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MACRO- AND MICROSTRUCTURAL HEART ARRANGEMENT IN WHITE RATS IN HEALTH

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Key words:
rat, myocardium, histological structure.

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The aim of research *– to study the histological structure of the heart of a white rat in health for a comparative analysis of possible changes during further experimental interventions.*
Material and methods. *The material for the study was heart biopsies of male rats with a body weight of 200±30 g. For the production of histological preparations, heart samples were taken randomly from different parts at least of 5 fragments. The thickness of samples for histological preparations was no more than 4 mm. For morphometry, the sections were stained with hematoxylin-eosin, azan and fuxilin and picrofuxin.*
The results. *Myocardial muscle fibers are formed by mono- and binucleate cells of rectangular shape in cross-section, which contact with each other in the form of chains and provide a contractile function. The myocardium of the ventricles is three-layered: it has superficial, middle and deep layers. The fibers of the superficial layer are located longitudinally. Bundles of the middle layer envelop each ventricle separately. The muscle bundles of the inner layer form the trabeculae of the ventricles of the heart. The atrial myocardium consists of two muscle layers – superficial and deep. The superficial layer is made of oblique-circular muscle bundles that continuously cover the two atria. The deep layer is made of longitudinal muscle bundles, and is separate for each atrium.*
Conclusions. *The histological structure of the white rat's heart in health has been specified, as a basis for a comparative analysis of possible changes during experimental interventions, accompanied by morphofunctional changes of the myocardium.*

Ключові слова:
щур, міокард, гістологічна будова.

Клінічна та експериментальна патологія 2022. Т.21, №4 (82). С. 60-64.

МАКРО- ТА МІКРОСТРУКТУРНА ОРГАНІЗАЦІЯ СЕРЦЯ БІЛОГО ЩУРА В НОРМІ

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Мета дослідження – *вивчити гістологічну будову серця білого щура в нормі для порівняльного аналізу можливих змін при подальших експериментальних втручаннях.*
Матеріал та методи. *Матеріалом для дослідження були біоптати серця щурів самців із масою тіла 200±30 гр. Для виготовлення гістологічних препаратів забір зразків серця відбувався випадковим чином із різних частин не менше 5 фрагментів. Товщина зразків для гістологічних препаратів була не більше 4 мм. Для морфометрії зрізи забарвлювали гематоксилін-еозином, азаном та фуксиліном і пікрофуксином.*
Результати. *М'язові волокна міокарда утворені одно- та двоядерними клітинами прямокутної форми на зрізі, які контактують між собою у вигляді ланцюжків і забезпечують скоротливу функцію. Міокард шлуночків тришаровий: має поверхневий, середній та глибокий шари. Волокна поверхневого шару розташовуються поздовжньо. Пучки середнього шару окремо огортають кожний шлуночок. М'язові пучки внутрішнього шару утворюють трабекули шлуночків серця. Міокард передсердь складається з двох м'язових шарів – поверхневого і глибокого. Поверхневий шар побудований з косо-циркулярних м'язових пучків, що суцільно покривають два передсердя. Глибокий шар – з поздовжніх м'язових пучків, і є окремим для кожного передсердя.*
Висновки. *Уточнено гістологічну будову серця білого щура в нормі, як підгрунтя для порівняльного аналізу можливих змін при експериментальних втручаннях, що супроводжуються морфофункціональними змінами міокарда.*

Introduction

According to the WHO, the cardiovascular system diseases are one of the defining problems of modern medicine. Cardiovascular diseases (CVD) are the main cause of mortality and disability of population in most countries of the world [1-3]. It is expected that more than 23 million people will die from these diseases by 2030. In Europe, cardiovascular pathology accounts for about 40% of all deaths in persons under the age of 75, including more than 60% of sudden cardiac death. Ukraine occupies one of the first places in Europe in terms of mortality rates due to diseases of the cardiovascular system (459.48 per 100,000 population) [1-4].

The property of the structure of the rat heart is that 5-6 pulmonary veins end into the left atrium. One vein, sometimes two of veins arise from the left lung, and four

veins arise from the right one. The rat heart resembles the shape of a cone, the apex of which is directed downward towards the diaphragm, the heart is slightly shifted to the left relative to the midline, and is located almost in a horizontal plane. The property of the rat heart includes the presence of the left cranial vena cava and sinus valves. The cone of the right ventricle extends significantly upward. The parietal leaflets of the atrioventricular valves are, in most cases, not clearly distinguished. The aortic arch is very steep, 2-3 trunks arise from it [5].

There are 9 pairs of ribs attached to the sternum in the rat. Skeleton topically, the rat heart is located between the third and the seventh ribs. Cardiac-diaphragmatic ligament extends from the apex of the rat heart [4].

Wall of the rat heart consists of three layers: epicardium – the outer layer, myocardium – the middle layer, and endocardium – the inner layer. The heart itself is located in the fibrous pericardium. Endocardium overlays the heart chambers from the inside, and forms the leaflets and semilunar valves of the heart. The atrial endocardium is thicker than that of the ventricles, but is thickest in the left heart chambers. Myocardium is the thickest layer of the heart wall, and consists of striated cardiac muscle tissue. The rat myocardium consists of two types of muscle cells – cardiomyocytes: typical contractile and conducting ones. Cardiac myofibers are formed by uninuclear and binuclear muscle cells, the contractile cardiomyocytes. These cells are rectangular with lateral processes, their length is 50-120 μm, and diameter is 15-20 μm. The nucleus is located in the center of the cell. Cardiomyocytes are connected to one another, forming intercalated discs. The atrial myocardium consists of two muscle layers, the outer layer, which consists of circular muscle bundles, and the deep layer, which has longitudinally oriented muscle bundles. Myocardium of the ventricles consists of three layers: outer, middle and deep (inner) ones. The outer layer is thin, and its fibers are longitudinally oriented. Its muscle bundles originate from fibrous rings. At the apex of the heart, these bundles twist and extend into the inner longitudinal layer. The middle layer is located between the longitudinal outer and inner muscle layers. The rat epicardium is formed by a serous membrane [6,7].

Aim of the study

To study the histological structure of a white rat heart in health for a comparative analysis of possible changes during further experimental interventions.

Materials and methods

Rats were kept on a standard vivarium diet with free and unrestricted access to water. All animals were kept in the conditions of the vivarium of Danylo Halytskyi Lviv National Medical University, the experiments were conducted in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), Law of Ukraine No. 3447-IV «On the Protection of Animals from Cruelty». The experiments were conducted in accordance with Minutes No. 7 dated 29.08.2022 of the Commission on the ethics of scientific research, experimental developments and scientific papers of Danylo Halytskyi Lviv National Medical University. Material for research was collected after decapitation of animals under ether anesthesia. Heart biopsy specimens of male rats weighing 180-230 g were the material for the study. Since heart is a large organ, tissue samples from each animal were collected simultaneously for histological examination, which allowed optimization of the use of biological material and reduction in the number of animals required for the study. Heart samples were taken randomly, at least 5 fragments from different parts for making histological preparations. Thickness of samples for histological preparations did not exceed 4 mm. Samples for making histological preparations were fixed in 10% buffered formalin. Afterwards, these samples were dehydrated by processing in increasing concentration of ethyl alcohol (60%, 70%, 80%, 90%, 95%, for 2 hours, and twice 100% for 30 minutes), and embedded in paraffin in a thermostat at a temperature of 60 °C with an intermediate processing in alcohol-xylene (50 to 50) and 100% xylene, 30 minutes in each solution. For morphometry, sections with a thickness of 3 μm were stained with hematoxylin-eosin.

Research results and their discussion

Heart muscle of the rat consists of branching and overlapping myofibers, forming a peculiar kind of a mesh. Fissures between myofibers are filled with loose connective tissue (nuclei of fibrocytes are clearly seen) with blood vessels and nerves. Connective tissue in health is poorly pronounced. Myofibers of the myocardium are formed by cardiomyocytes that are rectangular in cross-section (Fig. 1).

The atrial myocardium consists of two muscle layers, the outer and deep ones. The outer layer consists of circular transverse muscle bundles that continuously cover the two atria. The deep layer consists of longitudinal muscle bundles, and is separate for each atrium. Myocardium of the ventricles consists of three layers: outer, middle and deep ones. Fibers of the outer layer are located longitudinally. There is a middle layer between the outer and inner muscle layers, the bundles of which wrap around each ventricle separately. Muscle bundles of the inner layer form trabeculae carneae of the ventricles of the heart. Myocardial myofibers are formed by uninuclear or binuclear muscle cells communicating with one another in the form of chains, and are called cardiomyocytes providing a contractile function (Fig. 2). Ventricular cardiomyocytes are cylindrical in shape, and atrial cardiomyocytes have processes.

Layers of the connective tissue were observed between cardiomyocytes, in the depth of which vessels of various diameters with a small number of erythrocytes were seen. The intima of arterial vessels is tortuous in some places, and is represented by thinned endotheliocytes with elongated hyperchromic nuclei (Fig. 3, 4).

The nuclei of cardiomyocytes are ovoid or spindle-shaped, and are usually located in the center of the cell. Ventricular cardiomyocytes contain fair amount of sarcoplasm, and a small amount of myofibrils.

The inside diameter of the myocardial arterioles in the white rat is 12.95 (9.49; 15.03) μm, the thickness of the arteriolar wall is 2.77 (2.45; 3.20) μm, the diameter of the capillaries is 4.34 (3.84; 5.41) μm.

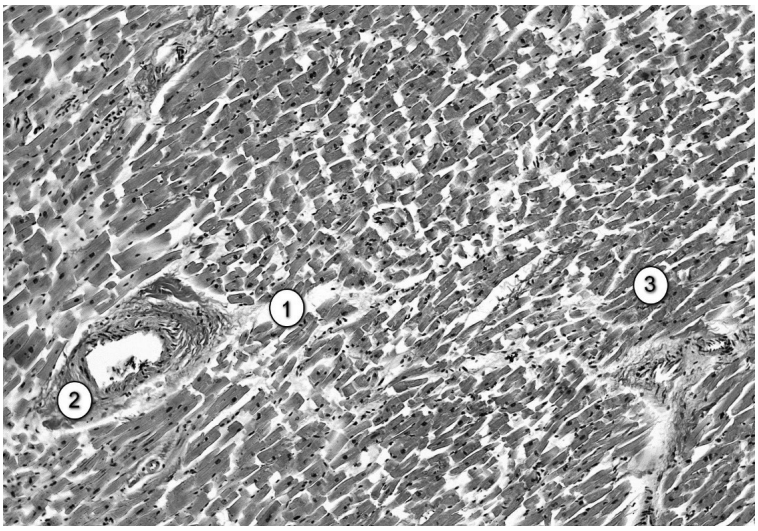

Fig. 1. Region of myocardium in intact rat. Micrograph. Fuchsilin and picrofuchsin staining. Magnification: × 100. Legend: 1 – layers of connective tissue; 2 – vessel; 3 – typical cardiomyocytes.

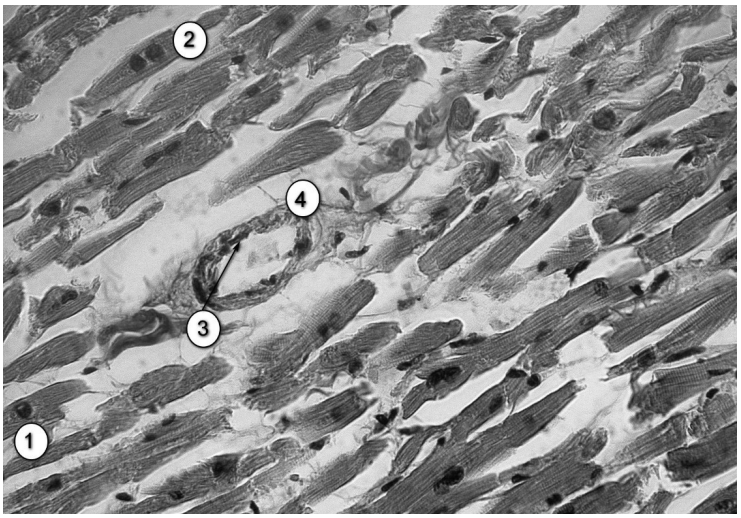

Fig. 2. Left ventricular region of myocardium in intact rat. Micrograph. Fuchsilin and picrofuchsin staining. Magnification: × 400. Legend: 1 – uninuclear cardiomyocyte; 2 – binuclear cardiomyocyte; 3 – nucleus of endotheliocyte; 4 – wall of arteriole.

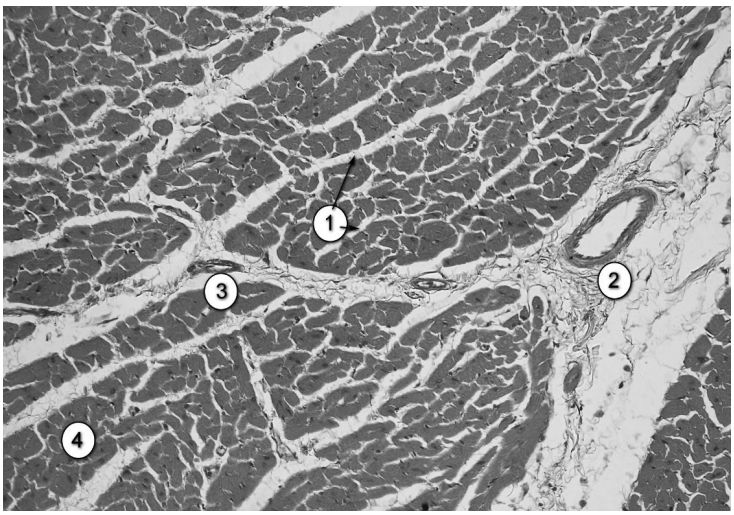

Fig. 3. Region of myocardium in intact rat. Micrograph. Hematoxylin and eosin staining. Magnification: × 200. Legend: 1 – layers of the connective tissue; 2 – arteriole; 3 – arteriolar capillary; 4 – cardiomyocytes.

Myofibrils on cross-section of muscular tissue are arranged in radial layers, the perinuclear spaces are free of fibrils, and are filled with sarcoplasm. Slightly stained nuclei located in the center of the cell were observed in the myocardial cells. Fibrocytes and capillaries were localized in a thin layer of the connective tissue between cardiomyocytes (Fig. 5).

In addition to contractile (typical) cardiomyocytes, we also distinguished another type of myocardial cells, conducting (atypical) cardiomyocytes, which formed cardiac

conducting system, and were divided into 3 types. The first type, P-cells (pacemaker cells) of small size, polygonal shape, had many pinocytotic vesicles and caveolae, they did not have a T-system, the sarcoplasmic reticulum was poorly developed. The second type, transitional cells are small elongated cells, smaller in diameter than typical contractile cardiomyocytes. Cells of the third type, the Purkinje fibers are large cells with peripheral location of myofibrils in the form of light cords, which contained fair amount of sarcoplasm, small amount of myofibrils, and much glycogen (Fig. 6).

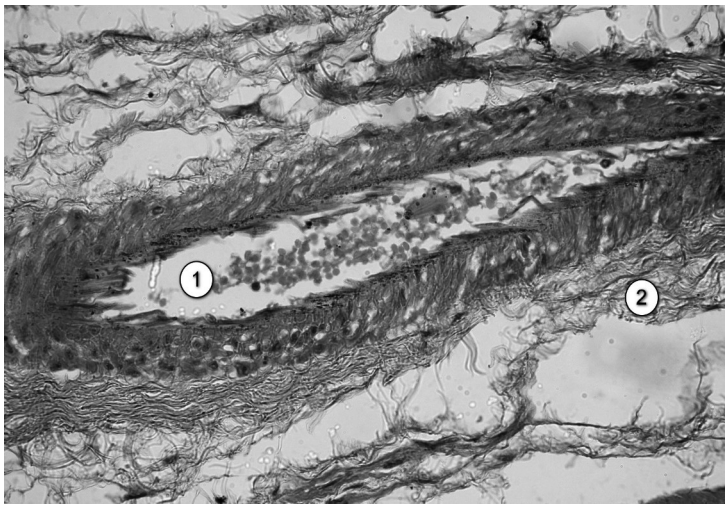

Fig. 4. Region of the myocardium in intact rat. Micrograph. Hematoxylin and eosin staining. Magnification: × 400. Legend: 1 – arterial lumen with blood corpuscles; 2 – interstitial connective tissue.

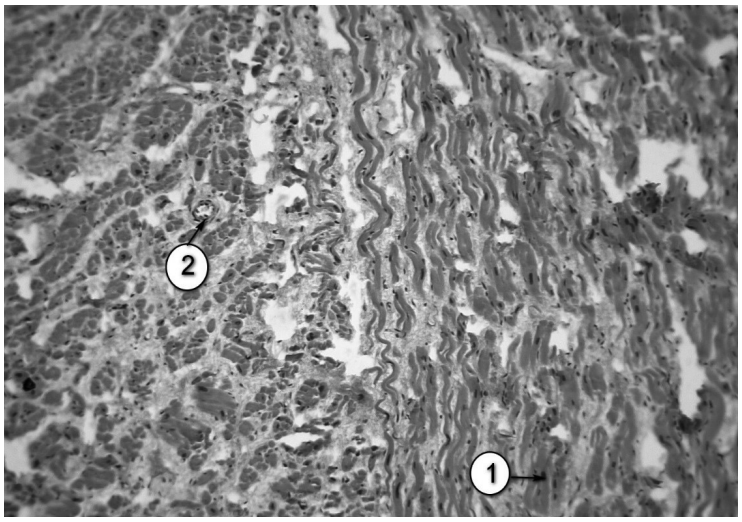

Fig. 5. Region of interatrial septum. Micrograph. Hematoxylin and eosin staining. Magnification: × 200. Legend: 1 – nucleus of cardiomyocyte; 2 – capillary.

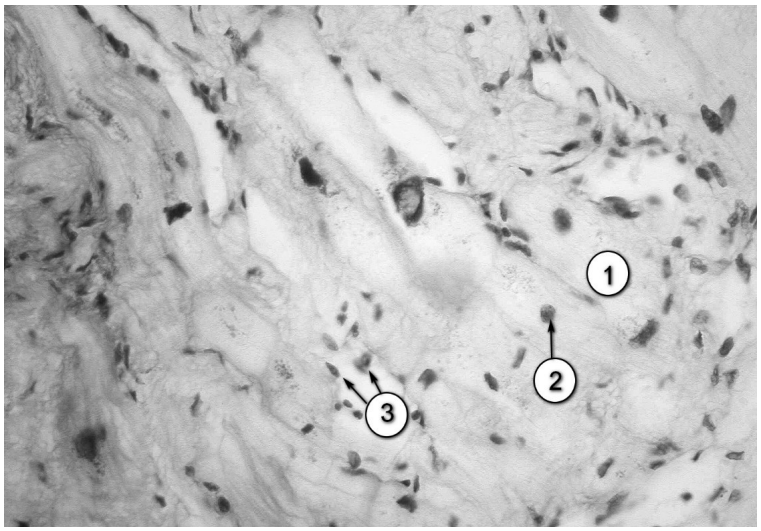

Fig. 6. Cells of A-V node. Micrograph. Azan saining. Magnification: × 400. Legend: 1 – atypical cardiomyocytes; 2 – nucleus of conducting cardiomyocyte; 3 – nuclei of fibroblasts.

Conclusions

1. Heart muscle of the white rat consists of branching and overlapping myofibers, forming a peculiar kind of a mesh.

2. Fissures between myofibers are filled with loose connective tissue with blood vessels and nerves.

3. The nuclei of cardiomyocytes are ovoid or spindle-shaped, and are usually located in the center of the cell.

4. Ventricular cardiomyocytes contain fair amount of sarcoplasm, and a small amount of myofibrils.

Directions for future research

Materials of our research will serve a morphological basis for further experimental studies.

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