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[1]Stetsuk E. V., [1]Shepitko V. I., [2]Nebesna Z. M., [1]Boruta N. V., [1]Yakushko O. S., [1]Rud M. V.

ELECTRONIC MICROSCOPIC SUBSTANTIATION OF POPULATIONS OF MACROPHAGES IN INTERSTITIAL SPACE ON THE SEEDS OF CONTROL AND INTACTIVE INTAINT RATS

[1]Poltava State Medical University (Poltava, Ukraine)
[2]Horbachevsky Ternopil National Medical University (Ternopil, Ukraine)
Stetsuk78@gmail.com

The reproductive system of mammals and humans was formed in the process of evolution in close interaction with external and internal environmental factors that affected all body systems. The equilibrium state between environments and living organisms, formed in the process of evolution, ensures their normal functioning and reproduction, as well as effective adaptation to changing environmental conditions due to various factors. The human immune system is a complex multi-level structure that responds to numerous factors of the external and internal environment. Modern research has shown the involvement of the immune system in the development of almost all pathological conditions in humans. They promote homeostasis by responding to internal and external changes in the body, not only as phagocytes in protection against microbes, but also in cleansing of dead and aging cells, as well as through the functions of trophic, cellular regulation and cell regeneration. Thus, the phagocytic system in comparison with the nervous and endocrine systems in that it can be adapted, regulated and able to perform trophic and protective functions, both locally and systemically. Based on this, macrophages can be considered as a dispersed homeostatic organ. The experimental study provided new information on the structural and functional features of the macrophage system of the interstitial space of the testes. Studying the cells of the macrophage series of connective tissue, we found that in the interstitial space, depending on the location, two groups of macrophages were clearly distinguished, both in the control group and in the intact group of animals. The first type of macrophages, these are cells that were mostly near blood vessels, located alone or in groups – interstitial macrophages. The second population consisted of macrophages, which we identified as parietal.

Key words: testis, interstitium, macrophage.

The connection of the publication with planned research works. The study is a fragment of the research project "Experimental morphological study of cryopreserved placenta transplants action of diphereline, ethanol and 1% methacrylic acid on the morphofunctional status in a number of internal organs", state registration № 0119U102925.

Introduction. None of the physiological systems of the body can be considered in isolation from others, because they are all components of integrity and work closely together [1, 2]. Reproductive function at all its stages actively mobilizes the body's regulatory systems in order to provide optimal conditions for the reproduction of the species [3, 4]. The transmission of genetic material to the next generation is the basic principle of species preservation.

The human immune system is a complex multi-level structure that responds to numerous factors of the external and internal environment. Modern research has shown the involvement of the immune system in the development of almost all pathological human conditions [5, 6]. In this case, the immune system performs the function of protection against genetically foreign material, and plays a crucial role in maintaining structural and functional homeostasis of the organism. It functions in close connection with the surrounding organs, tissues and cells, among which the main ones are macrophages, which are present in all mammalian tissues and form a widespread system in the body [7, 8, 9]. They help maintain homeostasis by responding to internal and external changes in the body, not only as phagocytes in protection against microbes, in the removal of dead and aging cells, but also through the functions of nutrition, cell regeneration and cell regulation. Based on this, macrophages can be considered as a dispersed homeostatic organ. Tissue macrophages are a distributed mononuclear phagocytic cell system that promotes the body's response to physiological changes in infectious conditions [10].

Thus, the phagocytic system, in comparison with the nervous and endocrine systems, can be adapted, regulated and able to perform trophic, protective functions, both locally and systemically.

The aim of this study was to establish the diversity of populations and to describe the electronograms of the interstitial space macrophages of the rat's testes in control and intact group of animals.

Object and methods of the research. The study was performed on 20 adult male rats. Animals were randomly divided into 2 groups: control (10 animals) and intact (10 animals). Animals in the control group were injected with saline in dosage 0.3 ml in the thigh. Animals of the intact and control groups were kept in standard conditions of the PDMU vivarium. Experimental animals were euthanized in strict accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the General Ethical Principles of Animal Experiments adopted by the First National Congress on Bioethics (Kyiv, 2001).

Preparation of material for electron microscopic studies of the interstitial space structures of the testis was performed according to the generally accepted method [11]. Animals anesthetized with ketamine were decapitated, and prepared small pieces of testes were

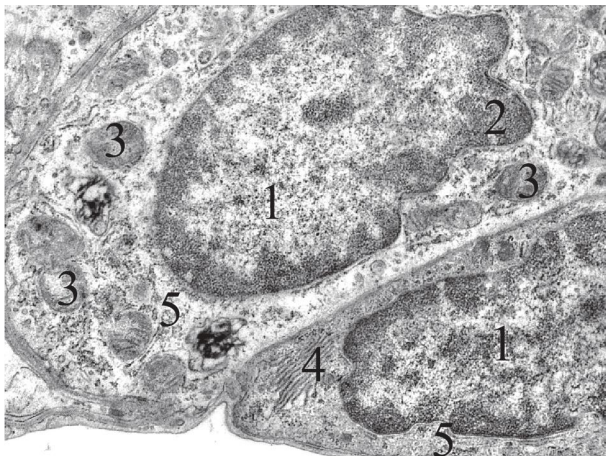

Figure 1 – Interstitial macrophage (IM) of rats of the control group of animals. Magnification: x12000. Marking: 1 – nucleus, 2 – chromatin, 3 – mitochondria, 4 – endoplasmic reticulum, 5 – cytoplasm.

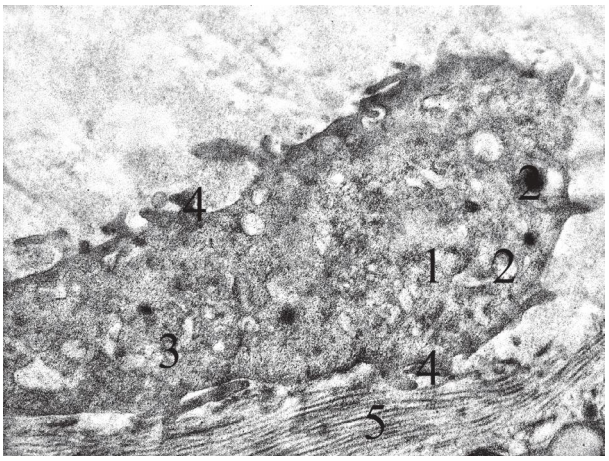

Figure 2 – Parietal macrophage of rats of the control group of animals. Magnification: x12000. Marking: 1 – cytoplasm, 2 – lysosomes, 3 – endoplasmic reticulum, 4 – pseudopodia, 5 – basement membrane of the convoluted tubule.

fixed in a 2.5% solution of glutaraldehyde with an active reaction pH 7.2-7.4, prepared on phosphate buffer. Post-fixation of the material was carried out with 1% solution of osmium tetroxide, after which it was dehydrated in propylene oxide and poured into a mixture of epoxy resins with araldite. Ultrathin sections made on an ultramicrotome LKB-3 (Sweden) were contrasted with 1% aqueous uranyl acetate and lead citrate according to the Reynolds method. Sections were studied using an electron microscope PEM-125 K, followed by photography at a magnification of 4000 to 16,000 times [12].

The results of the research and their discussion. Studying the electronograms of the control and intact groups of animals, we did not find a significant difference in the structure of the interstitial space and surrounding cells. Thus, the interstitial space of the animal's testis of control and intact groups was represented by loose connective tissue, in which blood vessels were clearly visualized, around which groups of interstitial endocrinocytes and cellular elements of connective tissue were found. Interstitial endocrinocytes had, for the most part, round nuclei, moderate number of lipid droplets were clearly visualized in the cytoplasm of the cells. The vessels are moderately full-blooded.

Studying the cells of the macrophage series of connective tissue, we found that in the interstitial space, depending on the location, two groups of macrophages were clearly distinguished, both in the control group and in the intact group of animals. To the first type of interstitial macrophages belong cells that are located alone or in groups of 1-3 in the field of view near the blood vessel **(fig. 1)**.

We will refer to them as interstitial macrophages (IM). These cells had large, electron-dense nuclei, with a predominance of heterochromatin. Karyolemma dense, two-layered, had a large number of pores. One nucleolus was determined in the karyoplasm. According to the amount of chromatin in the nuclei, the cell population could be divided into light and dark cell. Most cells encountered light nuclei. The cytoplasm of the cells was small in size, had a well-developed endoplasmic reticulum, which was represented by numerous tubes that branched and were filled with a fine-grained substance, and numerous ribosomes were located on the membranes.

Mitochondria are small, round and oval in shape, with an osmophilic matrix and a small number of cristae **(fig. 1)**. Also in the cytoplasm of cells are large number of lysosomes of different sizes and electron densities. Externally, an insignificant amount of pseudopodia and invagination is detected on the cell surface.

The second population consisted of macrophages, which we recognised as parietal (PM). Their number, compared with interstitial, was insignificant and amounted to approximately 8-12% of the total number of macrophages. They had a flattened nucleus of elongated, oval, sometimes flat shape with one or two nucleoli, karyoplasm electron-dense and karyolemma clear. Chromatin is heterogeneous. The cytoplasm was light and occupied a small part of the cell. A large number of pseudopodia and invagination are present on the cell surface. There is an endoplasmic reticulum and the Golgi complex in the cytoplasm of cells. Also a large number of lysosomes, varying in size and electron density are found in the cytoplasm. Numerous ribosomes are present in the hyaloplasm. Mitochondria are small, with an osmophilic matrix and a small number of cristae **(fig. 2)**.

Conclusions. Based on the evaluation of electronograms of the testes interstitial space, we found that macrophages, localized in the interstitial space, consisted of two groups, parietal and interstitial.

Prospects for further research. It is planned to study the macrophage system of the rat's testes by blocking the synthesis of luteinizing hormone for 360 days.

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ЕЛЕКТРОННОМІКРОСКОПИЧНЕ ОБГРУНТУВАННЯ ПОПУЛЯЦІЙ МАКРОФАГІВ ІНТЕРСТИЦІЙНОГО ПРОСТОРУ СІМ'ЯНИКІВ ЩУРІВ КОНТРОЛЬНОЇ ТА ІНТАКТНОЇ ГРУП ТВАРИН

Стецук Є. В., Шепітько В. І., Небесна З. М., Борута Н. В., Якушко О. С., Рудь М. В.

Резюме. *Вступ.* Передача генетичного матеріалу наступного покоління є основним принципом збереження образу. Імунна система людини – складно організована багаторівнева структура, що реагує на численні фактори зовнішнього та внутрішнього середовища. Сучасні дослідження довели участь імунної системи у розвитку практично всіх патологічних станів у людини. Вони сприяють гомеостазу, реагуючи внутрішні та зовнішні зміни в організмі, не тільки як фагоцити в захисті від мікробів, а також в очищенні від загиблих і старіючих клітин, а також через функції трофіки, клітинної регуляції і відновлення клітин. Таким чином, фагоцитарна система у порівнянні з нервовою та ендокринною системами в тому, що вона може бути пристосованою, регульованою та здатна виконувати трофічні, а також захисні функції, локально, так і системно. Виходячи з цього макрофаги можна розглядати як дисперсний гомеостатичний орган.

Метою даної роботи було встановлення різноманіття популяцій та опис електронограм макрофагів інтерстиційного простору сім'яників щурів контрольної та інтактної груп тварин.

Об'єкт і методи дослідження. Дослідження проведене на 20 статевозрілих щурах-самцях. Тварини були рандомізовано розподілені на 2 групи: контрольна (10 тварин) та інтактна (10 тварин). Тваринам контрольної групи вводили фізіологічний розчин (NaCl) – 0,3 мл в ділянку стегна. Забір матеріалу для електронномікроскопічних досліджень структур інтерстиційного простору яєчка проводили згідно із загальноприйнятою методикою.

Результати. Вивчаючи клітини макрофагального ряду сполучної тканини нами було встановлено що в інтерстиційному просторі в залежності від локалізації чітко виділялись дві групи макрофагів, як в групі контроля так і в інтактній групі тварин. Перший тип макрофагів, це клітини які знаходились здебільшого біля кровоносних судин, розташовувалися поодинці або групами – інтерстиційні макрофаги. Другу популяцію становили макрофагів які ми виділяли як пристінкові.

Висновки. На підставці оцінки електронограм інтерстиційного простору сім'яників нами встановлено, що макрофаги складали дві групи клітин за локалізацією в інтерстиційному просторі, це пристінкові та інтерстиційні.

Ключові слова: сім'яник, інтерстиція, макрофаг.

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Stetsuk E. V., Shepitko V. I., Nebesna Z. M., Boruta N. V., Yakushko O. S., Rud M. V.

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Key words: testis, interstitium, macrophage.

ORCID and contributionship:
Stetsuk E. V.: 0000-0002-4239-2618 [BCD]
Shepitko V. I.: 0000-0001-5570-795X [F]
Nebesna Z. M.: 0000-0002-6869-0859 [E]
Boruta N. V.: 0000-0002-9262-8967 [A]
Yakushko O. S.: 0000-0001-6247-340X [A]
Rud M. V.: 0000-0001-5241-1264 [A]

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

Corresponding author
Stetsuk Yevhen Valeriiovych
Poltava State Medical University
Ukraine, 36024, Poltava, 23 Shevchenko st.
Tel.: +380677576793
Email: stetsuk78@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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