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MORPHOFUNCTIONAL CHANGES IN RAT LUNGS UNDER THE INFLUENCE OF AFLATOXIN B1

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Morphofunctional changes in the lungs of rats under the influence of aflatoxin B1 were studied. The data obtained indicate that aflatoxicosis causes marked regressive changes due to pulmonary insufficiency, which are accompanied by massive destruction of lung cells, the presence of altered fields of view of emphysema, diffuse inflammatory foci, as well as an increase in the number of mast cells.

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***Keywords*:** Aflatoxin B1, lung, histological changes.

Introduction. Mycoses are diseases caused by pathogenic fungi. Currently, there are 100 known species of pathogenic fungi and 400 species of opportunistic pathogenic fungi. These fungi are found in the soil: mold spores and fragments of mycelium are constantly present in the air of cities, in apartments, open spaces. It has been shown that fragments of mycelium that often detach from contaminated surfaces retain their viability and can grow. The number of fungal particles in the air of buildings can reach 29 to 146 per cubic meter [1–6]. Under appropriate conditions the spores and mycelium of pathogenic fungi are implanted in human tissues and cause mycotic processes. Fungi produce toxic substances – mycotoxins, which become the cause of the development of various pathological phenomena in the organism [5, 7–11].

There is evidence in the literature that mold spores, fragments of mycelium, in addition to infectious and toxic effects on the human organism, can provoke the development of *immunopathological processes*, such as allergic reactions [5, 7–12].

There is evidence that mold antigens may have an adjuvant effect, that is, enhance the unique immune response of patients sensitized to other allergens such as tick antigens [8, 10, 12].

The morphological patterns of the effects of pathogenic mycogenic factors on the respiratory mucosa have not been sufficiently studied. There are only unique data in the literature on the possible provocative effects of mold cells and fragments on the development of autoimmune diseases [8, 10, 12].

Our study aimed to detect morphofunctional changes in the lungs of rats under the influence of aflatoxin B1 [1, 3, 13].

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Materials and Methods. The lungs of 12 rats weighing 150–200 *g* were used for work as a material. The group of experimental animals of 6 rats, received aflatoxin B1 at a dose of 0.0257 *mg* with food for 30 days. The lungs of 6 rats fed unpolluted feed served as controls. At the end of the experiments, under general anesthesia, the animals were weighed and killed by decapitation. Then, the sample taken from the lungs of each animal was fixed in Bouin and Carnoy's solutions. The material fixed in Bouin and Carnoy's solutions was subjected to histological processing [14]. Paraffin sections (4–5 *μm* thick) were prepared and stained with hematoxylin and eosin, as well as with blue toluidine solution prepared in acetate buffer.

The number of mast cells was counted on the blue toluidine processed microsamples of lung, in the same number of microscope fields at different periods of the experiment.

The data obtained during the studies were processed by Statistica 7 computer programm, the average arithmetic values of the indicators and the average errors were determined. The degree of probability of differences between the experimental and control animal groups was determined using the Student's table.

Results and Discussion. The structural and functional units of the respiratory tract of rats are the acini. Each acinus contains three types of respiratory bronchioles, alveolar ducts, and alveolar sacs.

The acini are separated by thin connective tissue layers. Several acini combine to form the pulmonary lobule (Fig. 1). The respiratory bronchioles are covered with a single layer of cuboidal epithelium. The epithelium is composed of Clara cells and individual ciliated cells. The wall of the bronchioles is made up of alveoli formed by flattened cells.

The alveolar ducts branch off from the respiratory bronchioles. They are lined with alveoli, between which you can see a ring of smooth muscle cells bundles (Fig. 2).

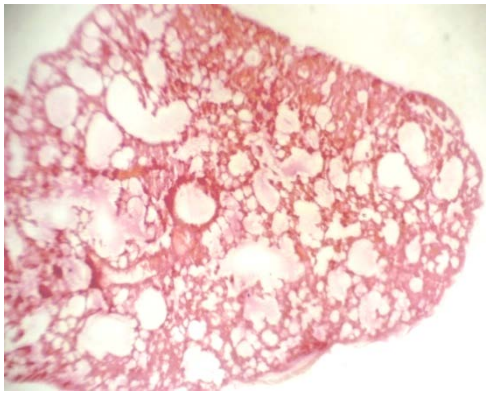

Fig. 1. Rat lung incision. Hematoxylin-eosin staining. Magnification ×40.

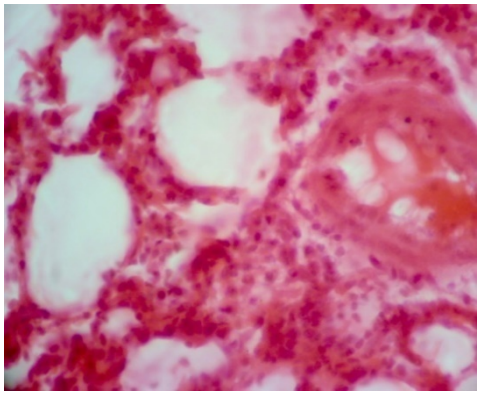

Fig. 2. Rat lung incision. Hematoxylin-eosin staining. Magnification ×400.

Alveolar sacs are clusters of alveoli. Alveoli are round formations covered with a flat epithelium surrounded by a dense capillary network. There are 2 types of cells in the epithelium: 1) flat or respiratory; 2) large or granular.

The first type, flat cells, contain a large number of pinocytotic vesicles. It is known from the literature that these cells are part of the blood–air barrier and are highly sensitive to toxic substances.

The second type of cells are cuboidal and contains granules in the cytoplasm. From the content of these granules, a surfactant is formed, an intercellular substance of lipoprotein nature, which forms a layer on the surface of the alveolar epithelium. Adjacent alveoliare connected by alveolar septum. Fibroblasts, macrophages, mast cells, lymphocytes, and leukocytes can be found in the composition of this septum.

While the lungs of rats exposed to aflatoxin B1 were under study, we followed morphofunctional changes of this organ. According to our data obtained in this study, after the introduction of aflatoxin B1 with food, on the 30th day of the experiment, dystrophic changes were observed in the lungs of rats, which were accompanied by the development of a chronic inflammatory process. Macrophage reactions could be clearly seen on microsamples stained with hematoxylin-eosin and toluidine blue. Due to the sparse alignment of the lung tissue, many anodic spaces were formed, including many altered fields of view of emphysema (Fig. 3).

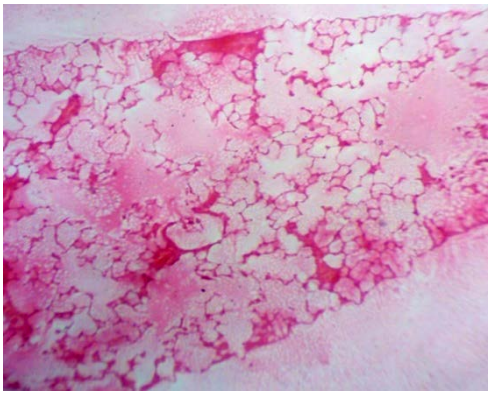

Fig. 3. Rat lung incision given aflatoxin B1 with feed. Staining with hematoxylin-eosin. Magnifcation ×40.

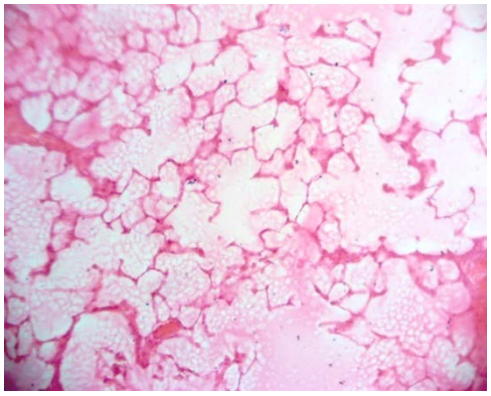

Fig. 4. Rat lung incision given aflatoxin B1 with feed. Staining with hematoxylin-eosin. Magnification ×100.

It can be assumed that these animals suffered from pulmonary insufficiency due to thinning of the lung wall, and violation of gas exchange.

The histomorphological picture of the lungs of animals with aflatoxicosis was significantly altered compared to the control. The ciliated cells were particularly structurally altered, and these epithelial cells were clearly inferior to the control animals. In some places, mass destruction of some areas of the cells that make up the wall of the alveoli and accumulation of necrotic cells could be seen (Fig. 4).

Along with the predominant retrograde, reactive changes, adaptive, compensatory phenomena were observed in the lungs of the experimental animals, which were manifested in an increase in the number of lymphocytes, granular leukocytes, and mast cells. These changes indirectly indicate the activation of humoral immune reactions.

After the introduction of aflatoxin B1 with food, on the 30th day of the experiment, the functional low level of alveoli observed in rat lungs was also

indicated by surfactant blurred images, presence of focal and diffuse inflammatory segments of connective tissue septa on incisions (Fig. 5).

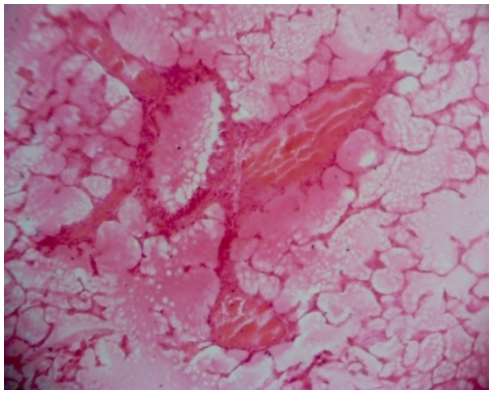

Fig. 5. Rat lung incision given aflatoxin B1 with feed. Staining with hematoxylin-eosin. Magnification ×100

In order to evaluate the reactive changes in the lungs of rats under daily intake of the above mentioned poison given with feed, we studied the changes in the number of mast cells.

Mast cells are known to be one of the specialized cell populations in the tissues of the internal environment. They are actively involved in processes of inflammation, immunogenesis, blood coagulation, blood circulation, helping to maintain local homeostasis. These cells perform their protective and regulatory function through special mediators. The latter play an important role in regulating the migration of effector cells from blood vessels to tissues, promoting selective contact between them and endothelial cells, i.e. adhesion.

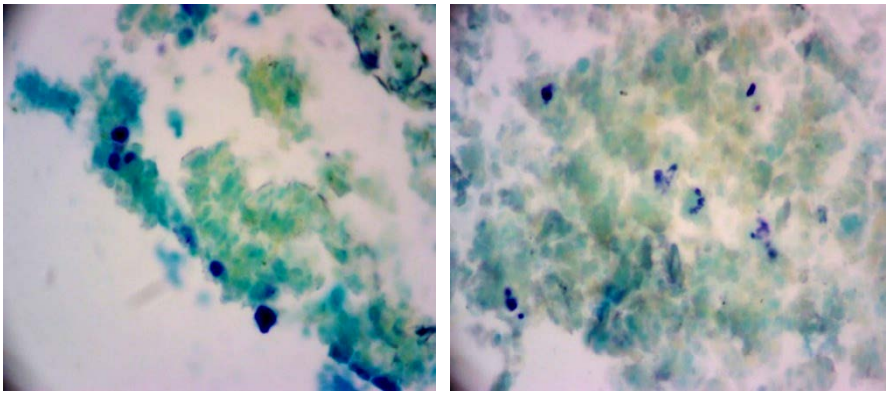

Fig. 6. Rat lung incision. Staining with toluidine blue. Magnification ×400.

Mast cells are located in the lung in the interlobular connective tissue. In the lungs of rats, the mast cells are quite large cells. The large granules in the cytoplasm have the property of metachromasia; they are dyed dark purple on the preparations treated with toluidine blue (Fig. 6). They stand out by a significant polymorphism,

which is manifested in the variety of their size and form, in different densities of granules in the cytoplasm. Due to the dense arrangement of the granules, the cell nucleus is usually camouflaged.

The table presents the data obtained in our work on rats that were fed feed contaminated with mentioned mycotoxin with daily feeding for 30 days.

Quantitative changes in mast cells under the conditions of introduction of mycotoxins with feed

Experimental animals	Number of mast cells, %
Control	6.60±0.62
Aflatoxin B1	13.10±0.75 ($p<0.05$)

As can be seen from the data in the Table, the number of fat cells in the lungs increases with the introduction of mycotoxins.

Conclusion. To sum up, histomorphological examination of the lungs of rats suffering from aflatoxicosis reveals noticeable retrograde changes. This is due to pulmonary insufficiency, which are manifested in the presence of mass destruction of cells, altered fields of view of emphysema, diffuse inflammatory foci. In the lungs, food contaminated with aflatoxin B1 increases the number of mast cells as an indicator of increased reactivity.

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ԱՌՆԵՏՆԵՐԻ ԹՈՔԵՐԻ ՄՈՐՖՈՖՈՒՆԿՑԻՈՆԱԼ ՓՈՓՈԽՈՒԹՅՈՒՆՆԵՐՆ ԱՖԼԱՏՈՔՍԻՆ B1-Ի ԱԶԴԵՑՈՒԹՅԱՆ ՊԱՅՄԱՆՆԵՐՈՒՄ

Ուսումնասիրվել են առնետների թոքերի մորֆոֆունկցիոնալ փոփոխություններն աֆլատոքսին B1-ի ազդեցության պայմաններում: Ստացված արդյունքները վկայում են այն մասին, որ աֆլատոքսիկոզը հարուցում է թոքային անբավարարությամբ պայմանավորված նկատելի հետադիմական փոփոխություններ, որոնք ուղեկցվում են թոքային բջիջների զանգվածային քայքայմամբ, էմֆիզեմատոզային փոփոխված տեսադաշտերի, դիֆուզ բորբոքային օջախների առկայությամբ, ինչպես նաև պարարտ բջիջների քանակության ավելացմամբ:

А. Ф. КАРАПЕТЯН, А. В. ГРИГОРЯН, Г. А. АВАГЯН

МОРФОФУНКЦИОНАЛЬНЫЕ ИЗМЕНЕНИЯ ЛЕГКИХ КРЫС ПОД ДЕЙСТВИЕМ АФЛАТОКСИНА B1

Изучены морфофункциональные изменения в легких крыс под действием афлатоксина B1. Полученные данные свидетельствуют о том, что афлатоксикоз вызывает обусловленные легочной недостаточностью выраженные деструктивные изменения, которые сопровождаются массивным разрушением клеток легких, наличием эмфизематозных очагов, диффузных воспалительных очагов, а также увеличением количества тучных клеток.