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РЕСПУБЛИКИ КАЗАХСТАН

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АГРАРНЫЙ УНИВЕРСИТЕТ

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**THE EFFECT OF TOXICANTS
ON THE MEMBRANE HYDROLYSIS
OF THE DIGESTIVE TRACT IN ANIMALS**

Abstract. The article deals with the effect of cadmium on abdominal and membrane hydrolysis of the digestive tract in rats. Continuous worsening of the ecological condition, increase in environmentally harmful factors, deterioration of ecological condition in the food industry in the world determines the high relevance of this research topic. Salts of heavy metals, including ions of cadmium are dangerous; they cause activity disorders of liver, kidneys and in blood circulation. In our studies we determined the effect of toxicants on the abdominal and membrane hydrolysis of the digestive tract on the activity of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT)ferments. As a result of the study, the activity of enzymes in the stomach was determined, both in the small and large intestines, in normal condition and after poisoning with toxicant.

Under poisoning, the increase of the amount of alkaline phosphatase is observed; the level of enzymes of aminotransferase has increased which is related to liver activity disorder; in pancreas and liver there is a strengthening of cytologic processes; the synthesis of protein has decreased and the transport function of metabolism gets disrupted. The obtained results have practical significance in ecology, medicine, agriculture, and the food industry. The results derived from the research make it possible to create a database of the effect of cadmium on the activity of the digestive system of mammals, and can be the basis for finding ways to solve this problem.

Key words: rats, toxicants, membrane hydrolysis, enzymes, digestive tract.

Introduction. Chemicals that pose a threat to human health and pollute the environment are industrial toxicants, such as cadmium, lead, mercury, and other volatile organic compounds. Knowledge of the mechanisms of the involvement of the circulatory lymphatic system, the preservation of balance in case of a disturbance in the adaptation reaction in the living organism will make it possible to reduce the negative influence of extreme factors on the visceral function, and also take preventive measures against them [1].

As a result of the conducted studies [1, 2], the effect of cadmium ions on the lymphatic flow and on the contractile activity of lymphatic vessels was clarified, along with deriving information on the negative effect of cadmium salts on the composition of blood and lymph proteins, as well as on the prothiolytic activity of cells [3, 4].

Cadmium salts also affect other cells and organs of a human. Conducted laboratory tests on animals have shown that when cadmium salts enter the body via different paths, in a short time - in around 12-16 weeks, the liver happens to increase while other organs and tissues of the body get damaged [5, 6].

Salts of heavy metals, and among them cadmium ions, are considered dangerous, as they cause disturbances in liver and kidney activity, as well as in blood circulation. Under poisoning with salts of heavy metals, and among them with salts of cadmium, now it is necessary to clarify the mechanism of the negative influence of cadmium ions on the body; at the same time in the modern period it has become relevant to study ways to restore the activity of organs after damage. The substances that poison the human body lead to various diseases. Poisoning with salts of heavy metals leads to cardiovascular

diseases, endoarthritis, thrombosis, gastrointestinal diseases, which can lead to both temporary and full-disability [7, 8].

The most vulnerable to various toxic substances are cells, subcells of membranes of organelles, important compounds of the biosynthetic system, for example, histamine, hormones, as well as oxidation of lipids, as one of the modern mechanisms of biomembrane damage. Damage to the cell begins with the release of a toxic substance into the organism and its appearance in the environment of the cell membrane [9, 10]. Cadmium changes the blood-brain conductivity of external systems and microvessels. Cadmium ions are absorbed in the membrane, penetrate into the red blood cells, then connect with the plasma protein and penetrate into other tissues. In erythrocytes, 70% of cadmium is retained [11].

In connection with this, the study of the function of the gastrointestinal tract, the functions of its exchange and drainage and the penetration of cadmium ions into the intercellular space and into the membrane exchange has great theoretical and practical significance. The common vital physiological and biochemical processes are based on the function of the biological membrane; in connection with this, under the poisoning of organs and tissues with various toxic substances, there is no information on the transport function of the gastrointestinal tract. In consequence of damage to the function of biomembrane, a chain of pathological processes appears in organism [12].

In the modern period, researchers are much interested in studying the ways of the transport function of the gastrointestinal tract, their biochemical indices and properties. In this regard, the main goal of our research was to study the effect of cadmium salts on the organism, in particular, investigating the changes occurring in the digestive system.

The aim of the research: to determine the activity of enzymes involved in the membrane hydrolysis of the digestive tract of rats in normal conditions and under intake of cadmium salts.

Materials of the research. In our research, the effect of salts of heavy metals, in particular cadmium salts on the membrane transport of the digestive system, was studied. The research was conducted on 50 adult white rats with an average weight of 230-250 grams.

Method of research. In order to study the effect of cadmium ions on membrane and cavity transport through the digestive tract, the rats were daily given a 200-ml cadmium chloride solution throughout 30 days. To determine the effect of the toxicant on the enzymatic activity, the experimental rats were decapitated after anesthesia. To determine the toxicity of heavy metal salts, laboratory rats were divided into two groups: the first group (20 rats) and the second experimental group (30 rats). To poison the rats, aqueous solutions of heavy metals were used. The salts of heavy metals, namely, an aqueous solution of cadmium chloride, were given to the experimental group in the amount of 200 ml per day for 30 days. An aqueous solution of cadmium chloride was prepared at a concentration of 0.05 mg/l (0.02 mg/kg) [13].

Determination of membrane digestion was carried out by the method of A.M. Ugolev. The tissues of the thin, large intestines and stomach were rinsed in Ringer-Locke solution (1:1), ground three times for 120 seconds each in a Polytron homogenizer; the homogenate was first centrifuged at the speed of 10,000 g for 30 minutes, then at the speed of 30,000 g for 60 minutes. Biochemical indices were derived from the obtained homogenates of the small, large intestines and stomach.

In membrane digestion, the enzymes alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) contained in the small and large intestines and stomach were determined by the Raitman-Frenkel method; the activity of alkaline phosphatase based on the hydrolysis of p-glycerophosphate was determined by the Bodansky method; the amount of α -amylase was determined by the amyloclastic method [14] with the help of the clinical diagnostic complex "Bio-Lachema-Test" (Czech Republic) using a biochemical analyzer "Biochem FC-360" (USA).

The obtained indicators were processed by a computer program Microsoft Excel, and also statistical data processing by the Student and Fisher criterion was used, performing calculations in the interval of $p < 0.05$ and $p < 0.001$.

Analysis and discussion of the data obtained. As a result of the study, during poisoning, the influence on the process of digestion was observed along with the disturbance of the equilibrium of water metabolism in the animals' organism, the water retention in organs and tissues was also noted. This led to an increase in the weight of some organs. In the organism, protein metabolism, lipid metabolism and the exchange of enzymatic processes are violated; the interstitial fluid in the liver and in the kidneys decreases. These processes are likely to be associated with the accumulation of cadmium ions in the organ tissues.

Table 1 – Effect of cadmium salts on biochemical indices of membrane digestion.

Indicator name	Observation group	Poisoning with cadmiumsalts
Smallintestine		
ALAT, IU/l	272,0±9,7	291,3±8,5*
ASAT, IU/l	263,9±7,2	287,4±6,9*
Alkalinephosphatase, IU/l	48,9±3,8	89,1±4,6*
Totalamelase, IU/l	46,4±3,3	60,1±4,1*
Largeintestine		
ALAT, IU/l	264,1±4,2	289,7±6,1*
ASAT, IU/l	212,7±3,5	238,9±5,4*
Alkalinephosphatase, IU/l	52,4±2,1	64,6±3,9*
Totalamelase, IU/l	7,13±0,3	10,2±0,1*
Stomach		
ALAT, IU/l	223,9±2,6	287,3±5,3*
ASAT, IU/l	231,4±4,1	299,6±2,5*
Alkalinephosphatase, IU/l	105,5±1,8	164,1±3,7**
Totalamelase, IU/l	52,8±2,2	87,3±2,9**
Note: the purity of observation when comparing: *P < 0,05; **P<0,01.		

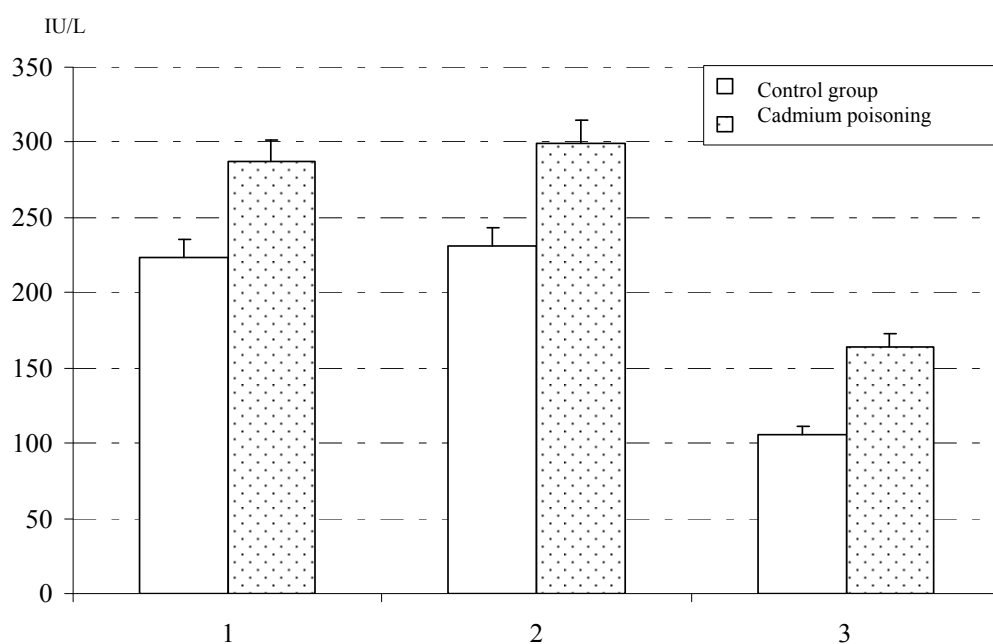
In the process of digestion by A. M. Ugolev, the membrane one is the second period of digestion. As a result of our studies, a negative effect of cadmium ions on membrane exchange was revealed (table 1).

In the course of the study, under both normal conditions and when poisoned with cadmium salts, chymes were taken from various areas and placed in the measuring tube; the mass of the mucosa was measured: the mass of small intestine was 0.048±0.0001 mg, of the large intestine - 0.065±0.0003 mg, of the stomach - 0.092±0.0002 mg.

The general membrane digestion proceeds due to the peculiarities of the structure of mucosa of the small intestine. We noted the presence of villi and microvilli in the intestinal mucosa [15]. The structure of the membrane is surrounded by a complex enzymatic system, at the same time, in the direction of the membrane, there is a holdup of enzymes from the intestinal cavity. Underpoisoning with cadmium salts, the membrane metabolism in the digestive system has a different directionality. This, in time, can lead to changes in enzymes of aminotransferase. During the study process, an increase in the parameters of alkaline phosphatase in the small intestine was observed at 82%, 89.1±4.6* IU/l; in the control group, these values were at 48.9±3.8 IU/l (table 1, figure 1).

Aspartate aminotransferase is one of the main enzymes of protein metabolism in the body. This enzyme is involved in the synthesis of amino acids, is part of the cell membrane and tissue. ASAT is active in all organs. In connection with such feature, aminotransferase can be attributed to specific enzymes. Alanine aminotransferase is synthesized in many human organs: in the kidneys, in the heart muscle, in the liver, and even in the skeletal musculature. One of the main functions of the enzyme is to participate in the turnover of amino acids. This enzyme is a catalyst in the transition of alaninamic acid to alpha-ketogluthorate. As a result of the conversion of the amino group, glutamine and pyruvic acid are synthesized.

ASAT mainly occurs in the myocardium (heart muscle), hepatocyte (hepatic cell), muscle tissue and brain neurons. The higher the activity of cytolysis, the higher the value of ASAT. When the cell structure is destroyed, the plasma activity decreases. The analysis of ALAT helps to determine changes in myocardial cells (cytolysis) or various deviations of the hepatic cell. The results of ALAT show not only specific cell abnormalities, but also serve as an indicator for differential diagnosis of heart and liver pathology. Based on anatomical, morphological, histological studies we are aware about the presence of villi and microvilli in the mucous membrane of the stomach and intestines. There is a complex enzymatic system in the structure of the membrane surrounding the mucous membrane; at the same time the enzymes

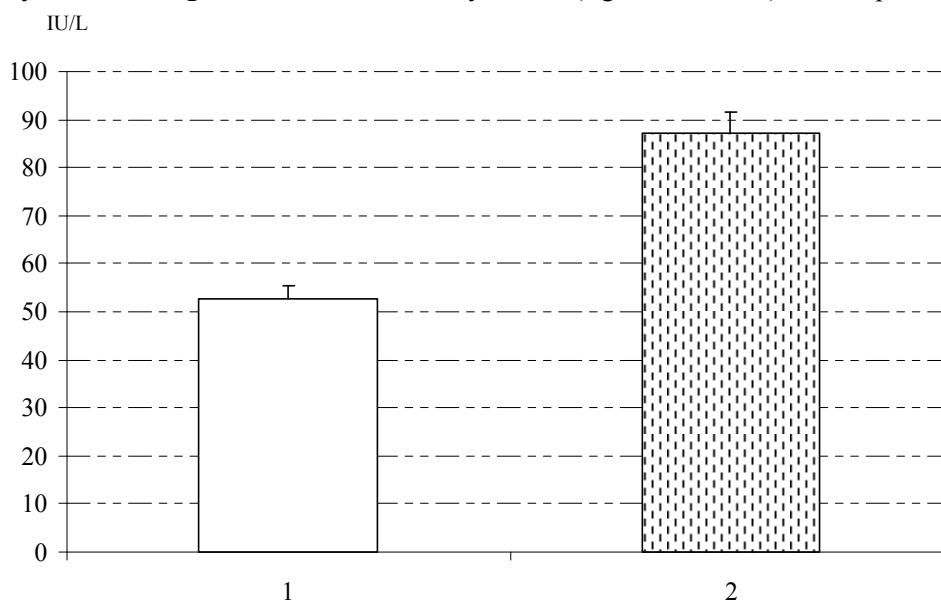


Designations: along the ordinate axis - ALAT, ASAT, alkaline phosphatase in IU/l, along the abscissa - 1 - ALAT, 2 - ASAT, 3 - indicators of alkaline phosphatase.

Figure 1 – Biochemical indices of membrane digestion in the stomach under control conditions and under poisoning with salts of heavy metals

are hold up from the inside of the membrane and from the intestinal cavity. Microvilli and enzymes in the membrane composition are involved in the digestion of food. And thus, the processes of splitting, digesting and influencing the biochemical and physiological processes proceed.

As a result of the analysis of literature data, information was obtained that under poisoning with cadmium salts, there are changes in the contents of ALAT, ASAT, trypsin, amylase, lipase, total protein, urea, creatinine, bilirubin and hematological parameters. When poisoning with salts of heavy metals, the content of amylase in the digestive tract increased by 29.5% (figure 6, table 2). In comparison with norm,



Designations: along the ordinate axis - amylase level in IU/l, along the abscissa - 1 - control group, 2 - group of animals poisoned with cadmium salt.

Figure 2 – Indices of the level of amylase in the stomach of rats under membrane digestion

the content of amylase in the small intestine increased by 1.2 times, 60.1 ± 4.1 * IU/l (table 1). An increase in the content of enzymes in the gastrointestinal tract is also observed through biochemical indicators.

An increase in the level of α -amylase in the small intestine indicates a rapid poisoning by the toxicant. According to the literature [159-161], an increase in the level of α -amylase explains the activity of enzymes and their appearance in the blood in a high concentration, and this all indicates a rapid poisoning of the organism and the onset of pathological processes.

The cadmium metal that enters the body is found in the blood in the form of a colloid phosphate or as albumins. First it enters through the blood in all the tissues and accumulates in the bones, in the liver tissues and kidneys. At such a moment, the formation of trivalent phosphate occurs. Due to the action of the cadmium metal, as a toxic substance, on the body as a whole, the poison also affects the nervous system. As a result of intoxication with the poison of the central nervous system, dizziness, insomnia, epileptic convulsions, inattention, visual and movement coordination impairment, and muscle fatigue are observed.

An increase in the level of amylase and alkaline phosphatase in the small intestine in the animals' organism indicates a rapid poisoning. According to the literature [16, 17], the increase in the level of amylase is explained by the activity of this enzyme and its appearance in the bloodstream and, in the whole, in the blood circulation of the body, and this, in turn, indicates a rapid onset of poisoning of the organism and the course of pathological processes in the organs.

In our research work, we drew attention to possible changes in the stomach when animals were poisoned with cadmium salts. A well-known to all of us mechanism of membrane digestion has a great biological significance. This stage of digestion generally proceeds in a pure, non-microbial state. This is due to the goblet structure of the mucosa.

From the biochemical parameters of the structure of the stomach the following information was obtained. In the control group of animals, the concentration of alanine aminotransferase and aspartate aminotransferase was 223.9 ± 2.6 and 231.4 ± 4.1 IU/l respectively; under poisoning by salts of cadmium the indicators were 287.3 ± 5.3 * and 299.6 ± 2.5 * IU/l respectively. After poisoning, the indices in the stomach rose by 28-29% ($P < 0.05$). In the digestive tract, most of the food appears, and here the accumulation of ions of cadmium salts is possible. Therefore, when the gastric mucosa is poisoned, as compared to other parts of the stomach, the phosphatase indicators tend to increase.

In rural animal husbandry, cadmium poisoning is rare. Poisoning occurs in most cases through drinking water. In the experiment, it was found that 600-800 mg/kg of cadmium is needed for acute poisoning with lethal outcome of cattle, and 30-40 mg/kg during 1 month is necessary for sluggish poisoning. According to the information obtained from the literature, it is known that salts of heavy metals cause a decrease in arterial pressure, inflammation of the gastrointestinal tract, stomach and duodenum, wrinkling of them, and possibly the appearance of ulcers, although these statements require further investigation.

According to the information obtained from the scientific literature, the weight of rats decreases under their poisoning with heavy metals, moreover, there occur changes in protein metabolism, biochemical, physiological, histochemical parameters, in particular, an increase in the nitrogenous amine, inhibition of the activity of alkaline phosphatase and enzymes, and insignificant liver dystrophy [18, 19].

In mammals, disintegration of food and membrane digestion occurs in the intestinal epithelium, and this serves as the reason for the research. In animals of the experimental group, compared with the norm, the activity of amine metabolic enzymes is revealed in the gastrointestinal tract, and the level of amylase production undergoes certain changes. The activity of ALAT and ASAT in the small and large intestines and in stomach increased evenly by 9-12%. The levels of amylase and alkaline phosphatase in the stomach are also increasing.

In the animals of the control group, the membrane digestion in the stomach is 52.8 ± 2.2 IU/l, and in the group receiving the cadmium salt, the indices are higher and are 87.3 ± 2.9 ** IU/l. As a result of intoxication with cadmium, there is a prolonged edema, inflammation and a decrease in the function of the stomach and intestines, heart and blood vessels. It is known from the scientific literature that poisoning influences the blood plasma elements (hematocrit), the specific weight of blood, and the viscosity of blood [20, 21].

Under the experimental study of the digestive system, we found an increase in ALAT and ASAT, which, on the one hand, is associated with the strengthening of gluconeogenesis, on the other hand,

possibly with a violation of the liver parenchyma cells and its structure. Throughout our studies, as compared with the control group, the ASAT indicators doubled and the ALAT level tripled. An increase in the rates of ALAT and ASAT in the digestive system underpoisoning of rats with cadmium chloride indicates an increase in the rate of cytological activity.

Based on the results of biochemical studies, it can be stated that not only the parameters of the gastrointestinal tract undergo the change, but also the parameters of blood plasma. Accumulating in the environment, heavy metal ions descend from the atmosphere and enter the water and the human organism. Entering the gastrointestinal tract, accumulating for a long time, heavy metals lead to a change in the function of both the individual organ and the whole organism [22, 23].

According to the results of our research, it can be stated that the increase in ALAT and ASAT is associated with both the strengthening of gluconeogenesis and violation of the function of the parenchymal cells of the liver and its structure. In our studies, as compared with the control group, the ASAT indicators increased twofold, and the ALAT level tripled. In the digestive system, an increase in the rates of ALAT and ASAT under cadmium salt poisoning showed an increase in the rate of cytological activity. Compared with the control group, in the experimental group of rats the level of biochemical indices in the small, large intestines and in the stomach increases several fold. This leads to disruption of water-salt homeostasis, as well as pancreas, a decrease in diuresis and electrolytes in the blood and protein levels in biological fluids.

The conclusion. Compared with the control group, in animals receiving cadmium salts, biochemical indices of the digestive system indicated a metabolic disturbance in the intestine and stomach. Compared with the control group, in the animals receiving the cadmium salt there was an increase in membrane digestion in the small and large intestines and in the stomach, there were high rates of ALAT, ASAT, amylase and alkaline phosphatase. In the control group, the indices of amylase in the small and large intestines corresponded to 46.4 ± 3.3 IU/l and 7.13 ± 0.3 IU/l, and in the stomach - 52.8 ± 2.2 IU/l, and under the poisoning with cadmium salt, these parameters in the small intestine increased by 29.5%, in the large intestine increased to 10.2 ± 0.1 IU/l and in the stomach by 65%. When poisoned with cadmium salt, alkaline phosphatase increased from 22% to 65%, along with an increase in the level of the enzyme aminotransferase, all of which indicated a violation of liver function, increased cytological activity in the liver and pancreas, decrease of protein synthesis and metabolic disorder.

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ЖАНУАРЛАРДЫҢ АС ҚОРЫТУ ЖОЛДАРЫНДА МЕМБРАНАЛЫҚ ГИДРОЛИЗИНЕ ТОКСИКАНТТЫҢ ӘСЕРІ

Аннотация. Мақалада жануарлардың ас қорыту жолында мембраналық гидролизіне кадмий иондарының әсері анықталды. Әлемде орын алып отырған экологиялық жағдайдың, зиянды факторлардың, экологиялық ахуалдың күн санап ушығып отырғандығының өзі, аталған мәселенің ерекше өзекті екенін айқындайды. Ауыр металл тұздары, оның ішінде, кадмий иондары да қауіпті болып саналады, олар қанайналымына, бауыр мен бүйрек қызметінің бұзылуын тудырады. Біз өз зерттеулерімізде ас қорыту жолының мембраналық гидролизіне, АсАТ және АлАТ, амилаза ферменттерінің және сілтілі фосфатаза белсенділігіне токсиканттың әсер етуін анықтадық. Зерттеу нәтижесінде егеуқұйрықтардың қалыпты және токсикантпен уланғаннан кейінгі қарын, аш ішек және тоқ ішектегі ферменттердің белсенділігі анықталды.

Улану кезінде сілтілі фосфатаза мөлшерінің жоғарлағаны, сонымен бірге аминотрансфераза ферменттерінің деңгейінің артқандығы байқалды, мұның барлығы бауыр қызметінің бұзылып, бауыр мен ұйқы безіндегі цитологиялық үдерістердің күшейгенін, белок синтезінің төмендегенін білдіреді және зат алмасудың тасмалдануының бұзылғандығын көрсетеді. Алынған нәтижелердің экология, медицина, ауыл шаруашылығы және тағам өнеркәсібі салаларында тәжірибелік маңыздылығы жоғары. Зерттеу барысында қол жеткізілген нәтижелер сүтқоректілердің ас қорыту жолының қызметіне кадмийдің әсері туралы мәліметтер базасын қалыптастыруға мүмкіндік берді және аталған мәселені шешудің жолдарын іздестіруге ғылыми негіз бола алады.

Түйін сөздер: егеуқұйрықтар, токсиканттар, мембраналық гидролиз, ферменттер, ас қорыту жолы.

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ВЛИЯНИЕ ТОКСИКАНТОВ НА МЕМБРАННЫЙ ГИДРОЛИЗ ПИЩЕВАРИТЕЛЬНОГО ТРАКТА У ЖИВОТНЫХ

Аннотация. В статье рассмотрены влияние кадмия на полостной и мембранный гидролиз пищеварительного тракта у животных. Ухудшение экологического состояния, экологически вредных факторов, экологического состояния в пищевой промышленности в мире определяет высокую актуальность данной темы исследования. Мире определяют соли тяжелых металлов, в том числе, ионы кадмия являются опасными, они вызывают нарушения деятельности печени и почек, а также кровообращения. Мы исследованиях определили действие токсикантов на полостной и мембранный гидролиз пищеварительного тракта, на показатели активности ферментов АлАт, АсАТ. В результате исследования были определены активность ферментов в желудке, в тонком и толстом кишечнике в норме и после отравления токсикантом.

При отравлении показано повышение количества щелочной фосфатазы, уровня ферментов аминотрансферазы, что связано с нарушением деятельности печени. В поджелудочной железе и печени происходит усиление цитологических процессов, также снижается синтез белка и нарушается транспортная функция обмена веществ. Полученные результаты имеют практическую значимость в экологии, медицине, сельском хозяйстве и в пищевой промышленности. Достигнутые результаты в ходе исследования дают возможность формировать базу данных о влиянии кадмия на деятельность пищеварительной системы млекопитающих, и может быть основой для поиска путей решения данной проблемы.

Ключевые слова: крысы, токсиканты, мембранный гидролиз, ферменты, пищеварительный тракт.

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