ISSN NO: 2770-2936
July 2024

The Structure Of The Rat Thymus During Late Postnatal Ontogenesis And Its Changes When Exposed To An Energy Drink.

At the Military Innovation University, Doctor of Biological Sciences,
Professor of the Department of Exact and Natural Sciences. **Ilyasov A.S.**
A free applicant. Bukhara State Medical Institute, pathologist at the specialized Scientific and Practical Center of Oncology and Radiology of the Bukhara branch. **Rakhmanova K.E**

Abstract: This article presents data on the structure of the thymus capsule and its trabeculae in the 1,3,6-month period of white laboratory rats. The best results were obtained by combining protocols such as staining with the van Ginzon technique for the detection of collagen fibers and histochemical staining with toluidine blue for the detection of mast cells. The article presents data from the new literature on the structure of the thymus, its cellular composition and the surrounding capsule of this organ and its changes under the energy drink influence.
Key words: Rats, Thymus, Energy Drinks, Mast Cells.

Introduction. It is known that the thymus is the central organ of immunogenesis, which protects the body from infections. It is known as the "main gland of immunity", which regulates the immune system by producing various chemicals known as cytokines, which enhance the migration of T-lymphocytes and thereby enhance immunity [Amirakulov M.M.2013].

According to Arbab Sikandar (2020), the thymus gland is considered one of the most important organs in maintaining immunity and protecting the host from aging and the development of diseases. The size, architecture and function of this gland decreases with age.

At an unspecified point, the thymus gland begins to show involution, which leads to fatty degeneration of the organ; hence the process is called the involution of adipose tissue. This aging process is accompanied by a stepwise disorganization of thymus compartments [Krisztian Kvell 2019].

Consequently, human death can occur mainly due to complications from infections and lack of immunity. The thymus atrophies over time, which is caused by many factors, namely growth and aging, infectious and endocrine instability, which cause non-standard release of T cells and, as a result, weaken the immune system [Polevshchikov A.V., 2017].

In rodents, the thymus gland performs at least two functions. This is the main site of lymphopoiesis in the embryo and newborn, as a result of which lymphocytes migrate from the thymus gland to the spleen, lymph nodes and other lymphoid organs. In addition, the thymus gland produces a hormone with an immunotrophic effect, that is, it endows cells with immunological potential with immunological competence [Vasendin D.In 2015, Asimova S.B 2023].

Over the past 10-15 years, smart products have become popular on the market and the so-called energy drinks (EN) have become especially widespread among them. At the same time, there are suggestions about their possible negative impact on the human body.[Campbell B 2013]. At the same time, attention should be paid to the fact that any substance, including ordinary water or table salt, with excessive consumption can lead to a toxiletal outcome.

The longest tests on the consumption of energy drinks within the established norms, conducted over several weeks, did not reveal any changes in the studied values of biosafety markers in healthy subjects [Roberts, M.D 2008]. These conflicting opinions in the works of researchers have prompted us to study this global problem.

Thus, it can be noted that EN are not physiologically necessary for normal human life. The prescription composition of EN does not have a deep scientific basis to date. In this regard, the study of the structure of the rat thymus during late postnatal ontogenesis and its changes when exposed to an energy drink is relevant.

The purpose of the study. The aim was to study the dynamics of formation and organization of thymus wall membranes and mast cells in white rats during late postnatal development and its changes during poisoning with an energy drink (Adrenaline Rush).

ISSN NO: 2770-2936
July 2024

Materials and methods of research. The object of the study was white laboratory rats from the vivarium of the Bukhara Medical Institute named after Abu Ali ibn Sina.

The thymus of a rat was taken for research. The isolated organ was fixed in a 10% neutral formalin solution, then the blocks were placed in an automated leash station KD-TS3D1 Automatic Tissue Processor. The tissue was dehydrated, filled with paraffin and thin sections 4-6 microns thick were prepared on a rotary microtome Semi Automatic Rotary Microtome KD-3358.

Histological staining of the tissue was performed with hematoxylin-eosin paint on the KD-RS2 Automatic Slide Stainer carousel device, Van Gieson staining was performed to identify collagen fibers, as well as histochemical staining with toluidine blue to identify mast cells.

The obtained thymus sections were microscopically documented using a Nikon eclipse E200 MV research microscope using a LEICA ICC50 E color camera.

The results and their discussions. The thymus in rats at puberty is located in the anterior mediastinum behind the sternum. Its shapes are different: rounded, cone-shaped, leaf-shaped, trapezoidal. During puberty, the average weight is 24.9±2.05 [Khasanova D.A., 2021]

By the age of one month, the thickness of the membranes of the organ wall is on average 28.0 ± 1.3 microns; in the experimental group, in rats receiving energy drinks, the thickness of the wall shells was on average 34.9 ± 0.92 microns. Figure 1 shows the capsule and trabeculae of the thymus of rats in the control group of animals.

The outer capsule of the thymus consists of loose connective tissue, in which bundles of collagen fibers envelop the organ and its proximal part, located above the parenchyma of the organ. These bundles of collagen fibers, changing their direction, penetrate deep into the organ, where they form trabeculae of the parenchyma of the thymus (Fig. 2).

The thickness of the bundles of collagen fibers of the outer shell of the thymus averaged 16.5 ± 0.6 microns, at this age in the experimental group it was on average 21.1 ±0.74.

At the age of one month, the wall thickness of the trabecula is on average 9.5 ± 0.44 microns. In case of poisoning with an energy drink at this age, the wall thickness of the trabeculae averaged 12.1±0.34 microns. Figure 3 shows the capsule and trabeculae of the thymus of rats in the experimental group.

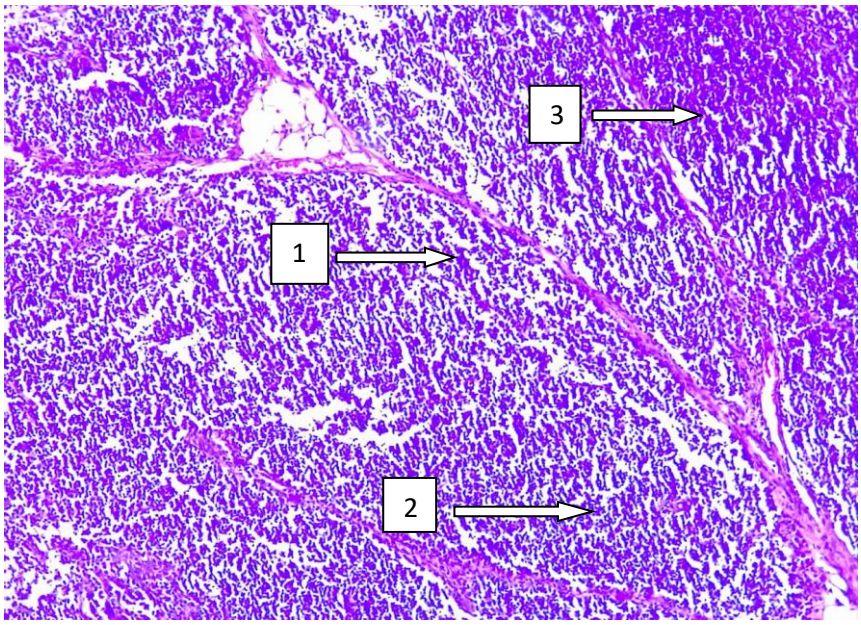

Figure 1 .Capsule and trabeculae of the thymus of rats in the control group.1- trabeculae 2 -parenchyma of the thymus 3 -cortical substance. Hematoxylin-eosin staining volume 10.approx.10

ISSN NO: 2770-2936
July 2024

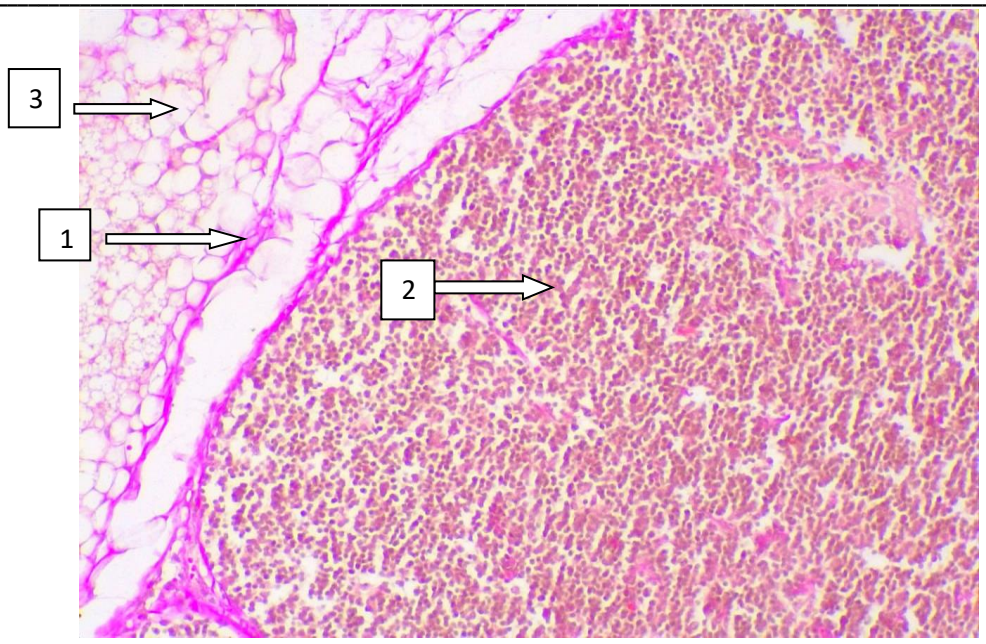

Figure 3 .Capsule of the thymus of rats of the control group. Collagen fibers.1-Capsule 2 -Parenchyma 3 - Adipose tissue.

Van Gieson staining vol.10. approx.10

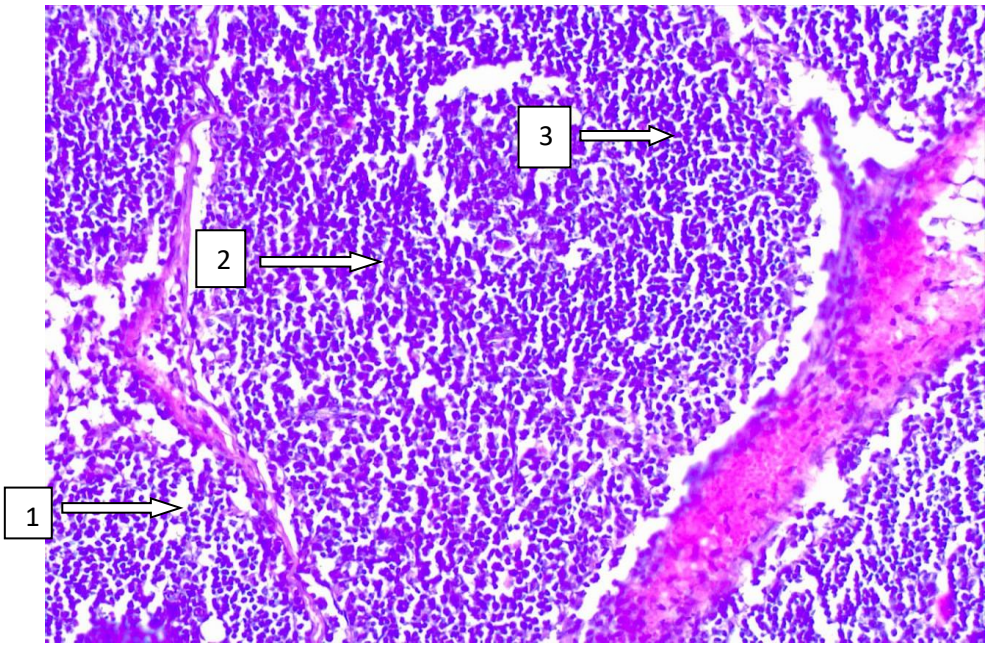

Figure 3 .Capsule and trabeculae of the thymus of rats in the experimental group.1 -trabeculae 2 - Parenchyma of the thymus 3 -cortical substance. Hematoxylin-eosin staining volume 10.approx.20

The thickness of the bundles of collagen fibers in the trabeculae averaged 6.2±0.44 microns. In case of poisoning with an energy drink, the thickness of collagen fibers in trabeculae is on average 9.2 ± 0.44 microns. In case of poisoning with energy drinks at the age of one month, the wall thickness of the trabeculae is on average 12.1 ± 0.37 microns. The thickness of the bundles of collagen fibers in the trabeculae is on average 6.2 ± 0.44 microns. In case of poisoning with energy drinks, the thickness of collagen fiber bundles in trabeculae averaged 9.2±0.4 microns.

At 3 months of age, the thickness of the thymus capsule averaged 32.5 ±1.3 microns, in the first experimental group, those who received energy drinks had an average capsule wall thickness of 39.5 ± 0.92 microns. The thickness of the bundles of collagen fibers in the capsule of the thymus at 3 months of age averaged 19.3 ±

ISSN NO: 2770-2936
July 2024

0.6 microns, when exposed to energy drinks (in the experimental group), the thickness of the bundles of collagen fibers in the capsule of the organ is on average 25.8 ± 0.83 microns.

The wall thickness of the trabeculae at 3 months of age is on average 11.0 ±0.52 microns, in the experiment the thickness is on average 14.2 ± 0.37 microns. The thickness of the bundles of collagen fibers in trabeculae at 3 months of age is on average 8.5 ± 0.6, the thickness of the bundles of collagen fibers in the experimental group is on average 10.3 ± 0.46 microns.

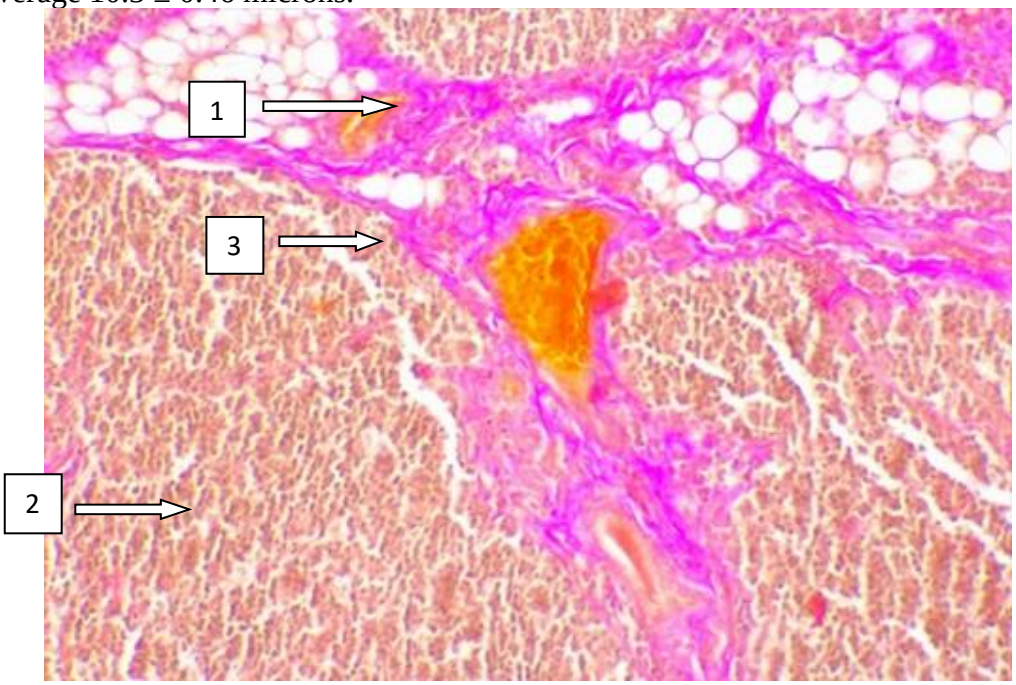

Figure 4 .The capsule of the rat thymus in the experimental group. Bundles of collagen fibers.1.Collagen fibers in capsule 2.Parenchyma of the thymus 3. Intima of the blood vessel. Van Gieson staining Vol.10hok.20

At the age of 6 months, the wall thickness of the thymus membrane in the control group of rats averaged 25.5±1.13microns, in the experimental group it averaged 12.7±1.3 microns, the thickness of the thymus capsule shell in the experimental group was 28.7±1.3 microns. The thickness of the collagen fiber bundles in the capsule at the age of 6 months averaged 20.9 ± 0.7 microns, in the experimental group an average of 24.1 ± 0.83 microns.

At the age of 6 months, the trabeculae that depart from the capsule wall are on average 8.5±0.44 microns thick, in the experimental group with exposure to energy drinks, the thickness of the thymus trabeculae is 0.3+0.55 microns.

The thickness of the bundles of collagen fibers in the trabeculae at 6 months of age is on average 5.8±0.34 microns, the thickness of the bundles of collagen fibers in the trabeculae in this experimental group was on average 7.2±0.37 microns.Figure 5 shows a comparative analysis of the structural parameters of the rat thymus during postnatal ontogenesis and in case of EN poisoning (Adrenaline Rush).

The structure of the rat thymus during postnatal ontogenesis in the control group and in case of EN poisoning (Adrenaline Rush)

ISSN NO: 2770-2936
July 2024

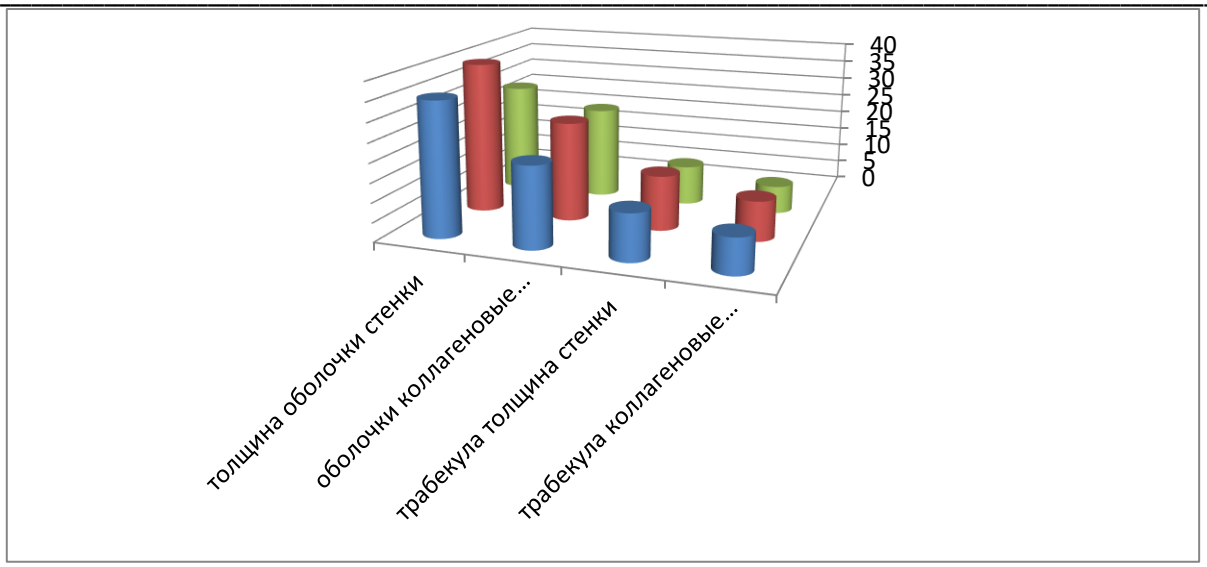

Figure 5.

When studying the cellular structures of the rat thymus, it was revealed that mast cells in immune organogenesis have long attracted the attention of researchers. The functional features of mast cells make it possible to identify pathological, physiological, and allergic reactions that implement innate or adaptive immunity.

When staining the thymus of rats with toluidine blue, mast cells are rare, mainly their location in interstitial tissue is single in the amount of 2-6 in the field of view. The cells are colored dark purple, the contours are smooth and irregularly rounded. Figure 6 shows the mast cells of the thymus of rats in the control group.

Histochemical reaction with toluidine blue, thymus of month-old rats in the experiment with EN showed successful staining, which revealed the number of mast cells 6-12. They turned purple, the nuclei are multiple with a basophilic granular cytoplasm, whose contours are indistinct with a stellate structure (Fig.7). Such a number of mast cells indicates that pathophysiological processes and inflammatory and allergic reactions occur intensively in this body. The reaction of 3-month-old rats to toluidine blue revealed mast cells during a histochemical reaction.

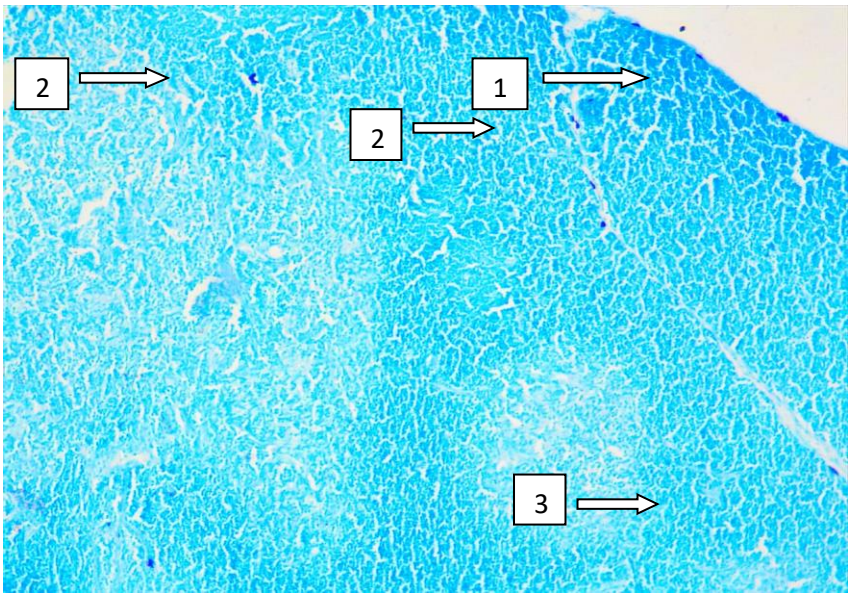

Fig. 6. The structure of the rat thymus in the control group: 1. Thymus capsule 2.Mast cells. 3. Parenchyma of the thymus. Technique: staining of mast cells with toluidine blue. About 10hok.10

ISSN NO: 2770-2936
July 2024

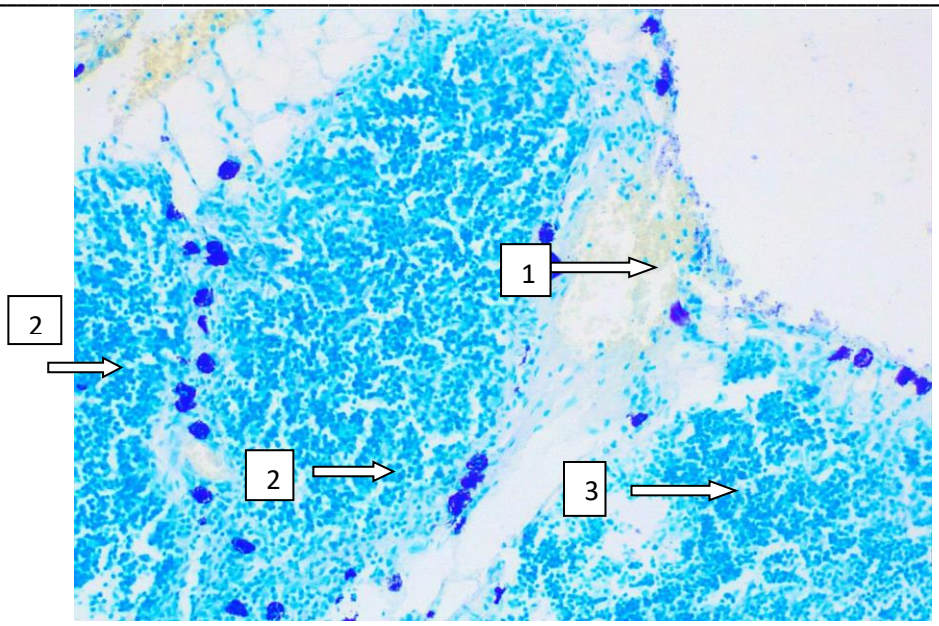

Fig.7. The cortical zone of the thymus of month-old rats in the experimental group with EN poisoning: 1. Thymus capsule 2. Mast cells. 3. Parenchyma of the thymus. Technique: staining of mast cells with toluidine blue. About 10hok.20

The parenchyma of the tissue is colored blue, mast granular cells with intense staining, the number of mast cells from 14-22 in one field of view, are mainly located along the periphery of the tissue with basophilic granularity of the cytoplasm.

Mast cells stained with toluidine blue were detected in the thymus of 6-month-old rats. The data obtained during histochemical examination: the number of mast cells averages from 4-10 in one field of view, their structure changes significantly, the cytoplasm is diffusely enlarged in volume, has the shape of a cone with small nucleoli and basophilic granularity.

A combination of histochemical studies under the influence of EN on the central organ of immunogenesis, the thymus, has revealed a number of pathological and physiological processes in this tissue that can be used to identify immune processes occurring in the body.

Conclusion: The study revealed that the outer shell of the capsule and trabeculae of the thymus of rats increase with age and the greatest increase was observed at the age of one month.

By the end of postnatal ontogenesis (6 months), due to involutive processes, the organ shell and trabeculae thin out. Bundles of collagen fibers in the control group envelop the organ in a dense ring, their basal structures, changing their direction in places, penetrate into the parenchyma of the thymus forming trabeculae.

In the experimental group, when EN (Adrenaline Rush) poisoning occurs, an increase in the thickness of the capsule and its trabeculae, thickening, violation of the integrity of the bundles of collagen fibers and their loosening is observed.

Histochemical reaction with toluidine blue staining showed that the number of mast cells increases dynamically and the structure of mast cells changes significantly when exposed to EN on the thymus of rats.

The conducted studies make it possible to better understand the patterns of structure and development of organs of immunogenesis, to standardize morphological data in the process of physiological ontogenesis.

References

1. Полевщиков А.В., Прозоровская Е.Л., Кудрявцев И.В., Серебрякова М. К. //Формирование иммунного ответа на тимус-зависимые и тимус-независимые антигены: роль тимуса// Медицинская экология. Наука-2017, С. 73-75
2. Полевщиков А.В.,Гурова О.В.,Зассеева М.Д.,Старская И.С.,Гусельникова В.В.//Динамика морфологических изменений в тимусе мыши после иммунизации// Тихоокеанский медицинский журнал 2014, С.42

ISSN NO: 2770-2936
July 2024

3. Амиракулов, М. М. Возрастная морфология иммунной системы индеек юга Кыргызстана: автореф. дис. канд. биол. наук: 03.00.00 / М. М. Амиракулов. – Бишкек, 2013 26 с.
4. Васендин Д.В., Мичурина С.В., Ищенко И.Ю//Структурные изменения в тимусе в анаболической фазе после воздействия экспериментальной гипертермии// Ученые записки СПбГМУ им. И. П. Павлова-2015 г. С.23-24
5. Азимова С.Б Международный научный журнал. «Научный импульс» № 10(100), часть 1.2023.459-463 с.
6. Arbab Sikandar, Shahzaib and Naeem Ullah (January 29th 2020). Microarchitecture of the Thymus Gland; Its Age and Disease-Associated Morphological Alterations, and Possible Means to Prolong Its Physiological Activity:227-228
7. Krisztian Kvell (July 2nd 2019). Thymic Senescence:126-127
8. Азимова, С. Б., & Хасанов, Б. Б. (2021). Токсический гепатит матери и структурно-функциональное формирование тимуса потомства в динамике раннего постнатального онтогенеза. Eurasian Journal of Academic Research, 1(9), 426-429.
9. Bahodurovna, A. S. (2022). Structural-Functional Properties of Stress and Thymus. Research Journal of Trauma and Disability Studies, 1(9), 54-59.
10. Davronov, R.D., & Davronova, Sh. R.(2021).Modern views on the particicpation of the thymus in the processes of immunogenesis. European Journal of Molecular & Clinical Medicine, Volume 07, Issue 09,1359-1366
11. Azimova, S.,& Oripova, N. (2021, March). FORMATION OF SPIRITUAL EDUCATION IS ONE OF THE PRIORITIES IN HUMAN LIFE AND SOCIETY. In Конференции
12. Kuchar, E. Review of guidance on immunization in persons with defective or deficient splenic function // Br. J. Haematol.- 2015. -Vol.171(5).- P. 683–694
13. Lusk RW. Diverse and widespread contamination evident in the unmapped depths of high throughput sequencing data. PLoS ONE. 2014;9:e110808.
14. Marx-Stoelting P, Braeuning A, Buhrke T, Lampen A, Niemann L, Oelgeschlaeger M, Rieke S, Schmidt F, Heise T, Pfeil R, Solecki R. Application of omics data in regulatory toxicology: Report of an international BfR expert workshop. Archives in Toxicology. 2015;89:2177–2184.
15. Хасанова Д.А Морфологические аспекты селезенки и тимуса в норме при влиянии генно-модифицированного продукта в эксперименте 2021 87 стр
16. Roberts, M.D. Efficacy and safety of a popular thermogenic drink after 28 days of ingestion / M.D. Roberts, V.J. Dalbo, S.E. Hassell [et al.] // Journal of the Intrnational Society of Sports Nutrition. — 2008. — Vol. 5: 19 (11 pp)
17. Campbell, B. International Society of Sports Nutrition position stand: energy drinks / B. Campbell, C. Wilborn, P. Bounty [et al] // Journal of the Intrnational Society of Sports Nutrition.— 2013.— Vol. 10:14 (6 pp)