

## ORIGINAL ARTICLE

# Morphofunctional features of small intestine remodeling under the influence of cadmium chloride in an experiment

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## ABSTRACT

**Aim:** The aim of the study was to determine the characteristics of structural changes in the jejunum and ileum of experimental animals of different ages following cadmium chloride intoxication.

**Materials and Methods:** Experimental studies were conducted on 60 white laboratory male rats, both prepubertal and pubertal, with body weights of 80–100 g and 190–220 g, respectively. All animals were divided into three groups. Of these, 12 animals constituted the intact control group, which was divided into two subgroups: 6 sexually immature white rats and 6 sexually mature animals. The experimental animals comprised two groups, each with two subgroups. The second group included 24 immature rats, of which 12 were removed from the experiment on the 7th day and 12 on the 14th day after cadmium chloride poisoning. Group 3 consisted of 24 experimental animals, of which 10 were removed from the experiment on day 7 and 14 two weeks after cadmium intoxication. Poisoning of the experimental white rats with a cadmium chloride solution was performed intraperitoneally at a dose of 6 mg/kg body weight. It was used electron microscopic examination.

**Results:** Cadmium chloride exposure causes structural changes in the jejunum and ileum. The observed phenomena are more pronounced in the jejunum, which is explained by its greater functional activity compared to the ileal segment of the small intestine. Exotoxin disrupts the function of proteins and cell membranes, affects metabolic processes in the small intestine and its epithelium. Under electron microscopy, the surface of the ciliated epithelial cells appeared as a series of microvilli closely arranged next to one another. A functional feature of the apical membrane of epithelial cells is a pronounced endocytosis, manifested by the detachment of endocytic vesicles containing substrates from the base of the microvilli. Changes in the ultrastructure of the microvilli of the brush border at the apical pole of columnar epithelial cells may indicate disturbances in parietal digestion and absorption processes. On the 7th day after cadmium chloride poisoning, exposure to exogenous factors results in thickening of the subepithelial mucus layer, which may be interpreted as the organ's protective response to a chemical irritant. Cadmium chloride intoxication in experimental animals leads to the suppression of regenerative processes against the background of morphological changes in the structure of ciliated epithelial cells, which is associated with cadmium accumulation in the cells.

**Conclusion:** The destructive changes in the organization of organelles, the cytoplasmic membrane of epithelial cells, and the basement membrane indicate disturbances in intracellular bioenergetics, the protein-synthesizing apparatus, and the transcellular transport of substances through the capillary network, leading to changes in the properties of the capillaries themselves.

**KEY WORDS:** small intestine, jejunum, cadmium chloride, ileum, morphology, electron microscopy, experiment

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## INTRODUCTION

The adverse environmental conditions in Ukraine's regions have led to an increase in the number of pathological lesions of the gastrointestinal tract, one of the main causes of which is the impact of environmental chemical factors on the body, particularly heavy metals and their salts, since the digestive system is one of the primary routes through which they enter and are excreted from the body [1, 2].

Cadmium ranks first in terms of toxicity and danger; once it enters the body, it is characterized by extremely

slow excretion and accumulates in various organs [3, 4]. It is known that cadmium cations are capable of interacting with biomolecules, causing damage to their structure, exerting a destructive effect on nuclear components, biomembranes, and cytoplasmic structures of cells, and influencing the processes of proliferation and apoptosis [5, 6, 7].

The small intestine plays an extremely important role in the digestive process and is therefore almost always involved in the pathological process when pollutants affect the body. The local effect on the organ is due to

the fact that most toxic chemical complexes enter the body through active absorption in the jejunum and ileum, thereby exerting a destructive effect on the morphological state of the organ walls, resulting in functional disorders [8, 9, 10].

A prerequisite for the disruption of metabolic processes in tissues and organs is impaired blood supply. In the event of any toxic effect on the gastrointestinal tract, the pathological process involves not only the structures of the mucosa, submucosa, muscular layer and serosa of the small and large intestines, but also the vessels of the microcirculatory bed, which constitute an important functional system that fully ensures homeostasis in accordance with the morphological characteristics and physiological or pathological states of the tissue or organ [11]. It is extremely important in morphological and toxicological studies to consider the age-related aspect, as each life stage is characterized by specific features of the action, distribution, and accumulation levels of chemical substances. With age, the body's resistance, sensitivity, and adaptation to the effects of xenobiotics change [12].

Thus, studying the process of structural changes in the small intestine wall during pathological conditions resulting from the influence of exogenous factors, as well as across different age periods, remains a relevant issue in experimental and clinical medicine.

## AIM

To determine the characteristics of structural changes in the jejunum and ileum of experimental animals of different ages following cadmium chloride intoxication.

## MATERIALS AND METHODS

Experimental studies were conducted on 60 white laboratory male rats, both prepubertal and pubertal, with body weights of 80–100 g and 190–220 g, respectively.

The care and handling (experimental studies and euthanasia), morphological and biochemical investigations of laboratory animals were conducted in accordance with the "Scientific and Practical Recommendations for the Care and Handling of Laboratory Animals" and Order No. 690 of the Ministry of Health of Ukraine dated September 23, 2009 in accordance with the provisions of the "General Ethical Principles for Animal Experiments," adopted by the First National Congress on Bioethics (Kyiv, 2001), and in compliance with the rules established by the European Commission for the Supervision of Laboratory and Other Experiments Involving Experimental Animals

of Various Species (European Convention, 1986) [13].

All animals were divided into three groups. Of these, 12 animals constituted the intact control group, which was divided into two subgroups: 6 sexually immature white rats and 6 sexually mature animals. The experimental animals comprised two groups, each with two subgroups. The second group included 24 immature rats, of which 12 were removed from the experiment on the 7th day and 12 on the 14th day after cadmium chloride poisoning. Group 3 consisted of 24 experimental animals, of which 10 were removed from the experiment on day 7 and 14 two weeks after cadmium intoxication.

Poisoning of the experimental white rats with a cadmium chloride solution was performed intraperitoneally at a dose of 6 mg/kg body weight. Animals in the control and experimental groups were kept under identical conditions, and the material collected from them for electron microscopic examination was processed simultaneously.

For electron microscopic examination, tissue sections from the specified parts of the small intestine were taken from the animals immediately after respiratory and cardiac arrest. The small intestine section was fixed in a 2% solution of osmium tetroxide in phosphate buffer with a pH of 7.4 and a molar concentration of 0.1 M. Initially, semi-thin sections were prepared to supplement the histological examination of the material, and sites for electron microscopic examination were selected from these sections. Ultrathin sections were prepared on an UMTF-7 ultramicrotome and mounted on 1-mm-diameter copper blanks with a Formvar base. Sections 30–50 nm thick were contrasted in a 2% solution of uranyl acetate in 70% ethanol and Reynolds' mixture and examined using a PEM-125K electron microscope.

## RESULTS

Electron microscopic studies of the jejunum wall in sexually immature white rats on the 7th day after cadmium intoxication revealed destructive changes in its structure. The height and number of damaged microvilli on the apical surface of columnar epithelial cells were noticeably reduced. In the cytoplasm, organelles were destroyed, the endoplasmic reticulum became heteromorphic, and enlarged vacuoles were observed in some areas.

Plasmocytes were detected in the lamina propria of the mucosa, indicating an immune response by the body. The presence of damaged fibroblasts indicated a decrease in the regenerative capacity of the tissues of the organ under study (Fig. 1).

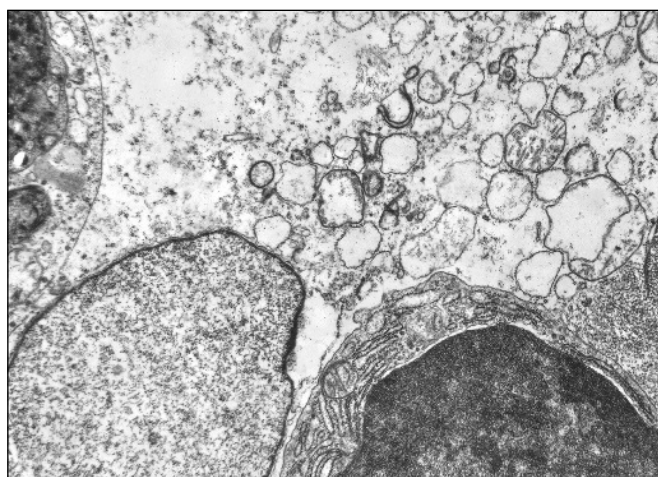


**Fig. 1.** Ultrastructural changes in the lamina propria of the small intestine mucosa of a sexually immature animal on the 7th day of observation. Cytoplasm of a damaged fibroblast with destructively altered organelles. Magnification:  $\times 14,000$ .

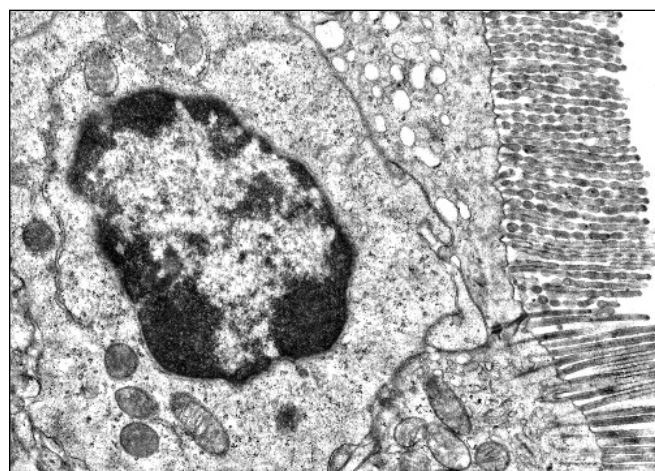


**Fig. 3.** Submicroscopic changes in the columnar epithelial cells of the ileal villi of a sexually immature animal 7 days after exposure to cadmium chloride. Partially fragmented microvilli, a nucleus with heterochromatin clusters, mitochondria. Magnification:  $\times 15,000$ .

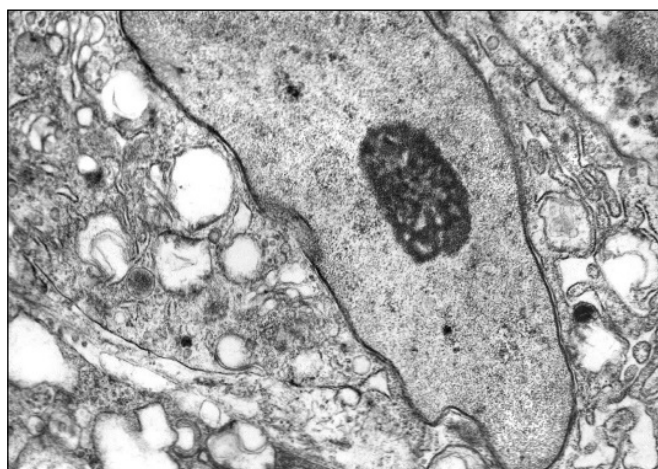


**Fig. 2.** Submicroscopic view of the lamina propria of the jejunum mucosa in a sexually immature white rat on the 14th day of observation. A nucleus with large nucleolus of a fibroblast, cytoplasm, and tubules of the granular endoplasmic reticulum. Magnification:  $\times 21,000$ .

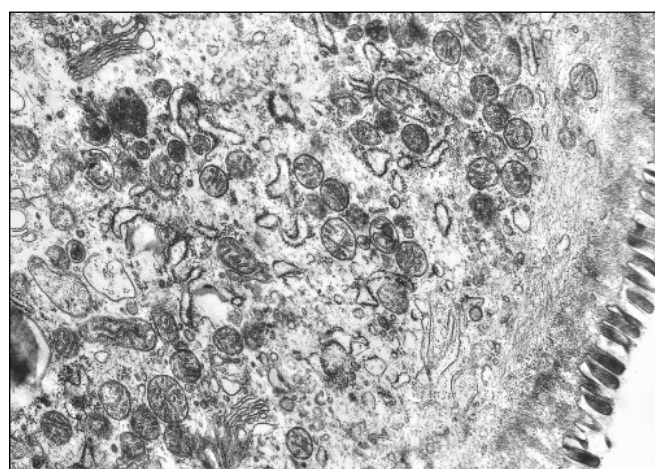


**Fig. 4.** Submicroscopic structure of a columnar epithelial cell in the lower part of a villus of the ileum of a sexually immature animal on the 14th day of observation. Short microvilli, epithelial cell cytoplasm, mitochondria, and a canaliculus of the granular endoplasmic reticulum. Magnification:  $\times 14,000$ .

On the 14th day following cadmium chloride poisoning in sexually immature white rats, signs of improvement in the condition of ciliated epithelial cells were observed. The plasma membranes were thickened and exhibited higher electron density, and the intercellular spaces were widened. Partial restoration of epithelial cell microvilli was observed, although signs of destructive processes were still present.

Organelle destruction was less pronounced: moderately dilated tubules of the granular endoplasmic reticulum were identified, and hypertrophy of the Golgi complex and primary lysosomes was detected. An increase in the number of mitochondria was observed, with organelles having a condensed matrix and a large

number of cristae. The elongated-oval nuclei were displaced apically. Euchromatin predominated in the karyoplasm. Leukocyte infiltration was observed near the damaged columnar epithelial cells (Fig. 2).

Morphometric, microscopic, and ultrastructural data on the condition of the epithelial cells of the villi in the mucosa of the affected small intestine indicated disturbances in the processes of parietal digestion and absorption.

Electron microscopic studies of the ileal wall in sexually immature animals poisoned with cadmium chloride revealed pronounced structural changes on the 7th day of observation. The intercellular spaces widened, leading to the extrusion of damaged cells

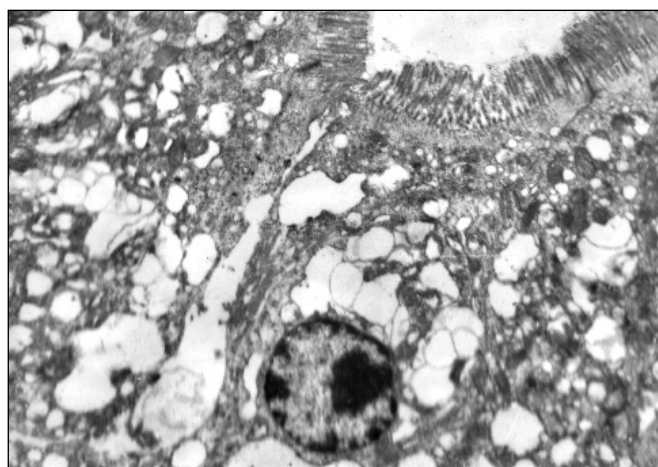


**Fig. 5.** Submicroscopic changes in the columnar epithelial cells of the villi of the small intestine mucosa of a sexually mature animal on the 7th day of observation. Fragmented microvilli, a nucleus with heterochromatin clumps, significantly dilated canaliculi of the granular endoplasmic reticulum, and an enlarged intercellular space. Magnification:  $\times 7000$ .

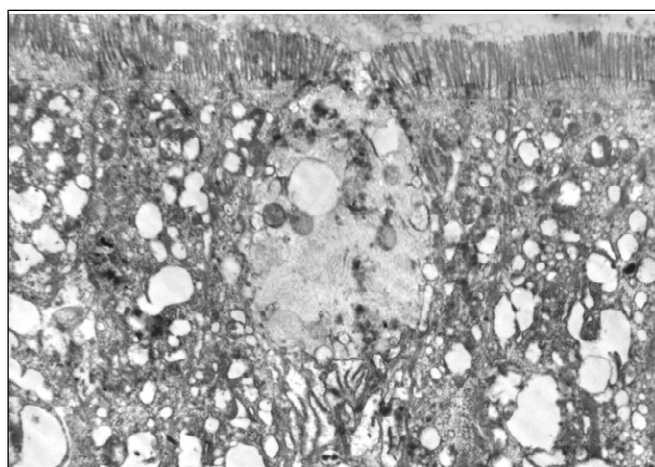


**Fig. 7.** Ultrastructural picture of the epithelial layer of the ileum mucosa villi in the sexually mature rat 7 days after exposure to cadmium chloride. Damaged microvilli on the apical surface of columnar epithelial cells, significantly dilated, vacuole-like tubules of the granular endoplasmic reticulum, damaged mitochondria, goblet cell. Magnification:  $\times 8000$ .

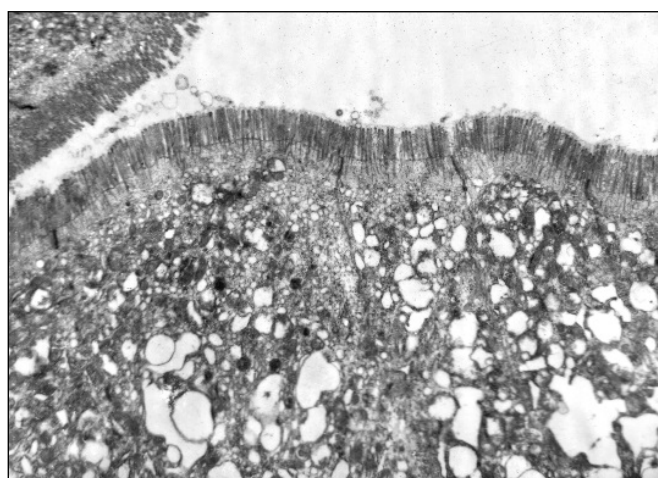


**Fig. 6.** Submicroscopic appearance of columnar epithelial cells in the villi of the small intestine of a sexually mature animal 14 days after exposure to cadmium chloride. The apical surface of the cell with microvilli. Magnification:  $\times 7000$ .

from the epithelial layer and, consequently, its destruction. Submicroscopic changes in columnar epithelial cells manifested as partial fragmentation of microvilli, thickening of the basement membrane, destruction of individual organelles, and an increase in the number of lysosomes (Fig. 3).

The basal orientation of the nucleus, characteristic of the cells under study, was lost. Clusters of heterochromatin were observed along the periphery of the nuclear matrix. Mitochondria were present in the nuclear zone, exhibiting the most significant changes. Mitochondrial membranes were preserved, but the cristae were not tightly arranged, and the mitochondrial matrix was

pale. Fine granulation was present in the cytoplasm, indicating protein dystrophy.

During the observation period, a large number of lysosomes appeared in the cytoplasm of epithelial cells, digesting organelles damaged and destroyed as a result of intoxication. Their appearance triggered the activation of the Golgi complex. The presence of autophagosomes in the cells indicated toxic stress, since autophagy is one of the ways cells rid themselves not only of damaged organelles but also remove dead cells from tissues. The appearance of lysosomes and autophagosomes in the cytoplasm indicated the activation of protective mechanisms in the studied organ. As a result of the studies, necrobiotically altered cells were identified among the epithelial cells.

On the 14th day after cadmium chloride poisoning of sexually immature white rats, signs of improvement in the condition of bordered epithelial cells were observed. Plasma membranes had higher electron density, and intercellular spaces were widened.

During this study period, partial restoration of epithelial cell microvilli was observed, but signs of destructive processes still persisted (Fig. 4).

The destruction of organelles displaced closer to the basal pole was less pronounced. Here, moderately dilated tubules of the granular endoplasmic reticulum were identified, and hypertrophy of the Golgi complex and primary lysosomes was observed. Mitochondrial hyperplasia was observed, with a condensed matrix and a large number of cristae. Nuclei were displaced apically, and euchromatin predominated in the karyoplasm. Lymphocytes were detected among the columnar epithelial cells.

In the intestinal villi, electron microscopy revealed columnar epithelial cells in which destructive changes were developing. Disruption of cell contouring and the basal arrangement of nuclei was observed; as a result of cadmium chloride exposure in sexually mature animals, the nuclei were localized in the center of the cell. Lymphocytes were detected near the altered cells.

The interconnection of columnar epithelial cells was maintained by a system of intercellular junctions, the spaces of which were locally expanded by the 7th day of the experiment.

A significant sign of impaired parietal digestion is a change in the structure of microvilli. On the apical surface of the examined cells, areas with deformation and destruction of microvilli were detected, manifested by their swelling and shortening, as well as partial fragmentation (Fig. 5).

Some of the microvilli underwent lysis, resulting in the complete exposure of certain areas. One of the functions of the apical membrane of columnar epithelial cells is endocytosis, which occurs through the budding of endocytic vesicles containing a specific substrate from the base of the microvilli. Lysis, fragmentation of the microvilli, and exposure of areas of the apical membrane led to a partial loss of its function.

Intestinal epithelial cells were in a state of protein dystrophy. An increased number of organelles was observed in the cytoplasm, concentrated in the perinuclear zone of the epithelial cells. Mitochondria were swollen, contained an electron-light matrix, and had isolated transparent vesicles. The cristae were damaged, and their number had significantly decreased; however, the mitochondrial membranes remained intact.

The Golgi apparatus was hypertrophied in some cells. Vacuole-like dilated cisternae were detected in this organelle. The vacuoles surrounding this structure had increased in size.

The granular endoplasmic reticulum underwent significant changes: the tubules were partially degranulated, fragmented, significantly dilated, and filled with a substance characterized by low electron density. On the 7th day of the experimental study, a significant decrease in the number of ribosomes localized on the membranes of the granular endoplasmic reticulum was observed, indicating a disruption in the functions of this cellular structure; its volume decreased at the basal poles of the cell. The membrane of this organelle lost its sharply defined outline and appeared thickened.

An increase in the number of lysosomes and autophagosomes was observed in the cytoplasm. In some intestinal columnar epithelial cells, large

autophagosomes were observed on the 7th day of the experimental study, inside which remnants of destroyed organelles were detected.

The oval-shaped nuclei increased in size. The nuclear envelope formed deep invaginations. In the partially disrupted nucleolus, areas undergoing lysis were observed. The perinuclear space was unevenly expanded. The nucleoplasm of the nuclei of the studied cells on the 7th day of the experimental study, under the influence of cadmium chloride, became diluted and lost its electron density. Clusters of condensed chromatin were concentrated along the periphery of the nuclear matrix.

On the 14th day of the experimental study, following the exposure of sexually mature animals to cadmium chloride, a partial restoration of the size of the microvilli of the mucosal epithelial cells of the hollow intestine was microscopically observed (Fig. 6).

Signs of destructive processes were observed in the epithelial layer of the villi: areas of epithelial detachment were found at the apical pole, and subepithelial edema was observed on the lateral surfaces and partially at the apex.

During this period of the study, heterogeneous changes in the columnar epithelial cells of the small intestine mucosa were observed. In a significant portion of the cells, the structural organization improved, although a large number of them remained significantly damaged.

The condition of the microvilli of some epithelial cells within the epithelial layer of the villi improved noticeably. Electron microscopic examination revealed that on the apical surface of the studied cells, the microvilli showed a tendency toward recovery, although some of them remained sparse and shortened. The intercellular spaces were not widened.

The cytoplasm of columnar epithelial cells, as the experimental study progressed, became fine-grained and clarified. Less pronounced destruction of organelles was observed within it, with these organelles displaced toward the basal pole of the columnar epithelial cells. Expanded tubules of the granular endoplasmic reticulum and cisternae of the Golgi complex are present. The cisternae of the endoplasmic reticulum varied in shape and size. Damaged mitochondria with a condensed matrix and a large number of cristae were observed. Primary lysosomes were present. The plasma membranes were thickened and had a higher electron density.

The elongated-oval nuclei were displaced apically. Euchromatin predominated in the karyoplasm. Morphological and ultrastructural changes in the villus epithelial cells indicated disturbances in paracellular digestion and absorption.

Electron microscopic studies of the ileal wall of sexually mature white rats on the 7th day after cadmium chloride exposure showed that destructive changes were observed in the epithelial lining of the villi (Fig. 7). As a result of intoxication, the nuclei of columnar epithelial cells were localized in the center of the cells. On the apical surface of the examined structures, areas with destruction of microvilli were detected, manifested by partial fragmentation, lysis, and exposure of certain zones of the apical membrane. The above indicates a disruption of paracellular digestion in the examined organ.

As of the 7th day of the study, the cytoplasm of the columnar epithelial cells is fine-grained and cloudy. Organelles are concentrated near the nucleus, which has increased in size. Certain areas of the karyolem have undergone lysis. As a result of the effect of cadmium chloride on cellular structures, the karyoplasm is diluted and has lost its electron density. An increased number of free ribosomes was detected in the cytoplasm of columnar epithelial cells, resulting from the degranulation of the granular endoplasmic reticulum. These changes indicate a dysfunction of the cellular structure under study. In certain areas, the tubules of the endoplasmic reticulum are dilated. Mitochondria, whose matrix is condensed, showed signs of swelling. The cristae of the studied cellular structures are partially destroyed, and, as a result, their number is reduced. The Golgi complex is surrounded by enlarged vesicles.

As of the 7th day after cadmium chloride intoxication in sexually mature animals, an increased number of autophagosomes and lysosomes was observed in the cells of the columnar epithelium of the ileum, indicating significant changes in the structure and disruption of the functions of cellular organelles. In particular, deformed and partially destroyed mitochondria were detected in some large autophagosomes.

On the 14th day of the experimental study, heterogeneous changes persisted in the epithelial cells with microvilli. In a certain portion of the cells, the structural organization improved, while signs of destructive processes persisted in a significant portion of the epitheliocytes.

On the apical surface of the studied cells with a microvilli, a tendency toward recovery was observed, indicating the restoration of epithelial cell functions, although a significant portion of the microvilli remained swollen, shortened, and partially fragmented. By the 14th day of observation, the cytoplasm was clear, with less pronounced destruction of organelles.

The mitochondria had partially restored their structure, as evidenced by an increased number of cristae compared to the previous stage of the experiment.

The plasma membranes were thickened and exhibited higher electron density. The nuclei occupied a basal position. Euchromatin predominated in the nucleoplasm.

Thus, the phenomena under study were more pronounced in the jejunum, which is explained by its greater functional activity compared to the ileum.

## DISCUSSION

According to the literature, it is known that the environment is constantly subjected to heavy metal pollution, which affects the human and animal organisms [14]. A common characteristic of heavy metals is their ability to slowly accumulate in living organisms [15, 16]. Excessive amounts of heavy metals can alter the structure and function of all body systems [17]. This disrupts the function of proteins and cell membranes, affects metabolic processes in the body, and has the potential to cause cellular mutations. It is also known that if the concentration of heavy metals in the body is higher, it is the great risk of viral activation [18]. Researchers have experimentally established that the intestinal epithelium is affected by the exotoxin [19]. This is confirmed by the data obtained in our study. Under electron microscopy, the surface of the ciliated epithelial cells appeared as a series of microvilli closely arranged next to one another.

A functional feature of the apical membrane of epithelial cells is a pronounced endocytosis, manifested by the detachment of endocytic vesicles containing substrates from the base of the microvilli. Changes in the ultrastructure of the microvilli of the brush border at the apical pole of columnar epithelial cells may indicate disturbances in parietal digestion and absorption processes [20].

The surface of the microvilli membrane is covered by the glycocalyx, which is the first structure to come into contact with intestinal contents; its glycoproteins serve as receptors for toxins, hormones, and antibodies, possess barrier properties, and retain a layer of mucus on the surface of the intestinal epithelium. The destruction of microvilli under the influence of exogenous factors indicates damage to the glycocalyx, which is an additional risk factor and creates conditions for the penetration of bacteria from the lumen of the small intestine into the mucosal tissue.

On the 7th day after cadmium chloride poisoning, edema of the villus stroma and increased desquamation of epithelial cells at their apical poles were observed in the mucosa of the jejunum and ileum of the experimental animals. According to scientific studies, exposure to exogenous factors results in thickening of the subepithelial mucus layer, which scientists interpret

as the organ's protective response to a chemical irritant [21, 22].

Some of the heavy metal salts in the lysosomes of bordered epithelial cells bind to metalloproteins to form complexes; these complexes, along with cells desquamated from the surface of the villi, enter the intestinal lumen, where some are reabsorbed and the rest are excreted in the feces. In addition, the thickened supramucosa layer of mucus, which plays a protective role by binding excess heavy metal salts in the lumen of the small intestine, prevents their absorption. According to researchers when the body is exposed to heavy metal compounds, particularly cadmium, hypertrophy and hyperplasia of goblet cells are observed, along with a significant thickening of the supraepithelial mucus, which plays a protective role by preventing further absorption of xenobiotics [16, 23].

Our results indicate that cadmium chloride intoxication in experimental animals leads to the suppression of regenerative processes against the background of morphological changes in the structure of ciliated epithelial cells, which is associated with cadmium accumulation in the cells.

As a result of electron microscopic studies of the walls of the jejunum and ileum in sexually immature and sexually mature white rats on the 7th day after cadmium chloride exposure, we observed destructive changes in the mitochondria, heteromorphism of the endoplasmic reticulum, an increase in the number of lysosomes, and damage to microvilli. According to researchers, morphological and functional disturbances of plasma and intracellular membranes are detected almost immediately upon exposure to external agents, even at relatively low doses [24]. The cause of impaired metabolic activity in epithelial cells may be structural disturbances in the elements of the endoplasmic reticulum during the course of intoxication. The detected destruction of the membranes of the granular endoplasmic reticulum indicates the predominance of catabolic processes over synthetic ones; disruption of the internal structural organization of mitochondria leads to the suppression of enzyme activity and a decrease in redox reactions occurring within the organelles. This, in turn, leads to an energy deficiency in the synthetic processes of intracellular metabolism. Disruption of mitochondrial function leads to a

decrease in cytosolic ATP content and to an energy deficit in cells, causing their necrosis.

The described destructive changes in the organization of organelles, the cytoplasmic membrane of epithelial cells, and the basement membrane indicate disturbances in intracellular bioenergetics, the protein-synthesizing apparatus, and the transcellular transport of substances through the capillary network, leading to changes in the properties of the capillaries themselves.

Electron microscopic examination of the walls of the jejunum and ileum of sexually immature and sexually mature white rats on the 14th day after cadmium chloride poisoning showed that the microvilli on the apical surface of columnar epithelial cells were clearly defined and closely adjacent to one another, indicating a probable improvement in membrane digestion and absorption.

During this observation period in experimental white rats of various age groups, we noted less pronounced destruction of organelles in the cytoplasm of bordered epithelial cells, indicating the predominance of anabolic processes over catabolic ones, as well as improvements in intracellular bioenergetics and the protein-synthesizing apparatus. However, data from electron microscopic studies of the structural components of the cell wall indicated that during the specified experimental period, the presence of free ribosomes, hypertrophic changes in the Golgi complex, and the granular endoplasmic reticulum - structures where the synthesis of enzymes, structural proteins, and other substances takes place - had not completely disappeared, which still indicated impaired intracellular digestion. The reactive changes we observed are consistent with the data of other authors [18, 24, 25].

The described dynamics indicate that by the 14th day of observation, a trend toward a decrease in destructive processes and an increase in regenerative processes was noted in the mucous membrane of the jejunum and ileum of experimental animals in various age groups.

## CONCLUSIONS

Cadmium chloride exposure causes structural changes in the jejunum and ileum. The observed phenomena are more pronounced in the jejunum, which is explained by its greater functional activity compared to the ileal segment of the small intestine.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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