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## THE ULTRASTRUCTURAL FEATURES OF THE ADRENAL GLANDS UNDER THE COMBINED EFFECT OF HYPOBARIC HYPOXIA AND STAPHYLOCOCCAL PERITONITIS

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The research work is devoted to the study of the pathogenesis and morphogenesis of ultrastructural changes of the adrenal glands under the combined influence of hypobaric hypoxia and staphylococcal infection. Hypoxia and infection develop ultrastructural changes in glandular tissue separately, as well as in combination, which are of a phase nature. In particular, the combined effect of both factors violates the structure of intracellular organelles, reduces their size, leads to deformation and atrophy of adrenocytes, focal growth of connective tissue, and in the course of the experiment, glandular cells adapt to a new environment.

**Keywords:** hypoxia, staphylococcal infection, adrenal glands, ultrastructure

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## УЛЬТРАСТРУКТУРНІ ОСОБЛИВОСТІ НАДНИРИКІВ ЗА ПОЄДНАНОГО ВПЛИВУ ГИПОБАРИЧНОЇ ГІПОКСІЇ І СТАФІЛОКОКОВОГО ПЕРИТОНІТУ

Робота присвячена вивченню патогенезу і морфогенезу ультраструктурних змін надниркових залоз за одночасного впливу гіпобаричної гіпоксії і стафілококової інфекції. Гіпоксія і інфекція розвивають ультраструктурні зміни залозистої тканини окремо, а також в поєднанні, які носять фазовий характер. Зокрема, спільна дія обох факторів порушує структуру внутрішньоклітинних органел, зменшує їх розмір, призводить до деформації і атрофії адреноцити, вогнищеве розростання сполучної тканини, а в ході експерименту залозисті клітини адаптуються до нового середовища.

**Ключові слова:** гіпоксія, стафілококова інфекція, наднирники, ультраструктура.

*The work is initiative.*

Various scientific studies in recent years have shown that microenvironmental factors alter cell morphology, cell surface marker expression, metabolic activity, viability, phagocytosis, and cytokine secretion by macrophages, as well as bacterial pathogenicity [4]. Any stress factor results in the activation of the hypothalamo-hypophyseal-adrenal axis (HHA) in the body, which increases the secretion of corticosteroids to optimize the stress response. According to research, the strongest irritants of the HHA axis are hypoxia, hypotension, infections of various origins, and sepsis [7].

A group of authors notes that hypoxia accelerates *S.aureus* killing by stimulated human neutrophils in vitro [6], and neutrophils isolated from subjects exposed to intermittent hypoxia have a strong bactericidal effect against *S.aureus* [9]. Other authors note that in vitro hypoxia weakens the bactericidal effect of neutrophils on *S.aureus* [10] and that the effective destruction of *S.aureus* depends on the formation of reactive oxygen species (ROS) [5].

During the period of widespread endocrine pathologies in the world, the morphofunctional changes occurring in the endocrine system, mainly in the adrenal tissues, under the influence of stress factors of various origins, the correction and practical significance of these changes, in particular, the dynamics of the restoration of anatomical structures during the processes of reparative regeneration and adaptation, have not been sufficiently studied. In this regard, despite the numerous research works devoted to the influence of stress factors on the endocrine organs, in particular, hypoxia and infection separately, the lack of information about the combined impact of these factors creates a significant information deficiency. Therefore, it is important to research this area.

**The purpose** of the study was to establish ultrastructural properties of the adrenal glands during hypobaric hypoxia and staphylococcal infection.

**Materials and methods.** The study was performed on 70 white rats weighing 180–200 grams according to the ethical rules and humanistic principles approved by the Ethics Rules Commission under the Ministry of Health of the Republic of Azerbaijan on 21.04.2008. Animals were divided into 4 groups – control, hypoxia, infection, and basic experimental groups.

Animals of the control group (n=10) were not subjected to intervention, animals included in the hypoxia group (Group II) (n=20) were placed in a special barochamber with a temperature of 19-20°C, atmospheric pressure equal to the pressure at an altitude of 2000-3000 m above sea level, 2 times a week for 5 hours every day. The culture of *S.aureus* dissolved in the concentration of  $1 \cdot 10^9$  microbial cells/kg (per kg) in the volume of 1 ml was injected into the peritoneal cavity of the animals included into the infection Group (Group III) (n=20) and the infection model (staphylococcal peritonitis) was created. The animals included in the main group (Group IV) (n=20) were exposed to the combined effects of hypoxia and infection. For this purpose, the animals were infected by injecting a suspension of *S.aureus* culture into the peritoneal cavity and kept in a barochamber for 2 hours every day, 5 times a week. The experiments were conducted during daylight hours, about 10–15<sup>00</sup>.

To study the combined effects of hypoxia and infection, the adrenal glands were removed after decapitation of the surviving rats under intraperitoneal anesthesia (subject to injection of 100 mg/kg 2–2.5 % solution of thiopental sodium) on the 15th and 30th days of the experiment, respectively and morphological features of the gland were studied by histological, electron microscopic and morphometric methods.

**Results of the study and their discussion.** To explain the combined effects of infection and hypoxia, we have tried in this article to provide a comparative analysis of the ultrastructural changes occurring in the adrenal gland under the isolated and combined effects of hypoxia and infection on the 15th and 30th days of the experiment.

During the electron microscopic examination of the adrenal glands of the animals included in the control group, the basement membrane of the adrenocytes is visible, the cytoplasm is transparent, the nuclei are located in the center of the cytoplasm, and the intracellular organelles are located around the nucleus. Vesicular cristae of mitochondria, small-sized an oval-shaped vesicles of the endoplasmic reticulum, cisternae of the Golgi complex, as well as a small number of lysosomes, ribosomes, and liposomes around the Golgi complex are observed.

On the 15th day of the experiment, an electron microscopic examination of the adrenal gland material taken from animals of the hypoxia group showed weak ultrastructural changes as a result of hypoxia. The basal membrane is visible, but the microvilli of the plasmalemma are slightly thickened. In some cells, the nuclear membrane is deformed, the nuclear chromatin is pale, and the nucleoli are poorly visible. Cytoplasmic organelles – the number of lysosomes and ribosomes decreases, there is a weak expansion of the cisterns of the endoplasmic reticulum, mitochondria undergo dystrophic changes (fig.1).

Under the influence of hypoxia, mitochondria have cristae with unclear contours and change their shape – from elongated to rounded. On the electronograms, the fenestrated endothelial cells of the capillaries are visible.

On the 30th day of hypoxic exposure, the electron microscopic picture of the adrenal cortex and medulla, as well as individual zones of the adrenal cortex, acquires the appearance characteristic of a normal organ structure. The cytoplasm of the cells is transparent, the nuclei are rounded, located mainly in

the center of the cytoplasm, and the nucleolus is eccentric. Condensed diffuse chromatin is observed inside the nucleus.

The endoplasmic reticulum is poorly visible; the Golgi complex is observed near the nucleus. Individual fibroblasts are visible in the stroma.

On the 15th day of the experiment, the heterogeneity of ultramicroscopic damage of cells and cellular organelles of the adrenal glands in animals included in the infection group draws attention. The nuclei of glandular cells, especially the nuclei of the cells of the adrenal medulla, are characterized by an irregular shape and eccentricity of the nucleolus due to numerous invaginations of the membrane. The condensation of heterochromatin in the form of lumps is observed mainly near the inner layer of the nuclear membrane.

Symptoms of adrenal depletion characterized by a decrease in the number and size of secretory vesicles and a decrease in the size of mitochondria and an increase in their electron density are observed. The cisterns of the endoplasmic reticulum significantly increase and in some places acquire a "spherical" shape. In the cytoplasm, along with mitochondria, lysosomes, and ribosomes, there are a small number of lipid droplets. In the adrenal parenchyma, there is a focal thickening and edema of the connective tissue strips between the endocrinocytes. Damage to the capillary endothelium and adhesion of erythrocytes in their lumen is observed.

On the 30th day of infection, ultrastructural changes in glandular tissue begin to develop in parallel with the processes of reparative regeneration. Around the paraadrenal cells, individual fibroblasts and collagen fibers of connective tissue are observed. At the same time, the size of the cell organelles decreases or even loses, and the involution of some cell organelles is visible (fig. 2).

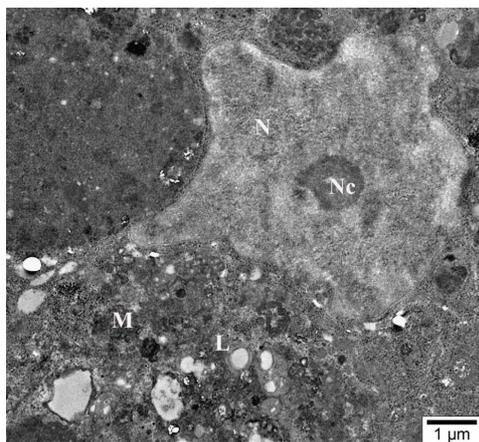


Fig. 1. An electron micrograph (TEM) of a section of the adrenal gland. 15th day of hypoxia model. Stain: uranium acetate and pure lead citrate. Scale: 1  $\mu\text{m}$ . N-nucleus; M-mitochondria; L-lipid droplets; Nc-nucleolus.

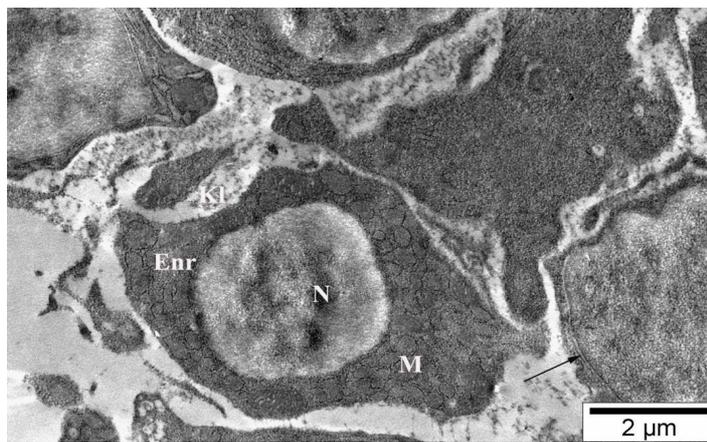


Fig. 2. An electron micrograph (TEM) of a section of the adrenal gland. 30th day of infection model. Stain: uranium acetate and pure lead citrate. Scale: 2  $\mu\text{m}$ . N-nucleus; M-mitochondria; Enr-endoplasmic reticulum; basement membrane (arrow).

In some endocrinocytes, secretory vesicles are electron-transparent and vacuolated, while in others they are invisible. Some vesicles are smaller, fill unevenly (concentrated in the center) with weak electron-density content, and are surrounded by a light border. The basement membrane of the capillaries is also unevenly thickened.

On the electronograms prepared from the adrenal gland preparations taken on the 15th day of the experiment in the main group of experimental animals, reflecting the combined effect of hypoxia and infection, attention is drawn to the hypertrophy of organelles, in particular mitochondria, the endoplasmic reticulum and the Golgi complex in the cytoplasm of the gland cells. In some cells, there is a decrease in lipid droplets and a small number of mitotic figures are found. In the cytoplasm of the cells are numerous, large, and round swollen mitochondria. At the same time as some mitochondria have a normal structure, some have a noticeable smoothing of the cristae. The expansion of the cisterns of the endoplasmic reticulum and the increase in the number of ribosomes is also noticeable (fig. 3).

By the end of the experiment (on the 30th day), electron microscopic changes acquire a milder character. Thus, it is possible to monitor hyperplasia of cytoplasmic organelles, thickening of the plasmalemma and the basal membrane of capillaries, as well as the contours of the nucleus and nuclei located in the center of the cell, and nuclear chromatin. There is an increase in the size and number of cytoplasmic organelles, mainly mitochondrial cristae, fibrocytes, and fine collagen fibers are observed. At

the ultrastructural level, the manifestation of this result can be justified by the rearrangement and adaptation of adrenal cells to new conditions (fig. 4).

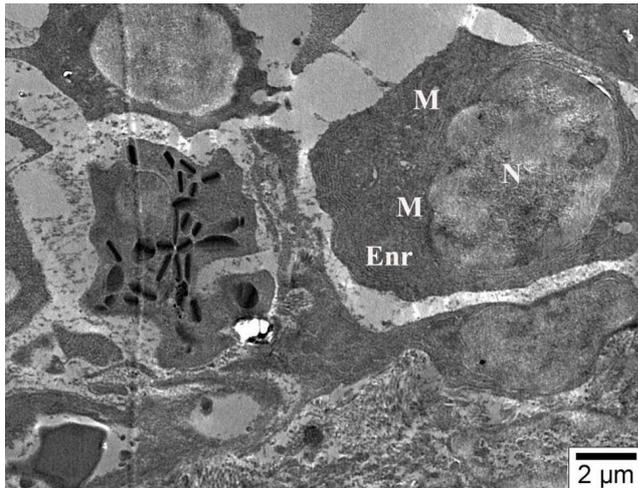


Fig. 3. An electron micrograph (TEM) of a section of the adrenal gland. 15th day of hypoxia and infection model. Stain: uranium acetate and pure lead citrate. Scale: 2  $\mu\text{m}$ . N-nucleus; M-mitochondria; Enr-endoplasmic reticulum.

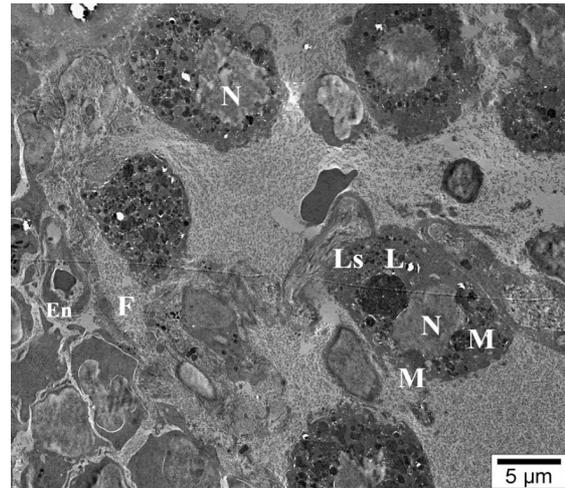


Fig. 4. An electron micrograph (TEM) of a section of the adrenal gland. 30th day of hypoxia and infection model. Stain: uranium acetate and pure lead citrate. Scale: 5  $\mu\text{m}$ . N-nucleus; M-mitochondria; L-lipid droplets; Ls-lysosomes; E-erythrocyte; En-endothelium; F- fibrocytes.

Thus, the analysis of our results shows that ultrastructural changes in the adrenal glands, caused by separate effects of both hypoxia and infection, are manifested in an increase in the number of collagen fibers in the intercellular space of glandular tissue and the structure of mitochondria. On the 15th day of hypoxia and infection, cell hypertrophy is observed, and on the 30th day, both hypertrophy and hyperplasia are observed. There is a partial reduction of adrenocytes and loss of parenchyma.

The body's adaptation to the effects of several stress factors depends on the intrasystemic restructuring of the structural elements of the adrenal glands, on the other hand, the adrenal glands play an important role in the regulation of processes under the influence of these factors. However, this regulation is complex and therefore requires special research [2]. So, under the influence of experimental hypoxia, as well as infection, according to the authors, morphofunctional changes occur in the adrenal cells, the lost parenchyma is replaced by the stroma, resulting in the formation of compensatory and adaptive processes, manifested by a change in the ratio of epithelium and connective tissue. During the transformation of the glandular tissue, the processes of stromal proliferation prevail, which, on the background of a decrease in the number of functioning adrenocytes, lead to a decrease in the concentration of produced hormones, a weakening of the functional activity of the gland [2, 8]. According to the results of our study, in all experimental animals under the influence of hypoxia and infection develop noticeable changes at the ultrastructural level, which lead to the morphological repairing of the gland structure. In particular, with the combined effect of both factors, along with deformation and atrophy of adrenocytes, a focal increase in connective tissue is observed, the structure of intracellular organelles is disrupted, their size decreases, and the glandular cells adapt to the newly formed environment. According to some authors [1], at this time in the cells of the gland proliferative activity weakens, endocrine pathologies develop in the body.

A comparative analysis of our results shows that during the combined effect of hypoxia and infection ultrastructural changes in glandular tissue are more pronounced. This suggests that hypoxia has a stimulating effect on staphylococcal infection. At the same time, the effect of hypoxia depends on several factors, including the nature of the pathogen and the characteristics of the infection [8]. We also believe that hypoxia causes the progression of the infection. This conclusion is also confirmed by the views of N.H. Hajdamowicz et al. [3].

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