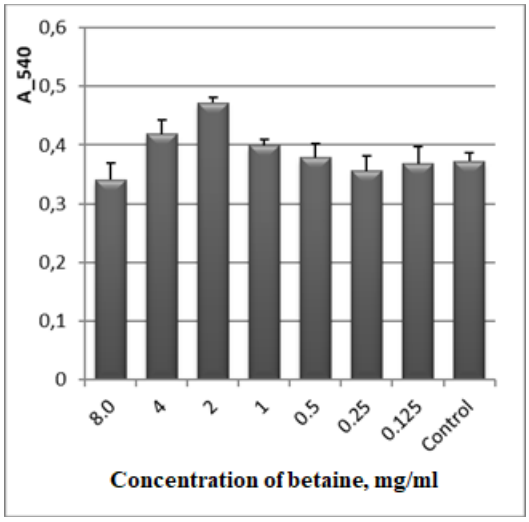
According to the above data, the concentration of cells under the influence of betaine did not differ from the control, and moreover, when incubating cells with 1

mg of betaine increased compared to the control by 1.2 times (p <0.5). Also, the percentage of dead cells at all concentrations studied did not differ from the control.

Table 2.6 - Concentration of PAE cells and the percentage of dead cells under the action of betaine

	4 mg / ml	2 mg / ml	1 mg / ml	0.5 mg / ml	0.125 mg / ml	Control
The concentration of cells (x1000)	56.2±8.9	71.3±11.7	85.5±4.8*	78.4±3.4	64.6±11.9	69.4±5.7
% dead cells (PAE)	2.3±0.4	8.4±4.2	4.9±1.6	2.3±0.6	6.4±1.2	3.5±0.6

*- p <0.5, compared to control



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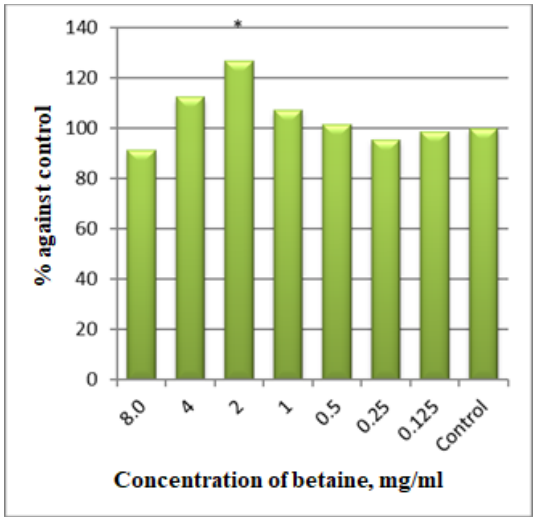


b

Figure 2.7 - Histograms of the optical uptake of endothelial cells (a) and the percentage of optical uptake compared to control (b) for betaine were determined in the MTT test.

Determination of mitochondrial enzyme activity in MTT test under the influence of betaine. When testing the effect of betaine on endothelial cells in the MTT test revealed a similar trend as in the routine counting in the Goryaev chamber. Optical uptake at most betaine concentrations in endothelial cell culture medium did not differ from optical uptake in controls (Fig. 2.7, a). Incubation with betaine at a concentration of 2 mg / ml betaine led to an increase in optical absorption in the MTT test compared to 26% control (Fig. 2.7, b) and a slight increase in this indicator when

adding betaine at a concentration of 4 mg / ml and 1 mg / ml.

Since the MTT test is based on the reduction of 3- (4,5-dimethylthiazol-2-yl) - 2,5-diphenyl-2H-tetrazolium bromide salt to formazan crystals by mitochondrial enzymes of living cells (mainly succinate dehydrogenase), this biochemical method is shown on the activity of mitochondrial respiration under the influence of various factors [69, 70]. Therefore, the next step in our study was to determine the activity of mitochondrial oxidoreductases per unit of living cells under the action of betaine. The obtained data indicate an increase in this indicator relative to the control of concentrations of 1 and 4 mg / ml betaine, while with decreasing concentration of the substance indicators of mitochondrial enzyme activity did not change (Table 2.7, Fig.2.7 (a)). According to the above data, at betaine concentrations of 4 mg / ml and 2 mg / ml in the incubation medium of endothelial cells, the activity of mitochondrial enzymes in optical units per unit of living cells increases 1.4 and 1.3 times ($p < 0.05$), respectively, compared with an indicator in the control (Table 2.7).

Determination of endothelial cell glucose uptake by betaine. The next step in the study was to test the effect of betaine on the level of glucose uptake by cultured cells. Since glucose is characterized by rapid penetration through the cell membrane by a concentration gradient, utilization with rapid energy production, including in the absence of oxygen, the determination of the level of absorbed glucose is a marker of their metabolism. The method is based on the use of glucose oxidase reaction - oxidation of glucose by the enzyme glucose oxidase using oxygen to gluconic acid and hydrogen peroxide, which in the presence of peroxidase reacts with phenol and 4-aminophenazone with the formation of quinonimine red-purple color that is spectrophotometrically determined.

When determining the uptake of glucose by cells at different concentrations of betaine, an increase in this indicator relative to control was found at concentrations of 0.125 mg / ml and 1 mg / ml, and at a concentration of 4 mg / ml the concentration of absorbed glucose decreased (Table 2.8).

Table 2.7 - Concentration of living cells, activity of mitochondrial enzymes as a function of reduction of formazan by endothelial cells under the action of betaine

The concentration of betaine in the culture medium	4 mg / ml	2 mg / ml	1 mg / ml	0.5 mg / ml	0.125 mg / ml	control
Concentration of living cells (x1000)	54.9±3.7	65.3±4,9	81.3±3.6	76.6±5.5	60.5±4.2	67.0±4.2
A_540 / 1000 living cells	$7.6x10^{-3*}$	$7.2\ x10^{-3*}$	$4.9\ x10^{-3}$	$5.0\ x10^{-3}$	$6.2\ x10^{-3}$	$5.5\ x10^{-3}$

*-p <0.05, against control

Table 2.8 - The level of glucose absorbed by endothelial cells under the action of betaine

The concentration of betaine in the culture medium	4 mg / ml	2 mg / ml	1 mg / ml	0.5 mg / ml	0.125 mg / ml	control
Concentration of living cells (x1000)	54.9±3.7	65.3±4,9	81.3±3.6	76.6±5.5	60.5±4.2	67.0±4.2
Glucose level / 1000 cells (mkM)	5.1±0.2	4.4±0.7	7.4±0.1*	5.5±0.4	7.6±0.3*	4.9±0.4

*-p <0.05, against control

At betaine concentrations of 0.125, 0.5 and 1 mg / ml, the optical absorption rates per 1000 living cells did not change compared to the control.

This tendency to change the level of glucose absorbed - a decrease at the highest concentration of betaine, may be associated with the impact on the processes of osmosis of betaine [71].

Determination of catalase activity, TBA-active products, and SH-groups in endothelial cells under the action of betaine. To determine the antioxidant system: catalase activity of TBA-active products and SH groups, PAE cells were cultured in 12-well plates and betaine was added at a concentration of 2 mg / ml for 24 hours.

At the end of the incubation period, the cells were removed from the substrate, the protein level, and the above values per mg of protein were determined. According

to the above data, catalase activity increased (Fig. 2.8, a), while the level of SH groups decreased (Fig. 2.8, b), and TBA-active products did not differ from the control (Fig. 2.8 c).

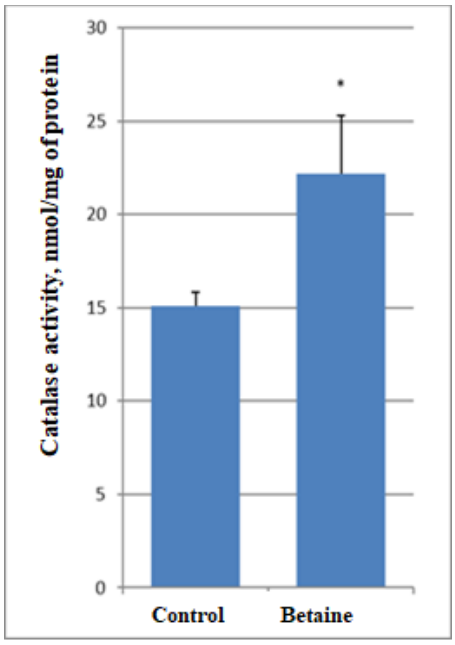
Thus, the definition of the main indicators of antioxidant protection indicates the normalization of these indicators by betaine. Cells normally synthesize reactive oxygen species. ROS are not only radicals, but also non-radical molecules with high reactivity. The most important free radicals include hydroxyl, superoxide-anion radical, nitrogen-containing radicals (nitrogen dioxide and monoxide), peroxyl. Other compounds - peroxynitrite, hypochlorous acid, hydrogen peroxide, singlet oxygen, ozone, nitric acid, dinitrogen trioxide are not free radicals, but easily cause free radical reactions in living organisms. The cardiovascular system is largely dependent on free radical reactions [72].

Therefore, inhibition of excessive ROS in the endothelium may be one of the mechanisms of normal metabolism through the endothelial barrier.

Determination of morphological characteristics of porcine endothelial cells under the action of betaine. Fixation of morphological features of porcine endothelium in culture was performed using an inverted microscope Axio Vert-40 with Axiovision software. To do this, the cells were photographed in real time under sterile conditions and after fixing the cells and staining the adherent cells with violet crystal.

Under the action of betaine in the cultivation of endothelium for 2 days recorded some differences from similar controls. morphological differences of cells from similar ones in the control (Fig. 2.9, 2.10), namely - elongation of cells (1), more processes (2), as well as the formation of structures that had signs of procapillary (3) (Fig. 2.9). As can be seen from the photos (Fig. 2.9) under the influence of betaine were more fixed on the substrate and spread cells.

a



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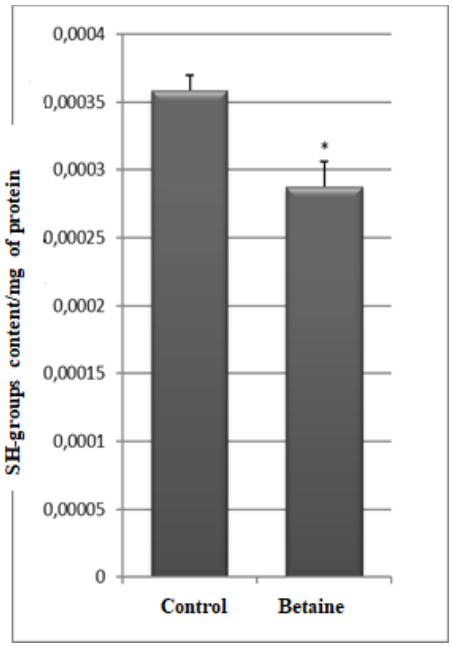

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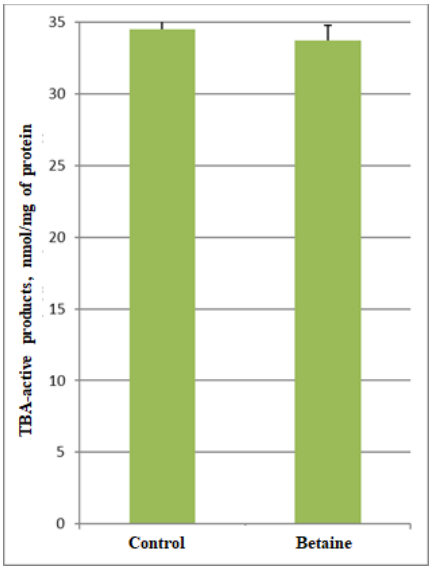


Figure 2.8 - The level of catalase activity (a), SH-groups (b) and TBA-active products (c) in endothelial cells under the action of betaine.

Typical photo (Fig. 2.10) with elongated cells and signs of formation of procapillary structures. At a betaine concentration of 1 mg / ml, the formation of 3D structures was observed, which may be a sign of morphogenesis in culture under the action of an osmolytic - betaine. Presented (Fig. 2.10) highlighted loci with typical differences from control.

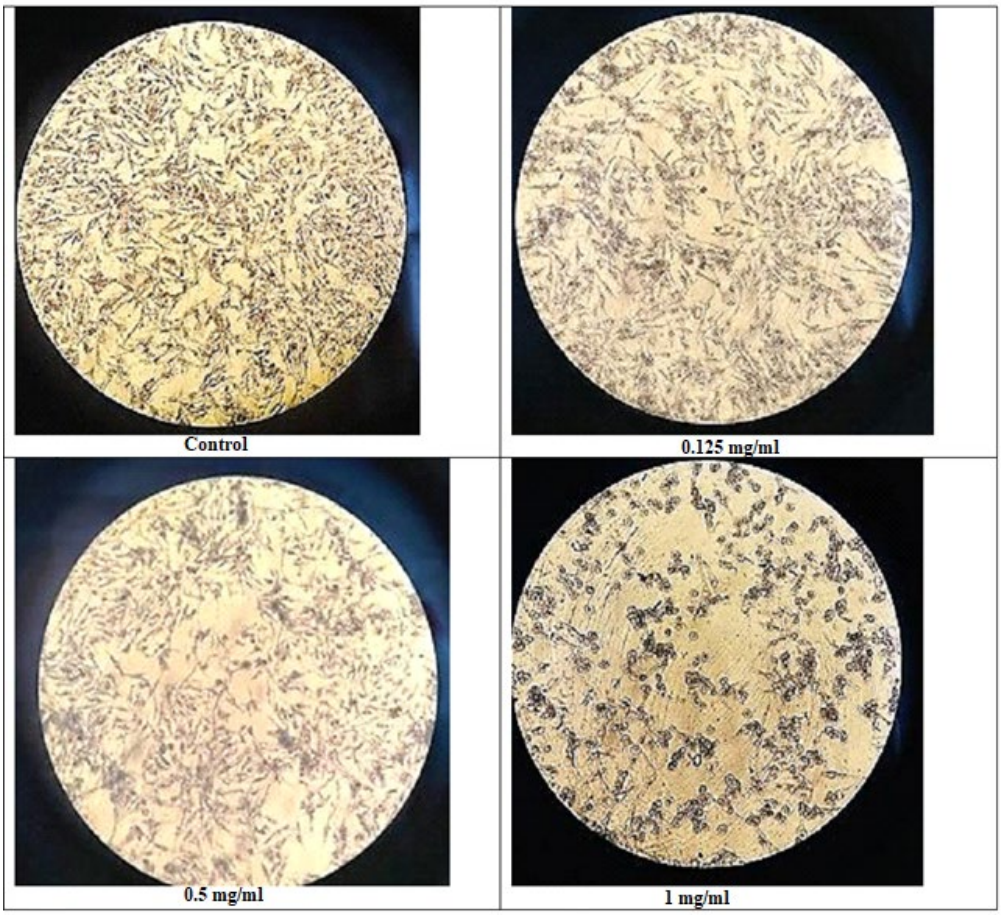
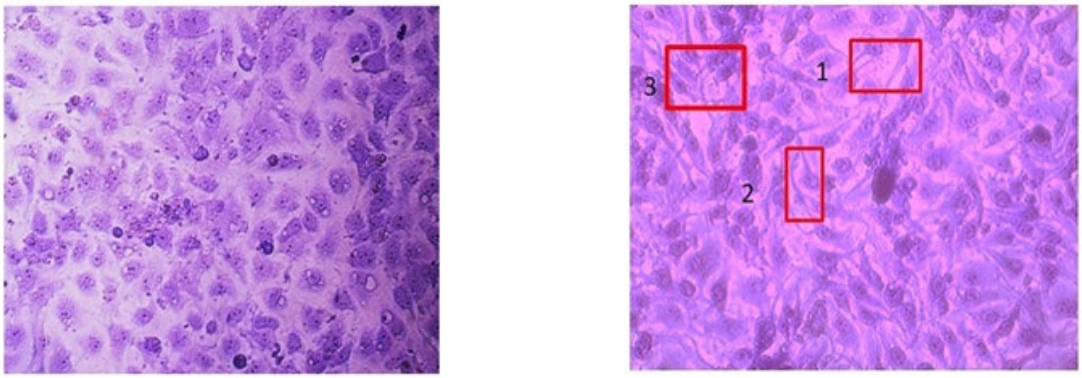


Figure 2.9 - Typical photos of PAE cells under the influence of betaine x50.



Control Betaine, 1 mg/ml

Figure 2.10 - Morphological features of endothelial cells under the influence of betaine; staining with crystalline purple dye.

Thus, comparing our results [77] with the literature of other authors [73 - 75] betaine or its derivatives can serve as a kind of substitute for anti-inflammatory anti-cytokine drugs to stabilize the endothelium. Previous studies [76] with the use of bioprotective substances (betaine and protein-rich supplements enriched with minerals in chelated form) in animal models of alcohol intoxication revealed recovery of marker enzymes of liver damage and normalization of redox processes and transamination intensity. Given the effects of betaine on endothelial cells, namely, enhanced cell growth and proliferation, increased survival, increased mitochondrial enzyme activity, further research is needed on cellular and possibly animal models with angiogenesis-mediated and immunomodulatory mechanisms.

Abschluss / Conclusions

According to the results of the research, therapeutic and prophylactic biological products with original organic / organic-mineral composition and immunomodulatory properties were created to correct metabolic abnormalities caused by factors of different nature (primarily anthropogenic, which have a significant impact on the environment); developed / improved methods of obtaining biological products based on macronutrients and some components of milk and approaches to their use with the appropriate patenting, as well as clarified a number of their biochemical, pharmacological and therapeutic properties.

1. Defined range of biochemical parameters (activity of enzymes such as ALT, AST, GGT, LDH, SOD, catalase, as well as total protein, creatinine, urea, MDA), which characterize the state of metabolism, show metabolic changes and individual pathomorphological abnormalities in rats under conditions of intoxication with alcohol and alcohol-carbohydrate mixture.

Potential bioprotectors for the correction of the above pathological abnormalities may be betaine and protein-containing supplements with minerals in the form of chelates, due to their significant effect on protein, lipid, and carbohydrate metabolism. These drugs are promising for further study and use because they are easily modified and can be used as raw material for the development of more complex bioprotectors.

2. The method of obtaining "Biofosfomag", which is an effective therapeutic and prophylactic and adaptogenic agent for widespread use in veterinary medicine and animal husbandry, as well as for further testing and evaluation of immunomodulatory properties, has been improved. Its effectiveness is associated with the stimulation and correction of metabolic processes.

3. According to the analysis of experimental data on the effect of betaine on endothelial cells of pigs of the PAE line, we obtained results indicating the normalizing effect of betaine on proliferative and metabolic parameters, and the presented scheme of its study can be used to assess immunomodulatory properties of

other biological products, OVA^+).

No toxic effects of betaine on porcine aortic endothelial cells were detected. An increase in the concentration of endothelial cells was recorded in comparison with the control for its level of 0.5 mg / ml and 1 mg / ml when stained with trypan blue. The MTT test showed an increase in optical absorption due to the reduction of formazan by mitochondrial enzymes of living cells within its concentrations of 1-4 mg / ml and showed an increase in the activity of mitochondrial oxidoreductases per unit of living cells. Betaine concentrations of 0.125 mg / ml and 1 mg / ml showed a significant increase in glucose uptake by cells. The determination of some indicators of antioxidant protection under the influence of betaine revealed an increase in catalase activity, a decrease in the level of SH groups, while the rate of TBA-active products remained unchanged compared to the control.

Verweise / References

Chapter 1

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Annotations / Anmerkungen

KALACHNIUK Liliia

SOME MEANS OF REGULATION IN AN ORGANISM OF ANIMALS AT ACTIONS OF FACTORS OF DIFFERENT NATURE

The monograph presents the theoretical and practical justification and development of methods for obtaining biological products, as well as the analysis of patent and market and applied base of foreign and domestic experience in creating therapeutic and prophylactic biological products with original organic-mineral composition to correct metabolic abnormalities. factors of different nature were developed methods of obtaining biologicals based on macronutrients and some components of milk and approaches to their use with appropriate patenting and tested the developed methods of obtaining biologicals with immunomodulatory properties and their evaluation.

The book presents the results of creating therapeutic and prophylactic biological products with original organic / organic-mineral composition and immunomodulatory properties to correct metabolic disorders caused by factors of different nature (primarily anthropogenic, which have a significant impact on the environment); Improving the methods of obtaining biological products based on macronutrients and some components of milk and approaches to their use with the appropriate patenting, as well as clarified a number of their biochemical, pharmacological and therapeutic properties.

For scientists, teachers, PhD-students, and applicants for higher education, interested in information on the means of regulating the vital processes of the organism and its adaptogenicity under the influence of factors of different nature.

SCIENTIFIC EDITION

MONOGRAPH

INTELLEKTUELLES KAPITAL - DIE GRUNDLAGE FÜR INNOVATIVE ENTWICKLUNG

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INTELLECTUAL CAPITAL IS THE FOUNDATION OF INNOVATIVE DEVELOPMENT

SOME MEANS OF REGULATION IN AN ORGANISM OF ANIMALS AT ACTIONS OF FACTORS OF DIFFERENT NATURE

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