DOI 10.29254/2077-4214-2023-2-169-381-386
UDC 611.452-024:613.29

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STRUCTURAL ORGANIZATION OF THE ADRENAL GLANDS MEDULLA OF RATS IN NORMAL AND AFTER INFLUENCE OF FOOD ADDITIVE COMPLEX

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Taste plays a significant role in the life of every person. Our taste buds are responsible for recognizing flavors, determining the suitability of foods, protecting against harmful substances, and regulating food intake. A person always gets pleasure from tasty food, and sweet dishes cause positive emotions. Unfortunately, we consume food that contains many food additives and preservatives daily, although we know that it can be harmful. Tasty but not healthy food has become extremely common and available, and it is difficult for us to resist the temptation to eat it. Many nutritional supplements have been created to meet the needs of the food industry, as mass production of products is different from home cooking. These additives are necessary to preserve the product's appearance but should not harm buyers' health or mislead them. Today, there are many food additives that consumers are unaware of but which can affect human organs, particularly the adrenal glands. Some of the most common food additives we study include monosodium glutamate (E621), sodium nitrite (E250) and Ponceau 4R (E124).

Consuming large amounts of dietary supplements can have the following effects: chest and abdominal pain, migraines, nausea, rapid heart rate (tachycardia), obesity, weakness, and allergic reactions. Particular attention should be paid to the effect of these supplements on the adrenal glands, as they can disrupt the function of these organs and contribute to the development of oncological diseases.

Research on the morphology of the adrenal glands has been ongoing for many years. In recent decades, there has been an increase in the pathology of the adrenal glands as the negative influence of external and internal factors increases, which leads to disturbances in the structure and functioning of the glands. Adrenal glands play an essential role not only in the hypothalamic-pituitary-adrenal system but also in the endocrine system. Interest in changes in the structure of the adrenal glands does not fade among experimental medicine and scientists. Thanks to the improvement of computer diagnostic methods, the detection of both hormonally active and inactive, benign and malignant neoplasms of the adrenal glands is increasing. The most common endocrine disorders are hyperaldosteronism, adrenergic and estrogenic balance disorders. Computed tomography significantly improves the detection of tumors of various origins in the adrenal glands. Diagnosis and treatment of diseases of the adrenal glands is an urgent medical and social problem.

Key words: *monosodium glutamate, morphology, medulla, adrenal glands, sodium nitrite, Ponceau 4R, reticular zone, cortical substance, hemomicrocirculatory bed, rats, morphological and functional changes, submicroscopic changes, endocrinocytes.*

Connection of the publication with planned research works.

The work is a fragment of the research work of the Poltava State Medical University "Regularities of the morphogenesis of organs, tissues and vascular and nervous formations in normal, pathological and under the influence of exogenous factors", state registration № 0118U004457.

Introduction.

The role of taste in human life is essential. It not only performs a protective function, helping us to recognize whether products are suitable for consumption but also gives us positive emotions from enjoying delicious food. In our daily diet, we consume foods that contain various food additives. These additives give particular food characteristics, ranging from a rich taste to an attractive appearance. We are in no hurry to give up such products, although it is known that food additives, even in small concentrations, can harm our health. However, with the growing popularity of delicious food, it is increasingly difficult to resist the temptation to eat at least a small but tasty piece.

Food additives are natural or synthetic substances added to products, raw materials and semi-finished products to improve their taste, appearance and shelf life. Many food additives appeared following the need to mass produce food products, which differ from home cooking. Although food additives are necessary to preserve the marketability of products, they should not mislead the consumer. Unscrupulous manufacturers often provide false information on labels, and we need to know what is contained in the products we choose on supermarket shelves [1].

Today, there are many food additives that the consumer does not think about or know. In European countries, the use and production of food additives increased by 2%. Various sweeteners are becoming especially popular. The presence of various food additives in products must be displayed using the "E" index following the European Community and identified according to the International Classification System (INS) [2].

The most common additives we study are monosodium glutamate (E621), sodium nitrite (E250) and Ponceau 4R (E124). These additives have a negative effect on human health and can also cause serious diseases. Among the most common negative consequences are migraine, anxiety, schizophrenia, epilepsy, depression, Alzheimer's and Parkinson's diseases, decreased muscle tension and blood pressure, asthma, abdominal pain, nausea, tachycardia, weakness, and others [3-4].

Food additives affect all organs and systems of the body, but in our case, the adrenal glands are susceptible

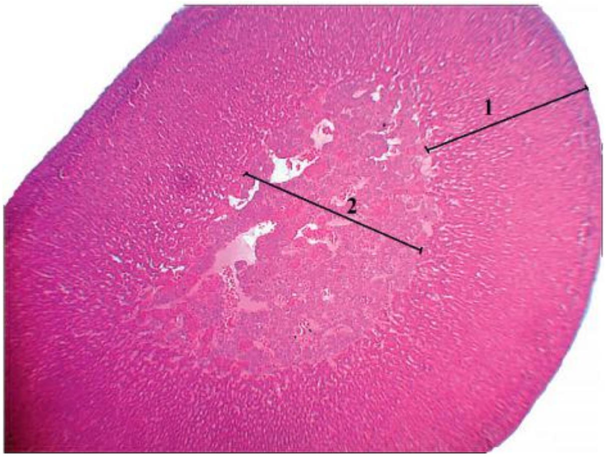

Figure 1 – Structural organization of the adrenal gland of the rat's control group. Hematoxylin and eosin staining. Magnification: okh. 10, obh. 10. Marking: 1 – cortex; 2 – medulla.

to these substances. Recently, the number of cases of adrenal gland diseases has been increasing.

The aim of the study.

Study the structural organization of the adrenal glands medulla of rats in a comparative-species aspect and obtain control data and structural-functional changes after exposure to a food additive complex.

Object and research methods.

To conduct the research, white outbred rats with a weight of 0.350±0.15 kg were used, which were kept under standard conditions in the vivarium of the Poltava State Medical University. During the experiment, the requirements of the humane treatment of animals, which are regulated by the Law of Ukraine "On the Protection of Animals from Cruelty Treatment" (№ 3447-IV dated 21.02.2006) and the European Convention on the Protection of Vertebrate Animals Used for Scientific Research and Other scientific goals (Strasbourg, 1986).

In the control group, saline and drinking water were administered orally. Rats from the experimental group were orally administered a 10% solution of sodium nitrite (E250). Monosodium glutamate (E621) was administered at 20 mg/kg in 0.5 ml of distilled water. Ponceau 4R was administered at 5 mg/kg in 0.5 ml of distilled water daily. Doses of food additives for rats were twice lower than the permissible norms in food products. Rats were removed from the experiment after 1, 4, 8, 12 and 16 weeks with the help of ether anesthesia and subsequent euthanasia. Fragments of rat adrenal glands were fixed in a neutral formalin solution and embedded in paraffin. After obtaining paraffin blocks, histological sections were made, stained with hematoxylin and eosin and examined using a Biorex light microscope with a DCM 900 digital photomicroscope. The examined material was fixed in glutaraldehyde and sealed in Epon-812 to obtain semi-thin sections. Prepared sections were stained using toluidine blue. The morphometric method measured the average total diameter of the cortex (glomerular, fasciculate, reticular zone) and medulla. A system of visual analysis of histological preparations was used during morphometric studies. Images of histological preparations of rats adrenal glands were displayed on a computer monitor using a microscope and a Vision CCD camera. Morphometric studies were performed on a personal computer using the VideoTest-5.0 program, KAAPA Image Base and Microsoft Excel. Statistica 10, BiostatPro 6, and Microsoft Excel 2019 software were used to process the obtained data statistically. Calculations and indicators in the samples were performed using the Shapiro-Wilk test. The quantitative assessment of the obtained data included the determination of the arithmetic mean of the variation series (M) and its standard error (m). The Student's t-test was used to compare quantitative values in paired series. The difference was considered significant at a value of $p<0.05$.

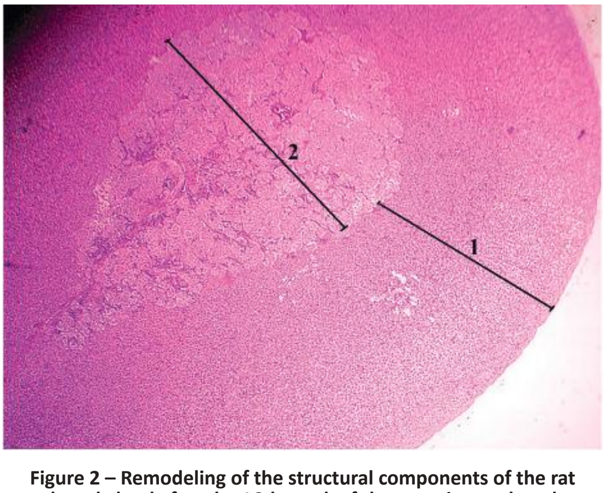

Figure 2 – Remodeling of the structural components of the rat adrenal gland after the 16th week of the experimental study. Hematoxylin and eosin staining. Magnification: okh. 10, obh. 10. Marking: 1 – cortex, 2 – medulla.

Research results and their discussion.

After conducting the research, it was established that the adrenal glands of rats consist of two parts that are different in origin, structure and functions: the surface – cortex and the central – medulla.

After measuring the size of the medulla of the adrenal glands of the control group, it was established that the average total diameter of the medulla was (382.22±3.61) μm (**fig. 1**).

After taking a complex of food supplements for one week, the diameter of the adrenal medulla increased to the value of (598.06±12.58) μm, an increase of 56.47%. This phenomenon can be caused by cell hypertrophy and swelling of the medulla of the adrenal glands. After 4th week of observation, the indicator was (515.64±4.79) μm, which exceeds the control value by 34.91%. After the 8th week, the total diameter of the medulla was (416.83±1.92) μm, which is 9.05% higher than the control value (**fig. 2**). At 12 and 16 weeks, the values were (585.29±8.43) μm and (512.81±5.55) μm, which exceeds the control values by 53.15% and 34.17%, respectively (**fig. 3**).

During the morphometric study, it was found that the hemomicrocirculatory bed (HMCB) of the adrenal glands of rats is well-developed and consists of several components. After we experimented with the effect of food additives on the adrenal glands of rats, we noticed systematic changes in the vessels of the medulla and their structural reorganization.

According to literature sources, the response of HMCB to external and internal environmental factors is quite specific. Therefore, during the morphological study of HMCB elements, we analyzed the total diameter of arteries, venules and capillaries, the lumen of these vessels and the average thickness of their walls [5-6]. In particular, we paid attention to the uneven thickening of the walls of arterioles, venules and capillaries, which manifested itself in the form of swelling and disorganization of the connective tissue surrounding these vessels. Hyperemia HMCB and vasodilatation were also recorded [7].

During the morphometric analysis of the general indicators of the vessels of the medulla of the adrenal glands of rats, it was established: the overall average diameter of the arterioles of the control group was (7.03±0.02) µm, after the 1st week this indicator was (6.91±0.04) µm, decreased by 1.71% from the initial value; after the 4th week, the indicator of the total average diameter of arterioles was (7.22±0.05) µm, which is 2.71% more than the control value; after the 8th week, the indicator was (6.95±0.06) µm and was 1.14% less than the initial values; after the 12th week of the experimental study, the indicator of the total average diameter of arterioles increased to (7.67±0.07) µm, which is 9.11% higher than the initial values; the most noticeable changes occurred in the 16th week, the index of the total mean diameter of arterioles was (7.86±0.08) µm, 11.81% higher than the control value.

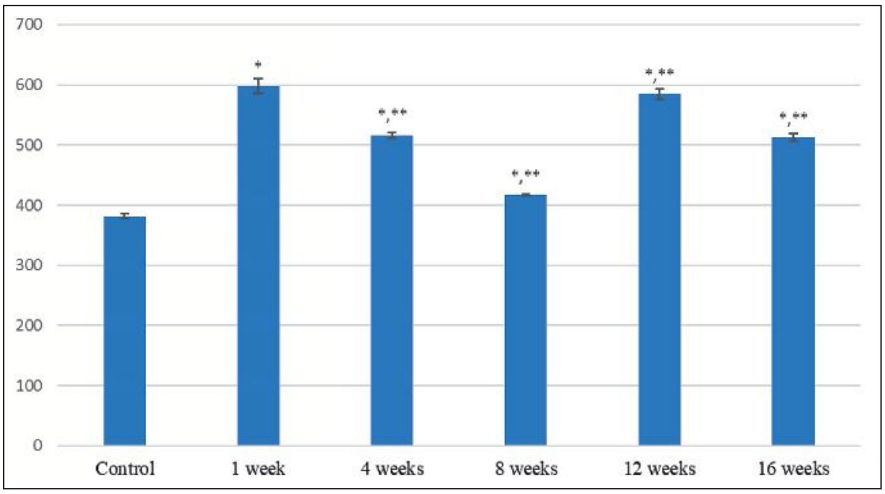

Figure 3 – The diameter of the medulla of the control group and after administration of the food additive complex.

Notes: * – p<0.05 compared to the control group, ** – p<0.05 compared to the last term of the experimental study.

The average diameter of the lumen of the arterioles of the control group was (5.47±0.02) µm. After the 1st week of taking a complex of food additives, the indicator was (5.38±0.03) µm, which is 1.56% less than the control value; after the 4th week of the experiment, the average diameter of the lumen of arterioles was (5.61±0.04) µm, which is 2.56% higher than the control value; after the 8th week, the indicator was (5.53±0.06) µm, 1.09% higher than the control values; after the 12th week of the experimental study, the average diameter of the lumen of the arterioles was (5.97±0.07) µm – 9.14% higher than the control values; the most significant changes occurred after the 16th week: the average diameter of the lumen of arterioles was (6.11±0.08) µm – 11.70% higher than the control value.

It was established that the average diameter of the arteriole wall of the control group was (1.54±0.01) µm. After the 1st week of the experimental study, the average diameter of the arteriole wall of rats was (1.51±0.03) µm, which is 1.95% less than the initial values. After the 4th and 8th weeks, the indicators were (1.58±0.04) µm – 2.59% more and (1.52±0.05) µm – 1.29% less than the control value. Starting from the 12th week, significant changes in the diameter of the arteriole wall were observed – (1.68±0.06) µm – 9.09% higher than the control values. After the 16th week, the indicator was (1.72±0.07) µm – 11.69% higher than the standard value.

The total diameter of the venules of the medulla of the adrenal glands of the control group was (16.53±0.12) µm. After the 1st week of the experiment, the indicator was (15.74±0.14) µm – 4.78% less than the control values. After the 4th and 8th weeks, the indicators of the total diameter of the venules were (16.19±0.15) µm and (19.07±0.17) µm – 2.06% less and 15.37% more than the initial values. After the 12th week, the indicators approached the initial values and amounted to (16.93±0.18) µm – 2.42% higher than the control values. After the 16th week, the indicator of the total diameter of the venules of the brain substance increased significantly and amounted to (19.65±0.19) µm – 18.87% higher than the control value.

The average diameter of the lumen of the venules of the medulla of the adrenal glands of the control group of rats was (15.93±0.11) µm. After the 1st week of using a food supplement complex, the indicator of the lumen of the venules of the medulla was (15.16±0.13) µm – 4.83% less. After the 4th and 8th weeks, the indicators were (15.61±0.15) µm – 2.01% less and (18.38±0.17) µm – 15.38% higher than the control values. After the 12th and 16th weeks, the indicators were (16.32±0.18) µm and (18.93±0.19) µm – 2.44% and 18.83% higher than the control values.

The diameter of the wall of the venules of the medulla of the adrenal glands of the control group of rats was (0.61±0.01) µm. After the 1st and 4th weeks, the indicators were (0.58±0.03) µm and (0.59±0.04) µm – by 4.92% and by 3.28%, respectively, less than the initial value. After the 8th and 12th weeks, the indicators were (0.71±0.05) µm and (0.62±0.06) µm – 16.39% and 1.64% higher than the control values. After the 16th week, the changes were most noticeable. The diameter of the venule wall was (0.73±0.07) µm – 19.67% higher than the control values.

The total average diameter of the capillaries of the medulla was (7.14±0.02) µm. After the first week of using a complex of nutritional supplements, the average diameter of capillaries was (6.64±0.04) µm – 7.01% less than the control values. After the 4th week, significant changes were observed in the average diameter indicator (11.54±0.12) µm – 60.62% higher than the initial values. After the 8th and 12th weeks, the indicators were (6.05±0.05) µm and (6.38±0.07) µm, respectively, which is 15.27% and 10.64% lower than the initial values.

The average diameter of the lumen of the capillaries of the control group medulla was equal to (6.05±0.02) µm. After the 1st week of using a complex of food additives, this indicator was (5.62±0.03) µm, which is 7.11% less than the control values. The most noticeable changes occurred after the 4th week of the experimental study: the average diameter of the lumen of the capillaries of the brain substance was (9.78±0.04) µm, which is 61.65% higher than the control value. After the 8th, 12th and 16th weeks, the indicators were (5.12±0.06) µm, (5.41±0.07) µm, (5.72±0.08) µm, which is less than the control values by 15, 37%, 10.58% and 5.45%, respectively.

The average diameter of the capillary wall of the control group was (1.07±0.01) µm. After the 1st week, this indicator was equal to (0.99±0.03) µm – 7.48% less than the previous indicators. After the 4th week, the average diameter of the capillary wall was (1.73±0.04) µm – 61.68% higher than the control values. After the 8th, 12th and 16th weeks, the indicators were equal to (0.91±0.05) µm, (0.96±0.06) µm and (1.01±0.07) µm, which is 14.95%, 10 .28% and 5.61% less than the initial values.

During the experiment, the influence of a food additive complex, which included monosodium glutamate, sodium nitrite and Ponceau 4R, in the early stages of the study revealed a decrease in the diameter of the blood vessels of the hemomicrocirculatory bed of the medulla of the adrenal glands in rats. This decrease is related to the influence of the components of food additives, similar to the reaction of the vascular bed to external factors [8-9]. Comparing changes in the average values of the total diameter and lumen of arterioles, capillaries, and venules of the medulla, it should be noted that these indicators correlate with each other in different periods of the experiment and reflect unidirectional morphological changes in these structures [10-11].

Arterioles responded by increasing the diameter of the lumen, starting mainly from the 4th week of the experiment [12]. Further development caused an inflammatory reaction with signs of edema, which led to a decrease in the lumen of arterioles and capillaries and an increase in the diameter of venules. Restorative and compensatory reactions aimed at neutralizing the source of alteration and restoring the vessels' morphofunctional state did not complete the restoration of normal hemodynamic parameters [13-14]. In the last weeks of the study, an increase in the lumen of the vessels was observed, which led to decompensation and a decrease in the diameter of the lumen of the vessels. Signs of hypoxia in the adrenal glands medulla accompanied this.

Conclusions.

The effect of a food additive complex on the vessels of the medulla of the adrenal glands in rats at the initial stages of the study was characterized by spasm of blood vessels of the hemomicrocirculatory bed, which arose due to the effect of the food additives, as well as an increase in the diameter of the lumen of the vessels as a result of hemodynamic disturbances. In the later stages of the experiment, inflammatory reactions and hypoxia occurred, which led to the development of compensatory and restorative reactions, but complete recovery did not occur. This indicates the need for further research and understanding of the impact of food additives on the vascular system and the development of measures to preserve health.

Prospects for further research.

An immunohistochemical study of rats' adrenal glands after consuming a food additive complex will be conducted.

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СТРУКТУРНА ОРГАНІЗАЦІЯ МОЗКОВОЇ РЕЧОВИНИ НАДНИРКОВИХ ЗАЛОЗ ЩУРІВ В НОРМІ ТА ПІСЛЯ ВПЛИВУ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК

Донченко С. В., Білаш С. М.

Резюме. Дана робота містить дослідження структур мозкової речовини надниркових залоз щурів в нормі та після вживання комплексу харчових добавок.

Для проведення даного експерименту ми використали біоптати надниркових залоз щурів. Отримані зразки піддавалися фарбуванню гематоксиліном та еозином, а також метиленовим синім та толуїдиновим синім. У ході нашого дослідження були застосовані гістологічні, морфометричні і статистичні методи.

В результаті даного дослідження було встановлено, що після вимірювання розмірів мозкової речовини надниркових залоз контрольної групи середня загальна товщина мозкової речовини становила (382,22±3,61) мкм. Після першого тижня, товщина мозкової речовини збільшилася до значення (598,06±12,58) мкм. Після 4 тижнів спостережень, показник становив (515,64±4,79) мкм, після восьмого тижня загальна товщина мозкової речовини становила (416,83±1,92) мкм; на дванадцятий та шістнадцятий тиждень, значення складали (585,29±8,43) мкм та (512,81±5,55) мкм, відповідно.

Отримані дані під час експерименту вказують на те, що комплекс харчових добавок, таких як глутамат натрію, нітрит натрію і Понсо 4R, спричиняє запалення та зміни структури гемомікроциркуляторної системи, зокрема артеріол, капілярів і венул.

Середні значення загального діаметра, діаметра просвіту та товщини стінок мікросудин змінювалися впродовж експерименту (з 4-го по 16-й тиждень) і відповідали фазам запальної реакції та процесам репарації і регенерації, що, очевидно, пов'язані з прямим впливом компонентів харчових добавок на стінки судин та реакцію судинної системи на зовнішні фактори. Судини великого калібру реагували збільшенням резистивного діаметру через порушення гемодинамічних умов. Надалі, внаслідок дії харчових добавок, виникає запальна реакція зі схильністю до набряку, що підтверджується зменшенням просвіту судин. Це спричиняє активацію артеріо-венулярних анастомозів, що призводить до збільшення діаметру венул мозкової речовини надниркових залоз у щурів.

Відновлювально-компенсаторні реакції, спрямовані на нейтралізацію джерела пошкодження та відновлення морфофункціонального стану судин, не призвели до повного відновлення нормальних гемодинамічних показників, що на останніх тижнях дослідження проявляється збільшенням просвіту судин і декомпенсацією, зменшенням діаметру просвіту судин обмінної ланки. Це супроводжується ознаками гіпоксії в мозковій речовині надниркових залоз, що свідчить про порушення гемодинамічних умов.

Ключові слова: глутамат натрію, морфологія, мозкова речовина, надниркові залози, нітрит натрію, Понсо 4R, сітчаста зона, кіркова речовина, гемомікроциркуляторне русло, щури, морфофункціональні зміни, субмікроскопічні зміни, ендокриноцити.

STRUCTURAL ORGANIZATION OF THE ADRENAL GLANDS MEDULLA OF RATS IN NORMAL AND AFTER INFLUENCE OF FOOD ADDITIVE COMPLEX

Donchenko S. V., Bilash S. M.

Abstract. This work contains a study of the structures of rats' adrenal glands' medulla in normal conditions and after using a food additive complex.

For this experiment, we used biopsies of rat adrenal glands. The obtained samples were stained with hematoxylin and eosin, methylene blue and toluidine blue. During our research, histological, morphometric and statistical methods were applied.

As a result of this study, it was established that after measuring the medulla of the adrenal glands of the control group, the average total thickness of the medulla was (382.22±3.61) µm. After the first week, the thickness of the medulla increased to the value of (598.06±12.58) µm. After the 4th week of observation, the indicator was (515.64±4.79) µm; after the 8th week, the total thickness of the medulla was (416.83±1.92) µm; for the 12th and 16th weeks, the values were (585.29±8.43) µm and (512.81±5.55) µm, respectively.

The data obtained during the experiment indicate that a complex of food additives, such as sodium glutamate, sodium nitrite and Ponceau 4R, causes inflammation and changes in the structure of the hemomicrocirculatory system, in particular arterioles, capillaries and venules.

The average values of the total diameter, the diameter of the lumen and the thickness of the walls of microvessels changed during the experiment (from the 4th to the 16th week) and corresponded to the phases of the inflammatory reaction and the processes of repair and regeneration, which are related to the direct effect of the components of food additives on vessel walls and the reaction of the vascular system to external factors. Large-caliber vessels reacted by increasing the resistive diameter due to hemodynamic conditions. In the future, as a result of the action of food additives, an inflammatory reaction occurs with a tendency to swelling, which is confirmed by a decrease in the lumen of the vessels. This causes the activation of arteriovenular anastomoses, which leads to an increase in the diameter of the venules of the adrenal medulla in rats.

Restorative and compensatory reactions, aimed at neutralizing the source of damage and restoring the morphofunctional state of the vessels, did not lead to a complete restoration of normal hemodynamic parameters, which in the last weeks of the study is manifested by an increase in the lumen of the vessels and decompensation, a decrease in the diameter of the lumen of the vessels of the exchange link. This is accompanied by signs of hypoxia in the medulla of the adrenal glands, which indicates a violation of hemodynamic conditions.

Key words: monosodium glutamate, morphology, medulla, adrenal glands, sodium nitrite, Ponceau 4R, reticular zone, cortical substance, hemomicrocirculatory bed, rats, morphological and functional changes, submicroscopic changes, endocrinocytes.

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Conflict of interest:
The Authors declare no conflict of interest.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

Received 27.11.2022
Accepted 05.05.2023

DOI 10.29254/2077-4214-2023-2-169-386-393
UDC 611.441-019

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COMPARATIVE MORPHOLOGY OF THE THYROID GLAND

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The thyroid gland has an extensive range of hormonal influences on the organs and systems of the body, as well as on the functioning and processes of adaptation of the body to environmental factors. Conducting experimental research on the reconstruction of the thyroid gland in various pathological conditions requires a deeper study of the features of the structural organization of the specified organ in experimental animals. The use of anatomical, morphometric and histological research methods made it possible to find out the peculiarity of its macrostructure in white rats, in particular, the absence of a pyramidal lobe, the presence of an inconspicuous glandular isthmus, the mass of the thyroid gland of a white rat, which was 0.035±0.05 mg, the dimensions of the gland, in particular its length was 6.55±0.05 mm, width – 3.75±0.02 mm, thickness – 2.65±0.002 mm, tight fit of the thyroid gland capsule to its parenchyma, the difference in the thickness of the interlobular septa of the gland in its central and peripheral parts glands (thinner on the periphery), changes in the shape of gland cells depending on their functional state, placement of C cells singly between the basal membrane and the basal pole of thyrocytes (parafollicular arrangement) and the surrounding of these cells with a dense network of capillaries, which, in turn, is also a related feature of the location of human C cells, various levels of metabolic processes in the cellular elements of the thyroid gland of laboratory rats. The obtained data will make it possible to create a base based on which it is possible to establish reactive changes in the thyroid gland and further use the data in modelling the effects of various factors for the prevention and treatment of human thyroid pathology.

Key words: *thyroid gland, macro – and microanatomy, rat.*

Connection of the publication with planned research works.

The article is part of the planned research work of the Department of Normal Anatomy and Operative Surgery with Topographic Anatomy of the Danylo Halytsky Lviv National Medical University «Morpho-functional features of organs in the pre – and postnatal periods of ontogenesis, under the influence of opioids, food additives, reconstructive operations and obesity» – state registration number 0120U002129.

Introduction.

Among the endocrine organs, the thyroid gland has unique features of morphological structure and functioning. It attracts considerable attention from researchers in various fields due to a wide range of hormonal influences on the development of a living organism, the formation and functioning of its systems, and the processes of adaptation to changing environmental factors. Receptors of thyroid hormones of the thyroid gland are expressed on the nuclear membranes of all body cells, which determines the wide spectrum of action of these hormones [1, 2, 3]. Thyroid hormones affect all types of metabolism, stimulate oxidative processes, affect the absorption of oxygen by cells, maintain water-salt balance, stimulate cell differentiation and proliferation, indirectly regulate carbohydrate metabolism, affect the contractility of the myocardium, the heart rate and the tone of blood vessels [4, 5, 6]. The wide variability of clinical manifestations of dysfunctions of the thyroid gland itself or disorders of the function of its hormones is associated with such a wide