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Donchenko S. V., Bilash S. M.

STRUCTURAL ORGANIZATION OF RATS ADRENAL GLANDS IN NORMAL STATE

Poltava State Medical University (Poltava, Ukraine)
donchsveta77@gmail.com

The study of the morphology of the adrenal glands has been carried out for many years. In recent decades, the pathology of the adrenal glands has been increasingly common because the negative influence of exogenous and endogenous factors is increasing, leading to the glands' morphofunctional disorders. Adrenal glands are not only essential elements of the hypothalamic-pituitary-adrenal system but are also endocrine organs. The interest of representatives of experimental medicine and scientists in changes in the structures of the adrenal glands does not subside. Thanks to the improvement of computer diagnostic methods, the percentage of detection of hormonally active and inactive, benign and malignant neoplasms of the adrenal glands has increased. The most common endocrine disorders are hyperaldosteronism, adrenergic and estrogenic balance disorders. The number of cases of detection of tumors of various genesis of the adrenal glands with the help of computer tomography is increasing significantly.

Diagnosing and treating adrenal gland diseases remains an urgent medical and social problem. Therefore, determining the morphology of the adrenal glands of rats in the control group will serve as controls for comparison in the experiment.

Key words: *adrenal glands, nutritional supplements, cortex, medulla, monosodium glutamate, sodium nitrite, Ponceau 4R.*

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 number 0118U004457.

Introduction.

Taste plays an essential role in human life. This is not only a protective mechanism, thanks to which we recognize the suitability of products for consumption, but also a way to get positive emotions from delicious food. Every day, a person consumes food products filled with various additives that give particular food properties: from a rich taste to an attractive appearance. However, we are in no hurry to give up these products, although everyone knows that food additives, even in small concentrations, are dangerous for human health. But tasty food is becoming increasingly common, and it often becomes difficult to resist the temptation to eat even a tiny but tasty piece [1].

Food additives are a group of natural or synthetic substances that are added to food raw materials, finished products, and semi-finished products to give them specific taste properties, a bright appearance and increase the duration of the shelf life [2]. Many food additives have appeared in response to the need to mass-produce products that are very different from those prepared at home. First, food additives are necessary to preserve the marketability of products but should not mislead the buyer [3, 4]. Often, an unscrupulous manufacturer indicates false information on the label, and we need to know what exactly is included in the products we choose on the supermarket shelves.

There are several thousand food additives that the buyer needs to think about or learn about. In European countries, the use and production of food additives increased by 2%. The number of various sweeteners is significantly growing [5]. The presence of various food additives in food must be marked with the E index within the European Community, identified according to the International Classification System (ICS).

The most common additives we consider are monosodium glutamate (E621), sodium nitrite (E250), and Ponceau 4R (E124). Unfortunately, these additives have a negative effect on human health and cause serious diseases [6]. The most frequent cases of adverse effects are: migraine, anxiety, schizophrenia, epilepsy, depression, Alzheimer's and Parkinson's disease, decreased muscle tone and blood pressure, asthma, abdominal pain, nausea, tachycardia, weakness, etc.

Food supplements affect all organs and systems, but in our case, it is precisely the adrenal glands that are easily affected by these substances. Recently, the number of cases of adrenal gland diseases has been increasing [7].

The aim of the study.

Study the structural organization of rat adrenal glands in a comparative-species aspect and obtain control data on their morphological features.

Object and research methods.

The study was performed on white outbred rats weighing 0.350±0.15 g, kept in the standard conditions of the vivarium of the Poltava State Medical University. The experimental research will be conducted in compliance with the requirements of humane treatment of experimental animals, regulated by the Law of Ukraine "On the Protection of Animals from Cruelty Treatment" (No. 3447-IV dated 21.02.2006) and the European Convention on the Protection of Vertebrate Animals Used for Research and Other scientific goals (Strasbourg, 1986).

The control group was kept in standard vivarium conditions and had round-the-clock access to drinking water.

Rats were removed from the experiment by using ether anesthesia followed by euthanasia. Fragments of rat adrenal glands fixed in a neutral formalin solution were embedded in paraffin. The histological sections were prepared from paraffin blocks and stained with hematoxylin and eosin and studied using a SEM-125K light microscope. To obtain semi-thin and ultra-thin sec-

tions, the studied material was fixed in glutaraldehyde and phosphate buffer and sealed in EPON-812. Sections from epoxy blocks were stained with toluidine and methylene blue. The morphometric method determined the average total thickness of the cortex (glomerular, fasciculate, and reticular zones) and medulla. When performing morphometric studies, we used a visual analysis system of histological preparations. Images of histological preparations of rat 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 Baseta Microsoft Excel. We studied the total thickness of the cortex (glomerular zone, fasciculate and reticular), the total thickness of the medulla, and the diameter of the vessels of the adrenal glands of rats and statistically processed the obtained data using the Statistica 10 BiostatPro 6 software and the Microsoft Excel 2019 program. Calculations and indicators in the samples were carried out using the Shapiro-Wilk test. The quantitative evaluation of the obtained data included the determination of the arithmetic mean of the variation series (M) and its standard error (m). We used Student's t-test to compare quantitative values in paired series. The difference was considered significant at values of $p<0.05$.

Research results and their discussion.

As a result of the research, it was established that the adrenal glands of rats consist of two parts that are different in origin, structure, and functions: the cortex surface substance and the central medulla. Endocrinocytes of the cortex of the adrenal glands formed fiber cords directed perpendicular to the surface of the adrenal gland. In the spaces between these cords, we observed loose connective tissue with blood capillaries and nerve fibers that entwined these cords. The cortex consists of three morphologically distinct zones: glomerular, fasciculate, and reticular [4, 8, 9].

After measuring the sizes of the component adrenal glands, it was established that the average total thickness of the capsule of the adrenal glands was (33.52±0.21) μm, the average total thickness of the cortex was (583.31±1.42) μm. The total average thickness of the glomerular zone was (44.27±0.21) μm, the total average thickness of the fasciculate zone was (401.87±2.68) μm, the total average thickness of the reticular zone was (131.56±0.86) μm. The average total thickness of the medulla was (382.22±3.61) μm.

Adrenal glands are well vascularized. As a result, the hemomicrocirculatory bed is well-developed and multicomponent **(fig. 1)**.

During the morphological study of the elements of the hemomicrocirculatory bed, we analyzed indicators of the total diameter of arteries, venules, capillaries, their lumen, and the average thickness of the walls [10, 11].

During the morphometric study of the vessels of the glomerular zone of the cortex of the control group, it was established that the total diameter of the arteries is (28.86±0.11) μm, the diameter of the lumen is (22.41±0.11) μm, and the wall thickness is (7, 01±0.02) μm. The total diameter of the venules of the glomerular zone of the cortex of the control group was (21.61±0.12) μm. The average diameter of the lumen of the venules of the glomerular layer of the cortical substance in the control group was (19.31±0.11) μm, and the diameter of the wall was (2.1±0.02) μm. During the morphological analysis of the total diameter of the capillaries of the glomerular zone of the cortex, control values were established – (7.14±0.01) μm. The average diameter of the lumen of the capillaries of the glomerular zone of the cortex in the control group was (5.67±0.02) μm, and the average diameter of the wall was (1.55±0.03) μm.

During the morphometric analysis of the indicators of the total diameter of the arteries of the hemomicrocirculatory bed of the fasciculate zone of the cortex of the adrenal glands of rats, the indicators of the control group were (8.04±0.01) μm; the diameter of the lumen of the arteries was (6.78±0.02) μm; wall diameter – (1.26±0.01) μm. Indicators of the total diameter of the venules of the fasciculate zone of the cortex of the adrenal glands of the control group were (7.14±0.02) μm. The indicators of the average diameter of the lumen of the venules of the fasciculate zone of the cortex of the adrenal glands in the control group were (5.59±0.02) μm; wall diameter – (1.55±0.02) μm. The total average diameter of the capillaries of the fasciculate zone of the cortex of the adrenal glands of the control group was (5.67±0.01) μm. Morphometric study of the average diameter of the lumen of the capillaries of the fasciculate zone of the cortex of the adrenal glands of rats in the control group was (5.04±0.01) μm; the average total wall diameter was (0.66±0.01) μm.

The average indicator of the total diameter of the hemomicrocirculatory bed of the arterioles of the reticular zone of the cortex of the adrenal glands of rats was (14.04±0.12) μm. The average total diameter of the lumen of arterioles was (12.74±0.11) μm. The average total diameter of the arteriole wall of the reticular zone was (1.29±0.02) μm. The average diameter of the venules of the reticular zone of the cortex of the control group was (10.29±0.02) μm. The average diameter of the lumen of the venules of the reticular

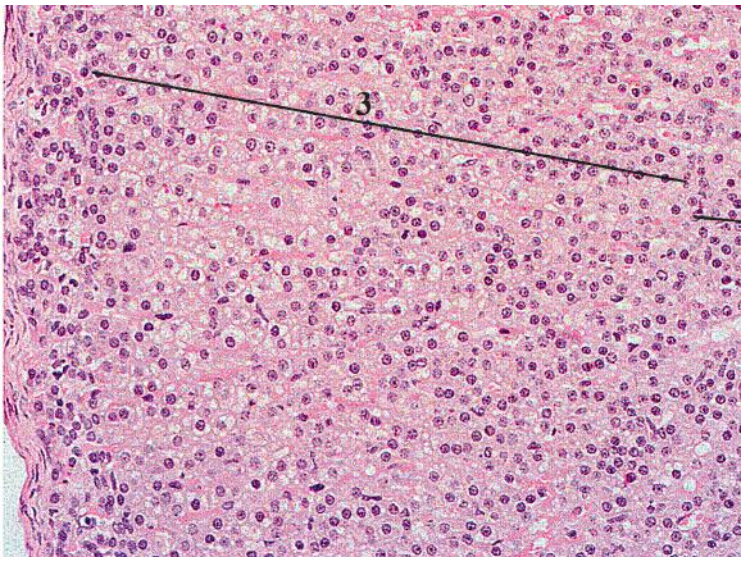

Figure 1 – Fenestrated capillaries with erythrocytes in the cortical substance of rat's adrenal glands. Hematoxylin and eosin staining. Magnification: okh. 10, obh. 40. Marking: 1 – capsule; 2 – glomerular zone; 3 – fasciculate zone; 4 – reticular zone; 5 – fenestrated capillaries with erythrocytes.

zone of the cortex of the adrenal glands of rats in the control group was (8.04±0.01) μm. The average diameter of the wall of the venules of the reticular zone of the cortex was (2.25±0.08) μm. The average diameter of the capillaries of the reticular zone of the cortex of the control group was (6.82±0.01) μm. The morphometric parameters of the average diameter of the lumen of the capillaries of the reticular zone of the cortex of the adrenal glands of rats were (5.25±0.02) μm. The total average diameter of the capillary wall of the reticular zone of the cortex was (1.57±0.01) μm.

During the morphometric analysis of the general indicators of the vessels of the medulla of the adrenal glands of rats, it was established: the total average diameter of arterioles was (7.03±0.02) μm, the average diameter of the lumen of arterioles was (5.47±0.02) μm, the diameter of the arteriole wall – (1.54±0.01) μm. The total diameter of the venules of the medulla of the adrenal glands was (16.53±0.12) μm; the lumen diameter was (15.93±0.11) μm, the wall diameter (0.61±0.01) μm. The total average diameter of the wall of capillaries of the medulla was (7.14±0.02) μm, the diameter of the lumen – (6.05±0.02) μm, the diameter of the wall – (1.07±0.01) μm.

Evaluating the obtained results, we established the morphology of human adrenal glands for comparative analysis.

The cells of the cortical substance of the glomerular zone were small in size, had a polygonal shape, and formed a rounded cluster in the form of glomeruli **(fig. 2)**.

The cells of the fasciculate zone were large, arranged in parallel rows, and formed bundles. The cells of this zone had light and dark cytoplasm and, depending on their functional state, had prismatic and cubic shapes. In the reticular zone, the cells had a rounded shape, smaller sizes than the cells of the fasciculate zone, and formed bundles that resembled a mesh. The cells of the fasciculate and reticular zones contained small lipid inclusions in their cytoplasm. Clusters of stem cells were observed between the zones of the cortical substance.

The medulla of the adrenal glands was separated from the cortical layer of connective tissue and formed by large polygonal, oval cells in the form of clusters and short interconnected cords – chromaffin or medulla cells **(fig. 3).**

Among the number of chromaffin cells, we distinguished: epinephrocytes (A-cells or adrenocytes), which made up the main part of the cell population, were filled with secretory granules and had light cytoplasm, and norepinephricocytes (H-cells or noradrenocytes), which were in the form of small clusters with dark cytoplasm and secretory granules.

Therefore, the data obtained on the structural organization of rat adrenal glands compared with human adrenal glands are almost identical. The obtained data on the average control values provide a perspective to use the obtained indicators during comparison with pathological conditions of the adrenal glands of rats.

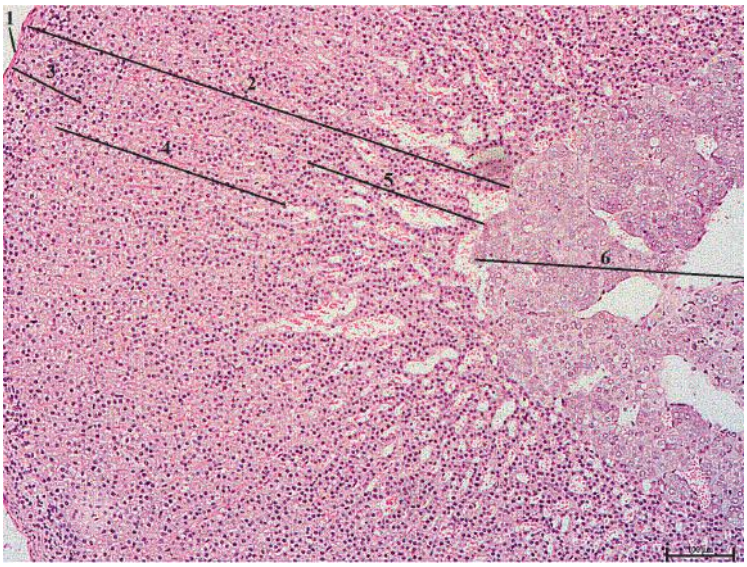

Figure 2 – The structure of the rat's adrenal glands components. Staining with hematoxylin and eosin. Magnification: okh. 10, obh. 10. Marking: 1 – capsule; 2 – cortex; 3 – glomerular zone; 4 – fasciculate zone; 5 – reticular zone; 6 – medulla.

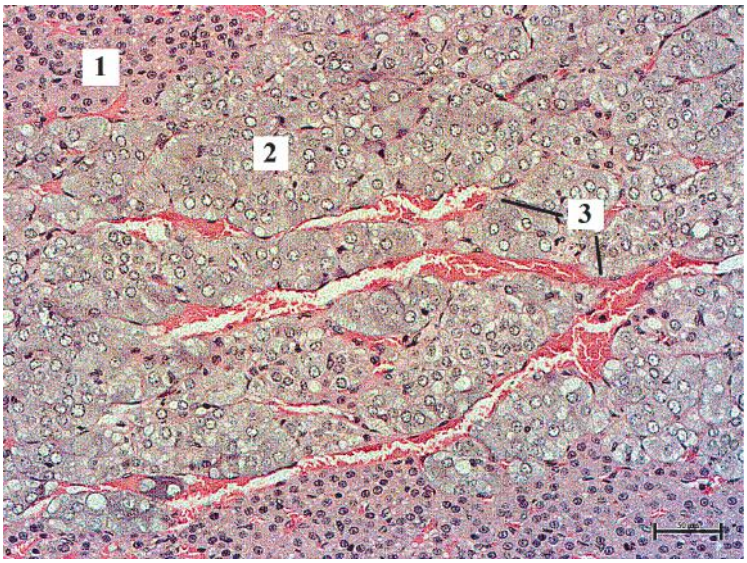

Figure 3 – The structure of rats' adrenal gland's medulla components. Staining with hematoxylin and eosin. Magnification: okh. 10, obh. 40. Marking: 1 – cells of the reticular zone; 2 – chromaffin cells; 3 – fenestrated capillaries with erythrocytes.

Conclusions.

It was established that rat adrenal glands correspond to the structure of human adrenal glands, which makes it possible to transfer the obtained results during experiments and studies of various origins. The obtained data on the average sizes of the structural components of the adrenal glands provide prospects for comparisons of adrenal glands after the negative influence of exogenous factors.

This study is the first step in studying the effect of a complex of food additives on the human adrenal glands with the prospect of transferring the obtained results to the human body.

Prospects for further research.

In the future, it is possible to compare the obtained data with the adrenal glands of rats exposed to a complex of food additives.

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СТРУКТУРНА ОРГАНІЗАЦІЯ НАДНИРКОВИХ ЗАЛОЗ ЩУРІВ В НОРМІ

Донченко С. В., Білаш С. М.

Резюме. Дана робота містить дослідження структури надниркових залоз щурів в нормі та порівняння їх із надниркoвими залозами у людини, що можна використовувати у подальших дослідженнях.

Для отримання даного експерименту ми використовували біоптат надниркових залоз щурів. Отримані зрізи були забарвлені гематоксиліном та еозином і метиленовим синім та толуїдиновим синім. Під час нашого дослідження були використані: гістологічний, морфометричний і статистичний методи.

В результаті даного дослідження було встановлено, що надниркові залози складаються із капсули, кіркової та мозкової речовини. Кіркова речовина в свою чергу поділялася на три морфологічно різні зони: клубочкову, пучкову та сітчасту. Клітини клубочкової зони мають полігональну форму та дрібні розміри; клітини пучкової зони розміщуються паралельними рядами, мають великі розміри та утворюють пучки; клітини сітчастої зони мають округлу форму та формують різнонаправлені пучки, що нагадують сітку. Мозкова речовина відмежована від кіркової речовини прошарком сполучної тканини. Клітини мозкової речовини утворені хромафіноцитами, серед яких виділили епінефроцити та норепінефроцити.

Після встановлення розмірів складових надниркових залоз ми отримали такі дані: середня загальна товщина капсули становила (33,52±0,21) мкм; середня товщина кіркової речовини становила (583,31±1,42) мкм; середня загальна товщина клубочкової зони – (44,27±0,21) мкм; середня загальна товщина пучкової зони становила (401,87±2,68) мкм; середня загальна товщина сітчастої зони становила (131,56±0,86) мкм. Середня загальна товщина мозкової речовини – (382,22±3,61) мкм.

Отримані дані ми можемо використати для інтерпретації отриманих результатів на людину під час досліджень та експериментів, а також в подальшому для дослідження впливу комплексу харчових добавок на морфофункціональний стан наднирників.

Ключові слова: надниркові залози, харчові добавки, кіркова речовина, мозкова речовина, глутамат натрію, нітрит натрію, Понсо 4R.

STRUCTURAL ORGANIZATION OF RATS ADRENAL GLANDS IN NORMAL STATE

Donchenko S. V., Bilash S. M.

Abstract. This work contains a study of the structure of the adrenal glands of rats in normal conditions and their comparison with the adrenal glands of humans, which can be used in further research.

For this experiment, we used a biopsy of rat adrenal glands. The resulting sections were stained with hematoxylin, eosin, methylene blue, and toluidine blue. During our research, we used: histological, morphometric, and statistical methods.

As a result of this study, it was established that the adrenal glands consist of a capsule, cortex, and medulla. The cortical substance, in turn, was divided into three morphologically different zones: glomerular, fascicular, and reticular. The cells of the glomerular zone are polygonal in shape and small in size; the cells of the fascicular zone are placed in parallel rows, have large sizes, and form bundles; the cells of the reticular zone have a rounded shape and form multidirectional bundles resembling a grid. The medulla is separated from the cortex substance by a layer of connective tissue. The medulla cells are formed by chromaffinocytes, among which epinephrocytes and norepinephocytes were identified.

After establishing the sizes of the component adrenal glands, we obtained the following data: the average total thickness of the capsule was (33.52±0.21) μm; the average thickness of the cortical substance was (583.31±1.42) μm; the average total thickness of the glomerular zone – (44.27±0.21) μm; the average total thickness of the fasciculate

zone was (401.87±2.68) μm; the average total thickness of the reticular zone was (131.56±0.86) μm. The average total thickness of the medulla is (382.22±3.61) μm.

We can use the obtained data to interpret the obtained results for a person during research and experiments, as well as in the future, to study the effect of a complex of food additives on the morphofunctional state of the adrenal glands.

Key words: adrenal glands, nutritional supplements, cortex, medulla, monosodium glutamate, sodium nitrite, Ponceau 4R.

ORCID and contributionship:
Donchenko S. V.: 0000-0001-7411-9897 ABCDEF
Bilash S. M.: 0000-0002-8351-6090 ABCEF

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

Corresponding author
Donchenko Svitlana Vladyslavivna
Poltava State Medical University
Ukraine, 36000, Poltava, 23 Shevchenko str.
Tel.: +380955699434
E-mail: donchsveta77@gmail.com

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

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