ORIGINAL ARTICLE

CONTENTS

Peculiarities of ultrastructural remodeling of the respiratory portion of rat lungs caused by consumption of complex of food additives

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ABSTRACT

Aim: The aim of the study was to investigate the ultrastructural changes in the components of the respiratory portion of the lungs in rats under normal conditions and under the impact of the complex of food additives: monosodium glutamate, sodium nitrite and Ponceau 4R.

Materials and Methods: Rats in the experimental group were administered 0.6 mg/kg of sodium nitrite, 20 mg/kg of monosodium glutamate, and 5 mg/kg of Ponceau 4R in 0.5 ml of distilled water once daily orally. The animals were removed from the experiment after 1, 4, 8, 12 and 16 weeks. An electron microscopic examination was performed.

Results: The respiratory portion of the rat lungs has a typical structure and can be extrapolated to the human body. At the early stages, vasospasm of the capacitance vessels was observed, accompanied by erythrocyte stasis, which led to the initial impairment of blood perfusion through the vessels. This was reflected in the condition of the interalveolar connective tissue and manifested as edema. Destructive and dystrophic changes were observed in the alveolar type II cells, leading to a disruption of the normal surfactant structure, as well as destructive changes in alveolar type I cells and the hemocapillary wall. Additionally, signs of activation of the humoral branch of the immune system were noted, evidenced by the presence of actively phagocytizing alveolar macrophages.

Conclusions: The consumption of the complex of food additives leads to destructive-dystrophic changes in the structural components of the lungs in rats and reorganization of the components of the intercellular substance.

KEY WORDS: food additives, monosodium glutamate, sodium nitrite, Ponceau 4R, lungs, rats

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INTRODUCTION

Experimental modeling of various diseases in animals is one of the primary methods for studying the patterns of pathological processes that frequently occur in clinical practice. For an objective comparative evaluation of experimental data and their subsequent extrapolation to humans, it is important to know the key structural features of organs and tissues in the normal state [1].

Recent scientific studies explore the features of changes in the macro- and microelement composition of the lungs in young rats under the impact of various exogenous factors [2, 3]. However, the issue of the impact of food additives on the structure and function of the respiratory system organs, specifically the respiratory portion of the lungs, remains unresolved and requires further research, especially under conditions of their combined action.

Given the unprecedented scale of food additive use today, the relevance of studying their impact on the body is unquestionable. Our analysis of food additives in products from both domestic and foreign manufacturers revealed that monosodium glutamate, sodium nitrite and the synthetic dye Ponceau 4R are the most commonly used additives by producers

Preclinical studies by domestic and foreign researchers have linked the intake of monosodium glutamate to cardiotoxicity, hepatotoxicity, neurotoxicity, low-grade inflammation, metabolic disorders, precancerous conditions and behavioral changes. Furthermore, reports have suggested a connection between the consumption of monosodium glutamate and tumor processes, as well as increased oxidative stress. Therefore, it was concluded that further clinical and epidemiological research is needed. A critical review of the existing literature indicates that many of the negative reports on the health effects of monosodium glutamate are poorly informative, as they are based on excessive doses that do not correspond to the standard levels typically used in food products [4].

A number of studies on experimental animals investigate the impact of sodium nitrite on the development of

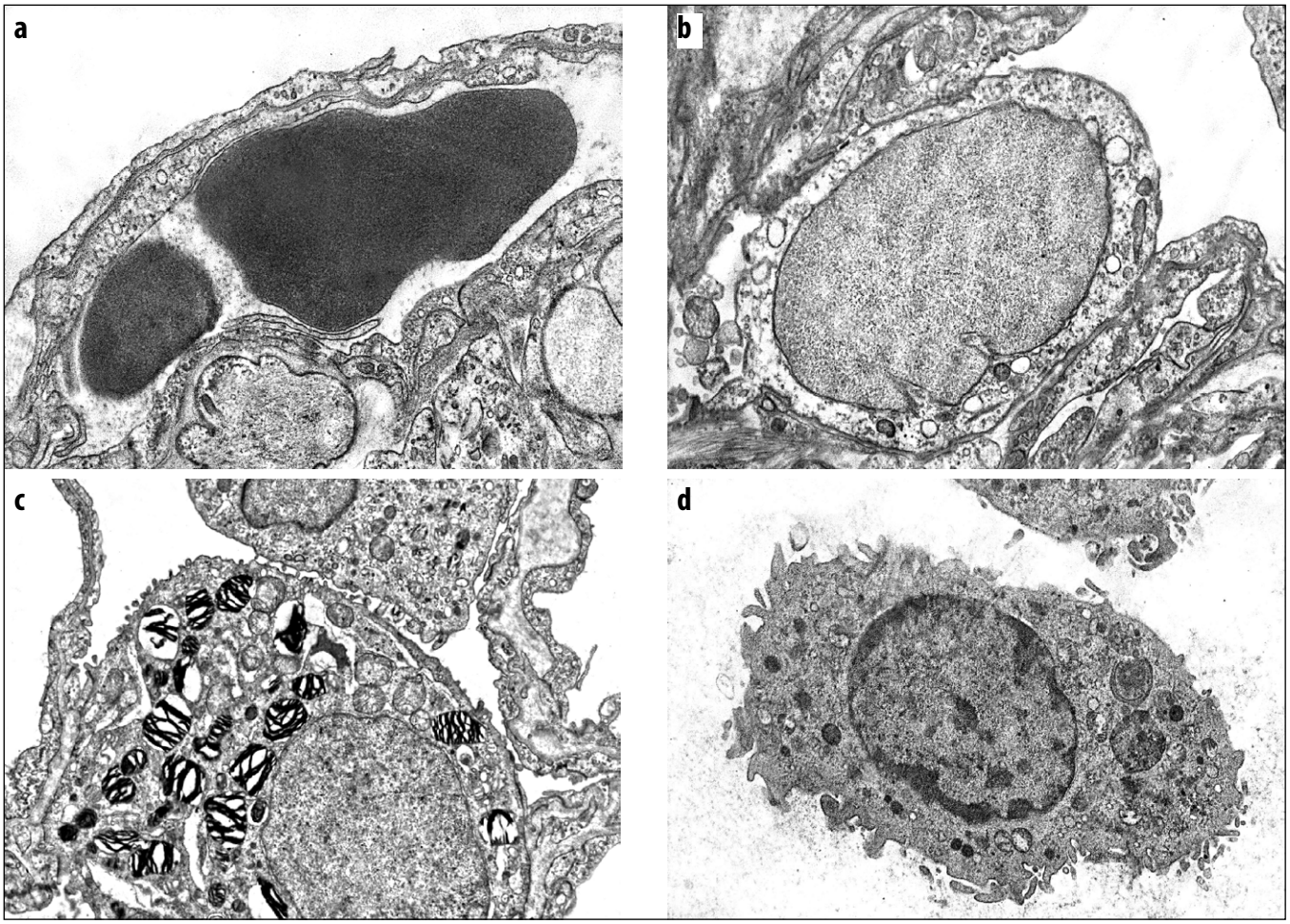

Fig. 1: a). Capillary as part of the air-blood barrier; b). Alveolar Type I cell; c). Alveolar Type II cell. d). Intra-alveolar macrophage of rats from the control group. Electron micrograph. Magnification ×8000
Picture taken by the authors

pulmonary hypertension induced by monocrotaline [5], as well as how the nebulization of acidified sodium nitrite reduces acute hypoxic pulmonary vasoconstriction [6].

Studies on the effects of food colorants have described numerous allergic reactions to food additives, such as urticaria, Quincke's edema, rhinitis, bronchitis and bronchial asthma [7-10]. Therefore, a thorough evaluation of the effects of azo dyes is needed.

AIM

The aim of the study was to investigate the ultrastructural changes in the components of the respiratory portion of the lungs in rats under normal conditions and under the impact of the complex of food additives: monosodium glutamate, sodium nitrite and Ponceau 4R.

MATERIALS AND METHODS

The study involved 84 sexually mature male rats. The animals in the control group received drinking water and were orally administered a physiological saline solution. The rats in the experimental group, with free access to water, were administered 0.6 mg/kg of sodium nitrite, 20 mg/kg of monosodium glutamate, and 5 mg/kg of Ponceau 4R in 0.5 ml of distilled water once daily. The doses of food additives were half of the maximum allowable norm. The adaptive behavior of the rats was assessed using the open field test [11].

The animals were euthanized by overdose of thiopental anesthesia at 1, 4, 8, 12, and 16 weeks. After euthanasia, fragments of the rats' lungs were fixed in 2.5% glutaraldehyde solution. Subsequently, lung tissue samples were embedded in Epon-812 using a conventional technique [12]. Electron microscopy studies were carried out at the electron microscopy laboratory of the Institute of Morphology at I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine. Ultrathin sections were prepared using the ultramicrotome LKB-3 (Sweden). The sections were contrasted first in a 1% uranyl acetate solution in methanol, and then with lead citrate according to Reynolds [13]. The

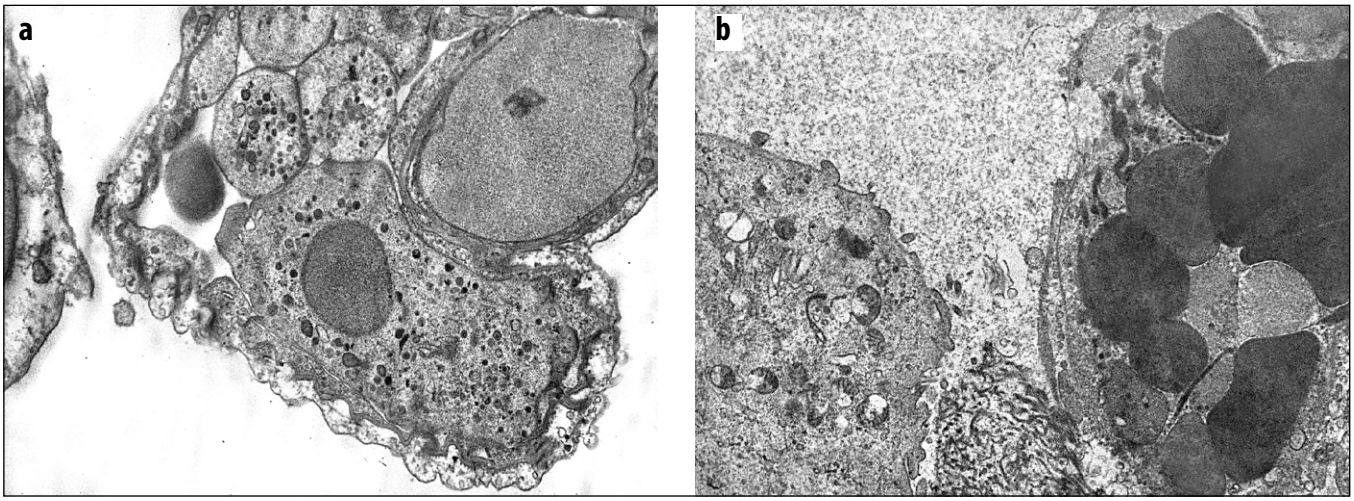

Fig. 2: a). Alveolar Type I cell; b). Stasis of erythrocytes in the venules of the respiratory portion of the lungs on week 1 of the experimental consumption of monosodium glutamate, sodium nitrite and Ponceau 4R. Electron micrograph. Magnification ×8000
Picture taken by the authors

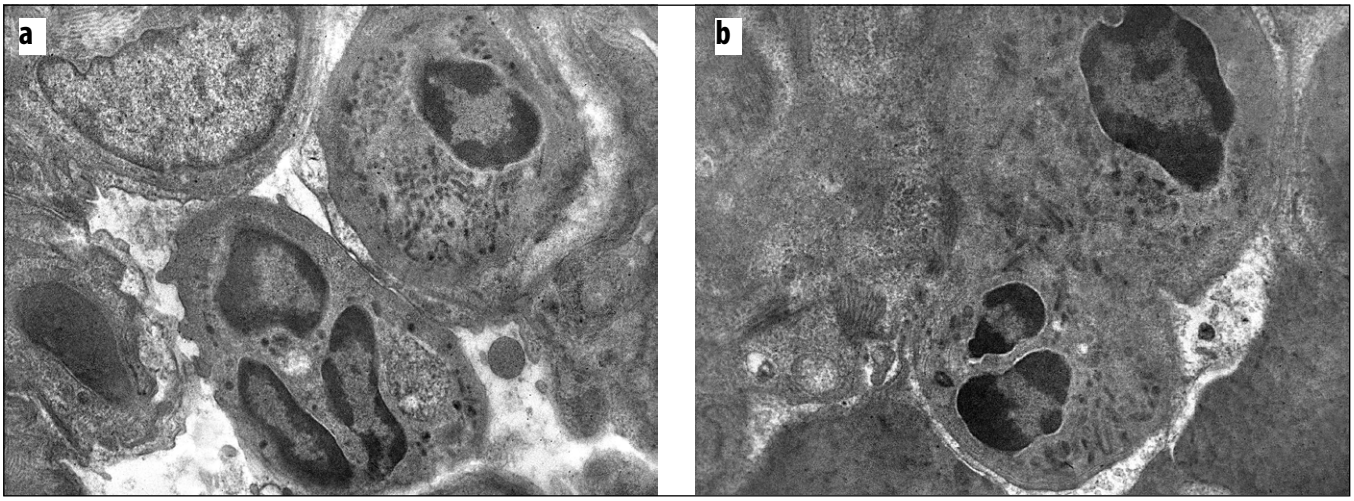

Fig. 3: a) Migration of neutrophils and macrophages through the vascular endothelium on week 4 of the experiment. Electron micrograph. Magnification ×8000. b) Migration of neutrophils and macrophages through the vascular endothelium on week 4 of the experiment. Electron micrograph. Magnification ×8000
Picture taken by the authors

specimens were analyzed under an electron microscope PEM-125 K (serial number 38-76, TU 25-07-871-70) at an accelerating voltage of 50-75 kW.

All animal experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986), in accordance with the rules for keeping experimental animals established by European Parliament and Council Directive (2010/63/EU) and the Order №134 of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 "On approval of the procedure for conducting tests, experiments on animals by research institutions", as well as the recommendations of the First National Congress of Ukraine on Bioethics (2001).

RESULTS

The study of the electron-microscopic structure of the respiratory portion of the lungs revealed that the alveolar walls of rats in the control group were formed by the alveolar type I and II cells, a basal membrane and endothelial cells of somatic-type capillaries. The peripheral part of the endothelial cells tightly adheres to the basal membrane, and the cytoplasm contains a large number of pinocytic vesicles. The nuclei of the endothelial cells have clear contours, and their internal content is almost homogeneous, represented by euchromatin. Erythrocytes are freely located in the lumen of the vessels. This arrangement of structures in the air-blood barrier ensures more efficient gas diffusion between the alveolar lumen and the blood vessels.

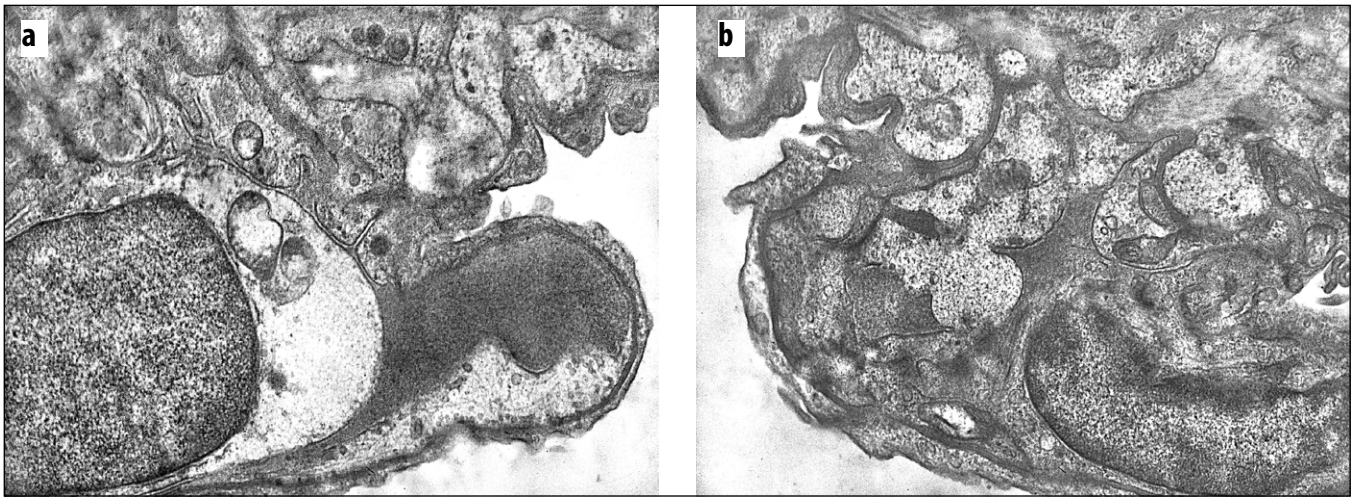

Fig. 4: a). Protrusion of the nucleus-containing part of capillary endothelial cells; b). Edema of the interalveolar interstitial tissue on week 4 under the impact of the complex of food additives. Electron micrograph. Magnification ×8000
Picture taken by the authors

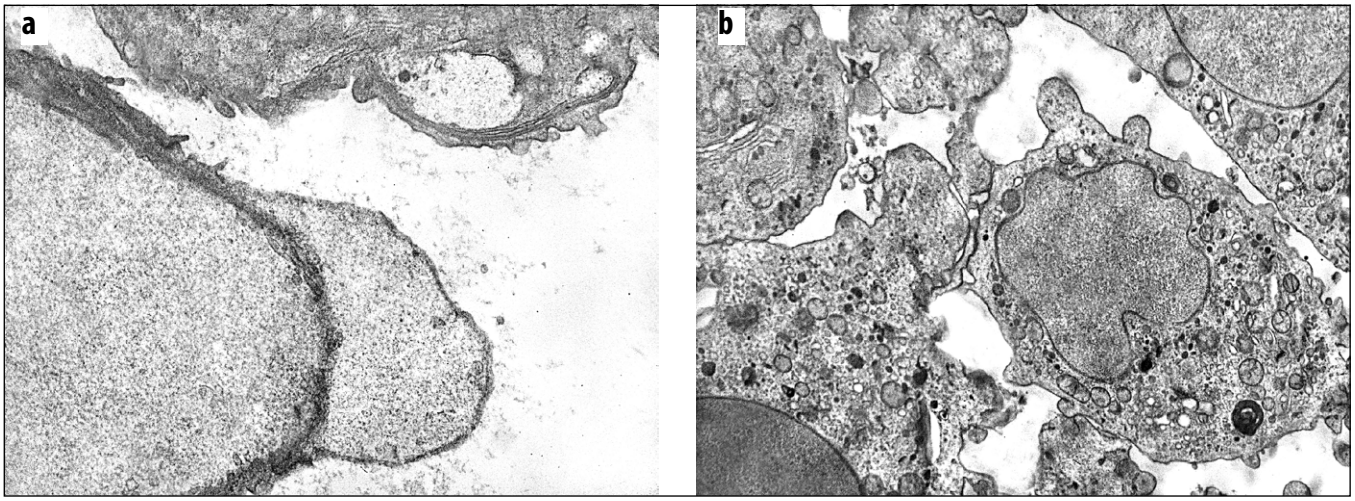

Fig. 5: a). Vacuolization of the cytoplasm in alveolar type II cells; b). Deformation of lamellar body membranes in alveolar type II cells on week 8 of the experiment. Electron micrograph. Magnification ×10000
Picture taken by the authors

Alveolar type I cells, which cover a large surface area of the alveolar wall, are involved in the formation of the air-blood barrier. They have a central region containing the nucleus and a peripheral region that is elongated and occupies a significant portion of the respiratory area of the alveolar cell, extending along the basal membrane. The cytoplasm of the peripheral part showed almost no organelles but contained a large number of pinocytic vesicles. Alveolar type II cells were located on the basal membrane, in such a way that they made contact with adjacent alveoli. On the apical surface, microvilli were present. The nucleus had an eccentric position, and the cytoplasm contained a large number of mitochondria, well-developed endoplasmic reticulum, and Golgi complex. Lamellar bodies, which exhibited osmiophilic properties, were found throughout the cytoplasm. Intra-alveolar macrophages of the control group of rats had varying shapes, from elongated to more rounded. The cytolemma was heterogenous due to the formation of various folds and invaginations, and the surface contained microvilli. The cytoplasm contained a large number of lysosomes with osmiophilic properties. The nucleus was centrally located, with euchromatin predominating in its composition (Fig. 1a, Fig. 1b, Fig. 1c, Fig. 1d).

After one week of consumption of the food additive complex, the nuclei of alveolar type I cells acquired an irregular shape, with invaginations present. Among the electron-dense euchromatin, dense areas of heterochromatin appeared. In the cytoplasm of the nuclear-containing portion, areas of transparency and darkening were observed. In the peripheral regions of alveolar type I cells, an increased number of pinocytic

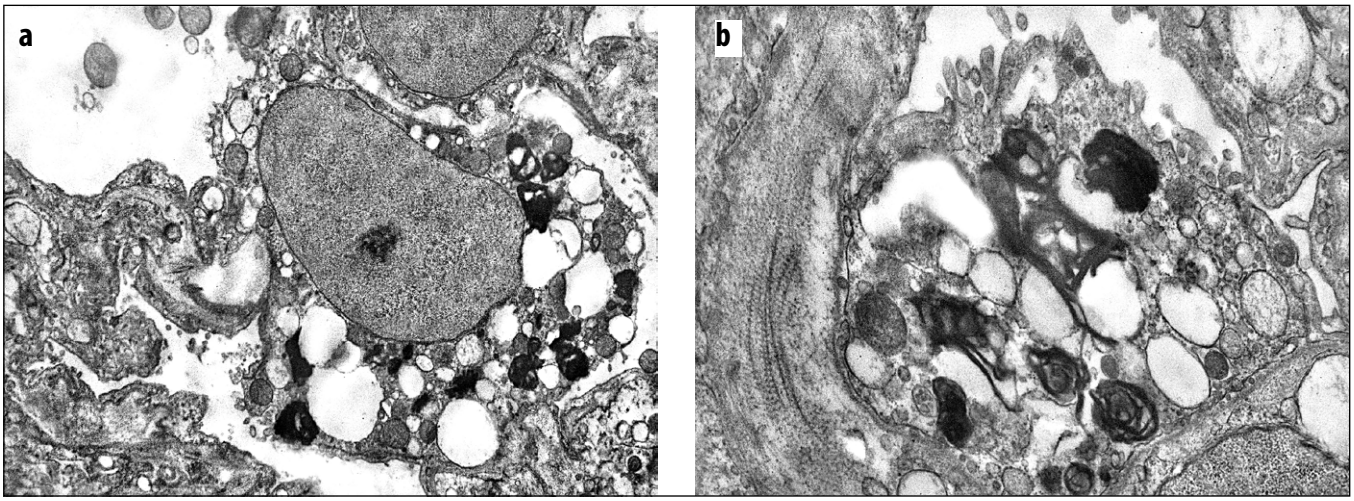

Fig. 6: a) Dilation of the capillary lumen in the alveoli of rat lungs; b). Phenomenon of vessel emptiness in the capacitance segment of the respiratory portion of the lungs in rats of the experimental group on week 12. Electron micrograph. Magnification ×8000
Picture taken by the authors

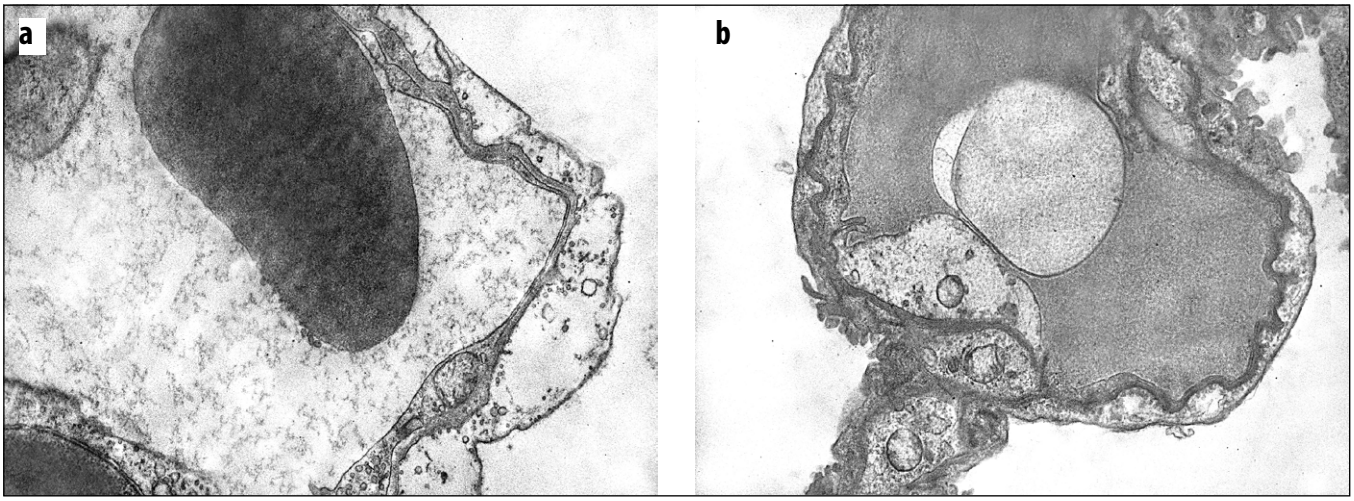

Fig. 7: a). Protrusion of the cytoplasmic portion of the alveolar type I cell into the alveolar lumen; b). Activation of macrophages on week 12 of consumption of monosodium glutamate, sodium nitrite, and Ponceau 4R. Electron micrograph. Magnification ×10000
Picture taken by the authors

vesicles was detected. The basal membrane mostly retained its normal structure, but focal thickening with indistinct contours and uneven course was noted. In the alveolar lumen, a significant increase in the number of intra-alveolar macrophages was observed, with cytoplasm containing a large number of osmiophilic primary lysosomes and a small number of secondary lysosomes. In the alveolar type II cells, a decrease in the number of lamellar bodies, which were in the process of degranulation, was noted, exhibiting signs of reduced osmiophilic properties. These bodies were of irregular shape with signs of local degeneration. A decrease in the number of microvilli on the plasmalemma and the appearance of numerous protrusions and invaginations were observed. A picture of vascular spasms in the capacitance vessels with erythrocyte stasis inside the vessels was evident. Collagen fibers in the adventitial layer of venules had a wavy shape (Fig. 2a, Fig. 2b).

By the 4th week of consumption of the chemical compound complex, folding and migration of leukocytes occurred in the vessels of the interalveolar interstitial tissue, with a predominance of neutrophils and macrophages. The connective tissue of the interalveolar septa showed signs of edema, with a large number of migratory cells whose cytoplasm was filled with granules of varying shape, size and electron density. A large number of primary lysosomes exhibited osmiophilic properties. In the segmented nuclei of neutrophils, euchromatin predominated, while the nuclei of macrophages had an oval shape, mostly with uneven edges, and their internal content was also filled with euchromatin (Fig. 3a, Fig. 3b).

Due to the increasing edema, the shape of alveolar

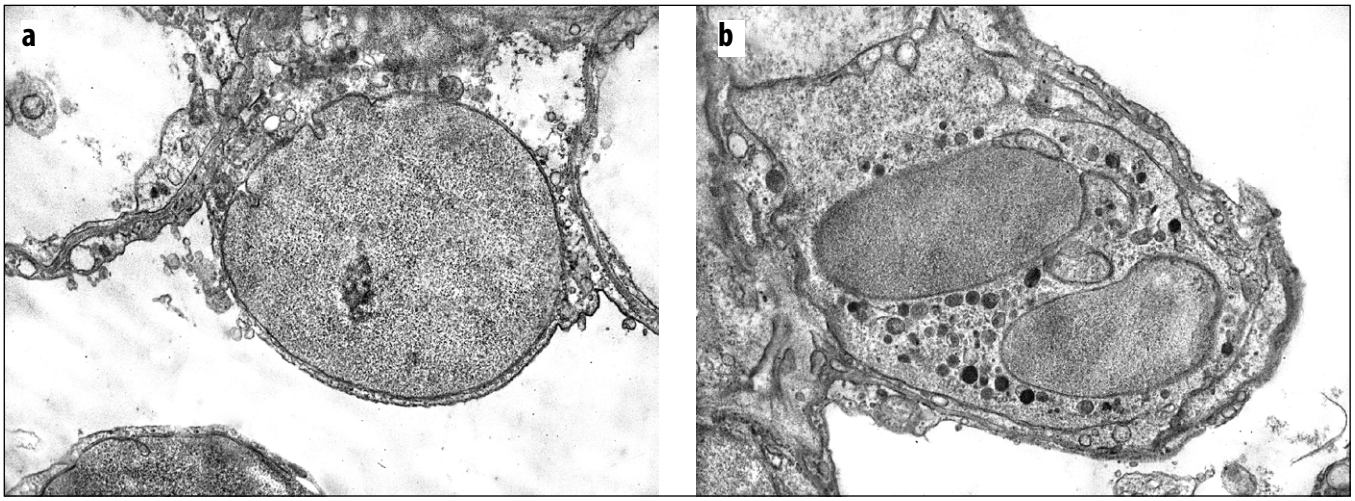

Fig. 8: a). Destructive phenomena in alveolar type I cells; b). Migration of eosinophils into the alveolar lumen on week 16 of consumption of monosodium glutamate, sodium nitrite and Ponceau 4R. Electron micrograph. Magnification ×10000
Picture taken by the authors

type I cells changed. The nuclei were hypertrophied, predominantly filled with heterochromatin. The cytoplasm contained a small number of organelles and pinocytic vesicles, appeared swollen, and translucent. The nuclear-containing portion of the endothelial cells of the capillaries in the aerogematic barrier was bulging. The basal membrane contained areas of thinning and thickening. The interstitial tissue showed signs of intensified edema. Locally, areas of electron-transparent material were identified, filling the gaps between cells in the interstitium and forming conglomerates. The aerogematic barrier thickened, with the basal membrane showing uneven thickness and indistinct edges on the interstitial side. The nuclei of endothelial cells were elongated and contained a moderate amount of euchromatin (Fig. 4a, Fig. 4b).

By the 8th week of consuming the complex of monosodium glutamate, sodium nitrite, and Ponceau 4R, changes were observed in the cytoplasm of alveolar type II cells, manifested by the appearance of numerous vacuoles and cavities containing remnants of lamellar material. The vacuoles varied in diameter, predominantly oval, and sometimes polygonal in shape, with differing electron density. The number of lamellar bodies was reduced, with some being deformed and partially filled with phospholipid material, exhibiting disoriented and deformed membranes, indicating a stage of destruction. The apical surface of secretory alveolar cells contained a small number of microvilli. The interstitial tissue showed a reduction in the intensity of edema (Fig. 5a and Fig. 5b).

By the 12th week of the experiment, in the blood vessels of the microcirculation, there was noticeable dilation of the capillary lumen, which was predominantly filled with blood plasma showing signs of low electron density. Sporadic erythrocytes were freely present within the lumen. The cytoplasm of endothelial cells was moderately edematous and contained a small number of pinocytotic vesicles. Similar changes were observed in the peripheral regions of the cytoplasm of respiratory alveolar cells, which were filled with electron-lucent substance. The basal membrane displayed an irregular profile with areas of local thickening and thinning. The venules exhibited signs of collapse, with regions of emptiness interspersed with substance of low electron density. Blood cellular elements were absent (Fig. 6a, Fig. 6b).

By the 12th week of the experiment, alveolar type I cells exhibited mild destruction of their structural components. Their cytoplasm appeared homogeneous and filled with an electron-lucent, almost transparent substance, indicating intracellular edema. This led to a noticeable bulging of cytoplasmic segments of the respiratory alveolar cells into the alveolar lumen. The cytoplasm of the secretory (type II) alveolar cells was heterogeneous, and their apical surfaces showed a near-total absence of microvilli. At this stage, an increased number of alveolar macrophages was observed, suggesting enhanced functional activity. Their surfaces were covered with numerous cytoplasmic projections. The nuclei were irregularly shaped with multiple invaginations, and euchromatin predominated within. The cytoplasm contained osmiophilic primary lysosomes as well as secondary ones. The matrix of small, variably shaped mitochondria exhibited moderate electron density. Cisternae of the rough endoplasmic reticulum were shortened and thickened, containing a moderate number of ribosomes (Fig. 7a, Fig. 7b).

At the 16th week of pollutant consumption, elec-

tron microscopy revealed alveoli with both collapsed lumens and emphysematously expanded ones. The peripheral regions of alveolar type I cells were locally edematous, showed destructive changes, and contained a small number of damaged organelles. A large number of eosinophils were observed in the alveolar lumen, characterized by increased functional activity. The nuclei were predominantly filled with euchromatin. The cytoplasm contained numerous osmiophilic primary lysosomes and specific granules (Fig. 8a, Fig. 8b).

DISCUSSION

Thus, electron microscopy of the respiratory apparatus of the lungs in rats from the control group revealed that the alveolar wall was composed of alveolar type I and type II cells, a basal membrane, and somatic-type capillary endothelial cells. Type I alveolar cells possessed a nucleus-containing region and a thin cytoplasmic extension that spread as a delicate layer along the basal membrane, facilitating efficient gas exchange. The cytoplasm contained a small number of organelles and numerous pinocytic vesicles. The cytoplasm of alveolar type II cells was rich in synthetic organelles and mitochondria. A large number of lamellar bodies and a well-developed Golgi complex indicated active surfactant synthesis. The apical surface of alveolar type II cells was covered with numerous microvilli. In the alveolar lumen, solitary macrophages were noted, containing osmiophilic primary lysosomes in their cytoplasm. Therefore, the respiratory portion of rat lungs has a typical structure and can be extrapolated to the human body.

At the 1st week of the experiment, disturbances were observed in the blood vessels of the microcirculation [14], particularly in the capacitance section, which manifested as vascular spasm with erythrocyte stasis inside the vessels. This led to initial perfusion disorders in the vascular system and was reflected in the condition of the interalveolar connective tissue throughout the experiment, becoming evident by week 4 as interstitial edema and disorganization of stromal components. Local areas of electron-lucent substance were detected, filling the intercellular spaces and forming conglomerates. Thickening of the air-blood barrier was detected, with the basal membrane being uneven, widened, and having blurred edges. Previous studies [15] on the state of diffuse lymphoid tissue in the lungs indicated its hyperplasia, reflecting activation of compensatory reactions, which in turn led to stress in the nonspecific and humoral components of the immune system. This was confirmed by an increase in the number of intra-alveolar macrophages, as components of food additives, particularly colorants, can act as haptens and exhibit antigenic properties [7; 8; 9], thereby increasing their activity and promoting leukocyte migration, predominantly neutrophils. Edematous changes caused deformation of respiratory alveolar cells and endothelial cells, as confirmed by electron microscopic data, and also led to thickening of the air-blood barrier. This triggered hyperfunction of secretory alveolocytes and further disruption of gas exchange processes. By the 8th week, destructive and dystrophic changes in alveolar type II cells were observed, leading to disruption of the normal surfactant structure, destructive changes in alveolar type I cells, and damage to the hemocapillary wall. As the experiment progressed, alongside increasing destruction of the air-blood barrier components, compensatory-reparative reactions were also noted, leading to improvement in the condition of alveolar epithelial components. However, signs of humoral immunity activation were simultaneously observed, manifested by the presence of actively phagocytizing alveolar macrophages. Impaired gas exchange processes led to the formation of so-called emphysematous zones in the alveolar apparatus and further destruction of the alveolar epithelium. Consumption of the complex of food additives also activated nonspecific immune responses, resulting in active migration of eosinophils into the alveolar lumen [16].

CONCLUSIONS

Thus, the consumption of the complex of food additives leads to destructive-dystrophic changes in the structural components of the lungs in rats and a reorganization of the intercellular substance components. The alveoli exhibited both collapsed lumens and emphysematously dilated areas due to the destruction of synthesis organelles and disruption of gas exchange processes, with lamellar bodies being significantly altered. The prolonged action of the components of the food complex resulted in the activation of alveolar macrophages, which were in a state of increased activity and characterized by polymorphism in their structural organization, alongside the activation of the nonspecific arm of the immune response.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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