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MORPHOFUNCTIONAL PECULIARITIES OF RATS' SMALL INTESTINE UNDER THE INFLUENCE OF MYCOTOXINS

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The purpose of the given work is by histological, cytological, morphological methods to study jet changes of a small intestine at rats in the conditions of influence of some mycotoxins, i.e. aflatoxin B1, ochratoxin A and and zearalenon. The obtained data will form a basis for revealing of cellular mechanisms of adaptive-compensatory processes and pathological changes occurred in this organ during noted conditions.

***Keywords*:** mycotoxins, rats, small intestine.

Introduction. Chemical toxic substances as mycotoxins appearing in different food chains as a result of mold fungi vital activity, pose a serious threat to humans and agricultural animals [1–3]. A high level mold fungi infection of grain and other food is observed in all countries at present. About 25% of cereal crops are being infected with mycotoxins. Vegetable crops (food) are contaminated by mycotoxins not only as a result of pathogenic fungi growth but also as a result of saprophytic fungi growth activity on stored food. Animal food, especially meat and dairy products, are contaminated by mycotoxins mainly because of the pollution of animals' feed [2, 4]. Thus, 5 *h* after feeding chickens with aflatoxin, 31% of the poison is detected in muscles, 11% in blood and 10% in liver. It has been found out that only. One point of 1200 points of aflatoxins absorbed with the feed passes into the chickens' liver. This ratio in milk is 75:1, this indicator is unstable in egg, but in egg yolk it is higher than in protein. It must be noted that most of mycotoxins are heat-resistant and they do not collapse even at 100°*C*, it is also resistant to bases and acids. Another feature of these substances is the state of being hydrophobic i.e. fat-soluble. And this is the reason why even if a small amount of it passing into human or animal organisms, are not removed, but are accumulated in different tissues [5, 6].

The study of reactive changes in different tissues and cells of animals that have been adversely affected by polluted environment and toxic food is one of the contemporary key issues in biology. It is known that the digestive system is extremely sensitive to environmental factors and that it cannot but react on the food contamination adverse effects caused by mycotoxins. Based on the above

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mentioned, we aimed to study rats' small intestine several morphofunctional peculiarities after the import/invasion of mycotoxins, i.e. aflatoxin B1, ochratoxin A and Zearalenone (ZEN) with feed, in different stages of the study.

Materials and Methods. The small intestines of 32 rats with 150–200 *g* body weight were used for the study, which had acquired mycotoxins: aflatoxin B1; ochratoxin A; ZEN mixed with the food. The daily rates of mycotoxins were: for aflatoxin B1 15–25 *mg*/*kg*; for ochratoxin A 0,1–5 *mg*/*kg*; for ZEN 5–10 *g*/*kg*. It is known that, the presence of mycotoxins in these doses leads to chronic diseases. Rats kept under the same conditions but acquiring feed without being contaminated with mycotoxins will serve as control group. The experimental animals were divided into 3 groups, which differed from each other by the duration of the experiment. The first group animals ate contaminated feed for 15 days, the second group for 30 days and the third group for 60 days. Eight intestines of rats acquiring uncontaminated feed served as controllers. Under general anesthesia the animals were weighed and killed by beheading.

For histological processing the intestinal samples were fixed in P. Bouin and Telesnitskiy solutions. The fixed material has undergone histological processing and was enclosed in paraffin. 5–6 μm thick paraffin consistent cuts were painted with Hematoxylin-eosin, Toluidine blue, Giemsa and May-Grunwald solution according to Papenheim, these cuts were also processed by silver nitrate according to Foote.

Histomorphological peculiarites of the small intestine were described during different stages of the experiment with the help of micropreparations painted with Hematoxylin-eosin and silver nitrate, the given features were also studied in the control/controllers. 1000 enterocytes were counted on the experimental animals' consistent cuts and the quantity of division with mitotis in them was defined. On the basis of these data the enterocytes mitotic index was determined for each animal. Besides this, the number of mast cells were calculated on a certain surface of the cuttings. The obtained numerical data were processed by statistical methods of average values (*M*) and average errors/mistakes (*m*). The reliability of the differences between the values obtained in the experimental and control materials were determined using the computer program "Statistica 8".

Results and Discussion. The data obtained from histomorphological study of the experimental animals 15 days after feeding the rats with mycotoxin contaminated food comes to witness the fact that with the import of the above mentioned toxins the intestine reacts with a number of changes. They have a predominantly retrospective nature during this period of the experiment and are displayed particularly in the mucous membrane structure.

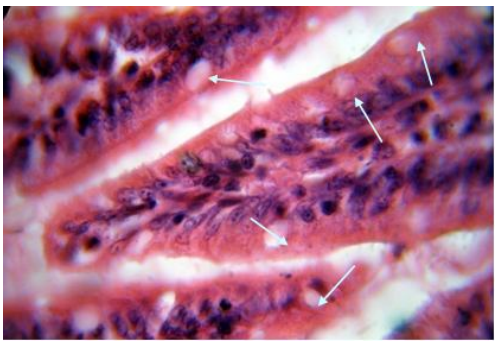


Fig. 1. Rat's small intestine: goblet cells. Painted with Hematoxylin-eosin. M ×1000.

Attention is drawn to the reduction of the height of the epithelial cells, which absorb the intestinal villi (villi intestinals). Enterocytes are predominantly cube-shaped in some parts even almost flattened. The number of squamous cells is quite high (Fig. 1).

Nuclei pycnosis changes and cells exposed to necrosis are often encountered in epithelium. A higher degree of cell death compared with the control became the main reason for deviation of epithelial physiological regeneration and in certain parts particularly in the apical parts of the intestinal villi, became the main reason of the epithelial layer integrity violation. The phenomenon of the epithelial physiological regeneration rhythm deviation and as a result degenerative changes in some parts of the intestine are most apparent.

In this parts of the mucous membrane the apical parts of the intestinal villi lost their epithelium coverage. Because of the growth of Connective tissue (CT) in these parts of the mucous membrane the intestinal villi are merged (Fig. 2).

Separate layers of epithelium coverage can be observed in the connective tissue between the intestinal villi. Despite the fact that the cells in these segments have maintained connection between each other certain retrospective changes are also obvious in them.

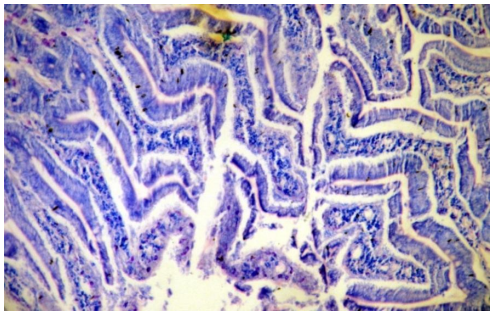


Fig. 2. Rat's small intestine. Intestinal villi merging. Painted according to Papenheim. M×200.

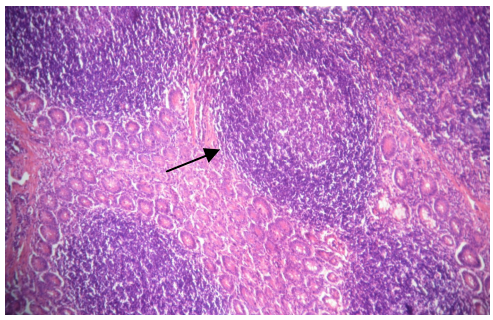


Fig. 3. Lymph nodes in rat's small intestine. Painted with Hematoxylin-eosin. M ×300.

Changes in the protective nature of rats' intestine fed by contamination with micro-toxins within 15 days are not particularly apparent. They find their reflection in the mucous membrane own villi, especially under the epithelium and between the epithelial cells within the increased number of lymphocytes. In some number of separates intestine, i.e in the intestinal villi own plate, sometimes in the apical parts of the intestinal villi lymph nodes (Fig. 3).

In the intestines of rats fed by mycotoxins for 60 days the retrospective changes become more apparent. These changes are evident in the intestinal mucous membrane, while muscle membrane and serosa (Tunica serosa) do not make any changes. During this period of the experiment in some parts of the intestinal villi apical parts have undergone necrosis. Oxyphil, amorphous mass is in the places in which connective tissue cells are diffused/scattered, parts of epithelium consisted of 3–4 not big cells. Interesting is the phenomenon of epithelial overgrowth in this period which covers some parts of the intestinal villi. As a result of an intensive multiplication of cells in the crypts in the proximal and apical parts of the villi the epithelium has become expressively multi-lane (Fig. 4). Epithelium cell bunch can be seen in those parts of the intestinal villi, tails made up of young, blister shaped nuclei, and of densely positioned cells at the same time epithelium consists of only enterosytes in those villi, there are almost no squamous cells in those parts.

During period of this experiment lymphocyte reaction is well expressed in the epithelium structure and a great number of lymphocytes under it is also well expressed. Plasma cells are clearly differentiated on the micro-preparations, which are processed according to Papenheim which appear quite often in the epithelium as CT, in the mucous membrane basic tissue structure.

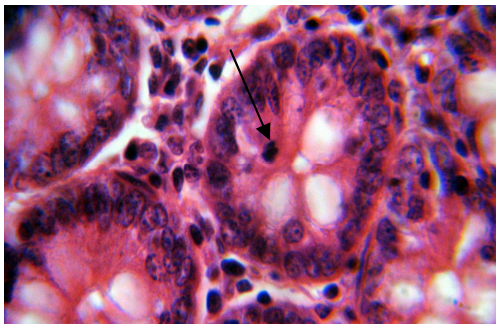
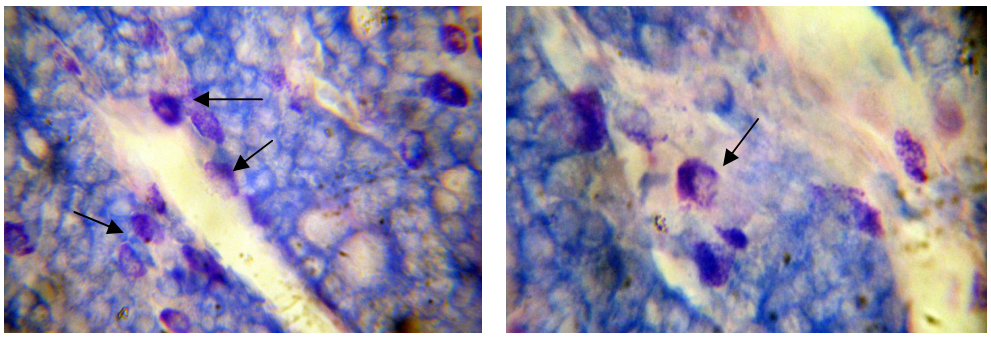


Fig. 4. Rat's small intestine, cell divided with mitotis. Hematoxylin-eosin. M×1000.

Plasma cells like lymphocytes often penetrate between the epithelium cells or stick directly to their base surface.

As a criteria for evaluation of morphofunctional changes in the rats' intestine that were fed with mycotoxin contaminated food the changes of mast cells quantity during different periods of the experiment were taken into consideration. As it is well known the mast cells are one of the specialized cellular populations of inner-tissues. They are actively involved in inflammation, immunogenesis, blood coagulation, blood circulation processes in the connective tissues supporting the maintenance of the local homeostasis. These cells perform their protective and regulating function through special mediators. The latter plays an important role in the migration regulation process of such effector cells as basophils, eosinophiles, neutrophils and macrophages from blood vessels to tissues, contribute to selective communication between them and endothelial cells i.e. adhaesio. The mast cells in rat's intestine are predominantly placed in the mucous membrane own plate in the intestinal villi and around the crypts.



a b

Fig. 5. The mast cells in rat's small intestine (a) and their degranulation (b). Painted according to Papenheim. M × 1000.

They are notable with considerable polymorphism which is expressed in the size and shape variety-ablong, sometimes hillock also degranulated in different density of the cytoplasm and etc. The cells as a rule contain large amounts of big granules, which are so dense in cytoplasm that the cell membrane is concealed, and usually it becomes invisible. On the 16th day of the experiment, the number of mast cells does not undergo considerable changes. However, the functional high activity of these cells can be recorded. Their degranulation is clearly seen: the cytoplasm

has a vivid expression of metachromasia but the grains are not expressed and the cytoplasm looks homogeneous.

The presence of microscopic particles with metachromasia in the intermedia around them come to witness that the mast cells emit their grain content under such conditions (Tab. 1, Fig. 5).

Table 1

Experiment duration	Number of mast cells (%) , $M \pm m$	P
control	24.3 ± 1.34	
15 days	26.74 ± 3.37	>0.05
30 days	37.85 ± 2.67	<0.05
60days	71.1 ± 6.19	<0.05

The changes of mast cells quantity in the rats' intestine fed with mycotoxin contaminated food during the different stages of the experiment.

During the 30 days period of feeding the rats with mycotoxin contaminated food brings to the increase of mast cells in the intestine's mucous membrane own plate.

They appear in the mucous membrane own plate between the crypts and also in the submucosal bases. They are quite large cells, sometimes hillock.

The activity of these cells, the release of inflammatory mediators, indicates a decrease in cytoplasmic grain growth as a result of which the large, light-colored painted core/nuclei is visible in the cells.

During the second month of our experiment the number of fertile cells in the experimental samples even grows, which illustrates the reactive state of the organism and the high activity of release of inflammatory mediators in the intestine under the influence of absorbed toxins.

Among the objectives were finding out the changes in the intensity of physiological regeneration of intestinal epithelium in the condition of feeding the rats with mikotoxin contaminated feeds within two months (Tab. 2).

Table 2

Experiment duration	Mitotic index (%), $M \pm m$	P
control	2.19 ± 0.415	
15 days	2.51 ± 0.39	>0.05
30 days	3.2 ± 0.546	>0.05
60 days	1.46 ± 0.058	<0.05

The mitotic index of the rats' intestinal epithelium fed with mycotoxin contaminated food during different stages of the experiment.

In the small intestine crypts the mitotic activity of enterocytes of the rats fed with mycotoxin mixture did not decrease during the first month of the experiment and it even declares growth tendency however, the difference to the controller indicator is not reliable.

Thus, we think that during first month despite of the mycotoxin invasion the intestinal epithelium natural regeneration was passing with its standard rhythm.

During the next period of experiment i.e. after feeding the animals with food contaminated with these data come to witness the fact of the weakening of the intestinal epithelium physiological regeneration intensity due to mycotoxins contaminated food which finds its reflection in the decrease of the epithelial cells' mitotic activity.

Conclusion. Summerizing the obtained data we can make general conclusions among which it is necessary to point out the fact that feeding the rats for two months with contaminated food by mycotoxins such as aflatoxin B1, ochratoxin A, and ZEN reactive changes are observed in the intestine which are manifested as destructive changes, as well as in the form of protective and adaptive ones.

Destructive changes are far more reflected in comparison with protective and adaptive ones during the two months feeding period with contaminated food.

During the early stages of the experiment i.e. in 16 days they find their reflection on the necrosis process of some enterocytes, picnotic changes in the nuclei cells, and in 30 and 60 days leads to necrosis of the apical parts, complete violation of the epithelial layer integrity in some parts even full violation of the epithelial layer.

The epithelial regeneration process decreases during the two months feeding process of the rats with mycotoxin contaminated food which is wintnessed in the low level of the enterocytes mitotic activity.

The protective-adaptive reaction in the rats' intestine is reflected by the lymphocyte growth in the epithelium and by the presence of relatively large amounts of mast cells and lymphoid tissues in submucosal layer.

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