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ENTEROCYTES MEMBRANES OF THE SMALL INTESTINE AT PATHOLOGY AND CONDITIONS OF HIBERNATION

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У монографії представлено результати досліджень особливостей обміну cAMP, cGMP і простагландинів в ізольованому епітелії тонкого кишечнику великої рогатої худоби залежно від віку та при ентеропатології, а також описано закономірності змін ліпідної компоненти мембран ентероцитів за цієї патології (спонтанної та експериментальної) й переведенні тварин у стан штучного вуглекислотного гіпобіозу, що лежать в основі адаптаційної відповіді організму за дії екопатогенного чинника на рівні клітинних мембран.

Для фахівців у галузі клінічної біохімії, клінічної діагностики, фізіології і патофізіології, терапії, морфології та фармакології, практичних лікарів ветеринарної медицини, а також для магістрантів, аспірантів і докторантів, науковців НДІ, науково-педагогічних працівників вищих навчальних закладів ветеринарного та біологічного профілю.

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Nowadays, in medicine, the key role of the violations of the structural organization of cell membranes in the development of severe liver diseases, cardiovascular and nervous systems, disorders of many functions of blood cells and the like is proved. In practical veterinary medicine, reparative therapy for internal non-contagious animal diseases is a new and relevant approach, since the restoration of the structural and functional state of the affected cells and the associated metabolic processes does not end, and three to five weeks after clinical recovery.

Membrane structures of cells of a living organism are exposed to numerous pathological factors of the external and internal environment. The interaction of the damaging agent with the surface of the cell plasmolemus triggers a cascade of interrelated biochemical processes proceeding both on the membrane and inside the cells.

The intensity of restoration of intracellular homeostasis in the course of development of pathology essentially depends on the duration of action of the pathogenic factor and the adaptive capabilities of the organism to a large extent determined by the degree of damage to the cell membranes. The main structural and functional components of the lipid bilayer of cells are known to be phospholipids. So, the functioning of cellular membrane systems depends on the integrity of their phospholipid structures. At the same time, the usefulness of metabolic processes in cells and their violation in the development of pathology is determined by the structural-functional state of membrane systems.

Pathogenic factors affect any body cells, is characterized by a number of regularities and features of the resulting changes at the molecular level. The complex conduct of not only biochemical blood tests, but also indicators characterizing the structural and functional state of cell membranes with their respective lesions is important from the point of view of the development of effective therapeutic remedial therapies, will help solve numerous complex problems with functional insufficiency of internal organs and the study of molecular mechanisms development of animal diseases. In this book, we describe the characteristic structural and functional changes

in cell membranes in the development of enteropathology and in the transfer of animals to the state of artificial carbon dioxide hypobiosis.

We express our sincere gratitude to all those who contributed to the conduct of relevant scientific research and the analysis of a significant amount of biochemical indicators, first of all, to the staff of the Department of Biochemistry and Physiology of Animals named after Academician M. F. Guliy.

Sincerely, the authors.

CHAPTER I
PECULIARITIES OF EXCHANGE OF cAMP, cGMP AND PROSTAGLANDIN IN THE ISOLATED ESTHELICS OF THE SMALL INTESTINE OF LARGE CATTLE IN DEPENDENCE ON AGE AND IN ENTEROPATHOLOGY

Cattle are distinguished among other animal and human species by the anatomical and functional features of the digestive canal [1–3]. Recent recent studies have established the characteristics of molecular organization [4] and enzyme activity [5, 6] in the cell membrane of the epithelium of the small intestine of cattle.

It is known that the hydrolytic and transport function of the cell membrane of the small intestine epithelium is regulated by the intracellular concentration of cyclic nucleotides – cAMP [7–9], cGMP [7] and prostaglandins [10], with predominantly E2 and F1α. But in view of the fact that the biochemical and physiological foundations of the processes of absorption and secretion in the small intestine of cattle, with the exception of several reports [11], are not well understood, the question of the state of biochemical regulatory systems (cyclic nucleotides and prostaglandins) in the epithelium of a thin intestines of cattle.

In addition, acute digestive disorders with signs of diarrhea are widespread in newborn calves in the first days of life, accompanied by dehydration of the body [11–13] and loss of electrolytes [14–16]. In this case, the mucosa of the intestine undergoes a significant disturbance of metabolic activity [17–19]. As is known, many diarrhea-provoking factors of non-infectious etiology [20, 21] or infectious nature – cholera [8, 13, 20, 22], salmonellosis [23], pathogenic *E. coli,* dysentery [24] develop with the participation of cyclic nucleotides. Distinguish diarrhea according to the type of action of cholera toxin (cAMP-mediated secretion of H_2O and electrolytes) or the action of thermostable toxin *E. coli* (cGMP-mediated secretion). The role of prostaglandins in the initiation of secretion in the small intestine is also known [25] and changes in their level in the mucous membrane in the pathology of the digestive canal. But I will not mention the absence of a final opinion on the etiology and

pathogenesis of alimentary diarrhea in newborn calves [10, 13], as well as a similar pathology in newborn children [26–28], and biochemical mechanisms (involving cyclic nucleotides and prostaglandins) of the emergence and development of enteropathology. At the same time, newborn calves, as an object of research, can act as a natural model for studying this pathology, searching for effective preventive and medicinal products.

It should be noted that in most of the available studies, the exchange of cyclic nucleotides and prostaglandins was carried out using biopsies, mucosal scrapings or intestinal sections, that is, in a material containing epithelial and other tissues in addition to the task of using homogeneous preparations of isolated epithelium.

Proceeding from the foregoing, the study of the exchange of cyclic nucleotides and prostaglandins in the epithelium of the small intestine of cattle, including healthy and dyspeptic neonatal calves, has theoretical, clinical and practical significance.

In this connection, in Chapter I of this work problems of the development of a technique for isolating isolated small intestinal epithelial cells of cattle, determining the level of cAMP, cGMP, and the activity of cyclic nucleotide exchange enzymes in the epithelium of the small intestine of adult cattle, newborn healthy and sick with enteropathology calves, as well as the study of the content of prostaglandins E2 and F1α in the epithelium of the small intestine of similar groups of animals.

1.1 Biochemical and physiological basis of the processes of absorption and secretion in the small intestine

The small intestine is one of the main organs of the digestive canal and performs a number of different functions: metabolic, secretory, transport-evacuation, depositing, hormonal, protective; which to varying degrees ensure the implementation of the two leading processes - hydrolysis and absorption of nutrients [29–36]. The variety of these functions is due to the uniqueness of the structure of the intestinal mucosa. To date, the structural and functional organization of the mucous membrane as a whole, and in particular the epithelium covering it, has been sufficiently well studied and described both in norm [29, 30, 37], and in the pathology of the digestive canal [11, 16, 18, 19, 38, 39]. Therefore, it is rational to outline the main features of the structure of the small intestinal mucosa and its role in the processes of hydrolysis and transport of nutrients.

1.1.1 Structural organization of the mucosa of the small intestine

The mucosa of the intestine (lamina mucosa intestinum) is a complex structural and morphological formation consisting of an interconnected complex of blood and lymphatic vessels, the nervous, connective and contractile tissues, bounded from the side of the intestinal lumen by the epithelium, and on the other hand by the muscular membrane [6, 30].

Three distinctly differentiated layers are distinguished in the mucosa:

1) epithelial - representing a layer of intestinal villi - outgrowth of the mucous membrane, protruding into the intestinal cough;

2) the layer of the mucous membrane proper, with indentations in it - intestinal crypts;

3) a thin layer of smooth muscle tissue - muscle plate [6, 30].

The epithelial layer and its own layer of the mucosa are formed by a loose connective tissue and are covered with a single-layered cylindrical epithelium.

Intestinal crypts are also lined with a single-layered epithelium, but its cells differ substantially in structural, cytochemical and functional parameters from the villous epithelium [6, 30]. According to the data obtained on the intestine of the rat, the epithelial layer occupies 73%, the layer of the mucous membrane proper is 22%, and the muscular shell – 5% of the volume of the intestinal mucosa.

The epithelial layer of the intestinal mucosa is the main component that provides the realization of the processes of hydrolysis and transport of nutrients. It is characterized by a folded surface in which the villous epithelium of the depression is isolated. Between the villous epithelium and the epithelium of the indentation are undifferentiated cells that have retained the ability to mitosis, from which the proliferation of cell populations of villi and depressions occurs [6, 30]. The main structural units of the epithelial layer of the intestinal mucosa are intestinal villi.

They are microorganisms with their vascular, muscular and nervous apparatus [30]. Three types of villi are distinguished in form: leaf-shaped, finger-shaped and linguiform [30]. At the base of the villi lies a layer of the mucous membrane proper, with depressions around the villi – crypts. On each villus it is necessary up to 5~ 9 crypts [6]. On the other hand, there are 10 to 40 villi per 1 mm² of the intestinal mucosa, which increases the surface of the epithelium 8 times [32, 40]. In addition, the surface area of the epithelium is increased by another 30–60 times due to a single-layered cylindrical epithelium covering the intestinal villi. Its main mass (about 90%) is made up of enterocytes with a narrow border formed by microvilli of the apical plasma membrane [29, 30, 32]. The rest (about 10%) falls on other types of epithelial cells [2]. Cellular elements of the mucosal layer proper are represented by reticular, plasmatic and mast cells, lymphocytes, fibroblasts, acidophilic leukocytes and macrophages [6]. In this layer, the presence of a significant amount of mucopolysaccharides and fibroblasts is established, which indicates a large plastic capacity of the mucosa [41].

The layer of smooth muscle tissue is represented by 2–5 crossing at an angle layers of muscle cells covered with fibrous connective tissue [6].

The presented data on the general characteristic of the structure of the intestinal mucosa testify to the significant importance of its constituent components in the realization of bowel functions. However, since the main role in the processes of hydrolysis and transport of nutrients belongs to the epithelial layer of the mucous membrane, namely its functional unit – the cell of the intestinal epithelium, it is necessary to dwell in more detail on its characteristics.

1.1.2 Functional-morphological characteristics of the epithelial cells of the small intestine

The epithelium of the small intestine is characterized by polymorphism and polyfunctionality even within one intestinal villi and one crypt [6]. It distinguishes the following main types of epithelial cells:

1) intestinal epitheliocyte with a striated cicatrix, also called a cylindrical, absorptive or main cell;

2) without bezel enterocyte intestinal crypt (undifferentiated, ancestral, stem, maternal or cambial cell);

3) goblet enterocyte (mucoid cell or Goblet cell);

4) enterocyte with acidophilic granules (Packet cell);

5) intestinal argytafinocyte (enterochromaffinocyte, Kulchitsky cell or endocrine cell) [15].

Intestinal epitheliocytes with striated edges have a cylindrical or columnar shape with dimensions of 22–31 μm in height and 6–9 μm in width [39]. A distinctive feature of these cells is the presence of two polar parts of the plasma membrane - the apical and basal [29, 30, 37, 42]. The apical surface of the plasma membrane faces the lumen of the intestine and is represented by a brush border, which consists of a set of finger-shaped outgrowths of the cell membrane called microvilli [2, 29, 30, 36, 43].

The number of brush jaw micromirrors (from 1700 to 4000 on one cell) and their sizes 0.8–1.5 microns in height and 0.05-0.1 microns in width vary

considerably, depending on the specific features of the intestinal cells, their level differentiation, type of nutrition, age and functional state of the organism [2, 3, 6, 30]. Microvilli are a complex structure in which the overmembrane glycoprotein layer - glycocalyx [2, 29, 30], the apical plasma membrane in the matrix is distinguished [37]. The presence of a large number of hydrolytic and transport enzymes in these structures ensures the unique function of the absorber cells of the intestine-the hydrolysis of nutrients and their entry into the enterocyte [29, 31–37]. It is generally accepted [31–36] that the digestive-transport complex of the apical plasma membrane is the key link of the entire transport system of enterocytes.

The apical membrane of an enterocyte with a striated border into the basal one, which faces the serous membrane of the intestine. It includes lateral areas, with which the enterocyte borders on neighboring cells, forming intercellular slits and, in fact, the basal part adjacent to the basal subepithelial membrane [6, 33, 42]. On the basal surface of the plasma membrane, various enzyme systems that transport the substances are detected mainly due to the energy liberated during the hydrolysis of ATP (transport ATPases) [17, 44]. These enzyme systems transport and exchange nutrients between the digestive system and the internal environment of the organism [5, 6]. In addition, the basal part of the plasma membrane has an important role in providing cell adhesion to the basal subepithelial layer [30]. Intensive carbohydrates and lipid metabolism in absorptive cells of the intestine cause high activity in them of biotransformation and detoxification systems [30]. These processes are provided by the active functioning of enzyme systems localized in well-developed intracellular organelles of enterocytes: mitochondria, the granular endoplasmic reticulum and the Golgi complex. With regard to lysosomes, there are relatively few of them in enterocytes and their main function is to release cells from the decay products of intracellular structures at the final stages of their life cycle, and in the early postnatal period to intracellular digestion [6, 30, 45].

Without bezel enterocytes are cells of intestinal crypts, from which mature absorbent epitheliocytes and goblet enterocytes develop [6]. They are predominantly cylindrical in shape and characterized by a weakly expressed striation, as well as a

few short and wide microvilli, which have a variable length and shape [46]. These cells significantly differ from enterocytes with striated activity and localization of enzymes in the plasma membrane. Thus, the activity of hydrolytic enzymes was virtually absent in the rim of undifferentiated enterocytes [6]. However, these cells have high proliferative activity, divide mitotically and replenish intestinal cell loss at the tops of intestinal villi [6].

Goblet enterocytes are unicellular glands located both on intestinal villi and in crypts [6]. The apical surface of these enterocytes is striated, as in absorbent cells. At the same time, the number of microvilli on the plasma membrane of goblet enterocytes is much smaller and they are of unequal length [47]. In addition, the activity of enzymes in goblet enteronites is weaker than in the absorbent ones, and the activity of hydrolytic enzymes has not been observed in their striated cortex [6]. The function of goblet enterocytes is secretion into the lumen of the intestinal mucus, which is rich in acidic and neutral mucopolysaccharides and poor in protein [6].

At the bottom of intestinal crypts are located enterocytes with acidophilic granules (Panet cells). They have the shape of a truncated cone, wider at the base and tapered to the apex [22]. Microvilli of these cells are rudimentary, very rare and contain fibrils that penetrate not a few microns into the apical cytoplasm. Filling the entire cytoplasm of Panet cells with large (from 2 to 4 microns) secretory granules with a homogeneous material indicates their secretory properties. However, the functional role of these cells has not been fully established [6].

Intestinal argytafinocytes (Kulchitsky cells) are triangular endocrine cells that occur both among the epithelial cells of the intestinal crypts and among the epithelium of the intestinal villi [6]. There are different variants of these cells. It is established that some of them secrete serotonin, other cholecystokinin. However, the functional role of intestinal argentafinocytes remains unclear [6]. So, the data presented above show that all types of intestinal epithelial cells participate to a greater or lesser extent in the implementation of complex processes of digestion, absorption and transport of nutrients. The determining role in this undoubtedly is assigned to the absorptive cells of the intestine - enterocytes with striated margins.

Mechanisms of absorption (absorption) and transport of substances in the intestine include two interrelated processes - absorption and secretion. One of the main components of these processes is the absorption and secretion of water and electrolytes. The state of absorption in secretion within physiological limits is of vital importance to the body. At the same time, abnormalities occur in the pathological conditions of the digestive canal (diarrhea), which lead to a change in the permeability of the intestinal epithelium to water and electrolytes. By this, it will be logical to briefly dwell on the characteristics of the absorption and secretion processes in the small intestine in norm and in pathology.

1.1.3 Characteristics of the absorption and secretion processes in the small intestine in normal and pathological conditions

The digestive canal is a flow system in which there is an intense absorption of water and salts, mainly sodium chloride [2, 6]. Of all the liquid and salts entering the intestine, only 20% comes with food. The rest is formed as a result of endogenous secretion of digestive organs, including the intestine [2, 30, 48]. Thus, it has been established that water passes through the intestine of the cow 3–4 times, sodium in 6-7 times, and chlorine 8-10 times more than in the case of a scorm [6].

The overwhelming amount of water and electrolytes is absorbed and secreted in the small intestine [2].

Absorption and absorption of water is carried out by steady osmotic gradients. In this case, water enters the body through conjugated active transport with electrolytes, monosaccharides, amino acids, and also di- and tripeptides. The driving force behind these processes is active sodium transport.

The absorption of sodium and chlorine ions is a conjugate process and from the side of the apical membrane of the absorbing cells is realized by the electroneutral mechanism, in which the absorption of sodium is carried out together with chlorine. The sodium input is inhibited by the absence of chlorine in the gut lumen and vice versa. This mechanism also includes anti-port systems: Na^+/K^+ , Cl^-/HCO^{3-}, Cl^-/OH^-,

and also Cl^-/H^+ [49]. In addition, the absorption of sodium from the side of the apical membrane is carried out by an electrogenic mechanism, also called joint sodium transport with organic substances - glucose, amino acids. The driving forces of these processes are transport enzyme systems (in particular Na^+, K^+-ATP-ase) located on the basolateral membrane of the enterocyte and carrying out active transport due to the energy released during ATP hydrolysis [17].

In crypt cells that secrete chlorine ions, the transport process also depends on the electrochemical gradient that is produced by Na^+, K^+-ATP-ase of basolateral membranes. However, in crypts, the carrier protein, sensitive to the sodium gradient, is located in the basolateral membrane of the epithelium, and not in the apical membrane, as in the absorptive cell. This contributes to the accumulation of chlorine in the cell, which under normal conditions exits through the apical membrane along an electrochemical gradient.

The general mechanisms of transport of water and sodium chloride described above characterize the physiological state of the organism, in which the level of absorption exceeds the level of secretion. However, the pathological state of the digestive system (enteropathology with diarrhea phenomena) leads to serious disruptions in the absorption of secretion, which in a short period of time lead to dehydration [12, 15, 16], an acid-base balance, especially sodium [16]. Infectious diseases such as cholera, dysentery, salmonella infection, pathogenic E. coli, as well as pathologies of non-infectious nature-alimentary diarrhea, both in humans [20] and in animals-cattle [11–13]. It should be noted that the biochemical mechanisms of diarrhea ofinfectious nature have been sufficiently described to date, while the widespread diarrhea of newborn calves [11, 13] and the similar problem in newborn children have not been studied, which makes it difficult to conduct effective preventive and curative activities in veterinary medicine and pediatrics.

1.1.4 Intracellular systems for the regulation of absorption and secretion in the small intestine

Regulation of absorption and secretion in the small intestine is performed at the level of the plasma membrane of the enterocyte by special intracellular mediators, the main ones of which are cyclic nucleotides – cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), as well as intracellular free calcium [9, 50]. A unique feature of the epithelium of the small intestine is that all these intracellular mediators have an effect on the ion transport processes by a similar mechanism. Thus, an increase in the intracellular concentration of any of these mediators inhibits absorption and stimulates the secretion of ions in the small intestine of mammals.

The metabolites of polyunsaturated higher fatty acids, in particular prostaglandins, are also important and intracellular regulators of the transport of substances in the intestine [10].

In the context of the problem we are considering-digestive disorders in newborn calves-cyclic nucleotides (cAMP and cGMP), as well as prostaglandins E2 and F1α, whose activation by various humoral, microbial and pharmacological agents lead to a state of diarrhea [22–24]. Therefore, it is advisable to consider the function of these intracellular regulators in more detail.

1.1.4.1 The role of cAMP and cGMP in the regulation of absorption and secretion in the small intestine

Cyclic 3,5-adenosine monophosphate occupies a central place in intracellular regulatory mechanisms, taking part in the implementation of a variety of vital processes of the body; it controls cellular growth and differentiation, increases the permeability of cell membranes, mediates the action of many hormones, participates in the development of adaptive reactions and in the intracellular metabolism of carbohydrates, proteins, lipids and mineral salts, as well as in neuromuscular

transmission and in the functions of the central nervous system [9, 50]. Cyclic 3,5-adenosine monophosphate is formed in cells from ATP in the presence of Mg^{2+} ions as a result of activation of the enzyme adenylate cyclase. In intestinal cells, this enzyme is localized primarily on the basolateral membrane. Activation of adenylate cyclase is carried out only by peptide hormones and neurotransmitters, as well as toxins that do not penetrate the interior of the cell and bind to specific surface receptors [9]. Therefore, adenylate cyclase is an entire system for signaling, consisting of a hormone binding receptor, a catalytic component converting ATP to cyclic AMP, and a complex of transducer proteins that bind the receptor and catalytic components of the system. At the same time, the level of cAMP in the cell is controlled by another enzyme, various forms of phosphodiesterase that are localized both in the cytosolic fraction and in the membrane fraction and convert cAMP into an inactive metabolite, a noncyclic 5'-adenosine monophosphate [50].

The mechanism of action of cyclic AMP is due to the activation of cAMP-dependent protein kinases, which act as regulators of the corresponding metabolic pathways [51]. The mechanism of protein kinase activation is the binding of cAMP to the regulatory subunits of the enzyme, which leads to the release of catalytic subunits that use ATP to phosphorylate the corresponding substrates.

Cyclic 3,5-guanosine monophosphate has similar cAMP functions. In the intestine cGMP is formed in the brush border of enterocytes with the participation of the enzyme guanylate cyclase and activates tissue-specific isoenzymes with GMP-dependent protein kinase. The level of cGMP in the cell is also controlled by one of the forms of phosphodiesterase, which cleaves with GMP to an inactive metabolite, a non-cyclic 5'-guanosine monophosphate.

In the intestine, both cAMP and cGMP stimulate secretion. However, the strength of the action, these mediators have some differences. Thus, it has been established that cAMP and cGMP rabbits in the absorptive cells of the ileum inhibit cotransport of Na and Cl equally, but in crypt cells the effect of cAMP is more effective than cGMP. This can be explained by a decrease in the activity gradient dependent on cGMP protein kinase from the villi region to the crypt region.

Thus, the data presented above demonstrate the important role of cAMP and cGMP regulation of absorption and secretion in the intestine. At the same time, the formation of cyclic nucleotides themselves can be controlled by other specific substances called prostaglandins [50, 52], which perform a variety of functions, associated with regulatory processes at the cell level.

1.1.4.2 The role of prostaglandins in the regulation of absorption and secretion in the small intestine

Prostaglandins are biologically active substances that are cyclic hydroxy acids, which are derivatives of polyunsaturated fatty acids with 20 carbon atoms [10, 53]. The main and most important precursor of prostaglandins is arachidonic acid. The source of arachidonic acid is mainly the phospholipids of cell membranes [10]. The release of arachidonic acid from the phospholipid pool of cell membranes is carried out by phospholipases, in particular phospholipase A2. Further formation of prostaglandins from arachidonic acid is accomplished by the operation of a special enzyme called prostaglandin synthetase [10]. Prostaglandins are synthesized by virtually all tissues of the body, but their number in different tissues and even in different populations of cells of the same tissue are different. Synthesis of prostaglandins occurs directly at the time of biological affect, and the action itself is limited mainly to the place of their formation, since prostaglandins have a short half-life and do not accumulate in the body [53]

Prostaglandins perform important functions, having different physiological and pharmacological effects: they cause stimulation or relaxation of the cell, cause strong diuretic and natriuretic effect, affect the tone of blood vessels, bronchi, blood pressure level, cardiac activity, coronary blood flow, platelet aggregation, central and autonomic activity nervous systems, hormone secretion and modulation of their action in target tissues [10].

In the gastrointestinal tract there are mainly prostaglandins of the group E and P, with a predominance in quantitative terms and in terms of the biological activity of

the prostaglandins of group E [53]. In addition to these prostaglandins, prostaglandin A, D2 and prostacyclin have also been found in the intestine [53]. The biological role of prostaglandins in the gastrointestinal tract is extremely diverse and consists in the modulation of gastric acid and alkaline secretion, motor activity of the gastrointestinal tract, the protection of cells vasodilation, and the role of mediators in the inflammatory response in pathological conditions, as well as in local regulation of electrolyte transport in the intestine.

Prostaglandins of both group E and group F stimulate active secretion of electrolytes in the small intestine [53, 54], and the group F prostaglandin also regulates intestinal motility [54].

Thus, it is obvious that the physiological level of cyclic nucleotides (cAMP and cGMP) and prostaglandins in the epithelium of the small intestine plays an important role in regulating the processes of absorption and secretion. At the same time, as will be discussed below, a change in the concentration of these intracellular mediators can lead to significant disturbances in biochemical processes in the small intestine.

1.1.5 Biochemical basis for the development of acute digestive disorders in the small intestine

Acute digestive disorders in the small intestine with the phenomena of diarrhea are one of the most common pathologies of this organ. As a result of the development of the disease, the processes of secretion over absorption take place. Therefore, the severity of the disease, and often a fatal outcome, does not come from the direct factor that causes diarrhea, but as a result of secondary processes - dehydration and loss of electrolytes.

Factors that can cause diarrhea include: infectious diseases – cholera [8, 13, 20, 22], salmonellosis [23], E. coli, dysentery [24], viral; noninfectious – eating disorder [20], diabetic diarrhea [55], lactose intolerance [21], caused by antibiotics or laxatives. The existence of diarrhea of a neurohumoral nature is also described in

terms of the ability of certain hormones (vasoactive intestinal peptide, secretin, serotonin) to cause a state of diarrhea.

It should be noted that the mechanism of action of many of the listed pathological factors is now sufficiently well understood. By the type of effect realization, they can be classified into several groups: acting by increasing the intracellular concentration

1) cAMP;

2) withGMP;

3) acting through the system of Ca-calmodulin.

According to the cAMP-mediated pathway leading to diarrhea, cholera diarrhea has been studied and described at the biochemical and pathophysiological level, and diarrhea caused by thermostable toxin E. coli has been studied in the cGMP-mediated pathway.

The current concept of the effect on the intestine of cholera toxin is presented in the following form [8, 9].

The colonization of the small intestine by the bacterium of the cholera vibrio (Vibrio cholere) leads to the accumulation of protein nature in the lumen of the intestine. Cholera toxin consists of several subunits. The enzymatic subunit is in a complex with subunits that bind to the apical membrane through the ganglioside site. Subsequently, the subunit A is activated, giving peptide A1, which modulates the adenylate cyclase of the basolateral membranes [8] in the following way. Adenylate cyclase is a complex receptor-regulated enzyme complex, which consists of: 1 – a membrane receptor; 2 – GTP-binding protein, which binds and hydrolyses GTP; 3 – catalytic subunit of adenylate cyclase proper. Of these components, only the receptor is localized on the outer surface of the cell [9].

Cholera toxin subunit A1 is an ADP-reabolizing enzyme that catalyzes the addition of ADP-ribose (from the NAD+) to a GTP-binding protein, after which he is not able to inactivate adenylate cyclase.

The result of exposure to cholera toxin is a prolonged activation of the adonylate cyclase complex, which leads to a significant increase in the intracellular concentration of cAMP [8, 9].

cAMP activates protein kinases, and those phosphorylate intracellular and membrane substrates related to suction systems, modifications of passive permeability and active transport.

It is assumed that these membrane substrates responsible for the inhibition of absorption of Na^+ and Cl^- in the epithelium of the villi and the activation of C1 ~ secretion in the epithelium of the crypts.

E. coli thermostable toxin has a similar structure to the cholera toxin and probably likewise stimulates guanylate cyclase activity [56] is localized in the apical membrane. At the same time, the concentration of cGMP increases, resulting in a secretion of H_20 and electrolytes in the intestinal cavity.

At the same time, there are a number of factors that distinguish the cGMP-mediated mechanism of diarrhea from the mechanism involving cAMP.

First, the thermostable toxin of *E. coli* does not bind to membrane gangliosides. Secondly, the modulation of the secretion processes in the intestinal epithelium is carried out by mutually independent targets for cAMP and cGMP, presumably by the domains of the corresponding protein kinases. Third, in the presence of *E. coli* heat-stable toxin saturation plots for cGMP increases 5 times for 3–5 minutes incubation, cAMP and only 2 times within 2 pm. In addition, the thermostable toxin of *E. coli* has a more complex mechanism of action. It has been found that the thermostable toxin similar with *E. coli* diarrhea has the ability to induce the calcium ionophore A-23187, which is known to promote entry of Ca^{2+} into the cells by concentration gradient. Moreover, A-23I87 intensified the action of toxin, activated the activity of calmodulin and phosphodiesterase. The blocker of Ca-channels, verapamil, and the calmodulin inhibitor-trifluoropyrazine, removed the ionophore effect. The authors concluded that the increased secretion of Na^+ and Cl^- diarrhea stimulated thermostable toxin of *E. coli*, it may be associated with an

increased flow of Ca^{2+} and calmodulin activity in the microvilli of the intestinal epithelium.

It should be noted that the Ca-dependent mechanism has a significant place in the characterization of secretory diarrhea. This should include the already mentioned factors – A-23I87, as well as serotonin, neurotensin, carbachol, ricinoleic acid, deoxycholate. Being an activator of phosphodiesterase and an adenylate cyclase inhibitor [8], calcium ions together with calmodulin are able, without changing the level of cAMP and cGMP, to stimulate intestinal secretion by activating protein kinases. At the same time, it is assumed that the primary effect of cAMP and cGMP can also be manifested through an increase in intracellular Ca^{2+}.

Prostaglandins activate adenylate cyclase, increasing the intracellular content of cAMP, and are also capable of causing diarrhea. Intraperitoneal injection of prostaglandin E2 (4 μg/kg body weight) causes diarrhea as early as 15 min, whereas prostaglandin F2α caused a similar effect only after 30 min. The content of prostaglandin E2 in blood plasma for diarrhea is 894 pg/cm³ at 346 pg/cm³ in healthy. All this indicates that prostaglandin E2 relates to secretory diarrhea, then F2α refers to bowel motility. At the same time, the involvement of eicosanoids in the inflammatory processes and the presence of inflamed foci in the intestine with diarrhea casts doubt on the concept of their main causal role in the development of diarrheal syndrome.

Thus, regardless of the way in which the effect of the external factor is realized-cAMP or with the GMP-mediated mechanism, Ca^{2+} or Ca^{2+}-calmodulin-dependent pathway, the absorption processes are inhibited and/or H_2O, Na^+, CI^- secretion processes are stimulated apical and basolateral membranes.

As for other manifestations of acute digestive disorders of infectious origin - salmonellosis [57], dysentery [58], they are also characterized by an increase in cAMP in the epithelium, appear to follow the pathway described for cholera toxin.

Little studied is diarrhea of a neurohumoral nature and of an alimentary origin, associated with malnutrition and widespread in newborns, both in humans [20, 28, 42] and in animals [11, 29]. This raises the problem, on the one hand, of deciphering

the biochemical mechanisms of this pathology occurring at the level of the intestinal epithelium, and on the other hand, the search for effective preventive and therapeutic agents. As noted above, the main functional load in the processes of absorption and secretion is performed by the epithelial cell membrane - morphologically divided into the apical and basolateral parts (membranes). Although the technique of human small intestine biopsy has now developed sufficiently, the application of this procedure in pediatrics is not acceptable. An alternative is modeling diarrhea in laboratory animals or studying in vivo, using newborn calves as an object of study, in which the enteropathology of non-infectious nature is widespread [11, 29].

As was established earlier by our studies [16, 17, 39], the molecular organization of the apical and basolateral membranes of the epithelium of the small intestine of cattle, with the exception of some differences, is similar to that of monogastric animals. In the same studies, as well as by other authors, lipid composition changes were established [16, 59] and transport ATPases [16, 17, 39] in the cell membrane of the epithelium of the small intestine, which, apparently, is typical for acute digestive disorders, since similar changes have been described for selmonellosis infection [23, 59], or with the action of diphenyl laxatives. The study of diarrhea in newborn calves, besides this, is of practical importance for animal husbandry. Therefore, studies of the mechanisms of the development of enteropathology with diarrheal syndrome in newborn calves, including the possibility of participation in this process of cyclic nucleotide exchange systems – cAMP and cGMP, prostaglandins, or other biochemical systems described in this review, have important scientific, theoretical and practical implications.

In conclusion, it should be noted that the conclusions about the role of cAMP and cGMP in adsorption and absorption processes in the small intestine are often based on measuring the total content of these nucleotides in the mucosa including, in addition to the epithelial mucosa, other tissues of the mucous membrane – muscle, fibroblasts, blood, etc. Hence the difficulty in interpreting the results obtained – on the contribution of a tissue to the content of cGMP or cAMP. Therefore, to adequately study the possible role of cAMP, cGMP or prostaglandins in the processes

of absorption and secretion in the small intestine, it is necessary to use isolated epithelium, excluding the admixture of cells of other tissues.

1.2 Obtaining isolated cells of small intestine epithelium of cattle

As is known, for the study of biochemical parameters of the small intestine epithelium, the use of isolated epithelial cells is the most acceptable, which makes it possible to exclude the effect on the studied parameters of the contribution of cells of other tissues present in such complex formations as the intestinal mucosa,

To date, there are many methods developed for different experimental conditions and different research objects. At the same time, there are no reports in the literature of a method for obtaining cells from the small intestine of cattle. Therefore, in this part of the paper, the results of studies on the selection of optimal experimental conditions and the effective method of obtaining isolated cells of the small intestine epithelium of cattle are described.

The research used cattle of black and motley breed. Before the research in the educational establishment, experimental groups of animals were clinically healthy adult cattle aged 3–5 years, newborn calves aged 3–5 days – healthy and sick with acute digestive disorders.

Adult animals of cattle were used in experiments according to two schemes, depending on the tasks of the experiment:

a) to develop a method for producing isolated epithelial cells, small intestine sites were selected in a meat-packing plant, washed with 0.9% NaCI, pH 7.4 and transported to the place of studies in the cold at 4–8 ° C;

b) studies of metabolism, the state of exchange of cyclic nucleotides, enzymes, prostaglandins, were carried out in isolated epithelium obtained from experimental animals, as described below.

1.2.1 Preparation of intestinal epithelial cells by various methodological approaches

Originally, the chemical citrate-EDTA method was studied, as the most simple and economical. Sequential incubation with solution (A) containing sodium citrate

for 10 min, and then solution (B) for 15 min, allows a suspension of single epithelial cells from the mucous membrane that retain the morphological structure and polarity (apical and basal parts) when analyzed by light microscopy. We have also studied the possibility of using the enzymatic method of obtaining epithelial cells, since other methods-scraping, vibration, rotation of the intestine [33] are not currently applied.

The enzymes chosen were the most widely used enzymes - hyaluronidase, collagenase and the method developed [60] using the proteolytic complex Aspergillus oruzae under the brand name Acrizim-III ("Diagnosticum", Lviv).

Criteria for the effectiveness of the application of these enzymes were selected: a) the ability of enzymes to maximal release into the incubation environment of epithelial cells; b) the absence of large aggregates and epithelial beds; c) the homogeneity of the suspension; d) the nature of the cells obtained; e) satisfactory values of intravital metabolic parameters.

As a result, it has been established that Acrizim-III possesses the minimum efficiency in the separation of epithelial cells from 1 cm^2 of the intestinal wall (along the serous membrane), and the maximum – of collagenase. Hyaluronidase reached 81% of the collagenase efficiency for 5 min, 77% for 15 min and 69% for 30 min incubation, but at the same time, the presence of aggregates and epithelial layers was greatest when using hyaluronidase and especially the citrate/EDTA method, as was noted in [61]. In the case of the use of collagenase and, especially, Acrizim-III, the cell suspension was devoid of aggregates, was homogeneous, and after precipitation and washing by centrifugation (in the angular rotor) with light shaking, it again turned into a homogeneous suspension.

Thus, by the efficiency of cell separation, the homogeneity of their suspension, the amount of material, the most appropriate is the use of collagenase. Most cells retained a monologic polarity with a clearly distinguishable apical (Figure 1.1, 1.2) (macroverse) and a basolateral particle, an elongated cylindrical shape with a brush border on the top, structured by the cytoplasm.

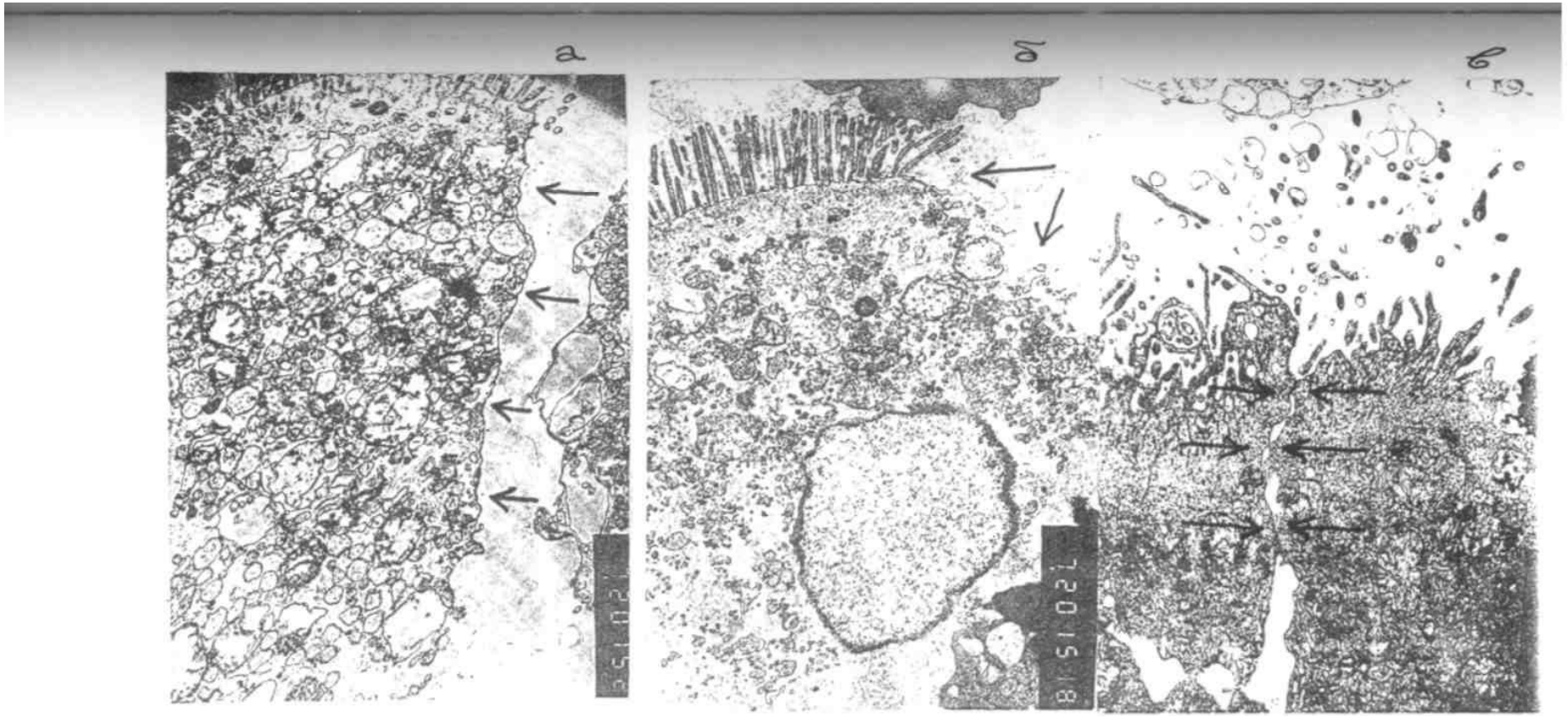

Fig. 1.1. Electron microscopic, characteristic of isolated small intestine cells of cattle, obtained by various methods: a – 0.05 % collagenase; b – 0.1 % of hyaluronidase; c – citrate/EDTA

The arrows indicate the high native activity of the apical and basal part of the cell membrane (a), the damage to the apical membrane (b), the presence of clusters of cells (c). x 7200.

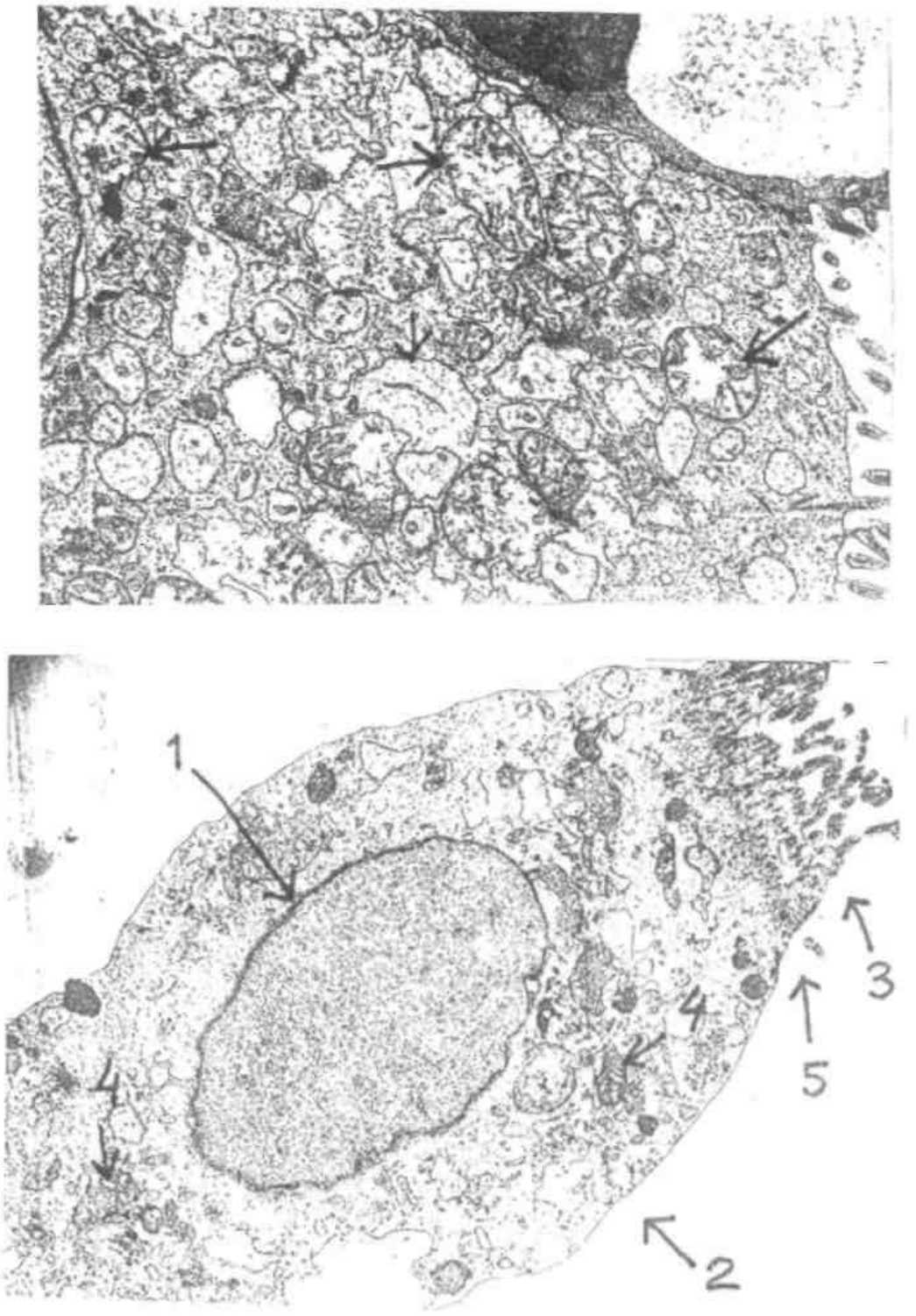

Fig. 1.2. Electron microscopic characteristics of isolated small intestine epithelial cells of cattle, obtained with 0.1% Acrizim-III (a).

A typical epithelial cell: 1 – nucleus; 2 – the basolateral membrane; 3 – apical membrane; 4 – mitochondria; 5 – area of apical intercellular contacts.

At the same time, the incubation of cells under physiological conditions for 120 min revealed a high yield of cytosolic enzyme lactate dehydrogenase from cells obtained with the help of Acrizim-III, which may indicate their low viability.

1.2.2 Evaluation of the metabolic activity of the epithelium obtained by various methodological approaches

The characteristics of the metabolic and functional characteristics of the isolated cells is crucial for the entire technique. This is especially important if it is necessary to maintain a culture of epithelial cells. The criteria for evaluating cells include a number of biochemical (lactate, pyruvate, citrate, etc.) and functional (transport of amino acids, sugars, inclusion of nucleotide bases) indicators. In some cases, the main goal is the speed of the study of the required indicators, and therefore there is no need for a thorough evaluation of the preparations obtained.

In view of the fact that epithelial cells were used immediately in our studies, the evaluation of their metabolic activity in obtaining enzymes differing was reduced to the study of glycolysis substrates as the main way of energy supply to the intestinal epithelium [62].

As might be expected, depending on the procedure, differences were found in the content of pyruvate, lactate, in the kinetics and specific activity of lactate dehydrogenase. It is known from the literature that the treatment of cells with hyaluronidase is more severe [25] than the use of collagenase [60]. As shown by studies using Akrizim-III [60] to isolate the pyramidal neurons of the rat hypocampus, the amplitude of the chemoactivated currents in the neuron was an order of magnitude higher (0.724 and 8.520 nA) compared to collagenase. The advantages of Acrizim-III before collagenase, in these studies, was confirmed by a similar difference in the registration of potential-dependent sodium currents.

The effect of these enzymes on the epithelial tissue of the small intestine of cattle has its own characteristics - the highest metabolic activity was detected by the action of Akrizim-III, and the smallest - with the use of collagenase. Taking into

account the data of electron microscopic studies and low cell viability during the time of incubation with the use of Akrizim-III, it can be concluded that the increase in metabolic activity in this case is aimed at replenishment of energy costs, which in turn are aimed at strengthening the life support systems of the cell (Na^+, K^+ pump).

In support of this view, similar collagenase effects of opposite direction are directed: with a high yield of cells, high plasma membrane safety for 120 min after the production of isolated epithelial cells, opposite metabolic activity is observed in comparison with Acrizim-III.

Thus, taking into account the indices of cell viability, the amount of material obtained, we propose two working schemes for isolating isolated cells of the small intestine epithelium of cattle using: a) 0.05% collagenase, as a highly specific method of cell disaggregation; b) citrate/EDTA, as a sufficiently effective and, at the same time, economical method.

1.2.3 Scheme of obtaining cells of the small intestine epithelium of cattle

The use of collagenase involves the following steps. After selection of the intestine and removal of chyme, it is washed with physiological saline, pH 7.4, which has been cooled to 4–6° C. A segment of the intestine 10–15 cm in length is turned out and placed in an incubation medium (see mat. and meth.) at 37° C, which is pre-blown to saturation (4–6 min) with 95% 0.2 and 5% $C0_2$, and before application of the intestinal region, the collagenase enzyme is added to a concentration of 0.05% and incubated for 15 min with a gentle shaking at a frequency of 45–60 times per minute.

At the end of the incubation, the intestinal tract is removed and the cell suspension is filtered through 4 layers with a pre-moistened gauze incubation medium. The resulting filtrate is centrifuged at 500 g in an angular rotor for 5 min. The supernatant is discarded, and the precipitate is diluted with an oxygenated incubation medium but not containing collagenase and centrifugation is repeated under the same conditions. The cell washing procedure is repeated 2–3 times. The

final precipitate of isolated epithelial cells is resuspended in isolation medium to a concentration of 1–4 mg protein cells/cm³.

Thus, the developed procedures for obtaining isolated cells of small intestine epithelium of cattle using collagenase and citrate/EDTA are quite acceptable for studying various intravital biochemical parameters, comparative studies in the age range or for studying pathologies of the digestive canal – enteropathology with diarrhea phenomena.

1.2.4 Characteristics of energy metabolism in the epithelium of the small intestine of adult cattle, healthy and sick enteropathology of newborn calves

Features of nutrition and digestion of cattle are now widely reflected in the research of many scientists, which makes it possible to identify cattle for organizing the digestive canal among other animals. Studies Melnichuk D. A. et al. [4, 5, 12, 16–19, 30, 39, 50, 63–74] established the structural and functional characteristics of subcellular elements of the small intestine epithelium. In this case, differences in the lipid composition of the epithelial cell membrane [4, 70] of enzyme activity [16, 17, 39] were established in norm and in pathology in acute digestive disorders. To date, there are a number of differences in biochemical parameters between newborns and adults [4, 37, 68]. Taking into account that the hydrolytic, transport, regulatory (cAMP and cGMP) function of the epithelium has a high dependence on energy supply, in this part of the work the characteristic of the state of energy metabolism in the epithelium of the small intestine of adult cattle, newborns healthy and sick with enteropathology of calves is given.

For the content of metabolites of glycolysis and the cycle of tricarboxylic acids in isolated small intestine epithelium of cattle, it is possible to characterize the degree of metabolism in adult animals, especially in newborn calves and changes in pathology. Thus, in adult cattle, a low level of pyruvate, characteristic for glycolysis, can be noted with a high lactate content. The lactate/pyruvate ratio is 37. Newborn healthy calves, compared with adults, significantly lower lactate levels, along with a

low content of pyruvate, citrate and lactate dehydrogenase activity. In newborn animals with enteropathology, in comparison with healthy animals, the content is increased: 3 times – pyruvate; 2.5 times lactate; in 18,8 times – citrate and 2,4 times lower the activity of lactate dehydrogenase.

It is known that the main energy source of the epithelial tissue of the small intestine is glycolysis. Despite the fact that the characterization of metabolic processes in the intestinal epithelium is widely covered in the literature, interpretation and comparative analysis of metabolic indicators are difficult due to the use of various drugs – intestinal or mucosal scrapings, which contain cells of other tissues . In this respect, the papers, where isolated epithelial cells were used, deserve attention. According to the data of, the concentration of lactate in the small intestine epithelium of the rat is 16 mM and, according to the authors, is 50 times higher than the pyruvate content.

In original studies using proton NMR spectroscopy in the chicken epithelium, the lactate level is 6.5 mM. If we take the intracellular volume of 1 mg of cells within 5–10 μl, then the level of lactate in the epithelium of bovine animals will be about 73–36.5 mM, according to our data. This can be explained either by the specific feature of the research object – cattle, the main product of which is the carbohydrate food [1–3, 26, 59], or reflect the typically high glycolysis level characteristic of this small intestine-jejunal section [23].

At the same time, the specific activity of lactate dehydrogenase in bovine epithelium is commensurate with the data obtained for isolated rat epithelium [47].

The low level of metabolites in healthy newborn calves, on the one hand, does not agree with sufficiently intensive transport processes during this period of development [29], but on the other hand it can be explained by the receipt of many components of energy metabolism from colostrum or characteristic of newborns state of carbohydrate metabolism. As shown [14], the rate of glucose oxidation in the intestinal sections of the intestines of newborn rats is very low until 21 days of development – during the period of feeding them with mother's milk. When switching to independent feeding, the rate of glucose oxidation and lactate production

increases 3–4 times [14]. This explains the low level of lactate, citrate, pyruvate in healthy newborn calves. The pathological state of the digestive canal leads to a violation of the electrolyte balance of the organism [12, 14–16] and affects the function of the epithelium [13, 18, 19, 24, 26, 38, 39, 41, 59], and according to our data changes intracellular homeostasis. The high level of citrate (18.8 times higher) in the epithelium of the small intestine of patients with enteropathology of newborn calves, in comparison with healthy calves, indicates the inhibition of oxidative processes in the mitochondria, which may be due either to insufficiency of the oxygen supply of the epithelium [36], or to inhibition by another mechanism. According to [30], suppression of oxidative phosphorylation by oligomycin or rotenone leads to a change in the course of glycolysis activation.

Proceeding from this, the increase in the level of lactate observed in the epithelium of newborn calves during diarrhea indicates activation of the glycolysis processes. In addition, the condition of animals during the period of illness can be conditionally regarded as a state of hunger, which can also lead to the activation of glycolysis [40]. At the same time, a decrease in the specific activity of lactate dehydrogovase in the epithelium in enteropathology can be explained by inhibition by a high level of pyruvate. In addition to the presented results from the calculated value for the NAD^+/NADH ratio, which is 242.7, 547.7 and 659.2 respectively for adult cattle, newborns healthy and diarrhea patients, the conclusion is that in the epithelium of patients animals are inhibited by reductive reactions.

Thus, isolated small hamstring epithelial cells isolated with collagenase or citrate/EDTA retain their metabolic parameters, native cell membrane, morphological polarity and can be recommended for studying various biochemical characteristics. Evaluation of the metabolic activity of the epithelium indicates differences in the state of energy metabolism in healthy newborn calves and significant changes in enteropathology. The use of isolated epithelial cells to study the state of exchange of cyclic nucleotides and prostaglandins, according to the procedure proposed by us, undoubtedly has a number of methodological advantages over other forms of drugs - mucosal scraping, intestinal wall.

1.3 Characteristics of intracellular regulatory systems in the epithelium of the small intestine of adult cattle, healthy and sick with enteropathology of newborn calves

The epithelium of the small intestine performs a variety of various and numerous functions associated with the processes of absorption and secretion of nutrients, water and electrolytes. To ensure these functions, a number of regulatory systems exist in the epithelium, including a classical scheme involving cyclic nucleotides – cAMP and cGMP, calcium, calmodulin and possibly prostaglandins. The functioning of these intracellular regulatory systems is based on maintaining their concentration within the cell at a certain basal level, which depends on the functional state of the organism, the intestinal tract and, apparently, the species of the animal.

It should be noted that there are no reports in the literature that characterize the state of exchange of cyclic nucleotides and prostaglandins in the epithelium of the small intestine of cattle. In addition, the biochemical mechanisms for the development of acute digestive disorders in newborn calves have not been fully investigated, although they are of great importance for modern animal husbandry for the purpose of preserving young animals, and may also be an alternative model for studying similar pathologies in pediatrics.

1.3.1 Cyclic nucleotide exchange state

When studying the state of cyclic nucleotide exchange, we studied the content of cAMP, cGMP and their exchange enzymes in freshly isolated isolated cells of the small intestine epithelium of adult cattle, newborns healthy and sick with enteropathology of calves. Given the likelihood that the gastrointestinal tract may be a source of cyclic nucleotides in the blood, and the possibility of filtering the cyclic nucleotides in the kidney [52, 74, 75], the content of cyclic nucleotides was also studied in blood plasma and urine experimental animals.

The content of cAMP in the epithelium of adult cattle is 59.4±10.9 pmol/mg protein. In healthy newborn calves, the value of this indicator was not significantly different from adult animals and was 46.21±20.6. Comparison of the obtained indices with analogous literature data is difficult, in view of the absence of studies using isolated cells. Although, according to [76], in the mucous membrane of developing rats, the level of cAMP is higher at a young age.

A high level of cAMP in the postnatal period is also characteristic of other tissues and organs - the liver, skeletal and cardiac muscles of adipose tissue [60]. In patients with diarrhea of calves, the level of cAMP decreases by 5.7 times. It is known that with enteropathology accompanied by diarrhea, on the contrary, there is an increase in the content of cAMP in the intestinal wall or mucosa in cholera [60, 75], salmonellosis [23], dysentery [74]. Thus, according to Bunin K.V. et al., the level of cAMP in biopsies of the human small intestine rises from 2346±310 pmoles/g of crude tissue in healthy to 4,583±789 in patients with dysentery. Similar changes from 817±23 to 1120±63 pmol/g of raw tissue were detected by Sidakov B.M. et al. in dysentery or other authors [23] for salmonella infection (from 335 to 1254 pmol/g crude tissue).

Along with this, it was shown that the level of cAMP in the intestine depends on the consumption of milk [60]. Deprivation of 5-day rats of milk leads to a significant decrease in the level of cAMP in their small intestine compared to the rats of the same age who receive milk. Even digested milk can serve as a source of cAMP in the intestinal mucosa. But these data are unlikely to be involved as the only explanation for the low level of cAMP in the intestinal epithelium of diarrhea-sick calves, who are often on a starvation diet and who are being reduced and sometimes given away colostrum. It is known that consumption of solid food in the period after weaning from milk increases the concentration of cAMP in the mucosa [60].

The content of cAMP in the blood plasma of adult cattle is more than 2 times higher than that of human [74] and rat [60] and is possibly determined by the high level of cAMP in the intestines of cattle at about 6,000 pmol/g wet weight cells, while newborn calves it is close: 15.5±2.2 pmol/ml in newborn calves, and 13–14 pmol/ml

in humans [23, 24, 74]. According to the data of [74], the content of cAMP in human plasma under dysentery did not differ between healthy and sick, and in the patients' urine was slightly higher.

The content of cAMP in the blood plasma of healthy and sick newborn calves is consistent with the indices in dysentery [74], whereas in the urine there are no significant differences. Moreover, the level of cAMP in the urine of adult animals is significantly (3 times) lower than in healthy newborn calves.

Comparison of the presented indices between groups of animals in the age aspect and in pathology, as well as in the intestine - blood - urine chain is advisable to conduct after considering the level of another cyclic nucleotide – cGMP and enzymes of their metabolism.

Thus, the content of cGMP in isolated small intestine epithelium of adult cattle and healthy newborn calves is in trace amounts, whereas in patients with enteropathology of newborn calves it is 10 pmol/mg protein, i.e. an order of magnitude higher than in healthy animals.

In the studies mentioned Bunin K.V. et al., when dysentery increased the content of cAMP in the epithelium, there was no increase in cGMP, and even vice versa, its decrease was observed. As we see, a similar but opposite direction in the content of cAMP and cGMP was established by us in the enteropathology of newborn calves.

In the blood plasma of adult cattle and healthy newborns, unlike humans (3–9 pmol/ml), the content of cGMP has trace amounts, and only in patients with enteropathology approaches 2.7±0.8.

At the same time, the results on the content of cGMP in the urine of cattle are fully interpreted. The high content of cGMP in the urine of adult cattle (10.3±2.1 nmol/ml) and healthy newborns (2.3±0.6 nmol/ml) is 20 times higher than the content of cAMP in human urine. There are in view of the peculiarities of feeding adult cattle (high water content in plant foods) and feeding newborn calves (colostrum, milk), and there is a need for intensive cGMP-dependent glomerular fluid filtration [7]. In newborn calves with diarrhea, intensive dehydration of the body

through the intestine occurs, and diuresis, in all probability, is inhibited, as is observed in enteropathology of infectious origin [74]. Hence, the content of cGMP in urine is 44 times lower than in adult cattle, and 10 times lower than in healthy newborn calves.

Thus, the obtained results indicate that cattle, as polygastric and ruminant animals, have a number of features in the content of intestinal epithelium, blood, and urine, with respect to the content of cAMP and cGMP, which are reduced to an increased cAMP content and a decreased cAMP level in blood plasma compared with other animals and humans, a high level (44 times) with cGMP in the urine.

For diabetic patients of newborn calves, the content of cGMP in isolated small intestinal epithelium increased by more than 10 times against the background of a lower content of cAMP in it. Moreover, a low level of cAMP in the epithelium can not be associated with a pool of adenyl nucleotides, as in the previous chapter it is shown that in patients with enteropathology of calves the state of energy metabolism (glycolysis) is in a sufficiently activated state.

Given the mechanisms of the development of enteropathology of infectious and non-infectious etiology, we are inclined to discuss the results obtained in terms of the mechanism of cGMP-mediated secretion in the small intestine in diarrhea of newborn calves.

1.3.2 The activity of adenylate cyclase, guacylate cyclase and phosphodiesterase

The activity of adenylate cyclase in isolated cattle small intestinal epithelial cells has a slightly lower activity than that described for other tissues [7, 9, 66, 77–79] and does not differ from that in healthy newborn calves. The established values of the activity of adenylate cilase correlate with the content of cAMP in the epithelium of these animals.

At the same time, in patients with enteropathology of newborn calves, the activity of adenylate cyclase is reduced by 35% compared to healthy calves (cAMP by 5.7 times).

It is known that the adenylate cyclase complex is sensitive to many regulatory factors: hormones, neurotransmitters, calcium and calmodulin, the phospholipid composition of membranes [78, 80–82], viscosity [52], the presence of an activating substrate-GTP , and also have age features. Thus, the interpretation of data on the change in the activity of adenylate cyclase in the epithelium of the small intestine of patients with enteropathology of calves can be very versatile.

First, the factor initiating diarrhea (increasing the level of cGMP) is unknown, since in the cAMP-mediated pathway of diarrhea development, the reverse is observed-activation of adenylate cyclase [23, 24, 74].

In our previous studies [4, 17, 74], it was established that in the basolateral membranes of the small intestine epithelium in diarrhea of calves there is a change in the phospholipid, fatty acid composition and cholesterol content, ie, precisely in that part of the cell membrane where adenylate cyclase is localized [32] . Changes in the lipid composition can lead to a change in the viscosity of the membranes, which in turn, can affect the activity of the enzyme [7, 9].

However, such an explanation is one-sided. It is likely that the factor that induces the production of cGMP can simultaneously be an inhibitor of cAMP production. Such phenomena include an increase in the concentration of intracellular calcium and the activation of calmodulin [81–83], which are capable of activating guanylate cyclase.

Indeed, the activity of guanylate cyclase in the epithelium of the small intestine of patients with enteropathology of calves (14.3±0.61 pmol/mg protein per 1 minute) is 2.2 times higher than in healthy calves. Based on these data and high-level data in the cGMP epithelium, it can be concluded that the secretory processes in diarrhea of newborn calves go along the cGMP-mediated pathway.

The enzyme, hydrolysing cyclic nucleotides – phophodiesterase, also has features depending on age and enteropathology. Its specific activity in the epithelium

of cattle is close to the described activity in the homogenate of the mucosa of the rabbit jejunum [23]. In newborn calves, the activity of phosphodiesterase is more than 2 times lower than in adults, and in patients with enteropathology, on the contrary, it is associated with such activity. The latter could be explained by previous studies [4], where in the brush border and basolateral membranes of newborns, and especially patients, the presence of myristic acid, an increase in the content of oleic acid, which, as shown in the studies of Severin S. E. activate phosphodiesterase. But due to the low activity of phosphodiesterase in healthy newborns, a more reasonable explanation may be the assumption of a Ca^{2+}-activating phosphodiesterase mechanism [8] involving the phosphatidylinositol pathway [71]. This is confirmed by our data on the decrease in the brush border of the epithelium of the small intestine in patients with enteropathology of calfs containing phosphatidylinositol 2.4 times [4].

1.3.3 The intracellular content of the prostaglandins F1α and E2

In the gastrointestinal tract there are mainly prostaglandins of group E and F [53, 54]. It is generally accepted that group E prostaglandins stimulate active secretion of electrolytes in the intestine by stimulating adenylate cyclase, i.e., by increasing the concentration of cAMP, whereas the prostaglandins F1α, in all likelihood, along with the secretory effect, stimulate the motility of the small intestine.

The results on the content of prostaglandins E2 and F1α in isolated small intestine epithelium of bovine animals correspond to their biological level in tissues [44, 48]. In healthy newborn calves, the level of F1 is 3 times lower and 5 times lower than E2 in comparison with adult animals. In diabetic patients calves increase in 4.1 times the content and in 6.3 times – E2 in comparison with healthy newborn calves. Properties of prostaglandins to cause diarrhea are indicated by their parenteral use [48], oral [27] or intestinal perfusion [46]. At the same time, if the biological effect of exogenous prostaglandins is well understood, the functional significance of endogenous prostaglandins in the small intestine is still not clear. The involvement of

prostaglandins in the inflammatory processes [10] including inflammatory processes of the small intestine [46] made it possible to put forward the concept that in inflammatory bowel diseases there is an uncontrolled increase in the level of prostaglandins. In the opinion of [46], the high level of prostaglandins in diarrhea not of coins is the root cause of the disease. In favor of this concept are data on the level of prostaglandins and cAMP in isolated epithelium of the small intestine of patients with diarrhea of calves. With a high content of prostaglandin E2, which is an activator of adenylate cyclase, we observe the reverse, low cAMP. Therefore, the ability of prostaglandins to cause diarrhea can be considered as a convenient model for stimulating diarrhea, whereas their biological effect may be more versatile and requires detailed study. Thus, the ability of prostaglandins of group E2 and F1α to inhibit the activity of intestinal Na^+, K^+ -ATPase [27, 54], stimulate the secretion of HCO_3^- [14] and chlorine [54].

Our preliminary studies showed a 5.3-fold decrease in the activity of the Na^+, K^+- ATP-ase of basolateral epithelial membranes [17, 39, 68–70], the change in the acid-base balance of crocs towards acidosis [14–16], a decrease in the level of chlorine in the blood of patients with diarrhea in newborn calves.

Therefore, along with the data obtained on the participation of cyclic guanosine monophosphate in stimulating secretion in the small intestine of diabetic patients of newborn calves, it is possible to assume that this pathology of prostaglandins E2 and F1α, namely, their involvement in anion secretion, is also involved (Fig. 1.3)

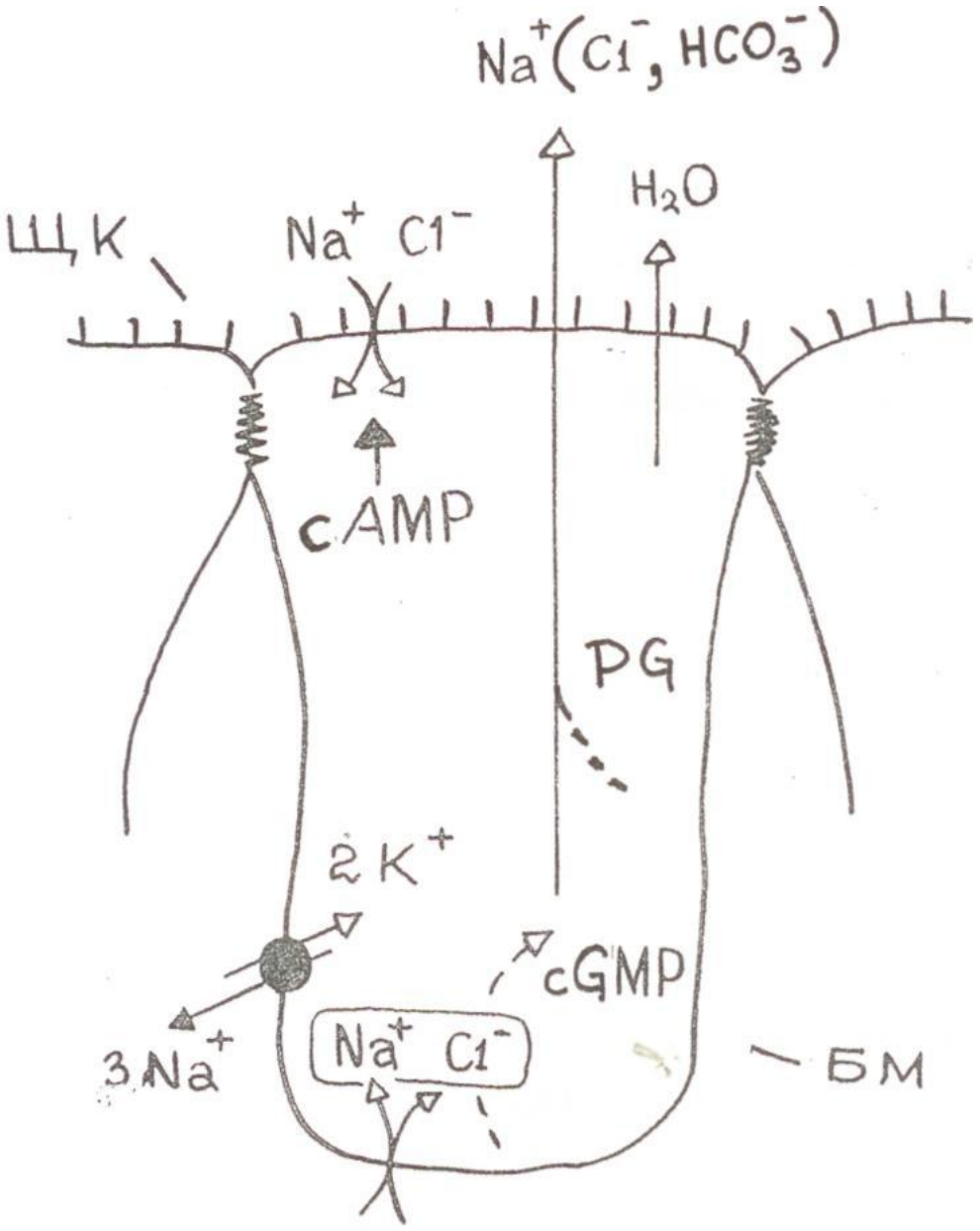

Fig. 1.3. Hypothetical mechanism of the secretion of electrolytes and H_2O in the epithelium of the small intestine, including the participation of cGMP and prostaglandins, with enteropathology with diarrheal syndrome in newborn calves

CONCLUSION TO CHAPTER I

The modern concept of the development of enteropathology with diarrheal syndrome (infectious and noninfectious nature) in the small intestine of animals and humans provides for the direct participation of cyclic nucleotides and prostaglandins in the initiation of secretory processes and inhibition of absorption.

Enteropathology of newborn calves is widespread, causing significant damage to livestock and represents a great economic and social problem. Uncertainty of the etiologic factor on the one hand, and a variety of causes that can lead to pathology (content, feeding, quality and quantity of colostrum, its temperature, etc.), on the other hand. Let us assume that the basis for the onset and development of diarrhea in newborn calves may be a single biochemical "trigger" mechanism of H_20 secretion and electrolytes localized in the epithelium of the small intestine. Obviously, for its investigation it is necessary to use a sufficiently pure experimental material-isolated epithelial cells, the procedure for obtaining which for cattle is not currently described. In addition, given the high sensitivity of individual tissues of the body to respond to fluctuations in cyclic nucleotides and prostaglandins on external influences (stresses, etc.), as well as the specificity of the object of our research, we faced diverse methodological problems in order to exclude the effect of inadequate factors and the manifestation of artifacts.

Using a variety of methodological approaches – chemical (citrate/EDTA) and enzymatic (collagenase, hyaduronidase and acryzyme III), we conducted a study on the selection of optimal experimental conditions for the purpose of obtaining viable isolated epithelium cells of the small intestine of cattle.

Criteria for the effectiveness of the method used were: a) the ability to maximize the release of cells in the incubation medium (histological control, the amount of cell protein); b) the homogeneity of the cell suspension, the absence of aggregates of cell clusters (light microscopy); c) preservation of morphological parameters (electron microscopy); d) cell nativity (Trypan blue staining, release into

the incubation medium of cytosolic lactate dehydrogenase); e) satisfactory values of intravital metabolic parameters (state of glycolysis, etc.).

As shown by the studies, the most optimal criterion is the use of 0.05% collagenase (enzymatic method), as well as the method using citrate/EDTA (chemical method). Moreover, with satisfactory parameters, citrate/EDTA method is economical.

But to study the level of cyclic nucleotides and prostaglandins this procedure is not acceptable in view of the possibility of changing the concentration of calcium.

Thus, in the present work, two procedures for the production of epithelial cells of the small intestine of adult cattle, newborn calves – healthy and patients with enteropathology with diarrhea, were developed and proposed, and their metabolic activity was assessed. The peculiarities of metabolic processes (a decreased state of glycolysis, a cycle of tricarboxylic acids) in isolated small intestine epithelium of healthy newborn calves are established, in comparison with adult animals, which can characterize a special (colostrum) feeding period. In patients with enteropathology with diarrhea syndrome of newborn calves, activation of glycolytic processes is observed with simultaneous inhibition of oxidative processes, which, apparently, reflects the pathological state of the tissue.

When studying the state of cyclic nucleotide exchange, we studied the content of cAMP, cGMP and enzymes of their metabolism (adenylate cyclase, guanylate-clase, phosphodiesterase) in freshly isolated isolated epithelial cells, as well as cAMP and cGMP levels in blood and urine.

For adult cattle, it has been established (epithelium/blood/urine): cAMP – 59.4±10.9/34.5±2.1/18.2±4.8 pmol/g protein (or ml of liquid); cGMP - traces/tracks/ 10.3±2.1 nmol/ml. Thus, the obtained value of the level of cAMP in the epithelium of the small intestine of bovines characterizes its intravital level in the epithelial tissue of this animal. The high level of cGMP in the urine reflects, apparently, the high diuretic activity of the kidneys, due to the high water content in the feed.

In the physiological limits, there is also the activity of adenylate cyclase, guanylate cyclase and phosphodiesterase of cyclic nucleotides in the epithelium of adult cattle.

The content of prostaglandin E2 and F1α in isolated small intestine epithelium of adult cattle is 168±36 and 205±55 ng/mg, respectively, which, apparently, reflects their physiological level in the tissue.

For healthy newborn calves, the level of cAMP, cGMP and adenylate cyclase activity is the same as for adult animals. The content of prostaglandins is significantly lower, F1α is 3 times, E2 is 5-fold, which may reflect the peculiarities of the state of the epithelium of the small intestine of newborn calves, as described for other animal species.

Acute digestive disorders of newborn calves are accompanied by significant changes in the studied indicators. Thus, in isolated epithelium of the small intestine of diabetic patients of newborn calves, the level of cAMP decreases by 5.7 times, but the level of cGMP rises tenfold. At the same time, a decrease in the activity of adenylate cyclase, but an increase in the activity of guanylate cyclase (by 2.2 times), and phosphodiesterase (possibly with the GMR of the stimulated isoform) has been established. The obtained data allow us to conclude that the emergence and development of enteropathology with diarrheal syndrome of alimentary nature in newborn calves takes place with the participation of a biochemical pathway involving cGMP-mediated secretion of H20 and electrolytes.

The high level in the epithelium of sick newborn animals of the prostaglandin F1α (4.1 times) and E2 (6.3 times) may indicate a profound pathological changes that are accompanied by damage to membranes, enhanced secretion of $HC0_3^-$ and Cl^-, inflammatory phenomena. Indeed, based on recent data, an increase in the level of prostaglandins in the pathology of the small intestine is a secondary process, but capable of stimulating additional secretion of anions. This allows us to explain the violation of the electrolyte balance that we received earlier in the body of sick newborn calves, in particular – the development of the acidosis state, a sharp decrease in $C1^-$ in the blood plasma.

Thus, as a result of the studies, the state of exchange of cyclic nucleotides and prostaglandins in epithelium of the small intestine of cattle as an animal species was characterized for the first time, the features of these biochemical regulatory systems for the neonatal period were established. Based on the known role of cAMP, cGMP and prostaglandins in the processes of secretion and absorption, the mechanisms of the development of enteropathology of infectious and noninfectious nature, and convincing data on the significantly high content of cGMP and prostaglandins in the epithelium of the small intestine, the pathology suggests a possible biochemical mechanism of diarrhea development in newborn calves, which included cGMP-mediated secretion of fluid and electrolytes:

- unidentified etiologic factor (*E. coli*, rotavirus, Ca^{2+}, etc.) lead to activation of guanylate cyclase and increase in cGMP level;

- an increase in the intracellular concentration of cGMP leads to the activation of secretory processes, as a result of which the secretion of Na^{+} predominates over its absorption;

- H_2O is secreted in parallel with sodium in its hydrate shells;

- development of the pathological process associated with the production of prostaglandins, stimulate the secretion of HCO_3^{-} and Cl^{-};

- the development of the disease leads to a violation of electrolyte blood and acidosis.

LIST OF CONVENTIONAL SYMBOLS AND ABBREVIATIONS

cAMP - cyclic adenosine monophosphate;

cGMP - cyclic guanosine monophosphate;

ATP - adenosine triphosphoric acid;

ATPasa - adenosine triphosphatases.

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CHAPTER II
LIPID COMPOSITION AND STRUCTURAL CHANGES IN THE PLASMOLEM OF CIRCUMFERENTIAL ENTEROCYTES AFTER SPONTANEOUS AND EXPERIMENTAL ENTEROPATHOLOGY OF ANIMALS

Membrane structures of the cells of the organs of the animal organism are the first to come into contact with the pathological factors of the external and internal environment [1, 2]. The interaction of the damaging agent with the receptors on the surface of the cell plasmolemus triggers a cascade of interrelated biochemical processes that occur not only on the membranes but also in the compartment cells, which is also characteristic for the manifestation of immune responses. Chain character of the development of metabolic homeostatic processes with the participation of secondary cellular messengers is an adequate response of target cells when they are in contact with the pathological factor underlying the manifestation of adaptive-compensatory changes on the part of the whole organism [3–5].

It is known [6] that the plasmolem of cells is a strategically important, stable and at the same time vulnerable feature between the intra- and extracellular environment. Upon contact with a harmful agent, the molecular structure of the cell membrane changes, primarily, and this is reflected in its physical and structural-dynamic characteristics.

The intensity of restoration of intracellular homeostasis in the course of development of pathology essentially depends on the duration of action of the pathogenic factor and the adaptive capabilities of the organism, primarily determined by the degree of involvement of plasmolemia and intracellular membranes. The results of clinical and experimental studies prove the key role of the lipid bilayer of membranes in the development of severe liver diseases, cardiovascular and nervous systems, violation of numerous functions of blood cells, epithelium and others [7–10].

The main structural components of the lipid bilayer of cell membranes are known to be phospholipids (PL) (about 65%) [11, 12]. Thus, the functioning of external and internal membrane systems depends on the integrity of their phospholipid structures [13]. So, the usefulness of metabolic processes in cells and their violation in the development of pathology is determined by the structural-functional state of membrane systems.

An important issue in the current state of the livestock sector is the preservation of the livestock of young animals and their productive qualities, the most intensive formation of which occurs in the early postnatal period of life. Applied value of these studies is confirmed by a high percentage of cases of young animals of different species of animals on enteropathology, as it is known, 60-90% of newborns are sick, of which 15 to 50% die [14].

Numerous studies have shown [15–22] that the metabolic status of the organism of newborn animals is distinguished by the significant lability of biochemical indices, which is related to the normalization of the acid-base state, the genetically determined process of substitution of the fetal type of blood proteins for an adult, the formation of a unique natural phenomenon – colostral immunity for endocytosis-pinocytosis mechanism of absorption of immune colostrum proteins in the gastrointestinal tract and a number of features of morphofunctional state of structural elements in cells and, above all, their membranes.

The appearance of dyspepsia during this "critical" period significantly disrupts the development of adaptive changes in the digestive canal and in other organs and systems of newborns. These animals show significant metabolic disturbances during their clinical recovery [23–25]. This indicates a profound disturbance of metabolism at the cellular level, which correlates with the intensity of recovery of the structural and functional state of cell membranes.

A significant and diverse arsenal of modern therapeutic and prophylactic drugs does not always give a high efficiency in the application of patients to enteropathology of newborn animals, which is explained by the lack of reparative action in therapeutic regimens. While the restoration of the metabolism and

structural-functional state of the epithelial cells of the intestinal mucosa does not end, and three weeks after the clinical recovery of the newborn calves [26].

This creates the problem of repeated relapses of enteropathology in clinically healthy calves, the development of a number of complications from other organs and systems (bronchopneumonia, nephritis, etc.), a decrease in the resistance state of the organism and productive qualities in animals who have experienced dyspepsia [27, 28].

The main source of PL for the restoration of the lipid structure of cell membranes in the early postnatal period of animal life is colostrum or milk. Taking into account that PLs not only have a structural role, but also stimulate the biological activity of the vast majority of membrane receptors that activate membrane-bound enzymes that regulate numerous metabolic processes between the intra- and intercellular environment, and also affect immune responses at the cellular level, the idea of creating biologically active additive (BAA) of reparative action based on natural PL, cheap raw materials for the production of which is an oil can (a byproduct of processing milk for oil). Under the guidance of Academician D. A. Melnichuk (1987), a method was developed for isolating PL from membranes of grease globules of the lubricator [29]. It is important that, according to their qualitative and quantitative spectrum, they correspond to the structure of plasma cell plasmolemia, especially hepatocytes.

The objectives of this study were to study the lipid composition, pro and antioxidant indices, which are interrelated with changes in the structural and dynamic characteristics of intestinal enterocyte membranes after spontaneous and experimental enteropathology, as well as the determination of the reparative efficacy of a complex scheme for PL milk «FLP-MD» is an author's development [30].

2.1 Lipid composition and structural characteristics of the epithelium of the small intestine mucosa in diseased calves with enteropathology

It is known that the stability of the organism under the action of various factors is determined by the peculiarities of lipid metabolism [31–33]. Since lipid components are involved in all vital physiological and biochemical processes of the body, they play an important role in compensatory reactions of the organism to changes in exogenous and endogenous conditions of existence and the negative influence of pathogenic factors.

Lipids in the body of living things, above all, are a source of energy. This function is performed by fatty acids (FA), which are released after the breakdown of fats. Thus, with the complete utilization of 1 g of fat, 9.3 kcal of energy is released, which is twice as much as the oxidation of carbohydrates and proteins. Lipids also play an important role in water metabolism (when oxidizing 100 g of fat, 107.1 g of water are formed). Phospholipids, glycolipids (GL) and cholesterol (ChS) are involved in the formation of cell membranes of all living things. Derivatives of some polyunsaturated fatty acids (PUFA) – prostaglandins perform a regulatory function. These LCDs belong to the category of essential (essential) factors of feeding and nutrition. Cholesterol is a structural component of biological membranes, as well as a precursor of bile acids and steroid hormones. In addition, along with fats, fat-soluble vitamins (A, E, D, K) and other lipotropic compounds enter the body [34–39].

Lipids, as components of biological membranes, play an important role in the provision of cell vital functions, the functioning of many enzymes and even chromosomes [40, 41].

Lipids also affect the structure of DNA, coordinate its replication [42].

Membrane lipids include PL, triacylglycerols (TAG), and cholesterol. The main PL membranes are phosphatidylcholine (PC, lecithin), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and cardiolipin (CL). The main sterulin of cell membranes in the body of animals is cholesterol. Glycolipids are contained only in plasma membranes. A significant

content of CR is found in mitochondria. The lipid bilayer in cell membranes is formed in the aqueous phase by means of amphiphilic PL molecules. These molecules are called amphiphilic because they consist of two parts, different in their solubility in water: a polar or hydrophilic "head" that has an affinity for water and a "tail", which is formed by nonpolar, hydrophobic carbon-hydrogen chains of FA. An example of an amphiphilic molecule may be a PE molecule. It should be noted that hydrophobicity is a common property of all lipids. But some lipids and their derivatives (GL, PL, bile acids) are amphiphilic, since they contain hydrophilic and hydrophobic parts [43].

In the cell membrane PL form a double layer in which hydrophobic LC chains are directed inside the membrane, and hydrophilic polar groups outward. Membrane proteins are attached peripherally due to polar or ionic interactions, and also form part of a lipid bilayer. Biological membranes have "liquid" properties, so on the edge of one layer individual lipid molecules are able to exchange places with neighboring ones at a speed of more than 1 million times per second. The exchange of lipid molecules between the layers (flip-flop) is quite rare. The cell membrane has an expressive asymmetry in terms of the distribution of various PLs in the outer and inner layers. Holincomposite neutral PLs, such as sphingomyelin (SM) and PC, are located on the outside of the membrane in combination with a small amount of PE. Its internal (cytosolic) part consists of a small amount of PC and SM, more significant – PE, as well as PS and PI. Thus, normal anionic PLs are not present on the external surface of biomembranes [44, 45].

Phospholipids not only form a lipid bilayer of cell membranes, but also participate in the excitation and transmission of nerve impulses, affect the processes of blood coagulation [46].

The main biological functions of lipids are also: structural (biomembrane component), protective (fat layer surrounding the internal organs), an important caloric component of the human and animal diet, the transport form of fat-soluble vitamins, regulatory (transport of water and salts, a regulator of the activity of certain

enzymes), immunomodulating, an integral part of some endohormones, mediator [47, 48].

Mechanisms for the regulation of lipid metabolism in mammals are closely related to the processes of cell growth and differentiation, the formation of eicosanoids, cytokines, adhesion molecules. Violation of the mechanisms of regulation of lipid biosynthesis can lead to the development of a pathological process. The scientific literature shows the results of studies that demonstrate the important role of complex lipids in the pathogenesis of various chronic diseases such as atherosclerosis, coronary heart disease, obesity, diabetes, malignant neoplasms, schizophrenia, etc. [49].

The list of biological functions of lipids increases as they study. In providing these functions, lipids of different classes participate in appropriate amounts. Therefore, to understand the essence of many biological processes in living organisms, one should have an idea of lipids at the same level as proteins, nucleic acids and carbohydrates.

2.1.1 Changes in the lipid composition of cellular structures in pathological conditions

The distribution of FA in the cell is interrelated with the content of cholesterol and the action of ecopathogenic environmental factors [49]. American scientists M. Sinensky and J. Hazel independently proposed a theory of homoviskous adaptation, which they developed as a result of studying the terminal adaptation of poikilothermic animals [49–51].

It has been experimentally proved that cholesterol increases the degree of ordering of the phospholipid bilayer of membranes in the liquid-crystalline phase and decreases in the gel phase. For physiological temperatures (about 37° C) in homoiothermic animals, cholesterol improves the ordering of the lipid bilayer of biological membranes. At the same time, the incorporation of cholesterol into the lipid bilayer of membranes is accompanied by a decrease in the degree of ordering of

the acyl residues of PL due to an increase in the degree of their unsaturation. An increase in the level of cholesterol in biological systems results in a decrease in the ratio of NLC/NLCLC. It has been established that individual PLs, such as PE, independently of the acyl composition due to the small size of the polar head, can reduce the degree of ordering of the PC liposomes [49, 50].

The theory of homoviskous adaptation does not give a complete idea of the functional role of changes in the lipid composition of membranes. However, it explains well why, when the body temperature of poikilothermic animals changes, the viscosity of biological membranes remains unchanged-this phenomenon is due to the modification of the degree of unsaturation of FA, esterified in PL membranes. Thus, as the temperature of the body of poikilothermic animals decreases, the content of PL with unsaturated acyl residues increases and the level of free cholesterol decreases, which prevents an increase in the viscosity of cell membranes; as the body temperature rises, the content of PL with unsaturated acyl residues decreases, and the level of free cholesterol increases, which prevents excessive fluid membrane growth [49–51].

Thus, the features of thermal adaptation in poikilothermic animals allowed us to formulate the theory of homoviskous adaptation, which for the first time attempted to reveal general patterns of lipid composition changes under extreme environmental conditions. This theory has revealed the biochemical mechanisms for maintaining the proper viscosity of biological membranes of poikilothermic [50, 51] and homeothermic animals [52, 53] as a result of modification of the lipid composition of biological systems. It has been shown that the increase in the level of cholesterol in the biological membranes of mammals is compensated by the increase in the degree of unsaturation of the PL and, conversely, the decrease in the level of cholesterol in the biological membranes is compensated by a decrease in the degree of unsaturation of the PL, so that the viscosity of the cell membranes does not change. Such a fundamental mechanism for compensating changes in the lipid composition allows maintaining homeostatic reactions in the cell at the proper level, ensuring its adaptation to the changing environmental conditions. Unfortunately, the authors who

investigated the homozygous adaptation of poikilo- and homoiothermal animals did not determine its place and role in the pathogenesis of acute and chronic diseases in humans and mammals [49].

An important role in world science is given to the study of lipids in the cells of the mammalian organism, primarily biopharmaceutical aspect. The significance of the latter is now especially important. This concerns the search for biologically active lipids and the development on their basis of new drugs for pharmacotherapy, especially the most common diseases. After all, the imbalance in the distribution of saturated and unsaturated FA, as well as in the content of ω-6 and ω-3 NEFLC is an important factor in the pathogenesis of various chronic diseases, in particular, atherosclerosis and coronary heart disease, diabetes mellitus, insulin resistance and obesity, malignant neoplasms, and bipolar disorders and schizophrenia, etc. [49, 54–56].

The theory of homoviskous adaptation entailed a number of important questions: are there certain general patterns of the formation of lipid imbalance in pathological conditions in humans and animals? What role does homozygous adaptation play in the emergence and course of acute and chronic pathological conditions? If homoviskous adaptation is protective in pathological conditions, is it possible to stimulate its course with the help of biologically active lipids? [49]

To date, it has been established that there are common patterns of disruption of the composition of FA membranes, cells and tissues in chronic cardiovascular and oncological diseases, and also in the remote period after the action of ionizing radiation. A universal sign of these chronic pathological conditions is the nonspecific increase in levels of PUFA and PL, in particular PE, in biological structures, which is compensated by an increase in the level of the total/free cholesterol index [49]. These changes provide compensation for lipid disorders in chronic diseases, as a result of which the increased content of compounds that reduce the ordering of the lipid bilayer (PUFA and non-lamilar PL) is leveled by cholesterol, which increases its ordering.

The biological essence of compensating lipid composite biomembrane disorders can consist in enhancing the protective reserves of the cell. This ensures its survival and adaptation to the action of the pathogenic factor by satisfying its increased need for PUFA as a factor in the regulation of membrane-bound functions and precursors of secondary messengers of the lipid nature, which mediate the action of stress hormones. As a result, a stable lipid imbalance is noted, which can lead to disruption of specific cell functions and the occurrence of chronic diseases [49].

If the level of PUFA in the cell increases, their availability for cyclooxygenases increases. As a result, large amounts of products can be formed in immunocompetent cells, which adversely affect the immune function, in particular monohydroxylated PUFA derivatives. This can be a key factor in the pathogenesis of various diseases. Pathogenetic mechanisms that are triggered during direct contact with a pathogenic factor (for example, ionizing radiation) can continue to function under the influence of further chronic effects of other unfavorable factors and the formation of a stable imbalance of FA. As described in the literature, the disadaptation syndrome, which develops under the influence of unfavorable factors of the external and internal environment, is realized in the pathology of the cardiovascular, endocrine, digestive and other systems of the organism with the acceleration of age involution and shortening of life expectancy [49, 57].

Meanwhile, pathological conditions, which are accompanied by a decrease in the level of total cholesterol in biological tissues, are associated with a decrease in the content of monounsaturated fatty acids (MUFA) and PUFA ω-6/ω-3 series, as well as general and some individual PL (experimental morphine dependence in rats).

The above changes were first interpreted from the viewpoint of the theory of homoviskous adaptation: an increase in the content of polyunsaturated lipids in the biological structures of humans and animals in chronic pathological conditions compensated for an increase in the level of cholesterol, and a decrease in their content by an increase in the level of cholesterol.

Remodeling of FA and PL are fundamental biochemical mechanisms, according to which homozoic adaptation is realized. Lipid remodeling is understood

as the conversion of one molecular lipid species to another [58]. In the cell, not fully functioning mechanisms function, which ensure the specific inclusion of acyl residues in membranous PL. A change in the acyl composition of PL leads to a modification of the functional activity of membranes [49, 59, 60].

It is known that the acyl groups in the glycerophospholipid composition are asymmetrically distributed. Saturated fatty acids (NLC) are usually located in sn-1-positions, unsaturated - in the sn-2-position of glycerophospholipids. The distribution of molecular species of PL with specific acyl residues in sn-1 and sn-2 positions at the cellular and subcellular levels differs depending on the type of tissue. The main enzymes that provide remodeling of the FA are desaturase and elongase, and PL - phospholipases and acyltransferases [58]. Violation of the process of deacylation/reacylation can cause an increase in the level of lysophospholipids (LPL) to cytotoxic concentrations [61]. Thus, the accumulation of LPL in acute myocardial ischemia is an important factor in the pathogenesis of cardiac arrhythmias [62]. In acute ischemia-reperfusion, there is a disruption in the processes of remodeling of the FA and PL in the tissues of the isolated heart and liver, the accumulation of free fatty acids (FFA), lyso-PL and the cholesterol esters, which adversely affect the functional activity of the organ [49].

Changes in the content of PUFA in the cell due to chronic pathological conditions are associated with the corresponding qualitative and quantitative transformations of PL and cholesterol. As a result, a persistent imbalance of liquid crystals develops [49].

Acute ischemia-reperfusion of the myocardium in humans and animals is accompanied by an increase in the content of free and esterified lipid PUFAs, as well as the accumulation of lyso-PL, which, however, unlike chronic pathological conditions, is not compensated by an increase in the level of total and free cholesterol. From the point of view of the existence of a certain latent period from the moment of increase in the PUFA content to the compensatory growth of the level of cholesterol with the corresponding diseases, it is assumed that after acute pathological conditions accompanied by ischemia-reperfusion, the cell does not have

time to start the reactions of homoviskous adaptation, is carried out at the level of gene expression, in particular under the influence of PUFA [49].

It is proved that FA can influence the genome, modifying the activity of nuclear receptors and the course of signal transduction processes. In fact, the FA can directly or indirectly act on the activity of various genes. It is now established that the effect of FA on gene expression is not limited to interaction with transcription factors. FA are capable of modifying mRNA and protein metabolism [63]. Together, these FA effects cause corresponding changes in the profile of gene expression, metabolism, growth and differentiation of cells [49, 58].

In addition, it has been found that FA and their metabolites can act like hormones, controlling the activity and distribution of specific transcription factors that interact with the corresponding genes and elements of the transcription machinery [64]. The fact that the synthesis of de novo lipids is suppressed by PUFA, which enter the body with food, has been known for more than 50 years [65]. Consumption of PUFA ω-3 or ω-6 series leads to a rapid (within a few hours) suppression of the activity of enzymes involved in the metabolism of carbohydrates and lipogenesis [66]. PUFA ω-3 and ω-6 series suppress the synthesis of de novo lipids in the liver of rodents and humans due to inhibition of the formation of mRNA, which encodes the biosynthesis of various enzymes involved in lipogenesis and glucose metabolism [63]. Mediated PUFA inhibition of acetyl-CoA carboxylase, stearoyl-CoA desaturase-1 and protein S14 occurs at the level of transcription, while suppression of glucose-6-phosphate dehydrogenase activity occurs as a result of post-translational modification reactions [49, 67].

Despite the fact that the rate of expression of a number of proteins participating in the de novo lipogenesis is stimulated by insulin, carbohydrates and triiodothyronine, PUFA suppress the effect of these activators, showing a pronounced negative effect on the expression rate of lipogenesis enzymes [63].

Free and esterified in PL, FA are important components of the cell, which determine the physico-chemical properties of biological membranes, provide nutrient inputs to the cell, serve as a source of energy, participate in signal transduction,

intercellular communications, and the like. It was established that in acute ischemia-reperfusion, the content of NLC (14:0, 16:0, 18:0) in the mitochondrial PL-cardiolipin content significantly decreases [49]. These changes in the acyl composition of cardiolipin is a manifestation of a disturbance in the mitochondrial function due to oxygen deficiency. Although the endoplasmic reticulum takes a leading place in the synthesis of FA and PL, mitochondria play an important role in regulating lipid homeostasis in lipid remodeling.

In the reperfusion of an isolated liver after a previous acute ischemia, an increase in the level of PS may be a consequence of stimulation of the base exchange reaction, during which this PL is formed from PC. It is known that the accumulation of PS in the inner sheet of the plasma membrane is an important molecular event that accompanies apoptosis. An increase in the content of PE in the case of the resumption of oxygen supply to hepatocytes is evidently a manifestation of the stimulation of the reaction of decarboxylation of PS in mitochondria with the formation of PE [49].

So, in acute pathological conditions, there is no compensatory increase in the level of total/free cholesterol in response to an increase in the content of PUFA, since the cell at the genome level does not have time to modify the metabolism of lipids. With chronic pathological conditions in the cell, there is enough time to compensate for abnormalities in the composition of the FA, which can be manifested by an increase in the level of cholesterol in biological systems. At the same time, cholesterol through the transcription factors can also influence the expression of desaturases of the FA [49].

As indicated in the literature [49], the cell has complex mechanisms for compensating for violations in the lipid composition. In fact, changes in the content of one class of lipids (for example, PUFA) after a while lead to compensatory qualitative and quantitative changes in others (for example, cholesterol).

Unbalanced changes in the distribution of FA and PL in damaged tissues cause a violation of the specific functions of the cell. A dualistic understanding of the biological significance of the imbalance of the LC and the compensatory role of cholesterol induces a review of the current therapeutic strategy for the

pharmacotherapy of hypercholesterolemia, develops differentiated approaches to the pharmacotherapy of dyslipidemia, which will take into account not only the level of cholesterol and lipoproteins in the serum/plasma, but also the content of FA, their monohydroxylated derivatives and PL in the shaped elements of the blood. This will effectively manage the risks of fatal cell damage as a result of the action of the pathogenic factor [49].

The given data theoretically substantiates the expediency of searching for biologically active substances (BAS), which stimulate the course of homoviskous adaptation of the cell and compensate for the imbalance of the FA in pathological conditions. After all, the fact that a cell under conditions of acute damage is capable of providing its own resources in a short period of time to ensure the reaction of homoviskous adaptation at the proper level dictates the necessity of introducing exogenous biologically active lipids capable of accelerating this process.

2.1.2 Structural characteristics of membranes of circumferential enterocytes in case of enteropathology of calves and their correction

Structural and dynamic properties of the preparations of the apical membrane (AM) of enterocytes of the mucous membrane of the jejunum of calves were studied with the help of fluorescent probes that are localized in different parts of the membrane: ANS (1-anilinonaphthalene-8-sulfonate) - mainly on the surface of the membrane bilayer and pyrene – in the zone of fatty acid chains of PL [68, 69]. The conformational state of the protein molecule in membranes was estimated from the value of tryptophan fluorescence and the effectiveness of its quenching with acrylamide [70]. In addition, the efficiency of inductive resonance energy transfer (IRET) in donor-acceptor pairs was determined under the conditions of their different localization in membranes [71].

The experiments used membrane preparations of enterocytes, which were characterized by a sufficient degree of purity. Groups of calves of 30-day age, 10 heads each were formed as follows: Group I – healthy calves (control); II group –

calves, who had dyspepsia and were treated according to the traditional scheme (TL); III group – calves, who had dyspepsia and were treated with additional application of liposomal form of dietary supplements based on PL milk, PUFA and vitamins A and E (TL + PL BAS «FLP-MD»).

Assessment of the state of the surface layer of membranes using ANS. When interacting with a membrane, a negatively charged ANS molecule can not sink deep into the lipid phase, but localizes at the lipid-water boundary. Therefore, the changes observed with the help of ANS are characteristic, mainly for the surface membrane layer [68].

Since almost all the measured fluorescence of the ANS is due only to the fluorescence of the bound probe, this makes it possible to determine the parameters of its binding to the membrane from the fluorescence data: the binding constant (K_{ANS}) and the number of binding sites (N_{ANS}). The parameter (N_{ANS}) indicates the maximum concentration of the probe in the membrane during its saturation with the probe, and the value of the (K_{ANS}) reflects changes in free energy during the transition of the 1 M probe from the aqueous phase to the membrane [68, 69].

As a result of the studies, the fluorescence intensity of the ANS was decreased by an average of 16–20% relative to the corresponding control values when binding to membrane preparations in all experimental groups. This may be due to changes in the binding of the probe to the membranes. It is established that the value of K_{ANS} increases for membrane preparations of group II animals by 21% relative to the control values. N_{ANS}, at the same time, is likely to decrease for groups II and III, respectively by 15 and 17%, relative to control values.

Changes in the association constant of the probe and the number of binding sites of the ANS with the membrane is a reflection of the integral processes (changes in the microenvironment of the probe, surface charge, structural rearrangements, etc.) that occur in the membrane for the actions of various factors, considering that the fluorescence of the ANS depends significantly on its composition [69]. Since the ANS molecule is localized in the polar region of the membrane, forming complexes with protein and lipid molecules, the results obtained on the interaction of the

fluorescent ANS probe with membranes indicate a change in the surface structure of the membrane, which may be due to the modification of both the lipid component in the zone of the polar heads of the glycerol residues of PL, and membrane proteins.

Microviscosity of the lipid component of membranes. Investigation of the microviscosity of the lipid component of membranes was carried out using a fluorescent pyrene probe, whose hydrophobic molecules are localized in the zone of fatty acid chains of PL [68, 69].

Absorbing a quantum of light, the pyrene molecule passes into an excited state. In this state, it emits at a wavelength of λ 390 nm or, in collision with another unexcited molecule, forms a pair-excimer, which emits a quantum of light in the region of λ 480 nm. At a given temperature and probe concentration, the degree of pyrene excimerization depends to a large extent on the microviscosity of its environment and can serve as its characteristic. With the increase in microviscosity, the diffusion of the probe molecules slows down, so the probability of collision between the two molecules decreases, and the degree of its excimerization decreases accordingly.

Embedding of pyrene and determination of the degree of its excimerization was carried out by the method [72]. To a membrane suspension (0.1 mg/cm³) in a volume 2.0 cm³ of buffer containing 0.1 M KC1, 25 mM HEPES-Tris (pH 7.0 at 25° C), 1 mM alcoholic pyrene solution was added in portions with continuous stirring to a final concentration of 5 μM. After 15 minutes of incubation at 25° C, fluorescence spectra were recorded. Under these conditions pyrene, practically insoluble in water, completely passes into the membrane.

To independently determine the microviscosity of anular (protein) lipids and the total lipid phase, excitation of the fluorescence of the probe was carried out by inductive resonance energy transfer (IRET) from tryptophan residues of membrane proteins at λ 280 nm (the viscosity of anular lipids), and then in the same samples as a result of pyrene excitation at λ 335 nm (the viscosity of the total lipid phase).

During the study, the relative changes in the degree of pyrene excimerization in the experimental samples were estimated in comparison with the control, taking into

account that the fluorescence of pyrene at λ 280 nm is largely due to the IRET with tryptophan protein residues (Table 2.1).

Under the experimental conditions, the increase in the N_{335} index by 16% and N_{280} by 10% relative to the control values was observed in the membrane preparations of the II group, which indicates a decrease in the microviscosity of the lipid phase and anular lipids. The degree of excimerization of pyrene in the studied membrane preparations of the animals of Group III practically did not change with respect to control, both for the lipid phase and anular lipids. The obtained data indicate a decrease in the structural ordering of the lipid component of the II group membranes.

Table 2.1

The degree of pyrene excimerization in membrane preparations for the total lipid phase (N_{335}) and anular lipids (N_{280}), res. units (M ± m, n = 10)

Group I (control)	N_{335}	N_{280}
II group of people (traditionally)	36.7±1.1	32.8±0.9
III group of people (complex)	41.7±1.2*	35.3±1.1*

The viscosity of membranes is known to be an integral value and depends on the composition of PL, the content of cholesterol that regulates the structure of the membrane, the amount of unsaturated LC, the degree of their unsaturation, and the intensity of the flow of lipid peroxidation (LPO) in membranes, etc. [73].

Structural state of protein molecules of membranes of enterocytes. The intrinsic fluorescence of proteins upon excitation in the ultraviolet region of the spectrum determines the presence of tryptophan, tyrosine and phenylalanine residues. However, the tryptophan residues of proteins make a dominant contribution to the fluorescence intensity at λ 340 nm. The intensity of tryptophan fluorescence is one of the main spectral indices characterizing the conformational state of the protein molecule, since the fluorescence maximums of individual tryptophan residues depend

on their location in the protein molecule and, accordingly, on the environment [70, 74].

The fluorescence intensity of tryptophan residues of membrane proteins was recorded at 338 nm, and the excitation wavelength was 296 nm [70]. The incubation medium contains 20 mM phosphate buffer (pH 7.4 at 25° C).

Changes in the fluorescence intensity of tryptophan residues of protein molecules can be associated with conformational rearrangements of their molecules, intramolecular protein dynamics, and the nature of the interaction of their tryptophan residues with neighboring groups, since the fluorescence of tryptophanyl is sensitive to the mobility of neighboring groups, etc. [70, 75].

It has been established that the fluorescence intensity of tryptophan residues of protein molecules in membrane preparations of Group II and III does not change in comparison with the control (Table 2.2).

Table 2.2

Tryptophan fluorescence of protein molecules of membrane preparations (M ± m, n = 10)

Group of animals	Tryptophan fluorescence, reducing units	The Stern-Volmer constant (K_{SV}), M^{-1}
Group I (control)	1,02±0,07	2,09±0,25
II group of people (traditionally)	1,07±0,06	1,39±0,10*
III group of people (complex)	1,03±0,08	1,22±0,13*

To assess the conformational changes in membrane proteins, tryptophan fluorescence quenching was studied by an external neutral polar quencher, acrylamide. A membrane suspension (0.1 mg/cm³ in a volume of 2 cm³) was titrated with a 1 M solution of acrylamide to a final concentration of 0.4 M [76].

An analysis of the results on the quenching of tryptophan fluorescence in preparations of the apical membrane indicates that the fraction of tryptophan residues

that can be quenched under the study conditions does not change significantly. At the same time, the value of the effective quenching constant decreases for groups II and III by 33 and 42 %, respectively, relative to the control values (Table 2.2).

It is known that the changes in K_{sv} reflect the intramolecular dynamics of protein molecules [70]. It should be noted that the decrease in the effectiveness of quenching of tryptophan fluorescence (Table 2.2), as evidenced by the decrease in K_{sv}, may be due to the growth of structural rigidity of membrane proteins [76].

The results obtained indicate that the conformational modification of protein molecules in membranes of groups II and III is due to a decrease in their intramolecular dynamics.

Evaluation of the spatial organization of protein-lipid complexes in membrane preparations. Spatial organization of protein-lipid complexes in the investigated membranes was evaluated using the method of inductive resonance energy transfer (IRPE) in a pair of donor-acceptor fluorophores. The effectiveness of the IRPE from the donor to the acceptor depends to a large extent on the relative location of the regions of preferential localization of fluorophores in the membrane [69], which makes it possible to estimate the changes in the distance between these sections of the membrane. The following donor-acceptor pairs were used: 1) tryptophan-pyrene. Fluorescence measurements were carried out at λ 280 nm and $\lambda_{фл}$ 340 nm [77]. The protein concentration was 0.1 mg/cm³, titration (quenching of fluorescence) was carried out with a probe to a final concentration of 5 μm. 3) tryptophan-ANS. The fluorescence was evaluated at λ 295 nm and λфл 340 nm [71]. The protein concentration was 0.1 mg/cm³, titration was performed with a probe at concentrations of 10–50 μm.

When analyzing the results, the IRPE took into account that the most probable localization sites for tryptophan residues are the hydrophobic regions of proteins that can be found in the membrane both in the protein and lipid environments [78], the fluorescent ANS probe is predominantly localized in the membrane at the lipid-water interface [69], pyrene – in the zone of fatty acid chains of PL [68].

The results of quenching of fluorescence were presented in modified Stern-Volmer coordinates. Given that cell membranes are complex protein-lipid systems, it is conventionally assumed that all donors are divided into two groups: 1) are from the acceptor at a distance of less critical, and, accordingly, participate in energy transfer; 2) remote at a distance greater than critical and not subject to extinction by an acceptor [68]. Based on the results of quenching of the fluorescence of the donor by the acceptor, the fraction of donor residues available for quenching by the acceptor and the value of F_o-F/F_o, (where F_o is the fluorescence intensity in the absence of a quencher, F in the presence of a quencher) are calculated, which indicates the effectiveness of the IRPE.

It is established that in membrane preparations for all donor-acceptor pairs a particle of donor molecules that are accessible to quenching by an acceptor does not change.

A significant decrease in the value of F_o-F/F_o for membrane preparations of Group II animals was established by 33% with respect to control in a tryptophan-pyrene pair, which indicates a decrease in the efficiency of energy transfer (Table 2.3).

Table 2.3

The efficiency of inductive-resonance energy transfer from tryptophan residues of membrane proteins to pyrene or ANS,

F_o-F/F_o, red. units (M ± m, n = 10)

Group of animals	Tryptophan res.-pyrene	Tryptophan res.-ANS
Group I (control)	1.02±0.03	1.89±0.05
II group of people (traditionally)	0.69±0.05*	2.24±0.08*
III group of people (complex)	0.99±0.05	1.78±0.08

As is known, the degree of immersion of proteins in the lipid phase of membranes can be estimated from the effectiveness of quenching by tryptophan fluorescence [77], that is, a decrease in the efficiency of the IRPE indicates an increase in the degree of exposure of proteins to the aqueous phase and/or aggregation of protein molecules both on the surface and in depth of the bilayer, which leads to an increase in the distance between the donor and the acceptor.

From such positions and taking into account the resulted results, changes are revealed to a greater extent due to the movement of protein molecules to the hydrophilic phase or their aggregation.

Investigation of the IRPE in the tryptophan-ANS pair showed that the F_o-F/F_o value also increases only in the preparations of the calves of group II by 19% with respect to the control. Increasing the efficiency of the IRPE in a pair of fluorophores indicates a decrease in the distance between them. Since the critical distance of the IRPE for the tryptophan-ANS pair is 2.0–3.5 nm [68] and the membrane thickness is about 4.0 nm [79], a more significant contribution to the energy transfer belongs to the fluorophore located on one side of the membrane in relation to the lipid phase. The results are presented, along with the data obtained in the study of the binding parameters of ANS with the membrane, indicate a structural modification of the surface regions of membranes.

The results of the conducted studies show that under the conditions of traditional treatment there are many-sided destructive changes in the membranes of enterocytes of the small intestine, namely: modification of the surface structure of membranes, reduction of the structural order of the lipid component and disruption of hydrophobic protein-lipid interactions and conformational modification of protein molecules. However, this structural modification is not observed in the case of membrane preparations obtained from calves of group III. That is, complex treatment with the inclusion of phospholipid-modifying dietary supplements of reparative action leads to the normalization of indicators characterizing the structural state of AM. This particularly applies to the lipid component, since the conformational state of the protein molecules in the membrane is not completely restored.

2.1.3 Lipid composition of the epithelium of the small intestinal mucosa in the course of enteropathology of calves and its correction

Dyslipidemia, which occurs in the blood of calves with the development of dyspepsia, due to the growth in the blood plasma levels of TAG and cholesterol is naturally accompanied by excessive formation and increased content in the blood and cells of lipid peroxidation products, increased permeability of cell membranes, the release of normal components and products of the disturbed blood into the peripheral blood stream metabolism of cells, disorders of their energy supply [80]. Membranopathy for dyspepsia of calves, along with the existing dysfunction of the immune system, leads to a decrease in regenerative processes in the tissues of sick animals [81]. This is confirmed by the inadequate restoration of the functional state of the intestine even in 30-day-old calves [82].

The data obtained show that the distribution of individual lipids in the animals that are dyspeptic with different treatment regimes is uneven.

Thus, the LP spectrum of blood plasma in calves under the traditional scheme of treatment of dyspepsia (group II) on the 30th day of life was characterized by hyperlipidemia, which was due to the probable growth (by 17%) of the total cholesterol (TC) content due to the free cholesterol fraction (CF) and TAG (1.7-fold), while a decrease in plasma levels of the level of FFA (by 22%) and PL (by 18%) was observed in (Table 2.4). This is a typical picture for the pathology of a similar genesis, indicating that there are existing disorders of lipid metabolism in calves, even three weeks after dyspepsia. At the same time, this situation reflects functional disorders on the part of the organs involved in the regulation of lipid metabolism - the intestine and the liver. Along with this, in the blood plasma of the calves of this group, the content of PC by 18% and SM by 16% reliably decrease, which are the main structural components of the outer layer of cell membranes, can slow down the intensity of their recovery.

Under the experimental conditions, the increase in the N_{335} index by 16% and N_{280} by 10% relative to the control values was observed in the membrane preparations

of the II group, which indicates a decrease in the microviscosity of the lipid phase and anular lipid. The degree of excimerization of pyrene in the studied membrane preparations of the animals of Group III practically did not change with respect to control, both for the lipid phase and anular lipids. The obtained data indicate a decrease in the structural ordering of the lipid component of the II group membranes.

Table 2.4

The lipid spectrum of the plasma of the experienced calves at the 30th day of life, mg% (M ± m, n = 8, quoted for [83])

Index	Group I (control)	Group II (traditional treatment)	Group III (complex treatment)
Total lipids	583.3±12.3	680.1±7.5*	602.5±13.1
Total cholesterol	249.2±4.8	286.9±6.9*	249.0±3.9
Free Cholesterol	121.2±3.0	140.1±7.3*	126.3±1.7
Esterified cholesterol	128.3±5.6	139.1±3.9	123.4±7.1
Free fatty acids	37.9±0.9	29.6±1.3*	28.7±0.7*
Triacylglycerols	36.2±2.5	61.2±1.1*	40.7±2.9
Phospholipids including:	264.1±4.9	233.1±7.5*	329.5±8.3*
Phosphatidylcholine	59.7±3.7	49.0±1.4*	73.2±4.3*
Lysophosphatidylcholine	1.9±0.1	1.6±0.1	2.5±0.2*
Phosphatidylethanolamine	52.4±1.9	49.9±3.1	66.1±2.3*
Lysophosphatidylethanolamine	2.3±0.3	2.5±0.1	3.3±0.1*
Phosphatidylserine	18.4±0.9	18.7±1.5	24.2±2.4*
Sphingomyelin	41.3±3.1	34.6±3.2*	49.5±6.4

Note: * - p <0.05, the results are probable in comparison with the values in animals of group I (control).

At the same time, in the blood plasma of the calves of group III, the fact of normalization of the content of both total lipids (TL) and individual fractions was established.

The exception is the reliably low content of FFA. It is likely that they are intensively used for the biosynthesis of PL, which was confirmed by the growth of their level in the blood plasma of these animals. In addition, a high content of PL in the blood plasma of calves, patients with dyspepsia, according to the complex treatment scheme is explained by the effective assimilation in the intestine of exogenous PL supplements. Among the PL in the blood plasma of these animals, the content of lysophosphothidylcholine (LPH), PH, PE, lysophosphatidylethanolamine (LPE) and PS was predominant compared to the level of calves I (control) in the blood.

As is known, most exogenous PL is delayed by the liver [84]. However, the intensity of their admission to this important organ directly depends on the structural and functional state of enterocytes in the small intestine [85].

It should be noted that calves under the traditional regimen of dyspepsia (II group) experienced a significant decrease in the intestinal mucosa of the small intestine by 11%, primarily TC by 17% and its bound form – esterified cholesterol (ECS) 2.3 times, TAG – 19% and PL – 9% (Table 2.5).

Changes in the PL-storage of small intestine epithelium in the calves of group II have similar trends, which were characterized by a significant decrease in the content of the main fractions of PL: PH by 19%, SM by 7% and PI by 13%. These trends may indicate insufficient recovery of digestion and absorption of lipids in the gastrointestinal canal of diseased calves on the 30th day of life, and also characterizes the disorders of the structural and functional state of enterocytes in the mucosa of the small intestine. Although the lipid/protein ratio corresponds to the control values, which proves the simultaneous decrease in the intensity of endogenous protein synthesis in these cells.

Along with this, in the complex scheme of treatment of calves (group III), patients with dyspepsia, recovery of most of the parameters of the LP spectrum of the epithelium of the small intestine was noted. First of all, this concerned the content of TL in the epithelium of the jejunum of the calves studied. Like a group of calves with a traditional treatment regimen, a significant decrease in the level of ECS was found -

1.6 times and TAG – by 22%. At the same time, the content of TC was within the normal range. There was also a significant decrease in the level of FFA – by 26% in the epithelium of the jejunum of these animals with a significantly high PL content.

Table 2.5

Lipid composition of the epithelium of the jejunal mucosa in experimental calves at the 30th day of life, mg% (M ± m, n = 6, quoted for [83])

Index	Group I (control)	Group II (traditional treatment)	Group III (complex treatment)
Total lipids	1218.9±35.1	1081.0±17.9*	1159.6±61.1
Total cholesterol	187.8±11.1	155.8±9.5*	170.7±8.7
Free Cholesterol	136.0±4.1	132.4±5.9	138.4±2.5
Esterified cholesterol	51.2±3.8	21.8±1.8*	32.3±1.5*
Free fatty acids	127.2±7.1	102.3±8.7	93.5±4.1*
Triacylglycerols	86.4±3.3	70.1±5.9*	67.5±2.3*
Phospholipids including:	816.8±11.3	750.7±9.5*	859.3±10.1*
Phosphatidylcholine	220.7±9.7	179.0±7.7*	234.7±2.3*
Lysophosphatidylcholine	19.7±1.6	19.2±0.9	27.9±2.3*
Phosphatidylethanolamine	16.3±0.9	15.1±0.7	15.5±1.2
Lysophosphatidylethanolamine	21.7±1.5	20.6±2.2	15.1±1.7*
Phosphatidylinositol	81.1±3.7	70.5±2.9*	80.0±5.1
"Lipid/protein"	0.88±0.13	0.89±0.09	0.69±0.05*

Note: * - p <0.05, the results are probable in comparison with the values in animals of group I (control).

This quantitative redistribution of lipid fractions is probably the result of intensive use of the described lipids in restoring the structural and functional state not only of the intestinal epithelium, but also of other organs and tissues that were affected by development in neonatal calves of dyspepsia.

Among the studied fractions of PL, the content of PC predominated, which is a positive factor in the course of reparative processes in the intestinal tissues. A significant decrease in the lipid/protein ratio was also studied, which may indicate an increase in the intensity of protein-synthesizing processes in the cells of the small intestine.

Thus, the results of studies of LP and PL spectra of blood and intestine testify to the presence of lipid metabolism disorders in calves under the traditional treatment schedule three weeks after the beginning of the rehabilitation period, which is explained by the inadequate restoration of the structural and functional state of this organ.

However, the use of such phospholipid maintenance nutrients, which activates reparative processes in the affected organs and tissues, positively affects the metabolism of lipids in the calves, especially in the complex scheme of use, which allows us to recommend it for correction of lipid metabolism disorders in the gastrointestinal pathology of newborn calves.

2.2 Lipid composition and structural characteristics of the small intestinal mucosa epithelium after experimental enteropathology

2.2.1 Biological mechanisms of the regulation of the functioning of the organs of the digestive system in experimental enteropathology

The functional capacity and morphological integrity of the digestive tract, and in particular the intestine, largely depend on the partial pressure of oxygen in the tissue and nutrients delivered by the blood microvessels. One of the consequences of oxygenation of the body is the development of hypoxia, which in turn can lead to epithelial cells, ulcers and even necrosis, due to the activation of free radical reactions, lipid peroxidation, an increase in the number of free forms of oxygen and the production of inflammatory mediators. Hypoxic changes are a key pathogenetic factor of bowel diseases (inflammatory bowel disease) and also a lethal factor in the development of intestinal graft necrosis with abdominal anal resections of the rectum and an unforeseen and almost completely lethal disease as a bowel infarction due to acute mesenteric ischemia [86–88].

Taking into account the scale and unquestionable importance of this problem for practical gastroenterology, abdominal surgery and oncology, scientists made a significant contribution to its solution [86–90].

Inflammatory bowel diseases, which include ulcerative colitis and Crohn’s disease, are characterized by chronic nonspecific inflammation and ulcers of the intestinal wall of uncertain etiology. In the last decade, according to experts from the World Health Organization, domestic and foreign literature, there has been a steady trend towards an increase in the incidence of inflammatory bowel disease. Each year, from 5 to 60 new patients per 100 thousand of the population are registered. More than 50% of patients are of working age. Due to the severity of the course and frequency of complications, which is often the reason for the increase in the period of incapacity for work, as well as disability and even death of patients, inflammatory

bowel diseases occupy one of the leading places among diseases of the gastrointestinal tract [90].

Modern therapy of inflammatory bowel diseases is aimed at reducing inflammation (5-aminosalicylate, steroid therapy), correction of the immunological response (anti-TNF-α "Infliximab") and suppression of possible pathogenic factors (antibiotics), which in most cases has a powerful side effect. At the same time, therapeutic measures aimed at restoring the intestinal barrier remain without attention [88–90].

The intestinal barrier consists of a layer of epithelial and endothelial cells. The epithelial barrier is formed by a continuous layer of superficial epithelial cells and prevents the penetration of bacteria and other antigens into the intestinal mucosa. Functional and morphological integrity of the epithelial barrier is essentially dependent on oxygen and nutrients delivered by the blood vessels of the intestinal mucosa. So, any pathological changes in the blood vessels lead to the development of hypoxia with the subsequent defeat of epithelial cells, the violation of the intestinal barrier, the development of erosions and ulcers [89, 90].

Violation of the process of formation of new blood vessels (angiogenesis) and functional activity of intestinal microvessels in conditions of chronic inflammatory bowel disease was demonstrated by numerous clinical and experimental studies. Despite a comprehensive study of anatomical, morphological, functional changes in the intestinal mucosa endothelium/microvessels in the pathogenesis of inflammatory bowel diseases and under the action of pro-inflammatory mediators, a direct answer to the question of the role of the endothelial barrier in the mechanisms of intestinal wall engorgement initiation, as well as the role of factors with angiogenic activity in the pathogenesis of inflammatory bowel diseases are absent or remain at the level of assumptions and hypotheses [89–91].

As a result, it was established [91, 92] that the initial stages of the development of spontaneous inflammatory bowel diseases (before the appearance of ulcers) are characterized by the presence of areas with signs of perivascular edema covered with an intact layer of superficial epitheliocytes. These data were obtained in relatively

young (6 weeks old) mice with a deficiency of the α-subunit gene of type 2 inhibitory G protein ($G\alpha\text{-}i2^{-/-}$). The later stages of chronic inflammatory bowel disease in these mice (aged 16 and 45 weeks), when ulcers and erosions were histologically diagnosed, were characterized by the presence of focal areas with perivascular edema that were infiltrated by leukocytes and covered with intact epithelium. Studies on another model of spontaneous inflammatory bowel diseases in mice aged 10 and 12 weeks against the background of a deficiency of IL-10 (mice $IL\text{-}10^{-/-)}$ confirmed these findings. Thus, in both age groups, in 100% of cases, parts of the mucous membrane of the large intestine with perivascular edema of the lamina propria lamina propria, completely covered with an intact layer of superficial epithelial cells, were found.

On this basis, it was suggested that the damage to endothelial cells and the increase in permeability of microvessels play a critical role in the development of erosions and ulcers in the pathogenesis of inflammatory bowel diseases and may be one of the reasons for the relapse of this disease [91].

To confirm the involvement of the endothelial barrier in the development of integrity disorders of the epithelium in inflammatory bowel diseases, changes in the permeability of the endothelial and epithelial layers of the colonic mucosa during the early stages of the development of chemically induced colitis (before the onset of lesions) which is characterized by a clearly defined time of development of various stages of inflammation/defeat. The studies were conducted on the model of iodoacetamide-mediated colitis, in which the damage to the mucous membrane of the colon develops already in the first hours after iodoacetamide injection. In addition, the dextran sodium sulfate (DSS) model-conditioned colitis was used, which proceeds more slowly and is characterized by the development of the first clinical signs of the disease in the form of fecal blood, the loss of body weight of animals only on day 3, and macroscopic lesions of the colon are observed only in the 7th day of administration of DSS. The results obtained for the first time showed that the damage to endothelial cells and the increase in the permeability of the endothelial layer of the microvessels of the colon mucosa against the background of iodoacetamide or DSS-mediated colitis develop much earlier than the increase in the

permeability of its epithelium, outstripping the development of erosions and ulcers. Increase in permeability of microvessels of the mucous membrane of the colon was accompanied by a gradual development of lamina propria edema, leading to detachment of the epithelium from the basal membrane. At the same time, there was no violation of the integrity of the epithelial layer and the infiltration of leukocytes on both models of chemically conditioned colitis [89–92] .

Ultrastructural studies of the endothelial and epithelial cells of the rat colon mucosa confirmed the quantitative analysis data and found unchanged surface epithelial cells with an intact brush border and dense contacts in the early stages of experimental colitis development. Under these conditions, a stasis of erythrocytes was found in the capillaries of the submucosal layer, which was associated with perivascular and interepithelial edema. This indicates an increase in vascular permeability, which was confirmed by the presence of gaps between the endothelial cells of microvessels. In accordance with changes in the capillaries, aggregation of platelets and their extravasation in the perivascular space increased, which was increased and filled with fibrillar structures (most likely fibrin) [91, 92].

One of the consequences of disturbance of the normal functioning of the vascular endothelium is the development of hypoxia, which, in turn, can lead to the defeat of the intestinal epitheliocytes due to activation of free radical reactions, lipid peroxidation, an increase in the number of free oxygen species and the production of inflammatory mediators. It has been established [93] that, in addition to endothelial damage and stasis of capillaries of the mucous membrane of the colon of experimental animals, an increase in hypoxia in superficial colonocytes is observed, which in dynamics precedes an increase in the permeability of the epithelial layer. The development of hypoxia was associated with an increase in the content of transcription factor HIF-1α, which is a key regulator of the response of cells to changes in oxygen homeostasis [89–92].

Since inflammatory bowel diseases are chronic diseases with frequent relapses, established data on early increase of permeability of blood microvessels can become a diagnostic marker of the site of lesions and predicting a possible relapse of the

disease. Place the extravasation of the paint by visualizing Evans (24-48 h for DSS- and 15–30 min for iodoacetamide-mediated colitis) as a blue spot that is located exclusively in the distal colon (where DSS- and iodoacetamide- caused by colitis), when macroscopic changes in the intestinal wall are not yet observed [93].

Thus, it was established that lamina propria edema and intestinal wall infiltration by leukocytes, which are characteristic signs of the pathogenesis of inflammatory bowel diseases in humans, arise against the background of increased permeability of the blood microvessels. An increase in the permeability of the microvascular vessels was accompanied by an increase in the level of the most potent stimulator of blood vessel permeability, VEGF [90–93].

It should also be emphasized that increased adhesion of platelets in the early stages of development of experimental colitis. Thrombocytosis is a concomitant pathology of patients on inflammatory bowel diseases; moreover, platelets of patients with inflammatory bowel disease are in an activated state that, first, increases their aggregation and adhesion, and secondly increases the release of pro-inflammatory mediators (CD40L, IL-1β , platelet factor-4, RANTES) and factors with angiogenic activity (VEGF). So, in conditions of remission, activated platelets are, so to speak, a "time bomb" that can cause an increase in vascular permeability due to excessive release of VEGF or cause hypoxia in the intestinal mucosa due to thrombosis of microvessels. These factors lead to a violation of the integrity of the epithelial layer and the recurrence of the disease [90–93].

Endothelial damage leads to significant changes in the functional activity of endothelial cells, increasing their adhesion to inflammation and platelet cells, as well as activation of angiogenesis processes. These events are based on changes in the transcriptional activity of cells and the activation of expression of genes associated with the development of vascular pathology [91, 93] .

It is believed that Egr-1 is the key regulatory protein in triggering a pro-inflammatory response to damaging stimuli in vascular pathology. Its expression is very quickly activated by a large number of different stimuli (growth factors, cytokines, oxidative stress, hypoxia, ionizing radiation, mechanical stress, vascular

lesions). In turn, Egr-1 itself, or through interaction with other transcription factors, is involved in the regulation of expression of pro-angiogenic factors (bFGF, PDGF-A, PDGF-B, VEGF, VEGFR-1, angiopoenthin-1, protease), and as well as pro-inflammatory mediators (ISAM-1, VCAM-1, TNF-α, IL-1β, IL-2, chemotactic monocyte-1 protein, tissue factor, GM-CSF) [90–93] .

It has been shown [94, 95] that the content of Egr-1 protein and mRNA level at different stages of development of experimental colitis caused by iodoacetamide has been increasing already in the 30th minute. The maximum values of these indicators are maintained during the first day, followed by a decrease to the 7th day of the experiment. These changes coincide in time with the activation of the Erk1/2-kinase signal transduction pathway in the colonic mucosa, which can trigger expression and activate Egr-1. The development of experimental colitis also causes rapid translocation of Egr-1 from the cytoplasm to the nucleus, where it binds efficiently to the corresponding cis-elements of the DNA [91–93].

A number of transcription factors forming protein complexes with Egr-1 have been established [93–95] in the pathogenesis of experimental colitis using 'TranSignal™ Protein/DNA Array'. According to the literature, the regulatory activity of Egr-1 is associated with another transcription factor-Sp1, their binding sites overlap partially in the common (-GGGCGG-) region of the promoter of a number of genes. These transcription factors can compete with each other in the binding sites in the promoter region, depending on their concentration in the nucleus of endothelial cells. It was found [96] that under normal conditions, Egr-1 forms a stable complex with Sp1. The development of inflammation followed by the formation of erosions and ulcers against the background of experimental colitis was accompanied by a decrease in the content of the protein Sp1 and, correspondingly, the level of its interaction with Egr-1 [89–92].

It has also been shown that Egr-1 forms protein complexes with other transcription factors, namely PPAR, GAS/ISRE, USF-1, AP-2, NF-E1, NF-E2, NF-κB, MEF-1, Myc-Max, PAR (DR5), E2F1, MRE, TR, GAG, ADR1, GATA-1/2, CREB-BP1, FKHR, the level of interaction with which varies depending on the stage

of colitis development. In the process of inflammation development against the background of colitis the strongest complex is formed between Egr-1 and NF-κB [93]. NF-κB is involved in the regulation of the expression of the immune response genes and the inflammatory process and is activated when the redox-homeostasis of cells is disturbed and the action of the pro-inflammatory cytokine TNF-α. NF-κB is considered to be the central pro-inflammatory transcription factor in the pathogenesis of inflammatory bowel diseases and with a connecting link between chronic ulcerative colitis and the development of colorectal cancer [89–93] .

Under normal conditions, Egr-1 forms a stable protein complex with PPAR, which practically does not change against the background of the development of experimental colitis [97]. It is known that PPAR plays a protective role in the pathogenesis of inflammatory bowel diseases, and the administration of PPAR agonists to animals significantly improves the condition in experimental colitis by reducing the expression of inflammatory markers, incl. NF-κB. The work assumes that the obtained stable interaction between Egr-1 and PPAR under normal conditions can be one of the protective mechanisms in the pathogenesis of inflammatory bowel diseases [89–93].

Egr-1 is a hypoxia-sensitive transcription factor that triggers the expression of a number of genes involved in the realization of a cellular response to a decrease in pO2; such as tissue factor, growth factors, cytokines/chemokines, and adhesion receptors. In addition, the binding site with Egr-1 was found in the promoter region of the VEGF gene, the main transcriptional regulator of which is HIF-1. It is established [93] that the regulatory activity of Egr-1 is independent of HIF-1 in the pathogenesis of inflammatory bowel diseases.

To further elucidate the role of Egr-1 as a possible pathogenetic factor in the development of lesions in inflammatory bowel diseases, a comparison of the morphological features of experimental colitis in mice with a homozygous (Egr-$1^{-/-}$) and heterozygous (Egr-$1^{+/-}$) mutation in the protein gene Egr-1 and wild type. It was found that the deficiency of Egr-1 leads to a significant decrease in the manifestations of morphological signs of iodoacetamide-mediated colitis. So, the activation of Egr-1

triggers the expression of damaging stimuli and is one of the reasons for the development of lesions in the pathogenesis of inflammatory bowel diseases [94, 95].

The development of any inflammatory process consists of two stages: acute and chronic. During the first hours of the development of the inflammatory process (acute phase), an increase in the permeability of the endothelial layer is observed, followed by an increase in adhesion to leukocytes, activation of coagulation processes, and at later stages (the phase of chronic inflammation) - the formation of new blood microvessels (angiogenesis). The initial stages of the development of inflammatory bowel diseases are characterized by the predominance of acute inflammation, which gradually turns into a chronic stage. VEGF is a unique factor capable, on the one hand, of enhancing the permeability of blood vessels and increasing the expression of adhesion proteins by endothelial cells, and on the other hand, stimulating angiogenesis [95–97].

Immunohistochemical staining of the wall of the colon revealed [98] that the main source of VEGF in the mucosa of the colon is endothelial, epithelial cells and leukocytes. With the help of immunoblot analysis, the growth of VEGFR-2 content was established [99], which is the main receptor in mediating VEGF-stimulated blood vessel permeability and their pro-angiogenic effects. The content of VEGFR-1 protein rises only at the stages of chronic course of the disease, can be explained by an increase in its expression by infiltrated monocytes/macrophages [100].

It is known [101, 102] that neutralization of VEGF activity by administration of an antibody to VEGF leads to a significant decrease in the clinical and morphological parameters of the experimental colitis and does not differ in the degree of efficacy from the common drug in the treatment of ulcerative colitis in humans with 5-aminosalicylic acid (mesalazine). It is important to note that the administration of an antibody to VEGF did not lead to a decrease in the number of blood vessels in the granulation tissue sites as compared to control animals, measured by the number of vessels positive for the von Willebrand endothelial cell marker.

Newly formed blood vessels in the pathogenesis of inflammatory bowel diseases are characterized by increased permeability and immaturity, which

contributes to infiltration of leukocytes and the transition of the disease to the chronic stage. Thus, it is proven that the administration of neutralizing antibodies to VEGF significantly reduces the transudation of Evans dye in the mucosa of the colon with iodoacetamide-mediated colitis. Thus, it was shown [101] that an increase in the level of VEGF leads to an increase in the permeability of the microvascular blood vessels of the colon of the large intestine. At the same time, healing of ulcers against the background of neutralizing antibody to VEGF was associated with a decrease in the number of leukocytes in the lesion area.

The effects of VEGF on the function of endothelial cells, namely proliferation, migration, survival and permeability of blood vessels, are mediated by various intracellular signaling pathways (Erk1/2, Akt, Src) through the activation of VEGFR-2.

The leading role of Src-tyrosine kinase in VEGF-mediated enhancement of blood vessel permeability in the pathogenesis of experimental colitis was established [101, 103] and the intracellular mechanism of this process was studied. The development of iodoacetamide-mediated colitis is accompanied by an increase in the total protein content of VEGFR-2 and its phosphorylation by the Tyr951 residue in the mucosa of the large intestine of rats. In parallel, activation of Src-tyrosine kinase and increased interprotein interaction between β-arrestin 2 and VE-cadherin are observed, while the total protein content of VE-cadherin remains unchanged.

The leading role of reinforcing the permeability of blood microvessels in the pathogenetic action of VEGF in inflammatory bowel diseases has been confirmed in the literature [94, 104]. And in this case, activation of Src-kinase, which plays a leading role in VEGF/VEGFR-2-mediated increase in blood vessel permeability, has been demonstrated. Phosphorylation of the kinase Akt, which also partially mediates the action of VEGF on the permeability of blood vessels and, to a large extent, predetermines the survival of endothelial cells, is also enhanced, but to a lesser extent. At the same time, the activation level of Erk1/2, on the contrary, is lower in IL-$10^{-/-}$ mice.

Another evidence of the important role of the VEGF/VEGFR-2 pathway in increasing the permeability of blood microvessels in the pathogenesis of inflammatory bowel diseases is literature data [105, 106] on the activation of D2-dopamine receptors. In particular, it was shown that the interaction of dopamine with D2-dopamine receptors of endothelial cells is accompanied by inhibition of VEGFR-2 phosphorylation and, as a result, a decrease in the permeability of blood vessels. According to the data obtained, the development of experimental colitis is associated with a violation of dopamine synthesis, which is determined by the level of tyrosine hydroxylase in the mucous membrane of the large intestine of rats. D2-dopamine receptor agonists, quinpyrol (1 mg/100 g) and bromocriptine (5 mg/100 g) almost halve the permeability of the blood vessels of the colonic mucosa of rats with iodoacetamide-mediated colitis. Since D2-dopamine receptor agonists are widely used in clinical practice to suppress lactation in women (cabergoline), as well as patients with Parkinson's disease (bromocriptine), they can become potentially new therapeutic agents in the therapy of inflammatory bowel diseases.

The scientific literature describes the mechanism of parallel increase in the level of pro-angiogenic and anti-angiogenic factors in the pathogenesis of inflammatory bowel diseases [107–109]. So, the role of endostatin, which is one of the most potent endogenous anti-angiogenic factors, has been established, has anti-carcinogenic properties. It is formed as a result of proteolytic degradation of collagen XVIII under the action of proteinases, including matrix metalloproteinases-9 (MMP-9).

An increase in the endostatin content in the large intestine of rats with both iodoacetamide-mediated colitis and a model of spontaneous inflammatory bowel disease in IL-$10^{-/-}$ mice was shown, which correlated with an increase in MMP-9 protein activity and content. Mice deficient in MMP-9 (MPP-$9^{-/-}$ mice) showed significantly lower endostatin levels in normal and DSS-mediated colitis relative to the corresponding values in wild-type mice. At the same time, the level of another anti-angiogenic factor, angiostatin, which can also be formed as a result of proteolytic degradation of plasminogen by MMP-9, did not change. Thus, it has been established

[108, 110] that MMP-9 plays a leading role in the formation of endostatin under normal conditions and in experimental colitis.

A parallel increase in endostatin and two pro-angiogenic factors of VEGF and PDGF in the model of iodoacetamide-mediated colitis and in IL-10$^{-/-}$ mice was demonstrated in the literature [110]. In addition, a positive correlation was found between the size of the colon lesions in experimental colitis and the content of endostatin. These data are consistent with the results of an increase in the level of pro-angiogenic factors of angiogenin and angiopoenthin-2 in the serum of patients for inflammatory bowel diseases and a higher level of endostatin in patients with progressive ulcerative colitis.

Simultaneous increase in the content of VEGF, PDGF and endostatin suggests the existence of a functional relationship between these factors in the pathogenesis of experimental inflammatory bowel diseases. Comparative studies in MMP-9$^{-/-}$ mice that had a reduced level of endostatin and wild-type mice with an elevated level in DSS-mediated colitis showed a significant decrease in the PDGF level in MMP-9$^{-/-}$ mice, which in its nature corresponded to changes at the level of endostatin.

The results of the above studies suggested the involvement of endostatin in the regulation of PDGF expression. Parallel elevation of PDGF and endostatin can positively influence the pathogenesis of inflammatory bowel diseases by restoring the processes of physiological angiogenesis. The opposite pattern was observed for VEGF: the level was even higher in MMP-9$^{-/-}$ mice compared to the wild type against a background of colitis caused by DSS. It is unlikely that MMP-9 directly affects the level of VEGF, since the induction of MMP-9 expression, by the introduction of a recombinant adenovirus, did not change this index. It is likely that an increase in the level of VEGF in MMP-9$^{-/-}$ mice is associated with a decrease in the level of endostatin. The literature shows that neutralizing VEGF activity with an antibody to VEGF results in a decrease in the level of not only VEGF, but also endostatin in the large intestine of rats with experimental colitis. Thus, the existence of a regulatory relationship between endostatin and VEGF has been established.

In review and experimental articles [101, 107, 108] it was shown that, in contrast to the pro-angiogenic factors bFGF, PDGF, HGF, the effects of VEGF are opposite in mechanisms of ulcer formation in peptic ulcer of gastroduodenal zone and inflammatory bowel diseases. The suppression of VEGF activity significantly improves the clinical and morphological parameters of the experimental colitis, and has the opposite effect in the experimental peptic ulcer of the gastroduodenal zone. While the administration of bFGF, PDGF or HGF significantly enhances the healing of lesions in both experimental inflammatory bowel diseases and gastroduodenal ulcer.

So, it is assumed that VEGF is capable of stimulating both normal/physiological angiogenesis (gastroduodenal zone ulcer) and excessive/pathological angiogenesis (inflammatory bowel disease) depending on the pathogenesis of the disease, and endostatin may be a kind of antagonist of VEGF-mediated effects. The simultaneous increase in the level of endostatin and VEGF in the mucosa of the duodenum is also shown in the model of experimental duodenal ulcer [109, 110]. In addition, it has been shown that administration of recombinant endostatin to rats significantly improves clinical (weight loss, stool consistency, blood in feces) and morphological signs of DSS-mediated colitis in mice. The introduction of endostatin to rats with a cysteamine-mediated duodenal ulcer, on the contrary, produces a negative effect on the healing rate of ulcers.

Thus, the parallel increase in endostatin in the pathogenesis of inflammatory bowel diseases (pathological angiogenesis) is an important link in the body's defense system against the pathological effects of overexpression of VEGF [110]. This position is confirmed by the specific efficacy of 5-aminosalicylic acid due to the improvement in the clinical and morphological parameters of experimental colitis when restoring the balance between VEGF and endostatin [110]. So the role of the endothelial barrier in the pathogenesis of inflammatory bowel diseases is defined.

A new concept of molecular mechanisms of the pathogenesis of inflammatory bowel diseases is formulated in terms of the role of the intestinal barrier in it. In particular, the factors that lead to an increase in the permeability of blood

microvessels (VEGF) and alter the functional activity of endothelial cells (hypoxia) can contribute to a relapse of the disease. In the literature, it is assumed that genetic or external factors that lead to spontaneous changes in the density of interendothelial adhesive contacts (VE-cadherin) or disruption of expression of endothelium-associated transcription factors (Egr-1) are simultaneously etiologic factors of propensity to inflammatory bowel diseases. Excessive activation of the pro-angiogenic potential in the pathogenesis of inflammatory bowel diseases is accompanied by a protective response of the body in the form of expression of anti-angiogenic factors, one of which is endostatin. Pharmacological correction of the balance between pro- and anti-angiogenic factors and bringing it to a physiological norm can be used in the treatment of patients for inflammatory bowel diseases. The diagnosis of increased permeability of blood microvessels in the intestinal mucosa of patients with PZK is recommended as a marker for the selection of effective therapy, and also as a prognostic marker for relapse of the disease [109, 110].

The chronic course of inflammatory bowel diseases associated with imbalance between pro- and anti-angiogenic factors is accompanied by the development of pathological angiogenesis and may be a prerequisite for the development of colitis-associated carcinogenesis [110].

In the literature, the results of a complex study of the functional state of the main elements of the Ca^{2+}-dependent signaling pathway (phospholipase C, PL, Ca^{2+}, protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase) and their functional links to individual units of other signaling systems (NO synthase, protein kinase A , protein kinase G and tyrosine protein kinase) in the epitheliocytes of the colonic mucosa under conditions of experimental colitis-associated carcinogenesis [107–110]. The participation of Ca^{2+} and NO-synthase in the formation of primary manifestations of inflammation in ulcerative colitis was noted. The accumulation of mitochondrial protein modulator proteins, apoptosis, pro- (B_{ax}) and anti-apoptotic (Bcl-2) proteins, has been shown in the late stages of oncology formation [110]. The obtained results are the theoretical basis for scientifically-based correction of the revealed metabolic disturbances.

Experimental reproduction of the acute form of enteropathology

The experimental work was carried out in three stages. At the first stage of the work, the task was to reproduce the acute form of dyspepsia in laboratory mice. The second stage involved the study of disorders of the LP spectrum of internal organs in laboratory mice with experimental dyspepsia, and the third - the determination of the effectiveness of various methods of correction of lipid metabolism disorders in the model of experimental dyspepsia in laboratory mice.

At all stages of the experiment, SVA mice of 5 weeks of age with an average body weight of 17–18 g were used, of which 8 groups of research groups were formed. For this, the animals were separately placed in cages. Before the experiment began, the mice were kept in quarantine with a clinical examination for a week [111–113]. 15 days prior to the beginning of the experiment, laboratory mice were placed in a vivarium with such zoogenic hygienic parameters: temperature 25–28° C, relative humidity 50–70%, photoperiod – 10 hours of daylight. During the experiment, the temperature, humidity and illumination in the room did not change. The mice had free access to feed and water. Monitoring of changes in body weight and feed intake by mice of research and control groups was monitored.

To reproduce the acute form of dyspepsia, four groups of animals were formed. Diseases were caused by the administration of a solution of diclofenac, which belongs to the group of nonsteroidal anti-inflammatory drugs (NSAIDs). To release the intestine from chyme and stool before application of diclofenac, animals were injected with guttalax. The main mechanism of action of diclofenac on the digestive organs is associated with its inhibitory effect on the synthesis of prostaglandins. It inhibits cyclooxygenase (COX) activity, but is not a selective drug. Diclofenac inhibits the activity of not only COX_2, which leads to a decrease in the inflammatory process, but also COX_1, which provides in the mucosal membrane the synthesis of prostaglandins – regulators of secretion of protective mucus, bicarbonates, free hydrochloric acid in the stomach and full blood supply [113]. The drug was administered orally, once a day, for two days at doses of 37.5 mg/kg, 25.0 and

12.5 mg/kg of body weight [113]. Observed changes in the general condition of animals, the presence of symptoms of intoxication. Every 24 hours of the experiment the mice were weighed, the amount of food consumed was monitored.

After the reproduction of an acute form of dyspepsia, the evidence of which was found clinical symptoms characteristic of this pathology [114, 115], assessed for behavior, appetite, body weight, skin and coat condition, stomach shape and size, stool consistency [116]. Under ether anesthesia, the mice were decapitated with a subsequent pathologic anatomical autopsy [117]. To confirm the diagnosis of the disease, a histological examination of the stomach, duodenum and liver was performed. The samples were fixed in a 10% aqueous solution of neutral formalin, washed in running water, dewatered in ethanol with increasing concentration (70º, 96 and 100º), kept in chloroform and later in paraffin and poured in plastic bottles. After cooling, the paraffin blocks were attached to wooden cubes. After fixation with the help of a sanitized microtome, sections of 10 μm thick were made which were stained with Karatz hematoxylin and eosin [118].

To conduct a comparative evaluation of the various methods of correction, four groups of mice were also formed in the form of para- analogs. Reconstructing the acute form of dyspepsia, the mice of group III used the solution of Essentiale forte (Germany), in 15 cm^3 of which contains 250 mg of "essential" PL: diglycerol esters, cholinephosphoric acid of vegetable origin, which contains an excess of unsaturated FA, predominantly linoleic (70%), linolenic and oleic [119].

Mice of Group IV used a 1% liposomal form of BAS «FLP-MD», made on the basis of PL milk, developed at the Department of Biochemistry and Physiology of Animals named after Academician M. F. Guliy of the NULES of Ukraine [119]. The analysis of the composition of the liquid crystal neutral and polar lipids of membranes of fatty globules of the oil cane indicates that palmitic, stearic, oleic and linoleic acids predominate in them, which constitute 84-90% of all FA. The most unsaturated polar lipids are PE and PS, which contain oleic (53.8–60.6%) and linoleic (9.7–18.7) acids, as well as palmitic and stearic (10–15%). In the FH fractions, 33–42% oleic, 28.2–39.0 palmitic and 10–22% stearic acids were found, whereas in SM

it was 31–67% tricosan and behenic [120, 121]. Mice of group II underwent self-rehabilitation during the period of treatment of animals of groups III and IV. Correction tools were administered orally once a day for 30 days. Doses of drugs were therapeutic.

Mice of control groups were kept on a traditional ration with a vivarium with oral administration of an equivalent volume of distilled water.

An acute form of dyspepsia was reproduced in laboratory mice of the SVA line of 5 weeks of age with an average body weight of 18.0 g.

Dyspepsia was caused by the stepwise oral administration of the drugs according to the following scheme: guttalax for five days twice a day, then diclofenac for two days once a day/

When Guttalax was administered for five days, a laxative effect was observed, which was characterized by a softening of the consistency of feces.

After the administration of diclofenac, the clinical signs characteristic of dyspepsia were observed in animals of groups II, III and IV, the degree of which depended on the dose of diclofenac.

In the second group, cases of mortality of animals on the second day of diclofenac were observed when its dose was high. At the same time, two animals died with signs of exhaustion. When patho-anatomical autopsy of these mice, diffuse hemorrhagic-catarrhal inflammation and edema of the mucous membranes of the stomach and the small intestine, myocardial dystrophy, liver and kidneys were detected.

The pathological and anatomical changes in the dead mice were characteristic of an acute form of dyspepsia. Therefore, all research animals under ether anesthesia were decapitated for pathoanatomical dissection and sampling of organs for histomorphological and biochemical studies.

When autopsy of research mice, pathological and anatomical changes were observed, characteristic of the acute form of dyspepsia, the degree of manifestation of which depended on the dose of diclofenac administered. This was also observed in the histomorphological study of the stomach, the small intestine and liver.

As a result of numerous experimental and laboratory studies, we determined that to reproduce the acute form of dyspepsia in laboratory mice, the optimal dose of diclofenac is 25 mg/kg of body weight of the animal, which was administered to mice of group III.

2.2.2 Structural characteristics of membranes of circumferential enterocytes and hepatocytes in experimental enteropathology and their correction

In the pathogenesis of inflammatory, dystrophic and degenerative processes in the development of enteropathology, the structural and functional state of the cellular membranes of the epitheliocytes of the intestine and liver is important, which is a consequence of enhanced peroxide oxidation of membrane lipids and phospholipid hydrolysis, which is naturally complicated by intracellular metabolism disorders [121].

As a result of the action of unfavorable factors of the external and internal environment on the animal organism, the phospholipid molecules are subjected to structural changes and are destroyed. Deficiency in the tissues of general lipids and PL slows the restoration of the membrane structure, which leads to various functional disorders in the affected organs. The question arises with respect to the intensity of recovery of the lipid composition of organs for which the characteristic endogenous synthesis and cumulation of exogenous phospholipids, especially when using preparations of reparative action on their basis.

To study the structural and functional changes in cell membranes, the fluorescent probe method was chosen, in particular, the use of a negatively charged ANS probe. The changes revealed by ANS are characteristic, mainly, for the surface membrane layer [122].

We conducted a comparative study of the fluorescent characteristics of the ANS during its binding to the plasmolemal enterocytes of the small intestine mucous membrane and hepatocytes under experimental enteropathology and the application

of the BAS «FLP-MD» supplements based on PL milk and Essentialial forte on the basis of PL soybean.

The study was carried out on lab rats with male body weight of 180–200 g, of which 4 groups of 7 animals were formed: I – control animals; II – experimental reproduction in rats of enteropathology with ulcerative erosion complicated by hepatitis (model) [123, 124]; III – reproduction in animals of the model of enteropathology and their treatment of Essentiale forte; IV – reproduction in rats of the model of enteropathology and correction of pathological changes by using liposomal form of BAS «FLP-MD» [119]. To reproduce the enteropathology model, rats of Group II–IV for 14 days were orally administered diclofenac at a dose of 12.5 mg/kg of body weight once a day. Further, group II animals remained without treatment (self-rehabilitation), Group III animals were orally administered Essenciale-Forte in a dose of 7.1 mg/kg body weight to group IV rats – 1% solution of phospholipid-based BAS «FLP-MD» at a dose of 13.5 mg/kg of body weight. After 14 days of reproducing the model (for group II) or after 30 days of treatment (for groups III and IV), the rats were decapitated under ether anesthesia.

In the experiments, preparations of the small intestine apical membrane (AM) of enterocytes of the small intestine were used, a component part of the enterocyte plasma membrane, microsomal (MC) preparations of hepatocytes – as part of the plasma membrane of hepatocytes, internal membrane of mitochondria of hepatocytes (IMMH) and internal membrane of mitochondria of enterocytes (IMME). Preparations of MC membranes of hepatocytes and SMJP were obtained by the method of [125]. Preparations of AM enterocytes of the small intestine mucosa were obtained, according to the procedure [126], and the internal membrane of mitochondria of enterocytes – submitochondrial parts (SMP), according to the procedure [127], with minor modifications.

Since almost all the fluorescence of the ANS under investigation is due only to the fluorescence of the membrane-bound probe, this makes it possible to determine the parameters of its binding to the membrane from the fluorescence data: the binding constant (K_{ANS}) and the number of binding sites (N_{ANS}). The N_{ANS} parameter indicates

the maximum concentration of the probe in the membrane during its saturation with the probe, and the value of the K_{ANS} reflects changes in free energy during the transition of 1 mole of the probe from the aqueous phase to the membrane [128].

Determination of the parameters of probe binding to membrane preparations was carried out according to the results of titration, according to [125]: 1) membrane preparations (0.1 mg/cm³) with a probe (2–40 μM) 2) probe solution (5 μM) with membrane preparations (0.025–0.30 mg/cm³). Representation of data in double inverse coordinates makes it possible to obtain rectilinear dependencies, and the parameters of probe binding to membrane preparations (K_{ANS} and N_{ANS}) were determined from the obtained graphs. The measurements were carried out in a medium containing 0.1 M KC1, 5 mM Tris-HCl (pH 7.0 at 25° C). The fluorescence intensity of the ANS was recorded at λ = 370 nm and λ = 480 nm.

To estimate the distance between different sections of the membrane, the method of inductive resonance energy transfer (IRET) was used from the donor to the acceptor. As donor-acceptor pairs, tryptophan-ANS was used. The fluorescence was evaluated at λ 295 and 340 nm. The protein concentration was 0.1 mg/ml, titration was performed with a probe at concentrations of 10–50 μM.

All fluorescence studies were performed on a "Shi-madzu-RF510" (Japan) spectrofluorimeter in quartz single-centimeter cells at t = 25° C.

Measurement and analysis of the results were carried out according to the groups: a) control (K); b) model (M) c) BAS «FLP-MD» (BAS) d) Essential forte preparation (E).

It is known that, when interacting with a biological membrane, a negatively charged ANS molecule is localized predominantly in the polar region [127], forming complexes with both the protein and lipid components of the membrane [128]. In the experiments we used preparations of AM enterocytes of the small intestine, MC membranes of hepatocytes and preparations of Mitochondrial parts whose structural and functional disturbances significantly affect the function of enterocytes and hepatocytes. As a result of the conducted studies, under the conditions of the model (M), changes in the binding parameters of ANS with AM preparations were revealed

(the values of K_{ANS} and N_{ANS} decrease by an average of 53 and 23%, respectively). The intensity of fluorescence of ANS associated with MK preparations under the conditions of the model decreases by an average of 26% relative to control. Under these conditions, the value characterizing the binding parameters of the ANS (K_{ANS} and N_{ANS}) also decrease by 28 and 30%, respectively.

The surface of the plasma membrane of cells is characterized by considerable heterogeneity, which causes the binding of ANS to sites that differ in their properties [126]. Moreover, the existence of not only different ANS binding sites in the membrane, but also various conformations of the probe, which are characterized by excellent fluorescence parameters, is known [128]. When analyzing the results obtained, it should be taken into account that the fluorescence intensity of ANS depends both on the number of binding sites and on their structure and conformation, and the heterogeneity of the membrane surface determines the existence of sorption centers for this fluorescent probe of various types [129]. Under the conditions of the applied model, the membrane surface of the AM and MC is reconstructed, which causes multidirectional changes in the fluorescence parameters of the membrane-bound probe, namely, a decrease in the number of binding sites and (or) their conformational changes. In this case, a decrease in the binding constant of the probe may indicate a change in the kinship of certain sections of membranes to the ANS.

Application of BAS «FLP-MD» of reparative action leads to partial restoration of the binding parameters of ANS with AM and MC. Under these conditions, the value of the binding constant of ANS with AM and MC, as well as the fluorescence intensity of the ANS associated with MC, does not differ from the reference values. However, the value characterizing the number of binding sites of the probe with AM and MC remains low on average by 32%, relative to control.

The use of the Essential-forte medicinal preparation for animals results in the restoration of only the value characterizing the binding constant of ANS with AM and MC. The parameters of other indicators differ from the control values, namely: the fluorescence intensity of the ANS associated with MC and AM is reduced by 18 and 24%, respectively, and the N_{ANS} value by 50 and 42%, respectively.

It should be noted that under conditions of experimental enteropathology, changes in the surface structure of membranes of enterocytes and hepatocytes are observed, which is manifested in changes in the binding parameters of a negatively charged ANS probe with these membranes. Therapy of different in origin PL preparations contributes to a partial recovery of both these parameters, and the value of K_{ANS}, which returns to the control values. The revealed changes in the surface structure of AM and MC are largely due to the modification of both membrane proteins and the lipid component, as evidenced by such an indicator as the number of ANS binding sites. Under the conditions of the study methods of treatment, this value does not acquire control values, it may take a long time. Although, in the conditions of using the supplements «FLP-MD», the recovery of the studied parameters is better expressed.

Similar studies have been conducted for preparations of the internal membrane of mitochondria of enterocytes (SMEs) and hepatocytes (SMJP).

It is established that under the conditions of the model the parameters of binding of ANS with SMCE products change: the fluorescence intensity of the ANS decreases by an average of 22%, the value of K_{ANS} increases by 28% and the N_{ANS} decreases by 20%. The nature of the changes in the spectral characteristics of the probe associated with SMEs indicates a variety of membrane modifications that lead to a decrease in the number of binding sites and/or their conformational changes, as well as a decrease in the affinity of the membrane to the ANS, since the value of K_{ANS} is likely to increase as a result of the appearance of negatively charged groups. As a result, the fluorescence intensity of the membrane-bound probe decreases. Treatment with both essential oil Forte and «FLP-MD» does not lead to the restoration of the studied parameters in the membranes of SMEs (fluorescence intensity is reduced by an average of 28%, the value of K_{ANS} increased by 50–57% and N_{ANS} is reduced by 30- 50%, concerning the control).

Under the conditions of the model used, mitochondrial membranes of hepatocytes also revealed changes in the studied parameters: the intensity of ANS fluorescence does not change, but the value of K_{ANS} decreases by 23% on average and

N_{ANS} by 7%. Moreover, under the conditions of treatment of rats, the intensity of fluorescence of the membrane-bound ANS remains low on average by 20%, and N_{ANS} by 20–40% compared to the control. These changes are similar to those found for drugs AM and MC.

In further studies, the IRET method in a pair of tryptophan-ANS fluorophores (donor-acceptor) was used to evaluate the modification of surface regions of membranes. The effectiveness of the IRET largely depends on the relative location of the regions of preferential localization of fluorophores in the membrane. The most probable locations for the localization of tryptophan residues are the hydrophobic regions of proteins that may be present in the membrane in both the protein and lipid environments [122], and the fluorescent ANS probe is predominantly localized in the membrane at the lipid-water interface. Since the critical distance of the IRET for the tryptophan-ANS pair is 2.0–3.5 nm, and the membrane thickness is about 4.0 nm, so a more significant contribution to energy transfer is made by fluorophores located on one side of the membrane with respect to lipid phase. The F_o-F/F_o (where F_o is the fluorescence intensity in the absence of a quencher, F in the presence of a quencher) is calculated from the results of quenching of donor fluorescence by an acceptor, which indicates the effectiveness of the IRET.

The results of the study of the IRET using the tryptophan-ANS pair showed that the value of (F_o-F/F_o decreases under model conditions for all types of membranes.) Reducing the efficiency of the IRET between the respective fluorophores under these conditions indicates an increase in the distance between them.

The obtained results on the study of the interaction of the fluorescent ANS probe with membranes indicate a change in the surface structure of all the membranes under study in conditions of experimental simulation of enteropathology.

Moreover, for different membranes it has a somewhat different character. The established parameters of binding of ANS to SMEs in the experimental model indicate stable changes in this membrane structure, unlike other membrane preparations, can not be restored under the conditions of the treatment.

Multidirectional changes in the association constant of the probe and the number of binding centers of the ANS with the membranes AM, MC and SMJP is a reflection of integral processes (changes in the microenvironment of the probe, structural rearrangements, etc.) that occur in the membrane, taking into account that the fluorescence of ANS essentially depends on its composition [122]. This may be due to the modification of both the lipid component and the membrane proteins, since the ANS molecule, localized in the polar region of the membrane, forms complexes with protein and lipid molecules. This, to a large extent, causes changes in the surface structure of AM and MC, since the recovery of membrane components requires more time. Confirmation of the presence of a structural modification of the surface regions of cell membranes of enterocytes and hepatocytes under the conditions of the model is also based on the results of the efficiency of the IRET in a tryptophan-ANS pair. Treatment of animals with liposomal form of BAS «FLP-MD» does not lead to a complete recovery of the studied parameters, although it is more effective than the use of Essentiale Fort, and the results indicate the need for a longer course of application of liposomal form of BAS «FLP-MD» to obtain the necessary corrective effect.

It should also be noted about the differences in the response of plasmolemia and mitochondrial membranes under the conditions of the modeling of enteropathology complicated by hepatitis, and their reaction to used preparations based on PL of various origins. This indicates a multifaceted change in the spatial organization of protein-lipid complexes, depending on the type of membrane.

2.2.3 Lipid composition of membranes of circumferential enterocytes of the small intestine in experimental enteropathology and its correction

An important structural and functional characteristic of the biomembrane is the qualitative and quantitative composition of its lipid component. The lipid composition of biological membranes determines the preservation of the ultrastructure, selective permeability, regulation of enzymatic activity, stability of the

membrane, transport of ions and molecules, etc. The main structural components of the lipid bilayer of cell membranes is PL. Thus, the functioning of membrane systems depends on the integrity of their phospholipid structures. To date, the key role of lipid bilayer membrane disorders in the development of severe liver diseases, cardiovascular and nervous systems, disorders of many functions of blood cells, epithelium, etc., has been proved, which involves the use of phospholipid-releasing reparative agents.

The efficacy of the therapeutic effect of the liposomal form of the BAS «FLP-MD» compared to the medicinal preparation essential balm for restoring the lipid composition of the inner mitochondrial and apical membranes of the peritoneal enterocytes of the small intestine using the experimental model of enteropathology was studied.

The study was conducted on white laboratory same-sex rats with a body weight of 180–200 g, of which 4 groups formed (6 animals each): I – control animals; II – reproduction of a model of enteropathology complicated by hepatitis (model) III – reproduction of the model of enteropathology and treatment of essential-forte; IV – reproduction of the model of enteropathology and correction of pathological changes by applying liposomal form of BAS «FLP-MD». To reproduce the model of the disease, rats of groups II–IV received NSAIDs diclofenac at a dose of 12.5 mg/kg body weight for 14 days. Further, group II animals remained untreated; Group III animals were orally administered the Essential-forte preparation (10–12 mg per animal) to the rats of group IV-oral «FLP-MD» (2.7 mg per animal) was administered orally. After 14 days of reproducing the model (for group II) and after 30 days of treatment (for groups III and IV), the rats were decapitated.

The lipid composition and content of individual lipids is an important structural and functional characteristic of membranes. The content of lipids in membranes is determined by the intensity of biosynthesis and decay, the rate of intracellular transport of lipids, etc., which leads to protein-lipid and lipid-lipid interactions.

Determination of the content of TLP and PL in the investigated membranes, indicates an unreliable decrease in their content under the conditions of the experimental model (Table 2.6). Under the experimental conditions, for the use of BAS «FLP-MD», the content of TLP and PL returns to the control values for the preparations of the SMP membranes, and for AM – even exceeds the control values by 30%. Against the background of the use of Essential-forte, the content of membrane lipids, in particular, in the preparations of SMP membranes, is reduced by an average of 25%, and in AM – by 20%, as compared with the control.

Table 2.6

The content of total lipids and phospholipids in membranes of peritoneal enterocytes of the small intestine (μg/mg protein) in the control and under the experimental conditions (M ± m, n = 6)

Index	Control	Model	Model + BAS «FLP-MD»	Model + preparation Essential-forte
Mitochondrial membrane				
Total lipids	438,8±42,7	356,3±39,4	441,9±42,5	328,7±33,2*
Phospholipids	289,7±25,1	231,2±25,9	285,3±22,4	216,8±27,7*
The apical membrane				
Total lipids	470,1±38,2	450,0±31,8	611,0±44,8*	373,8±33,6*
Phospholipids	273,9±18,4	267,7±25,6	359,0±19,7*	220,3±14,4*

Note: here and in Table. 2.16 * - p <0.05 relative to the control.

The phospholipid composition of the biological membrane is one of the main factors that determines its structural organization and functional state. The results obtained show that the most part is represented in the membranes PH and PE, the total content of which is on the average 62 and 77% of the total amount of PL, respectively, for the preparations of the SMP membranes and AM. The content of SM, PI and PS in these membranes is much lower, namely: in the fraction of the SMP membranes, respectively, they are 9.5%, 2.5 and 6.0%, and in AM – 9.3%, 3.2 and

7.9 %. It should be noted that for mitochondria, these PL play an important role as components involved in the regulation of the activity of respiratory chain enzymes, ion transport, etc.

A characteristic feature for SMP membranes is the CL content, which reaches almost 20%, which is important in maintaining a functionally active structures of mitochondrial enzymes. In the control preparations of membranes under the experimental conditions, the lysoforms of PL: LPH and LPE were not detected.

An analysis of the results indicates a quantitative redistribution of individual PL in all experimental conditions. In AM preparations, the contents of different PL fractions decrease insignificantly under the conditions of the experimental model. In the conditions of Essential-forte treatment, the content of PH, PE, SM, and PS remains low on average by 18–34%, relative to control. Treatment of animals with dietary supplements «FLP-MD» leads to an increase in the content of individual PLs, relative to control, in the content of individual PL: by 28%, PE by 22, SM by 31 and PI by 34%. The increase in their content is probably due to the acceleration of the synthesis or interconversion reactions of these compounds.

In the preparations of the SMP membranes under the conditions of the experimental model, the content of the PLs studied is decreased, and for all fractions other than PH. Thus, the content of the PE is reduced by 17%, cm by 46, PS by 43, PI by 21 and CL by 47%. Along with this, under the conditions of the experimental model, lipase forms PL (LPE, LPH) appear in the preparations of the SMP membranes, which is probably due to the activation of phospholipases. The results obtained indicate a violation of the structure of the SMP membranes under the conditions of the experimental model. Therapy of sick animals with the use of the Essentialial forte preparation only leads to a partial restoration to the level of control of the lysoform content of PL, and when using the liposomal form of BAS «FLP-MD» in the preparations of the SMP membranes, they were not detected. It should be noted that LPL, as monoacyl forms, are characterized by greater hydrophilicity, compared with diacyls, which contributes to the weakening of hydrophobic interactions in the membrane. In addition, an increase in the content of LPH in

membranes promotes the formation of a hexagonal type of packaging of phospholipid membrane components [123]. This, above all, causes an increase in the permeability of the latter, as well as a breakdown in the binding strength of proteins to the hydrophobic part of the lipid matrix, facilitates the migration of some of the lipid molecules to the polar surface layers. At the same time, the use of Essential-Fort does not cause restoration to control values of the content of individual PLs, except for PS. However, when using liposomal form of BAS «FLP-MD», the PL content is almost not different from the control, with the exception of PE and CL, which remains reduced by 12 and 24%, respectively. Along with this, the content of PI is increased 1.8 times. Under these conditions, an increase in the PI content may be due to an increase in the intensity of the reactions of the interconversion of individual PLs and is a manifestation of the compensatory reaction of the organism.

In addition, one of the reasons for the detected decrease in the content of the main fractions of the PL of the apical and mitochondrial membranes under the conditions of the experimental model can be the activation of oxidative processes, the main substrates of which are precisely these compounds [128, 129]. The decrease in the content of PH and PE and the formation of LPH and LPE characterizes the activation of phospholipase hydrolysis, which leads to the modification of cell membranes. The revealed regularities can also be the result of changes in the intensity of PL synthesis, against the background of a violation of their acylation or reacylation. In particular, the decrease in the content of PE can be a consequence of the inhibition of re-alkylation of LPE, as indicated by its accumulation in membranes.

Thus, changes in the PL composition of membranes under the experimental model of enteropathology, which are accompanied by a violation of the ratio of their individual representatives, can be caused by changes in the processes of reduction of the phospholipid component of membranes, damage to the PL structure under the influence of phospholipases or as a result of oxidation. A consequence of the revealed structural changes in the apical and mitochondrial membranes may be violations of their transport function. Moreover, deeper changes in the PL spectrum of the mitochondrial inner membrane of peritoneal enterocytes indicate a possible violation

of their energy function. Unlike therapy with Essenciale Fort, the use of the liposomal form of BAS «FLP-MD» leads to an almost complete recovery of the PL-storage of the apical and mitochondrial membranes of the peritoneal enterocytes of the small intestinal mucosa.

2.2.4 Pro- and antioxidant processes in the body under experimental enteropathology, their correction

Peroxide oxidation of lipids is a physiological process, and abnormalities lead to undesirable consequences [129–131]. The LPO system is a multi-stage chain mechanism, which is activated when various factors influence the body. In cells, the level of LPO is maintained due to the balance of the pro- and antioxidant system. The latter is multicomponent, which includes both enzyme complexes and endogenous antioxidants [132]. It has been shown [133] that the use of additives to the feed – vitamins, mineral elements, antioxidants, herbal preparations and other biologically active substances (BAS) – stimulates the system AOP of the organism and, in turn, contributes to its resistance, reproductive capacity, productivity. The BAS «FLP-MD», which contains PL milk, unsaturated fatty acids, retinol acetate and α-tocopherol, has been developed and is effective when used in the complex treatment of calves that have recovered from neonatal dyspepsia [121]. To determine the biological effect of the action of BAS «FLP-MD» it is important to study its effect on the intensity of the course of LPO and the activity of antioxidant enzymes of various organs and cells.

Thus, prooxis-antioxidant state of liver cells, small intestine mucosa and blood under conditions of enteropathology and for the therapeutic effect of liposomal form of BAS «FLP-MD» have been studied.

The study was carried out on laboratory mongrel male rats weighing 200–220 g, from which three groups of 8 individuals were formed: I (control) – intact animals; II – reproduction of the model of enteropathology with ulcerative erosion;

III – reproduction of the model of enteropathology and correction of pathological changes by applying liposomal form of BAS «FLP-MD».

Various pathological states of the body are characterized by the activation of LPO – a free-radical chain process, which leads to the formation of peroxides of organic and inorganic nature. It was found that under the conditions of the enteropathology model with ulcerative erosion, the content of TBA-active products in the liver cells decreased insignificantly, and in the cells of the small intestine mucosa by 23%. This may be due to the use of LPO products in oxygen-containing organic compounds. Along with this, the content of TBA-active products in blood serum increases significantly (by 3.2 times), possibly as a result of their intake from other tissues.

Control over the formation and neutralization of metabolites of free radical oxidation and their products is carried out with the participation of the AOP of the body system, which leads to the maintenance of intracellular oxygenase reactions at the optimal steady-state level, which is a prerequisite for the realization of various physiological functions. In cells and biological fluids a physiological antioxidant system functions. The most important components of anti-radical and antiperoxide protection are enzymes that catalyze the reactions between the active forms of oxygen [134, 135]. One of the key enzymes of the antioxidant defense system is SOD and KAT. The results obtained to determine the activity of SOD indicate that under the conditions of the model, the activity of the enzyme in the studied preparations is reduced, which for the liver is 23%.

The activity of KAT in liver and blood preparations is reduced by 10 and 45%, respectively, and the small intestine grows by 24% under the conditions of the model. The growth of enzyme activity can be considered as a reaction of the AOP system of cells of the small intestinal mucosa to the intensification of the processes of free radical oxidation and the activation of many metabolic processes. According to the therapeutic use of dietary supplements, the activity indices of these enzymes in the studied preparations are almost not different from the control values, and the activity

of blood KAT even exceeds them. The indicator of the content of TBA-active products in the studied preparations also almost returns to the control values.

However, in these conditions, in the preparations of the small intestine its activity increases by 200%. Multidirectional changes were detected for the drugs under study and in the activity of glutathione transferase (GT): in the blood, the activity of the enzyme decreases by 41%, and in liver and small intestine preparations it increases (by 21% for the latter). Along with this, the content of reduced glutathione (RG) drops sharply in blood preparations (by 92%), and in preparations of the liver and small intestine by 19 and 14%, respectively. Under conditions of therapeutic administration of dietary supplements, the activity of GT and GP of blood approaches the control values, while for small intestine preparations there is an increase, compared with the control, of the activity of the corresponding enzymes. With the introduction of BAS «FLP-MD» in enteropathology conditions, the content of RG in the studied preparations remains significantly lower with respect to control

Analyzing the received results it is necessary to note infringements of functioning of system AOP of an organism in conditions of model. However, the nature of these changes has features for various organs. This probably indicates a violation of the interaction of its functioning under these conditions. The greatest changes in the conditions of the model are observed for the studied blood indices. Under these conditions, TBA-active products accumulate in the blood, the content of RG and the activity of GT, as well as the activity of KAT and GP, accumulate, testifies to the oppression of the functioning of the OA-defense system of the organism.

The decrease in enzymatic and non-enzymatic links of AOP in the liver in experimental animals indicates a suppression of SOD activity and a decrease in the content of RG. Under these conditions, the activity of enzymes responsible for the detoxification of peroxide compounds (KAT and GP) is also slightly reduced. For preparations of the small intestine, there is an increase in the activity of KAT and GP. At the same time, the activity of SOD varies insignificantly, and the decrease in the content of RG is observed along with the growth of GT activity. The therapeutic administration of BAS «FLP-MD» under conditions of enteropathology has different

effects on the restoration of the studied parameters: the activity values of the AOP enzymes approach the control values or even grow (K_{AT} of the liver and blood or GP of the liver and small intestine). However, under the conditions of treatment of BAS «FLP-MD», the content of RG remains below the control values. Since RG participates in many metabolic processes, a decrease in its content for the therapeutic effect of BAS «FLP-MD» may also indicate the activation of restorative processes in the body. Thus, BAS «FLP-MD» has a protective effect on the course of oxidative processes under enteropathology, given that its main component is PL, which on one hand stabilize the cell membranes, and on the other hand, are oxidized for the actions of oxygen radicals, that is, natural antioxidants.

CONCLUSION TO CHAPTER II

The livestock sector of Ukraine has been suffering significant losses for a long time due to the development of gastrointestinal diseases in newborns and young animals. When there is enteropathology, the main changes occur in the epithelial layer of the mucous membrane of the organs of the digestive apparatus, liver and kidneys. First of all, this applies to cytoplasmic membranes, consisting mainly of PL, performing structural, regulatory, protective and other functions. Therefore, in conditions of practice, therapeutic and prophylactic drugs with a reparative effect of action should be used. However, all of them are predominantly of plant origin (for example, Essentiale forte), are costly and their therapeutic effect when applied to animals is relatively low. Developed at the Department of Biochemistry and Physiology of Animals. acad. M. F. Gulogo National University of Life and Environmental Sciences of Ukraine BAS «FLP-MD» contains PL of animal origin, obtained from affordable and environmentally safe raw materials (oil can). That is, its lipid components in terms of chemical structure and physico-chemical properties correspond to the membrane lipids of newborn calves to the maximum, in a complex it gives higher biological and clinical effects.

With the help of clinical, electron microscopic, physicochemical and biochemical methods of research, the effective effect of PL milk of «FLP-MD» supplements on restoring the blood, cells and tissues parameters characterizing the functional state of the intestine, liver and the organism as a whole is proved, which significantly distinguishes these animals from calves, who were treated according to the traditional scheme. It is proved that the best therapeutic effect is noted in the complex scheme of using traditional products for the treatment of dietary supplements «FLP-MD» based on milk PL.

Cells, like other living systems, are capable of regeneration, that is, to restore lost parts or renew elements of their external and internal structure. The basis of many pathological processes is the pathology of cell reproduction. Detention of their entry into mitosis occurs mainly due to metabolic disturbances: first of all, changes in

the synthesis of DNA, RNA and proteins. Intracellular synthesis of biopolymers directly depends on the structural and functional state of the cell membranes of the corresponding structures.

Interaction of the pathological factor with the surface of cytoplasmic membranes is always accompanied by a change in their physical and structural-dynamic characteristics. In calves of 30-day-old age who had recovered from enteropathology, a decrease in the fluorescence intensity of the ANS by 15–20% was established with respect to control when binding to membrane preparations of enterocytes of the small intestine. This may be due to changes in the binding of the probe to the membranes. The size of K_{ANS} increases for membrane preparations from animals with traditional therapy. At the same time N_{ANS} significantly decreases with both traditional and complex treatment regimens. The obtained results on the features of the interaction of the fluorescent ANS probe with membranes indicate a change in the surface structure of the membrane, which may be due to the modification of both the lipid component in the zone of the polar heads of glycerol residues of phospholipids and membrane proteins.

The degree of pyrene excimerization, the reciprocal of which characterizes the microviscosity of membranes, in the studied membrane preparations when using dietary supplements based on PL milk acquires control values for both the lipid phase and anular lipids. Only in membrane preparations for traditional treatment, an increase in N_{335} and N_{280} relative to the control values was observed, which indicates a decrease in the microviscosity of the lipid phase and anular lipids and indicates a decrease in the structural ordering of the lipid component of the membranes.

It has been established that in the membrane preparations both in the traditional and complex treatment regimens there are no significant changes in fluorescence intensity of tryptophan residues of protein molecules. To assess the conformational changes in membrane proteins, tryptophan fluorescence quenching was studied by an external neutral polar quencher, acrylamide. The value of the effective quenching constant decreased in all experimental groups under different therapeutic approaches. It should be noted that the decrease in the effectiveness of quenching of tryptophan

fluorescence, as evidenced by the decrease in K_{SV}, may be due to the growth of the structural stiffness of membrane proteins. The results indicate that the conformational modification of protein molecules in membranes is due to a decrease in their intramolecular dynamics.

In membrane preparations for all donor-acceptor pairs, the particle of donor molecules that are accessible to quenching by the acceptor did not change. For the first selected pair of fluorophores, a significant reduction in the amount of F_o-F/F_o membrane preparations from animals from traditional treatment by 33% relative to the corresponding control values indicates a decrease in the energy transfer efficiency in the tryptophan-pyrene pair. By the efficiency of pyrene quenching of tryptophan fluorescence, it is possible to estimate the degree of immersion of proteins in the lipid phase of membranes. From such positions one can assert about an increase in the degree of exposure of proteins to the aqueous phase and/or aggregation of protein molecules both on the surface and in the depth of the bilayer, which leads to an increase in the distance between the donor and the acceptor. Taking into account the results presented earlier, the changes are found to be largely due to the movement of proteins to the hydrophilic phase.

Investigation of the IRET in the tryptophan-ANS pair showed that the F_o-F/F_o value also increases only in calf preparations under the traditional regimen (by 19%). The increase in the efficiency of the IRET in a pair of fluorophores suggests a decrease in the distance between them. Since the critical distance of the IRET for the tryptophan-ANS pair is 2.0–3.5 nm and the membrane thickness is about 4.0 nm, a more significant contribution to energy transfer belongs to the fluorophores located on one side of the membrane with respect to the lipid phase. The results are presented, along with the data obtained in the study of the binding parameters of ANS with the membrane, indicate a structural modification of the surface regions of membranes.

So, under the conditions of the traditional treatment scheme, there are a variety of destructive changes in the membranes of small intestine enterocytes, namely: modification of the surface structure of membranes, reduction of the structural order

of the lipid component, and violation of hydrophobic protein-lipid interactions and conformational modification of protein molecules. However, this structural modification is not observed in the case of membrane preparations obtained from calves of group III. That is, the complex treatment scheme with the inclusion of phospholipid-modifying dietary supplements of reparative action accelerates the recovery of indicators characterizing the physical and structural-dynamic state of the apical membranes. This especially applies to the lipid component, since the conformational state of the protein molecules in the membrane is not completely restored.

An important role in the pathogenesis of the development of destructive changes in cell membranes, regardless of etiology, is the intensification of the destructive effect of LPO. Both free fatty acids and unsaturated fatty acid residues in the PL and glycolipids of cell membranes are subject to peroxide oxidation. This, as confirmed by the data obtained, leads to fragmentation of membranes, changes the lipid conformation, leads to the formation of covalent crosslinks between lipid molecules and lipids and proteins. The formation of lipid peroxides can lead to the oxidation of membrane proteins [14]. This leads to the development of irreversible changes that cause cell death.

Considering this, it becomes understandable to use therapeutic regimens of dietary supplements that are capable of stimulating the repair of cell membranes. Thus, in the sick animals immediately after recovery, significant inhibition of the activity of AOP enzymes: catalase, superoxide dismutase and glutathione peroxidase was detected, which may indicate depletion of the enzyme system of antioxidant protection in their diseases and, accordingly, ineffective changes in the disturbed prooxidant-antioxidant balance. However, an intensive decrease in the content of reduced glutathione indicates simultaneous activation in the calves of compensatory mechanisms aimed at the recovery and detoxification of organic peroxides, which are actively formed in reactions of free radical lipid oxidation. It should be noted that a high intensity of LPO was detected in the ill calves, which is confirmed by the growth in the blood of the concentration of TBA-active products. On the 28th and

30th days of life, the activity of superoxide dismutase and catalase and the increased intensity of LPO remain in these calves, as evidenced by a significant increase in the blood plasma content of TBA-active products. However, in these very animals, for the third week after recovery, the activity of glutathione peroxidase increases, which characterizes the development of regenerative processes. In addition, they also showed a tendency to reduce hemoglobin, which often leads to anemia. The latter, perhaps, is a consequence of the pathological changes that the erythrocyte membranes carry.

In the complex treatment scheme with additional inclusion of phospholipids milk BAS immediately after the disappearance of indigestion signs of increased activity of superoxide dismutase, catalase and glutathione peroxidase. At the same time, the concentration of lipid peroxidation products in the blood plasma decreases, which may indicate an effective effect of phospholipid-based supplements on the intensity of LPO flow and the functional activity of AOP factors. On the 30th day of life in these calves, the activity of AOP enzymes was restored and the intensity of LPO was reduced (the content of TBA-active products was reduced to a control level). This indicates a membrane-protective effect of PL milk, that is, their reparative efficacy in relation to the plasmolemal cells that are affected by the development of enteropathology in animals. In these calves, there was also a recovery in the blood of the hemoglobin level, which positively characterizes the biological efficacy of the PL fluids studied relative to the hemoglobin-synthetical function of the red bone marrow. This dietary supplement can be a means of preventing the development of hemic hypoxia, which is a characteristic phenomenon for calves of the rehabilitation period.

The process of restoring the structural and functional state of cells is important during the rehabilitation of animals.

The data of recent years convincingly show that dyslipidemia develops in patients with dyspepsia of calves, which causes a decrease in the reserves of regeneration in the affected tissues and organs. At the same time, as we have established, the normalization of the structural and functional state of the liver,

intestines and kidneys in calves that have recovered with dyspepsia does not end even three weeks after clinical recovery, which can also significantly impede the restoration of lipid metabolism in the body.

The distribution of individual lipids in the body of animals that are dyspeptic with different treatment regimes is uneven.

Thus, the lipid spectrum of blood plasma in calves with the traditional scheme of treatment of dyspepsia on the 30th day of life is characterized by hyperlipidemia, which arises from the probable increase in total cholesterol (due to the free cholesterol fraction) and triacylglycerols, while reducing the level of free fatty acids in the blood plasma and phospholipids. This is a typical picture for the pathology of a similar genesis, which indicates the existing disorders of lipid metabolism in calves, even three weeks after the disappearance of signs of dyspepsia. At the same time, this confirms the presence of functional disorders on the part of the liver, intestine and kidney organs involved in the regulation of lipid metabolism. Along with this, in the blood plasma of the calves of this group the content of phosphatidylcholine and sphingomyelin, which are the main structural components of the outer layer of cell membranes, can be reliably reduced, can slow down the intensity of their recovery. At the same time, in the tissues of calves, the content of both total lipids and individual fractions is normalized for a complex treatment regimen using phospholipid-adjuvant dietary supplements. The exception is a reliably low content of free fatty acids. This is probably due to their intensive use for the biosynthesis of endogenous PL, which is confirmed by the increase in their level in the blood plasma of these animals. In addition, a high content of phospholipids in the blood plasma of calves according to the complex treatment of dyspepsia is explained by the effective absorption of exogenous phospholipids in the intestine of dietary supplements. Among individual PL in the blood plasma of these animals, the content of lysophosphothidylcholine, phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylethanolamine and phosphatidylserine predominate.

The most exogenous phospholipids are delayed by the liver. However, the intensity of their admission to this important organ directly depends on the structural

and functional state of enterocytes in the small intestine. It should be noted that in calves under the traditional regimen of dyspepsia, there is a significant decrease in the content of total lipids in the intestine, primarily total cholesterol and its ester fraction, triacylglycerols and phospholipids. Changes in the PL spectrum of the intestine are also characterized by a possible decrease in the content of their main representatives: phosphatidylcholine, sphingomyelin and phosphatidylinositol. These trends may indicate a lack of recovery of digestion and absorption of lipids in the gastrointestinal tract of calves that have recovered on the 30th day of life. In this case, the lipid/protein ratio corresponds to a control level, which is confirmed by a simultaneous decrease in the intensity of endogenous protein synthesis in these cells.

In a complex treatment regimen in calves of dyspepsia, the recovery of most of the parameters of the lipid spectrum of epithelial tissue of the small intestine is observed. First of all, it concerns the content of common lipids. Similar to calves under the traditional treatment scheme, a significant decrease in the level of esterified cholesterol and triacylglycerols was established. The content of total cholesterol does not differ from the reference values. There was also a significant decrease in the level of free fatty acids against the background of a significantly high PL content. Among the individual phospholipids studied, phosphatidylcholine predominates, which is a positive factor in the course of reparative processes in the intestine. A significant decrease in the lipid/protein ratio was also observed, which indicates an increase in the intensity of protein-synthesizing processes in the epithelial cells of the small intestine.

The reparative effect of the action and recovery of lipid metabolism is noted when using a 1% solution of the liposome form of BAS «FLP-MD» in laboratory mice, patients with experimental enteropathology. Its course in mice is characterized by pronounced clinical and pathological-anatomical signs and changes in the walls of the stomach and intestines. It can be used in veterinary and humane medicine for carrying out experimental work with the purpose of studying the features of metabolism and ultrastructural changes in the organism of animals in the

development of this pathology with varying degrees of severity, as well as for clinical testing of new drugs and introduction of effective treatment regimens into medicine.

These facts give grounds to consider PL milk as attractive new molecules for the development of innovative medicines with reparative properties that can positively influence the course of restorative processes in the affected organs and tissues, and are therefore recommended for correcting lipid imbalances in the animals with enteropathology and other pathological conditions.

LIST OF SYMBOLS AND ABBREVIATIONS

AM	– apical membrane;
AAS	– 1-anilinonaphthalene-8-sulfonate;
AOP	– antioxidant protection;
ATA	– adenosine triphosphoric acid;
BAS «FLP-MD»	– biologically active additive «FLP-MD»;
FFA	– free fatty acids;
RC	– reconstituted cholesterol;
GL	– glycolipids;
ECh	– esterified cholesterol;
FA	– fatty acids(s);
CL	– common lipids;
CP	– common phospholipids;
TCh	– total cholesterol;
IRET	– inductive-resonant energy transfer;
CL	– cardiolipin;
LP	– lipid(s);
LPE	– lysophosphatidylethanolamine;
LPI	– lysophosphatidylinositol;
LPA	– lysophosphatidic acid;
LPP	– lysophospholipids;
LPC	– lysophosphatidylcholine;
MS	– microsomal;

MUFA	– monounsaturated fatty acids;
NLC	– saturated fatty acids;
NSAID	– non-steroidal anti-inflammatory drug;
NULES	– National University of Life and Environmental Sciences of Ukraine;
PUFA	– polyunsaturated fatty acids;
POL	– peroxide oxidation of lipids;
SM	– sphingomyelin;
SMP	– submitochondrial particles;
SMPE	– submitochondrial particles (enterocytes);
SMPL	– submitochondrial particles (liver);
TAG	– triacylglycerol(s);
TBA-active products	– thiobarbituric acid active products;
TT	– traditional treatment;
PG	– phosphatidylglycerol;
PE	– phosphatidylethanolamine;
PI	– phosphatidylinositol;
PA	– phosphatidic acid;
PL	– phospholipid(s);
PS	– phosphatidylserine;
PC	– phosphatidylcholine;
Ch	– cholesterol;
COX	– cyclooxygenase;
FI	– fluorescence intensity of ANS.

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CHAPTER III

LIPID COMPOSITION OF PLASMA MEMBRANES OF ENTEROCYTES OF THE SMALL INTESTINE OF A LEAVED GROUND SQUIRREL IN A STATE OF ACTIVE VITAL ACTIVITY AND GIBERNATION

Studies of animal adaptation to environmental factors are now being given considerable attention by researchers [1–4]. These factors are cold [5], excessive heat [6], shortage of water and food, etc. [7]. However, in these conditions, animals conduct an active lifestyle, combining it with the adaptive state of the organism, in which the intensity of vital activity and metabolism decreases sharply, often without visible manifestations (anabiosis, hypobiosis, hibernation, hypothermia).

The state of hypobiosis in animals is accompanied by characteristic changes in water-electrolyte metabolism [8]. Great importance in the emergence and residence of animals in the state of hypobiosis has the status of the hormonal system of the organism [9–11], as well as a factor such as the change in the concentration of the basic forms of carbon dioxide (pCO_2 i HCO_3^-) [12].

Considerable attention is paid to the study of lipid metabolism and their composition in various organs and tissues in hibernating animals, since lipids are the main energy substratum in their bodies during hibernation [13–14]. Known special role of brown adipose tissue in this period and during the release from it [4]. However, the features of quantitative and qualitative changes in structural lipids in the membranes of cells of hibernating animals, in particular, in membranes of the epithelium of the gastrointestinal tract, which does not function during the period of hibernation [13].

Now the lipid composition of the apical (AM) and basolateral (BM) membranes of enterocytes of the small intestine of animals and humans is described in sufficient detail [15–17]. However, the question of the lipid composition of the plasma membrane of the epithelium of the small intestine of animals during active life and in the state of hibernation remains unexplored. The existence of two forms of vital activity in animals (active state and hibernation) causes a number of peculiarities

in the organization of the structural and functional parameters of the cells of the digestive tract [18]. Although, animals during the hibernation of 6–7 months do not eat, the intestine greatly influences the metabolism in their body.

It has been established that parenteral administration to a warm-blooded animal of the gut extract of a hibernating ground squirrel causes changes in the metabolism and heat production in their body, similar to changes in the state of animal hibernation [19]. These effects are due to the appearance in the tissues of hibernating animals of so-called triggers and antitragers of hibernation, which are inherently polypeptides [8, 14, 20, 21], which indicates the important role of the gastrointestinal tract in the development of hibernation. This is the reason for the urgency of deepening the study of the features of the structure and functions of enterocytes of hibernating animals in an active state of vital activity, in a state of hibernation, and the period of emergence from it. One of the factors that plays an important role in membrane adaptation of animals to extreme conditions may be a change in lipid composition of membranes in tissues [22]. In this context, the relevance of the study of the lipid composition of the apical and basolateral parts of the membrane of enterocytes of the small intestine in animals falling into hibernation is considered. The data of such a plan will allow to expand existing representations on ultrastructural and metabolic features on adaptive processes in the organism of hibernating animals.

3.1 Obtaining fluffy enterocytes from the small intestine of a speckled ground squirrel and isolating apical and basolateral membranes from them

Methods for obtaining fluffy enterocytes [15, 23–24] from the small intestine of various animal species were tested for their effectiveness against the speckled ground squirrel, from which the simplest and most effective method was selected [26]. It should be noted that this method has been determined to be effective also with respect to other objects of investigation, for example, in obtaining small intestine cells of cattle [15], guinea pig [26], rat [27]. However, to conduct our experiments, there were no data on the production of enterocytes from the epithelium of the small intestine of animals that fall into a state of hibernation, including a ground squirrel.

The chemical method with the use of citrate and EDTA gives way to 90% of highly morphologically pure cells [26]. Morphological, biochemical and functional criteria are used to identify the obtained cell fractions. Thus, the differentiated cells from the villi have an elongated shape, a developed bristle border, a higher activity of marker enzymes, such as alkaline phosphatase and sucrose, a greater absorption of methyl glucose and retinol. In contrast, the incorporation of [^{3}H] thymidine in these cells is sharply reduced compared to the cryptal cells. Cell viability is also assessed by the inclusion test of trypan blue and labeled amino acids [23, 25–27].

Having received enterocytes of the small intestine of speckled ground squirrel [28], we used generally accepted methods to evaluate their integrity [29, 30]. As a result, the optimal time for incubation of enterocytes from the small intestine of speckled ground squirrel was established (10 min), which agrees with the literature data for guinea pigs [26] but differs from the isolation of these cells in cattle (15 min) [15].

It should also be noted that the chemical citrate/EDTA method can be used in animals with a low mucus content in the small intestine [15, 26]. Otherwise, the use of this method will result in obtaining an indivisible clot of cells and mucus [15]. Thus, the method used for the speckled ground squirrel meets the necessary requirements.

To isolate plasma membranes from the cells of the epithelium of the small intestine of various animal and human species, many schemes have been developed that differ in methodological approaches [31–35]. Their analysis indicates that in any case it is necessary to introduce into the already existing schemes specific stages of isolation of membrane preparations, determined by the particularity of the object of research, the purpose of the work and its tasks. Particularly noteworthy are the methods in which the separation and purification of AM and BM is achieved by a single isolation procedure [36, 37].

The isolation and separation of AM and BM largely depends on the methods of cell homogenization, homogenization medium selection and homogenate fractionation [17, 32, 36, 37]. However, all these methods of homogenization have their advantages and disadvantages. So, with insufficient homogenization, the cells do not completely degrade, which affects the low yield of the material. With excessive homogenization, the biological structures of the cells are destroyed, which, as a result, contaminate the final fractions of the membranes. These reasons are eliminated by an individual selection of the optimal conditions for homogenization, which are selected empirically. In the case of our experiment, the time for homogenization of mottle-borne ground squirrel was 25 s at a knife rotation speed of 9.5 thousand rpm. For comparison, during homogenization of enterocytes of small intestine of cattle at the same speed, the time was 20 seconds. The degree of homogenization was controlled by light microscopy of the homogenate for the maximum release into the nuclear medium and the absence of large membrane and other fragments [31, 36].

The ratio of homogenization medium to the number of tissues depends on the type of homogenization chosen and requires the selection of optimal conditions. Thus, some researchers use 1-2% homogenate [38], while others use 4-15% and even more [36, 37, 39]. In the experiment with enterocytes of the small intestine of the speckled ground squirrel, we used an 8 % homogenate, as the most optimal for obtaining highly purified membrane fractions.

To isolate membranes of the enterocyte, the following homogenization media are used: iso-osmotic (0.25-0.3 M) sucrose and 5 mM EDTA [16, 31, 36]; iso-osmotic (sucrose or mannitol) without the addition of EDTA and calcium and magnesium salts [17]; hypo-osmotic with EDTA [40]; hypoosmotic with the addition of calcium or magnesium salts to EDTA [32]. However, these homogenization media have some disadvantages: EDTA hypo-osmotic media induce aggregation of nuclei that interfere with the purification of AM; precipitation with calcium and magnesium salts leads to AM fragmentation; in isosmotic solutions, the nuclei are precipitated together with AM. However, only with the use of an iso-osmotic solution with the addition of EDTA can it be possible to separate the nuclei from AM, and the supernatant, which remains after precipitation, is fractionated into other organelles [31, 36].

An important stage in the isolation of AM and BM is fractionation of the homogenate. Thus, in a number of works [17, 32, 39, 40], membrane fractions were obtained by differential centrifugation, and in others [31, 33, 34, 36] – a combination of differential centrifugation and centrifugation in a density gradient. At the same time, we note that purification on a density gradient significantly prolongs the time for isolation of membranes and leads to the loss of a significant amount of the preparation. Therefore, the expediency of this procedure depends on the tasks of the experiment.

The value of the angular acceleration (g) is also important. Thus, in some experiments membrane deposition was performed at low values of g (350–1500) [31, 40, 41], whereas in others 10-12 thousand g and even higher [32, 33, 36, 39]. This is explained by the fact that at large values of g large AM fragments with a large amount of granular material are deposited. Further, vesicular preparations precipitate only at high values of g, which was also used in our work (Figure 3.1).

The degree of purification and contamination of the resulting membrane fractions, one by one and cell elements, is evaluated by the activity of marker enzymes, which are specific for this type of membranes and internal elements of the cell. To assess the purity of AM enterocytes, the activity of alkaline phosphatase is

most often used [31, 34, 37], more rarely – sucrose [33], maltase [40], lactase [42], aminopeptidase [43]. To determine the purity of BM enterocytes, the determination of Na^+, K^+-ATPase activities is more often used [16, 37, 38], less often 5–nucleotidase [43] and adenylate cyclase [32].

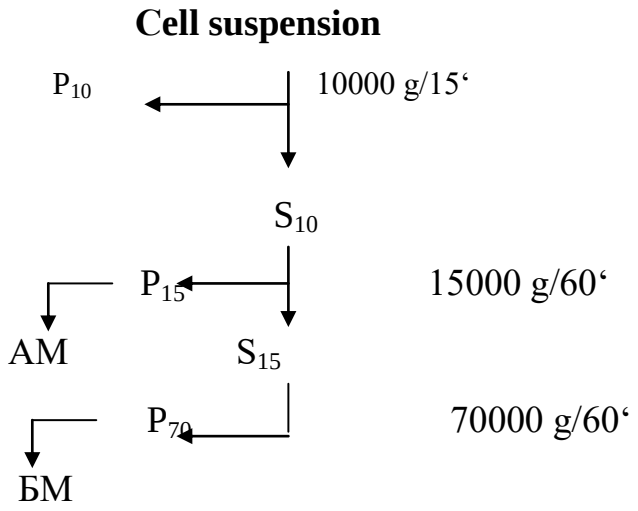

Fig. 3.1. Scheme of fractionation of a suspension of fluffy enterocytes of the epithelium of the small intestine of a speckled ground squirrel

However, not all of the above enzymes are reliable markers, since they can simultaneously be present in both the AM fraction and the BM fraction. To assess the degree of purification of AM and BM in our work, marker enzymes-alkaline phosphatase and Na^+, K^+-ATPase, respectively, were used. In this study, the degree of clearance of AM enterocytes of the small intestine of mottled ground squirrel was 5.0-5.3 times, and BM 5.4-5.6 times. Similar results were obtained with the isolation of plasma membranes of the small intestine of other animals [37, 42]. It should be noted that the contamination of the AM and BM preparations isolated by us with the components of the cell cytosol is insignificant and lies within the limits of the given literature for the membranes of enterocytes of the small intestine of cattle, rats and rabbits [36, 37, 44].

Thus, the qualitative production of AM and BM enterocytes of the small intestine depends on the selection of the homogenization separation medium,

differential centrifugation using high values of the rotor angular acceleration (g) and evaluation of the purity degree by the activity of marker enzymes.

3.2 Phospholipid composition of apical and basolateral membranes of enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity

It has been established that the content of phospholipids in AM and BM enterocytes of the small intestine of the speckled ground squirrel in the state of hibernation is much higher than in the state of its active vital activity (Figure 3.2), which confirms the literature data on the increase in the phospholipids content in membranes of organs and tissues of hibernating animals [13], whereas on awakening, their content is significantly reduced and reaches the indices that are inherent in animals during active life.

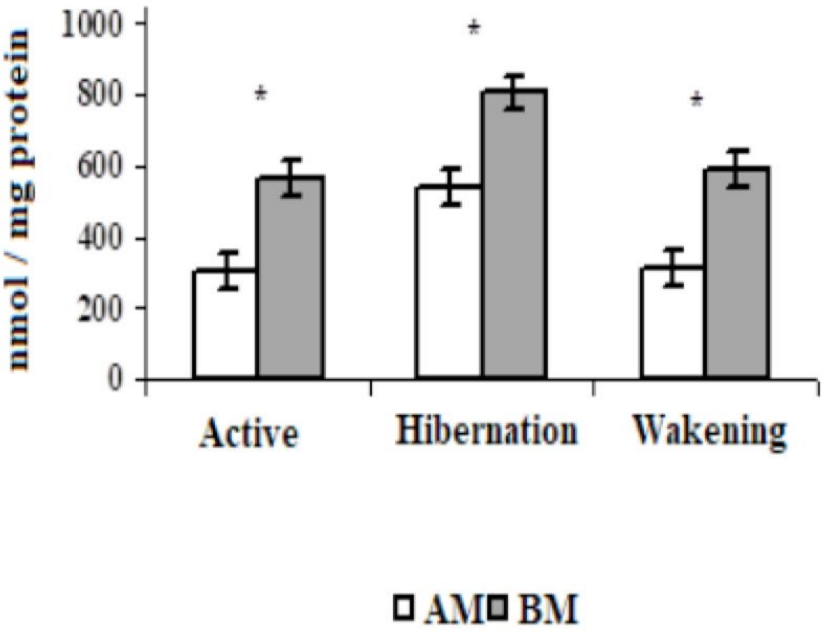

Fig. 3.2. The content of phospholipids in the apical (AM) and basolateral (BM) membranes of the enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity (M ± m, n = 5, p <0,05)

As a result of the study of AM and BM enterocytes of the small intestine of the speckled ground squirrel, four phospholipids were detected in both membranes:

phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and sphingomyelin (SM) (Table 3.1; 3.2).

Both in AM and in BM enterocytes of the small intestine of experimental animals, PC and PE are most abundant, which agrees with the data obtained in the study of the phospholipid composition of these membranes of bovine enterocytes [45], rat [16], guinea pig [26], mouse [46] and rabbit [17].

Changes in the qualitative composition of phospholipids in AM and BM enterocytes of the small intestine of the speckled ground squirrel were not detected during the period of hibernation.

Table 3.1

The content of phospholipids in the apical membranes of the enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity, nmol/mg protein (M ± m, n = 5)

Phospholipid	Active state	Hibernation	Awakening
Phosphatidylcholine	122.6±5.8	180.5±0.3*	106.1±6.3
Phosphatidylethanolamine	117.7±5.1	228.7±5.2*	135.3±8.4
Phosphatidylinositol	20.3±2.3	56.7±5.6*	28.2±3.0
Sphingomyelin	40.9±1.9	73.9±4.6*	44.2±2.8

Note: here and further * - the difference is reliable in comparison with the active state of animals, $p < 0.05$.

In the AM enterocytes of the small intestine of the speckled ground squirrel in a state of hibernation, in comparison with AM enterocytes of animals during active life, the content of all individual phospholipids significantly increases. Particularly increases the content of Fl, which can significantly affect the maintenance of the electrochemical gradient in the cell [47].

The content of all individual phospholipids in the BM enterocytes of the small intestine of experimental animals in the period of hibernation, as compared with this index during their active life, also increases significantly. Especially the SM content is increased (almost 3.2 times), which may be due to its connection with the

cholesterol pool and the formation of microdomains in the membrane [48]. Sfingomyelin can also reduce the ability of cholesterol to migrate to the membranes of cellular organelles [49].

The qualitative composition of the studied phospholipids in AM and BM enterocytes of the small intestine does not change after the awakening of the animals. However, upon the awakening of point ground squirrels, the content of phospholipids in AM and BM enterocytes of the small intestine is reliably reduced, and almost reaches the parameters that are characteristic of gophers during active life.

Table 3.2

The content of phospholipids in the basolateral membranes of the enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity, nmol/mg protein (M ± m, n = 5)

Phospholipid	Active state	Hibernation	Awakening
Phosphatidylcholine	229.3±14.4	267.4±13.1*	215.9±11.5
Phosphatidylethanolamine	242.8±15.9	332.5±24.1*	242.1±14.2
Phosphatidylinositol	60.7±5.4	92.4±9.1*	55.6±6.1
Sphingomyelin	36.2±3.5	114.8±5.3*	80.5±6.3*

In the BM enterocytes of the small intestine during the awakening of animals, the content of all individual phospholipids, with the exception of SM, reaches the indices that are characteristic of them in a state of active vital activity. The content of SM in BM enterocytes during the awakening period was 2.2 times higher than in the state of active vital activity. As a result, a large microviscosity can be stored in BM enterocytes of the ground squirrel during the awakening period. These data are of interest due to the fact that the microviscosity of the membranes controls the cellular metabolism [50]. On the other hand, the high content of SM in BM gnatocyte enterocytes during hibernation and on awakening can play an important role in immune cell protection.

In general, the data obtained by us on changes in the content of phospholipids in AM and BM enterocytes of the small intestine of the speckled ground squirrel, depending on the state of vital activity, attest to their important role in adaptation processes to conditions conditioned by seasonal factors.

3.3 The content of cholesterol in the apical and basolateral membranes of enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity

The data obtained indicate that the content of cholesterol in AM and BM enterocytes of the small intestine of point gophers, which are in an active state of vital activity, differ significantly. Thus, the cholesterol content in BM, in comparison with AM enterocytes, was almost 2.9 times higher. The same differences in cholesterol content in AM and BM remain in animals during and after hibernation (Table 3.3).

Table 3.3

The content of cholesterol in the apical (AM) and basolateral (BM) membranes of the enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity, nmol/mg protein (M ± m, n = 5)

Condition of the animal	The apical membrane	Basolateral membrane
Active state	155.0±22.2	443.3±31.3
Hibernation	249.3±15.7*	624.4±47.9*
Awakening	178.7±4.0*	500.8±19.7

In the period of hibernation of gophers in comparison with the active state of their vital activity, the content of cholesterol in AM and BM enterocytes of the small intestine is significantly increased. Similar results were obtained when studying the cholesterol content in the hepatocyte membranes of the liver of the ground squirrel small [52]. This indicates the influence of hibernation on the content of cholesterol in the cell membranes of animal tissues.

When awakening experienced animals, the cholesterol content in the plasma membranes of the enterocytes of their small intestine is reduced. However, the level of cholesterol in this period remains at a high level in comparison with the indices

characteristic of active life and affects, as shown below, the ratio of cholesterol/phospholipids in plasma membranes of enterocytes (Table 3.4).

Table 3.4

The molar ratio of cholesterol/phospholipids in the apical and basolateral membranes of the enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity (M ± m, n = 5)

Index		Cholesterol, nmol/mg protein	Phospholipids, nmol/mg protein	The molar ratio of cholesterol/ phospholipid
AM	Active state Hibernation Awakening	155.0±6.2 249.3±15.7* 178.7±4.0*	327.7±8.5 573.2±19.3* 339.9±10.9	0.47±0.01 0.43±0.02 0.53±0.03
БМ	Active state Hibernation Awakening	443.3±31.3 624.4±47.9* 500.8±19.7	613.2±16.0 857.1±17.6* 638.8±19.1	0.72±0.05 0.73±0.04 0.78±0.03

It is known that cholesterol has an important role in ensuring the physical state of biological membranes - their microviscosity [48, 53, 54]. However, microviscosity and permeability of cell membranes largely depend on the molar ratio between the content of cholesterol and phospholipids in them [45, 55].

The data obtained by us indicate that during the active state of activity of ground squirrels the ratio of cholesterol/phospholipids in AM enterocytes of the small intestine is lower than in BM almost 1.5 times and remains at this level during and after hibernation.

During the period of hibernation, the molar ratio of cholesterol/phospholipids in AM enterocytes of the small intestine of the speckled ground squirrel decreases slightly, but remains unchanged in BM. A decrease in the ratio of cholesterol/ phospholipids, while increasing the content of phospholipids, was also observed in

the bristle border of the small intestine of rats during fasting [56]. So it is possible that the process, which is found in the plasma membranes of the small intestine of the speckled ground squirrel in a state of hibernation, is similar to that which occurs during fasting in homothermal animals. On the other hand, increasing the phospholipid content in membranes during animal starvation can be considered as an adaptation mechanism in response to a change in body temperature below the threshold level that occurs in hungry animals [57].

When awakening ground squirrels the ratio of cholesterol/phospholipids in AM and BM enterocytes increases, which leads to a decrease in their permeability for ions and metabolites [55] and may be important in the transition from winter sleep to wakefulness of the state of vital activity [58].

Thus, the content of cholesterol in AM and BM enterocytes of the small intestine of the speckled ground squirrel increases in the period of hibernation in comparison with the active state of vital activity, and in the period of awakening it decreases to the values revealed in the state of active vital activity. However, the molar ratio of cholesterol/phospholipids during the hibernation of speckled ground squirrel decreases only in AM enterocytes, whereas in the awakening period compared to the active state of the animal's vital activity, this index in both membranes increases. Such changes in the content of structural lipids in AM and BM enterocytes of the small intestine of the speckled ground squirrel may indicate, first of all, structural and functional rearrangement caused by inhibition and restoration of metabolic processes in the animals.

3.4 The lipid/protein ratio in the apical and basolateral membranes of the small intestine enterocytes of the speckled ground squirrel at various states of vital activity

The conducted studies showed that the lipid/protein ratio in the active state of the mottled squirrel in AM enterocytes of the small intestine was lower than in BM almost 1.2 times, while in the period of hibernation this figure in AM was almost 1.4 times higher, than in BM. The same differences in lipid/protein ratio in these membranes persist even during the awakening of animals (Table 3.5).

Table 3.5

The lipid/protein ratio in the apical and basolateral membranes of the small intestine enterocytes of the speckled ground squirrel at various states of vital activity (M ± m, n = 5)

Index		Total lipids, mg	Total protein, mg	Ratio lipid/protein
AM	Active state	8.4±0.4	10.4±0.4	0.80±0.04
	Hibernation	9.0±0.3	5.9±0.2*	1.52±0.03*
	Awakening	8.6±0.3	7.1±0.3*	1.17±0.01*
BM	Active state	7.0±0.6	7.3±0.5	0.95±0.02
	Hibernation	7.2±0.6	6.5±0.4	1.10±0.01*
	Awakening	6.9±0.1	6.8±0.3	1.00±0.02

In AM enterocytes in the period of hibernation of experimental animals in comparison with the active state of vital activity, the lipid/protein ratio increases by 1.9 times, while in BM this index increases significantly less. During the awakening of the speckled squirrel, the lipid/protein ratio in AM enterocytes decreases significantly more than in BM, but does not reach the level found during active life.

The data obtained indicate a rapid reorganization of AM enterocytes of the small intestine of the speckled ground squirrel with changes in the physiological state. It is possible upon awakening of animals that it contributes to the rapid incorporation of enzyme systems, due to which the content of protein in membranes increases. As for lipids, their content in the membranes decreases, since a significant activation of the processes of lipid peroxidation during the awakening of the animal leads to the replacement of old membrane structures or their individual sites [59].

Thus, the change in the lipid/protein ratio at different periods of life of the mottled squirrel is characteristic only of AM enterocytes of the small intestine, and in BM, this index is stable throughout the life periods of animals.

3.5 Fatty acid composition of lipids of apical and basolateral membranes of enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity

Among fatty acids in lipid AM and BM enterocytes of the small intestine of the speckled ground squirrel in a state of hibernation and in the period of awakening in comparison with the active state of its vital activity, there are no qualitative changes in their composition. However, the total fatty acid content in lipids of AM enterocytes during the hibernation of animals increases almost 1.9 times, whereas in BM lipids their content decreases almost 1.3 times. During the awakening period, the total fatty acid content in AM enterocyte lipids is higher than in the active state by 1.2 times, and in BM lipids it decreases 1.6 times (Figure 3.3).

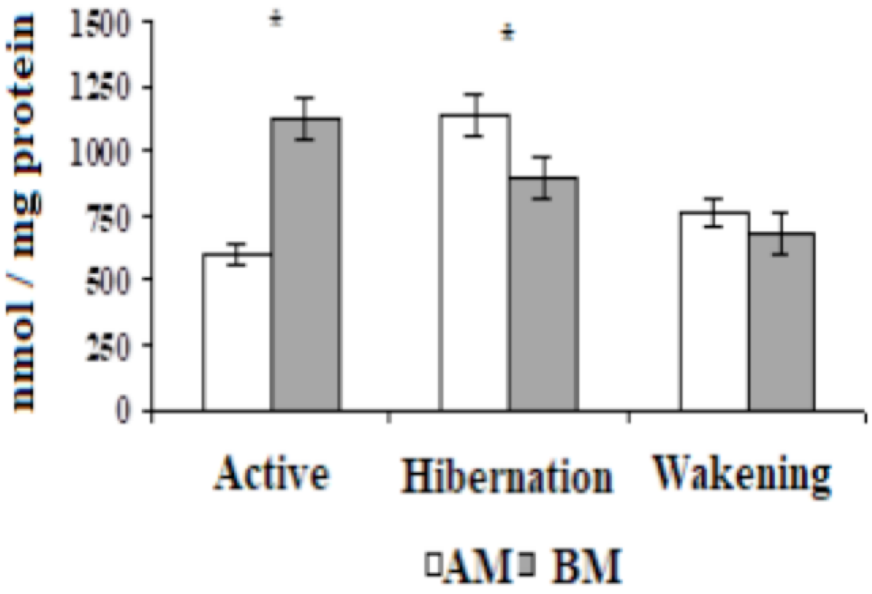

Fig. 3.3. The content of fatty acids in the lipids of the apical and basolateral membranes of the small intestine enterocytes of the speckled ground squirrel at various states of vital activity (M ± m, n = 5)

As a result of the study of lipids AM and BM enterocytes of the small intestine of the speckled ground squirrel, eight fractions of fatty acids were found in the state of active life: six saturated – lauric ($C_{12:0}$), myristic ($C_{14:0}$), pentadecane ($C_{15:0}$),

palmitic ($C_{16:0}$), heptadecane ($C_{17:0}$), stearic ($C_{18:0}$), and two unsaturated ones – palmitoleinic ($C_{16:1}$), and oleic ($C_{18:1}$), (Tables 3.5, 3.6).

Table 3.5

The content of fatty acids in lipids of the apical membrane of enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity, nmol/mg protein (M ± m, n = 5)

Fatty acid	Active state	Hibernation	Awakening
$C_{12:0}$	5.1±0.5	10.9±2.2*	8.3±1.0*
$C_{14:0}$	21.2±1.0	44.7±9.2*	34.7±6.1
$C_{15:0}$	12.3±0.9	44.7±9.8*	30.9±5.1*
$C_{16:1}$	32.3±2.6	98.9±17.5*	76.1±11.2*
$C_{16:0}$	193.7±10.8	324.1±33.8*	229.2±17.8
$C_{17:0}$	10.4±1.2	33.7±4.7*	23.5±2.9*
$C_{18:1}$	229.3±11.9	404.9±34.4*	240.0±16.1
$C_{18:0}$	99.2±8.9	173.6±24.5*	113.9±5.5

Lipids of AM and BM enterocytes of the small intestine of the speckled ground squirrel during the active period of their vital activity are characterized by a high content of palmitic and stearic fatty acids, which is consistent with the data obtained in the study of the fatty acid composition of lipids of plasma membranes of rat enterocytes [32], rabbit [60] and cattle [45]. Among fatty acids in lipids of plasma membranes of enterocytes, ground squirrels contain the most oleic acid, which can be considered as a feature of this species of animals.

The content of C12:0 fatty acid in AM lipids of small intestine enterocytes in the state of hibernation was 2.1 times higher, C14:0 – in 2.1, C15:0 – in 3.6, C16:1 – in 3,1, C16:0 – in 1,7, C17:0 – in 3,2, C18:1 and C18:0 – in 1,7 times. An essential feature of AM lipids is the high content of fatty acids with an odd number of carbon atoms C15:0, C17:0 and palmitooleic acid (C16:1). The important role of fatty acids with an odd number of carbon atoms in the lipids of a gopher body in the stage of

hibernation can be explained by the fact that, unlike fatty acids with an even number of carbon atoms, they can participate in gluconeogenesis, that is, they turn into glucose [61].

Table 3.6

The content of fatty acids in the lipids of the basolateral membrane of small intestine enterocytes of the speckled ground squirrel at various states of vital activity, nmol/mg protein (M ± m, n = 5)

Fatty acid	Active state	Hibernation	Awakening
$C_{12:0}$	9.3±0.6	13.1±1.0*	9.9±0.5
$C_{14:0}$	38.5±3.1	47.7±3.3*	32.5±1.9
$C_{15:0}$	37.6±3.1	49.1±5.8*	31.8±2.4
$C_{16:1}$	31.5±2.2	41.3±3.4*	36.3±2.3
$C_{16:0}$	317.4±13.6	242.7±15.7*	192.0±13.8*
$C_{17:0}$	55.8±5.2	86.6±4.6*	68.8±5.1
$C_{18:1}$	447.1±35.6	288.8±14.6*	213.0±18.1*
$C_{18:0}$	189.4±11.8	121.8±11.0*	99.1±9.8*

In the lipids of BM enterocytes, the content of some fatty acids in the period of hibernation was higher than in the active period of life: $C_{12:0}$ – 1.4 times; $C_{14:0}$ – in 1.2; $C_{15:0}$ – in 1,3; $C_{16:1}$ – in 1,3; $C_{17:0}$ – 1.5 times. At the same time, the content of $C_{16:0}$, $C_{18:1}$ and $C_{18:0}$ lipids of BM enterocytes of ground squirrels in the state of hibernation was accordingly 1.3 times, 1.5 and 1.5 times lower than in the active state of vital activity.

It should be emphasized that a decrease in the fatty acid content in the lipid of the BM enterocytes of the small intestine of the speckled ground squirrel during the hibernation does not affect the phospholipid content in this membrane, which can be considered a feature of lipid metabolism in these animals.

On the other hand, it is possible that the decrease in the fatty acid content in lipids of GM gnatocyte enterocytes during hibernation occurs due to a decrease in the glycolipid content [50] and an increase in the SM content, which has only one acyl residue in the structure [49, 62], which can also affect the content of fatty acids in the lipids of plasma membranes of enterocytes.

As a result of the study of fatty acid composition of lipids AM and BM enterocytes of the small intestine of the speckled ground squirrel upon waking, there are no changes in the qualitative composition of fatty acids. The content of fatty acids $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:1}$, $C_{16:0}$, $C_{17:0}$, $C_{18:1}$ and $C_{18:0}$ lipid AM was close to its content in lipid AM enterocytes of the small intestine gopher in the active state of life. In BM lipids, the content of $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:1}$, $C_{17:0}$ of fatty acids was close to its content in lipids of GM gopher enterocytes during the active state of vital activity, whereas the content of $C_{16:0}$ fatty acids, $C_{18:1}$, $C_{18:0}$ in this case was significantly lower.

Structural and functional changes in the lipid bilayer of cell membranes caused by changes in the ratio of saturated fatty acids to unsaturated in phospholipids [63], which affect the surface properties of phospholipids, protein-lipid and lipid-lipid interactions, as well as the functioning of membrane-bound enzymes [62].

Our studies have shown that in the active state of the suslik, the ratio of saturated/unsaturated fatty acids in the lipids of AM and BM enterocytes of the small intestine is almost the same (Table 3.7).

Reduction of body temperature of animals in a state of hibernation is accompanied by an increase in desaturase activity, as a result, the content of unsaturated fatty acids in the lipid composition of membranes increases [13]. However, in the period of hibernation of the ground squirrel, compared with the state of its active vital activity, the ratio of saturated/unsaturated fatty acids in the lipids of AM enterocytes of the small intestine decreases slightly, while in MB lipids it rises by 1.3 times.

In lipid membranes of kidney gopher cells during the period of hibernation and during the active period of vital activity, an increase in the content of unsaturated

fatty acids is also absent [64, 65]. This was also observed in membranes of hamster erythrocytes in the period of hibernation [66]. It is important to note that the role of unsaturated fatty acids in adaptive reactions of membranes of warm-blooded animals is less expressed than in cold-blooded animals [13].

Table 3.7

Fatty acids of lipids of the apical and basolateral membranes of enterocytes of the small intestine of the speckled ground squirrel at various states of vital activity, nmol/mg of protein (M ± m, n = 5)

Index		Fatty acid		The saturated / unsaturated
		saturated	unsaturated	
AM	Active state	341.9±17.7	261.7±9.4	1.31±0.08
	Hibernation	631.9±23.0	503.9±45.5*	1.30±0.14
	Awakening	440.7±17.8*	316.1±17.9*	1.40±0.09
BM	Active state	628.0±26.7	478.4±37.7	1.35±0.15
	Hibernation	561.0±32.7	330.1±15.8*	1.70±0.15
	Awakening	434.1±20.0*	249.3±20.4*	1.75±0.16

When awakening gophers, in comparison with the state of active life, as shown by our studies of the ratio of saturated/unsaturated fatty acids in the lipids of AM and BM enterocytes of the small intestine increase.

Consequently, the level of fatty acids in lipids AM and BM enterocytes of the small intestine of the speckled ground squirrel can explain some features of the structure of the lipids of the plasma membrane and their functions at different periods of the animal's vital activity.

Thus, the results of the study established the features of the lipid composition of the apical and basolateral membranes of small intestine enterocytes of the speckled ground squirrel in conditions of active state of vital activity, hibernation and when it

leaves it. The quantitative changes in the fatty acid composition and the ratio of individual classes of lipids are revealed, which expand the existing understanding of the organization of lipid membrane components in the structure of various parts of the plasma membrane of the epithelium of the small intestine of animals.

Optimal conditions for isolating isolated fluffy enterocytes and obtaining apical and basolateral parts of their plasma membrane from the epithelium of the small intestine of mottled ground squirrel were established.

The content of phospholipids in the apical and basolateral membranes of the enterocytes of the small intestine of the speckled ground squirrel increases significantly in the state of hibernation, and on waking it decreases to the level found during active life.

In the apical and basolateral membranes of the enterocytes of the small intestine of the speckled ground squirrel, four representatives of phospholipids were detected: PC, PE, PI, and SM; the content of all individual phospholipids in both membranes of enterocytes during the hibernation of animals is significantly higher than in the active state of life and on awakening; the content of SM in BM spotted gopher enterocytes remains high and on awakening.

The cholesterol content in AM and BM enterocytes of the small intestine of the speckled ground squirrel is significantly higher in the hibernation period than in the state of active vital activity and on awakening.

The molar ratio of cholesterol/phospholipids in the apical membrane of enterocytes of the small intestine of the speckled ground squirrel decreases during the hibernation period, but does not change in BM; When the animal wakes up, this ratio rises in both membranes.

The lipid/protein ratio in the AM enterocytes of the small intestine of the speckled ground squirrel during the hibernation and on awakening is 1.9 and 1.4 times higher, respectively, than in the active state, in BM this indicator does not change.

The content of fatty acids in the lipid fraction of AM enterocytes of the small intestine of the speckled ground squirrel during the period of hibernation is much

higher than in the state of active vital activity, whereas in BM there are no such differences; when waking animals, the content of fatty acids in AM lipids significantly decreases than in BM lipids.

In the lipid AM and BM enterocytes of the small intestine of the speckled ground squirrel, eight fatty acids were isolated: $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$ $C_{18:1}$. The content of fatty acids $C_{15:0}$, $C_{16:1}$ and $C_{17:0}$ lipids AM in the period of hibernation and when the suslik is awakened is 3 and 2 times higher respectively, than in the state of active vital activity; in lipids of BM during these periods, the content of $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ fatty acids is reliably reduced.

The content of saturated fatty acids in lipids AM and BM enterocytes of the small intestine of speckled ground squirrel in a state of active vital activity is dominated by the content of unsaturated fatty acids and remains unchanged during the period of hibernation and on the awakening of animals.

LIST OF CONVENTIONAL SYMBOLS AND ABBREVIATIONS

EDTA – Ethylenediaminetetraocetic acid;

AM – Apical membrane;

BM – Basolateral membrane;

PC – Phosphatidylcholine;

PE – Phosphatidylethanolamine;

PI – Phosphatidylinositol;

SM – Sphingomyelin.

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Монографія

МЕМБРАНИ ЕНТЕРОЦИТІВ ТОНКОГО КИШЕЧНИКУ ПРИ ПАТОЛОГІЇ ТА ЗА УМОВ ГІПОБІОЗУ