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### DYNAMICS OF MORPHOLOGICAL CHANGES IN THE GLOMERULAR ZONE OF THE ADRENAL CORTEX IN RATS DURING THE EARLY STAGES OF THE EXPERIMENT FOLLOWING ADMINISTRATION OF A COMPLEX OF DIETARY SUPPLEMENTS

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This article presents the results of an experimental study of changes in the glomerular zone of the adrenal cortex under the influence of a complex of food additives (monosodium glutamate, sodium nitrite, Ponceau 4R). The relevance of this work stems from the prevalence of endocrine pathology and the influence of exogenous factors on the hypothalamic-pituitary-adrenal axis. The study was conducted on 84 white rats using morphological and morphometric methods. It was established that the glomerular zone undergoes pronounced destructive-degenerative changes: cellular disorganization, loss of lipid inclusions, nuclear pyknosis, and the formation of apoptotic bodies. The detected changes indicate impaired mineralocorticoid synthesis and, consequently, disturbances in water-electrolyte balance. The results obtained highlight the sensitivity of the glomerular zone to dietary xenobiotics and emphasize the importance of dietary factors in maintaining endocrine homeostasis, laying the groundwork for preventive strategies in clinical practice.

**Key words:** rats, morphology, adrenal glands, glomerular zone, morphometry, microscopic changes.

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### ДИНАМІКА МОРФОЛОГІЧНИХ ЗМІН У КЛУБОЧКОВІЙ ЗОНІ КОРИ НАДНИРКОВИХ ЗАЛОЗ У ЩУРІВ НА РАННІХ ЕТАПАХ ЕКСПЕРИМЕНТУ ПІСЛЯ ВВЕДЕННЯ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

У статті висвітлено результати експериментального дослідження змін клубочкової зони кіркової речовини надниркових залоз під впливом комплексу харчових добавок (глутамат натрію, нітрит натрію, понсо 4R). Актуальність роботи зумовлена поширенням ендокринної патології та впливом екзогенних чинників на гіпоталамо-гіпофізарно-надниркову систему. Дослідження було проведено на 84 білих щурах із застосуванням морфологічних і морфометричних методів. Встановлено, що клубочкова зона зазнає виражених деструктивно-дегенеративних змін: дезорганізації клітин, втрати ліпідних включень, пікнозу ядер і формування апоптичних тілець. Виявлені зміни свідчать про порушення синтезу мінералокортикоїдів і, як наслідок, розлади водно-електролітного балансу. Отримані результати підкреслюють чутливість клубочкової зони до харчових ксенобіотиків і акцентують значення харчових факторів у підтриманні ендокринного гомеостазу, формуючи підґрунтя для профілактичних стратегій у клінічній практиці.

**Ключові слова:** щури, морфологія, надниркові залози, клубочкова зона, морфометрія, мікроскопічні зміни.

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The adrenal glands play a central role in the body's neuroendocrine regulatory system, acting as an integrative hub between the nervous and humoral components of adaptation [5]. Their complex structure, which combines cortical and medullary tissue of different histogenetic origins, provides a wide range of functions: from regulating metabolism to mediating the stress response. The adrenal glands form the basis of the hypothalamic-pituitary-adrenal system, which determines the body's ability to respond to changing conditions in the external and internal environments, maintain homeostasis, and ensure survival in the face of the constant challenges of the modern environment [9].

The adrenal cortex is a functionally differentiated structure divided into three zones: the glomerular, fascicular, and reticular zones, each of

which performs a specific hormonal function [4]. Among these, the glomerular zone, despite its relatively small proportion of the total cortical volume, plays a critically important role in maintaining the vital properties of the internal environment. It is the primary site of mineralocorticoid synthesis, particularly aldosterone - a hormone that regulates water-electrolyte balance, blood pressure, and circulating blood volume [4]. By acting on the distal tubules of the nephron, aldosterone facilitates the reabsorption of sodium, chloride, and water, and the excretion of potassium and hydrogen ions, thereby maintaining a delicate balance between the stability of the internal environment and its dynamic adaptation.

The functional activity of the glomerular zone is regulated by a complex network of neurohumoral

mechanisms. The renin-angiotensin-aldosterone system plays a leading role, responding to changes in blood pressure, blood volume, and electrolyte concentrations [7, 12]. Additional influences are exerted by pituitary adrenocorticotrophic hormone and plasma ion composition, particularly potassium levels. Such multilevel regulation underscores the glomerular zone's high sensitivity to changes in both the internal and external environments, making it an important indicator of adaptive processes in the body.

In modern human life, factors that were not previously considered key regulators of the endocrine system are becoming increasingly important. Chronic psychoemotional stress against the backdrop of war, urbanization, dietary irregularities, and the widespread use of dietary supplements create a new context for the functioning of the hypothalamic-pituitary-adrenal axis. The adrenal glands, as the central organ of the stress response, are the first to respond to these changes, which manifest in both functional and morphological adaptations.

Particular attention is drawn to the effects of exogenous chemicals, particularly food additives, which enter the human body daily [4]. Although most of them are considered safe at acceptable doses, their cumulative and combined effects remain insufficiently studied. Data from experimental studies indicate that certain chemical compounds can influence steroidogenesis, disrupt adrenal cortex cell function, and induce oxidative stress and cellular damage [3,6,8,10]. In this context, the glomerular zone is among the most vulnerable structures, as its functional activity depends directly on the state of cell membranes, enzymatic systems, and the microcirculation.

At the same time, clinical manifestations of adrenal dysfunction are often nonspecific and masked by other pathological conditions, which complicates timely diagnosis. Early changes may manifest only as minor deviations in electrolyte balance or blood pressure, which often go unnoticed by both patients and physicians. This is precisely why morphological studies aimed at detecting initial structural changes are of particular value, allowing us to understand the mechanisms of pathology development even before the onset of clinical symptoms.

Thus, the current understanding of adrenal gland function extends beyond the classical view of them as hormone-producing organs. They are emerging as a dynamic system sensitive to lifestyle, diet, and environmental influences. In this context, the study of structural and functional changes in the glomerular zone under the influence of a complex of dietary supplements is not only scientifically sound but also a socially significant area of research aimed at preserving public health and developing new approaches to preventing endocrine disorders.

**The purpose** of the study was to conduct a comprehensive investigation of the structural and functional characteristics of the glomerular zone of

the adrenal cortex and to determine the nature of its morphological and morphometric changes in the early stages of an experimental study under the influence of a complex of food additives (monosodium glutamate, sodium nitrite, and Ponceau 4R).

**Materials and methods.** The study was conducted on 84 outbred male rats as experimental laboratory animals. When choosing a model for the study, we settled on white rats, since this breed of animals is well-suited for group experiments, as they consistently reproduce the same types of reactions, ensuring the reliability and comparability of the results obtained. The weight of laboratory rats with an average weight of  $0.350 \pm 0.15$  kg, which were kept in the vivarium for animals of the Poltava State Medical University, with a standard diet, controlled lighting and free access to water. The animals were divided into a control group ( $n=14$ ) and 5 experimental groups of 14 rats each. The experimental study was conducted from September 2022 to January 2023.

Experimental animals were administered a complex of food additives (monosodium glutamate - 20 mg/kg, sodium nitrite - 0.6 mg/kg, Ponceau 4R - 5 mg/kg) orally daily in the form of a 0.5-mL aqueous solution prior to the main feeding. The control group received saline solution according to the same regimen. The selected concentrations were below the maximum permissible limits, simulating chronic exposure under real dietary conditions. Animals were euthanized by sodium thiopental overdose under ether anesthesia in accordance with the observation periods. Following euthanasia, the adrenal glands were harvested while maintaining the anatomical integrity of the organ, after which the tissue was fixed in 10 % neutral formalin, 2.5 % glutaraldehyde, or osmium tetroxide, depending on the subsequent research methods. Histological examinations were performed on paraffin and epoxy sections 1-5  $\mu$ m thick using standard staining methods (hematoxylin-eosin, Mayer's hematoxylin, methylene blue) [1]. Optical analysis was performed using an Olympus C 3040-ADU microscope equipped with a digital microphotography attachment and Olympus DP-Soft software, enabling high-precision imaging and morphometric analysis of the adrenal gland's structural components. Morphometric analysis included determining the thickness of the glomerular zone of the adrenal glands. Statistical analysis of the results was performed using Student's t-test with a significance level of  $p < 0.05$ . The study was approved by the Bioethics Committee of Poltava State Medical University (Protocol No. 208, September 22, 2022) and complies with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

**Results of the study.** At the light-microscopic level, as early as 1 week after the start of the experiment, a detailed analysis of semithin sections

revealed the presence of distinct and structurally significant morphological changes in the glomerular zone of the adrenal cortex, manifested by alterations in the architecture of the adrenocortical glomeruli, changes in their spatial organization, as well as the appearance of signs of cellular dystrophy and early degenerative processes. In particular, mineralocorticoid cells located in the adrenocortical glomeruli showed signs of compressive constriction, which was likely due to the general thinning of the glomerular zone. The most superficially located glomeruli took on a flattened, somewhat deformed shape, while the connective tissue septa separating the glomeruli were in a state of hyperhydration (Fig. 1).

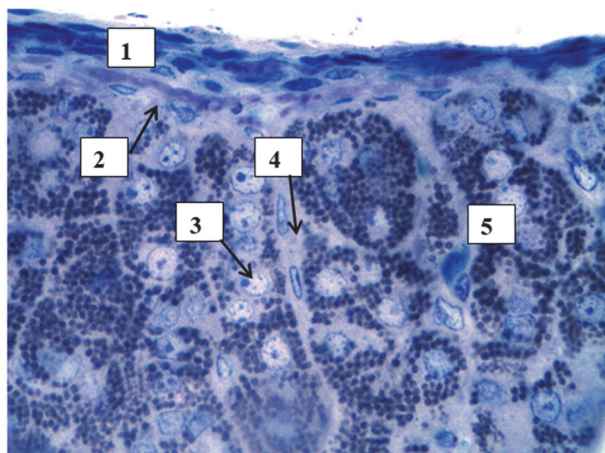


Fig. 1. Morphological organization of the glomerular zone of the adrenal cortex in white rats during the early stages of the experiment. Semi-thin section. Staining: toluidine blue. Magnification: okh. 10; obh. 40. 1. Capsule; 2. Glomerular zone; 3. Oxyphilic corticocytes; 4. Connective tissue septa; 5. Cortical substance.

Within these structures, adrenocorticytes at various stages of the secretory cycle were visualized. At the same time, the predominance of eosinophilic cells over basophilic ones was notable. The nuclei of eosinophilic corticocytes exhibited signs of karyopyknosis, indicating the initiation of apoptotic processes. A small number of basophilic corticosites contained lipid inclusions in the cytoplasm, whereas in eosinophilic cells these inclusions were significantly reduced or completely absent. Such a loss of the lipid component likely reflects the depletion of the cells steroidogenic potential and is accompanied by a decrease or complete loss of their functional activity.

The microvascular loops tightly enveloping the adrenocortical glomeruli were overflowing with blood cells, indicating stasis. This, in turn, could lead to impaired microcirculation and tissue trophism, exacerbating dystrophic and degenerative changes in the structures under study.

At the ultrastructural level, early destructive changes are already observed after just 1 week: corticocyte nuclei are optically pale with uniformly distributed fine-grained chromatin, and nucleoli are tightly adherent to the nucleole. In the cytoplasm, a

reduction in mitochondria, the appearance of myeloid bodies, and a significant decrease in lipid inclusions are observed, indicating impaired steroidogenesis and cellular energy metabolism.

By the 4th week of the experiment, the previously identified morphological changes not only persist but also become more pronounced and progressive. In particular, a persistent trend toward further thinning of the glomerular zone of the adrenal cortex is observed, accompanied by an intensification of the compression of adrenocortical cell complexes. Such changes are likely caused by both the progressive reduction of cortical substance and the pronounced hyperhydration of connective tissue elements, leading to mechanical compression of parenchymal structures.

The cell population within the glomerular zone is characterized by a clear predominance of eosinophilic corticocytes, whose nuclei show signs of karyopyknosis, indicating the activation of apoptotic processes. At the same time, a sharp decrease or complete elimination of lipid inclusions is observed in the cytoplasm of these cells, likely reflecting the depletion of their steroidogenic function. Basophilic corticosites, which remain in small numbers, exhibit pronounced signs of deformation, an irregular, sometimes elongated or flattened shape, and are in a state of mechanical compression against the eosinophilic cells. These morphological features indicate a disruption of cellular homeostasis, a loss of tissue structural organization, and the progression of dystrophic-degenerative changes in the examined area (Fig. 2).

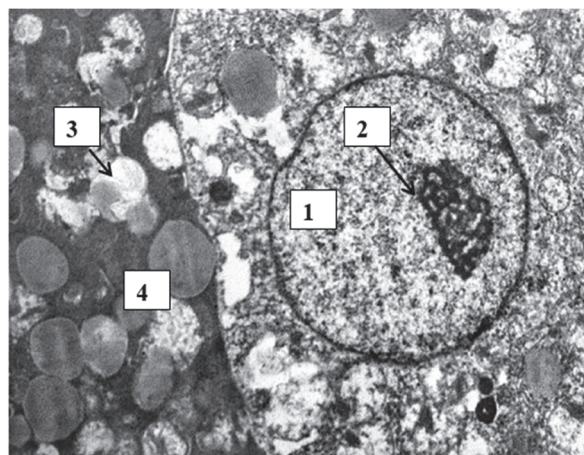


Fig. 2. Morphological and functional changes in corticocytes of the glomerular zone of the adrenal cortex in white rats after 4 weeks of experimental study. Electron micrograph. Magnification: 12000. 1. Nucleus; 2. Nucleolus with pyknotic changes; 3. Lipid inclusions; 4. Areas of cytoplasmic disorganization.

At this stage, submicroscopic examination reveals progressive disorganization of membrane organelles, hyperhydration of pericellular spaces, destruction of lipid inclusions, and the formation of apoptotic bodies, reflecting the depletion of cellular adaptive reserves and the activation of programmed cell death mechanisms.

Morphometric analysis confirms the morphological changes: the thickness of the glomerular zone after 1 week of the experiment is significantly ( $p < 0.05$ ) reduced by 1.16 - fold compared to the control (Fig. 3).

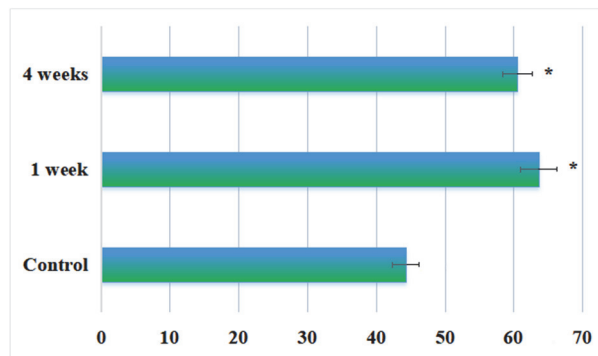


Fig. 3. Changes in the morphometric parameters of the thickness of the glomerular zone of the adrenal cortex during the early stages of the experiment.

Note: \* – statistically significant at  $p < 0.05$  compared to the control group.

After 4 weeks, this value is even lower - by a factor of 1.23 ( $p < 0.05$ ). Compared to the previous time point, there is a trend toward a further 1.06 - fold decrease in thickness, which is not statistically significant ( $p > 0.05$ ), suggesting a certain stabilization of the process against the backdrop of already established structural changes.

Thus, in the glomerular zone of the adrenal cortex, a complex of interrelated and pathogenetically significant morphological changes forms as early as the initial stages of the experiment. These changes include phenomena of compressive tissue remodeling, accompanied by disruption of the normal architecture of adrenocortical glomeruli, disorganization of cellular elements, and loss of their spatial order. In addition, a reduction or complete disappearance of lipid inclusions in the cytoplasm of corticocytes is observed, reflecting a depletion of their steroidogenic potential. Additionally, marked microcirculatory disturbances are noted, manifested by stasis of blood cells and, consequently, impaired tissue trophism. At the cellular level, these processes are accompanied by apoptosis, as evidenced by pyknotic nuclei.

The combination of these morphological changes indicates a significant suppression of the functional activity of mineralocorticoid-producing cells in the glomerular zone and may be regarded as the structural basis for the development of disturbances in the body's water-electrolyte homeostasis, particularly imbalances in sodium, potassium, and extracellular fluid volume.

**Discussion.** According to the literature, disruption of the structural organization of the glomerular zone is accompanied by the development of both functional and morphological changes [14]. In particular, under pathological influences of a complex of food additives (sodium

nitrite, monosodium glutamate, and Ponceau 4R), disorganization of glomerular cells, vacuolar dystrophy, necrotic changes in corticocytes, and impaired hemomicrocirculation are observed [2]. Such changes may underlie the development of clinically significant conditions, such as primary hyperaldosteronism (Conn's syndrome), which is caused by hyperfunction or neoplastic processes in the glomerular zone and is characterized by arterial hypertension, hypokalemia, and metabolic alkalosis [3].

Particular attention is drawn to the effects of various exogenous toxic factors on the glomerular region [15]. It has been demonstrated that a number of chemical substances, including organophosphates, pesticides, phthalates, and heavy metals, can inhibit steroidogenic enzyme systems, disrupt mitochondrial function, and cause structural changes in the adrenal cortex.

The key pathogenic mechanism of these changes is the development of oxidative stress, which leads to lipid peroxidation, damage to cell membranes, and intracellular organelles [7].

Similar effects have been described with food additives widely used in the modern food industry. In particular, aspartame and sodium benzoate can induce the formation of free radicals, leading to the destruction of adrenal gland cells and a decrease in their functional activity [4, 9, 16]. Monosodium glutamate, by affecting the hypothalamic-pituitary-adrenal axis, can disrupt steroidogenesis regulation, cause degenerative changes in cells, and promote lipid inclusion accumulation, indicating dysfunction of metabolic processes [11]. Also important is its ability to deplete the cells' antioxidant system by disrupting glutathione homeostasis, which exacerbates the damaging effects of oxidative stress.

In addition to chemical factors, stress and metabolic factors have a significant impact on the structural organization of the glomerular zone [6]. Hyperthermia, hypothermia, physical overload, and hyperglycemia are accompanied by impaired microcirculation, the development of edema, cell destruction, and vascular remodeling [13]. Such changes can lead to impaired tissue trophism and the progression of destructive processes in the adrenal cortex [14].

The results obtained in this study are consistent with the literature data cited. The observed thinning of the glomerular zone, compressive constriction of adrenocortical glomeruli, predominance of eosinophilic cells with pyknotic nuclei, reduction of lipid inclusions, and impaired microcirculation indicate the development of destructive-degenerative processes [8]. Ultrastructural changes, in particular a reduction in mitochondria, the appearance of myeloid-like bodies, and the disorganization of cytoplasmic structures, confirm disturbances in steroidogenesis and cellular energy metabolism.

## Conclusion

Thus, even in the early stages of the experiment, pronounced dystrophic-degenerative changes develop in the glomerular zone of the adrenal cortex, affecting both the cellular and tissue levels of organization. These manifest as architectural disruption, reduced lipid inclusions, activation of apoptosis, and microcirculatory disorders, which collectively lead to the suppression of mineralocorticoid cell function. Such morphological changes provide the structural basis for disturbances in the water-electrolyte homeostasis of the adrenal glands. At the same time, the combination of the identified structural changes with functional disturbances only confirms the adverse effect of dietary supplements and their prolonged action on the body.

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**Conflict of interest.** The authors have no conflicts of interest to declare.

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