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STRUCTURAL PECULIARITIES OF MYOCARDIUM IN RATS UNDER CONDITIONS OF IODINE DEFICIENCY AND OBESITY

Abstract. *The article represents the results of the study of the structural peculiarities of myocardium in rats under conditions of iodine deficiency and obesity in the experiment. Under the conditions of iodine deficiency there were observed: an increase of interstitium in the myocardium due to its edema, in the arterioles – swelling and vacuolation of the endothelial cell cytoplasm, in medial membrane of small arteries – small vacuoles. More significant changes in the structural organization of the myocardium were found in the rats that were on a high-calorie diet. Fragmentation of parenchymal cells of the heart is determined, where there could be observed small amount of vacuoles which are small, of round form and transparent. In the tiny arteries, the solitary focal lucent areas of the cytoplasm of the innercellular membrane are visualized due to the presence of small transparent vacuoles, as well as around solitary tiny arteries there is a slight expansion of connective tissue fibers.*
Key words: *iodine deficiency, obesity, cardiovascular system, myocardium*

Introduction. Lack of iodine in the environment for many countries of the world creates an significant medical and social problem. According to WHO experts, one third of the Earth's population is at risk of iodine deficiency (ID) Approximately one billion inhabitants of the planet have clinical manifestations of ID [1]. Deficiency of hormones of the thyroid gland in the body leads to a breakdown of carbohydrate, water-electrolyte, lipid, protein metabolism, causing morphofunctional and biochemical changes in various organs and systems. In particular, ID are associated with an increase in cardiovascular morbidity [2-3]. In spite of the successes in the prevention of cardiovascular events, cardiovascular diseases occupy a leading place among the causes of mortality in the industrialized world. An important factor in the development of cardiovascular disease is obesity, which is one of the most common metabolic diseases in the world [4]. In most countries, between 30% and 60% of adults have excess body weight. It is known that excess body weight reduces the standard of living, human capacity, complicates physical and intellectual activity, leads to premature aging, early disability, reduction in life expectancy [5].

The purpose of the study. Investigate the structural features of myocardium in rats under iodine deficiency and obesity in the experiment.

Material and methods. The study was conducted on 60 white adult mature rats weighing 150-180 g that were divided into two experimental groups: animals with iodine deficiency (1st experimental group, n = 20) and obese animals (2nd experimental group, n = 20). For comparison, similar studies were performed on intact animals (control group, n = 20). The condition of ID was reproduced by a two-month iodine-deficient diet [6]. To simulate obesity animals were kept on a high-calorie diet [7]. To assess the thyroid status of animals, the content of thyroid hormones in blood serum and urinary iodine concentration were determined. Control over the reconstruction of alimentary obesity was performed by calculating the body mass index (BMI). Histologic cuts of the heart were stained with hematoxylin and eosin, according to Shabadash (identification of glycogen), alcian blue according to Stidmen (determination of nonsulfated glycosaminoglycans), and also PAS-staining (verification of glycoproteins) was performed. Histological studies were carried out on the Leica DME light-optical microscope. In

order to objectivize the quantitative studies, computer morphometry of objects in histological preparations was performed. Morphometric analysis of the myocardium was performed taking into account the following parameters: the thickness of the cardiomyocyte (TC), the average perimeter of the cardiomyocyte nucleus (APCN), the average area of the cardiomyocyte nucleus (AACN), the parenchyma-interstitial index (PII) was calculated. Euthanasia of animals was carried out by decapitation under ketamine quenching (100 mg / kg body weight). The keeping, feeding and euthanasia of animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes (Strasbourg, 1986). The statistical analysis of the results was performed using Microsoft Excel and Statistica 5.5 computer programs.

Results of research and discussion. As a result of the study in animals with iodine deficiency there is a tendency to increase TC, APCN with a clear visualization of the borders of most muscular cells of the heart. The nuclei are mainly localized in the central part of the cells, somewhere in the paracentral. The shape of the nuclei is ovoid, somewhere round and spindle-shaped. The nuclei are mostly with a clear karyolemma, chromatin of nuclei is finely dispersed, moderately basophilic, uniformly distributed in the nucleoplasm. Some cells contain central and paracentral tiny, rounded nucleoli. Focal muscular cells are unevenly enlarged in diameter, have a wavy appearance, plasmolemma is veiled, the cytoplasm is unevenly stained with eosin, with moderate lucent areas. The nuclei of such parenchymal cells of the myocardium are slightly lumenized, some of them – are reduced in size, with a wavy karyolemma, and somewhere nuclei are not visualized due to karyolysis. There is an increase in interstitium due to its edema, which is evidenced by a sharp decrease in PII at 76% ($p<0.001$) in relation to control (Table 1). At the same time, the loose connective tissue of the interstitium is visualized by an lumenized, somewhere vacuolated, there are some macrophages and lymphocytes in a small number. When staining of histological sections with alcian blue according to Stidman in the extended interstitial zones the accumulation of nonsulfated glycosaminoglycans of greenish color is visualized, which is a reflection of deficiency of thyroid hormones on the basis of iodine deficiency with the development of mucinous edema (Fig. 1). An increase in the interstitial layers is observed, which is accompanied by a violation of the ordered structure of the muscular cells of the heart, which appear to be flaky in this case. Somewhere there is a fragmentation of cardiomyocytes into separate areas. In the arterioles of the myocardium, insignificant condensation of the chromatin of the endothelial cells nuclei is observed, in some of them – there are swelling and vacuolation of the endothelial cells cytoplasm, single macrophages and lymphocytes are periarteriolar. In the lumen of arterioles, in most cases, there are red cells located compact.

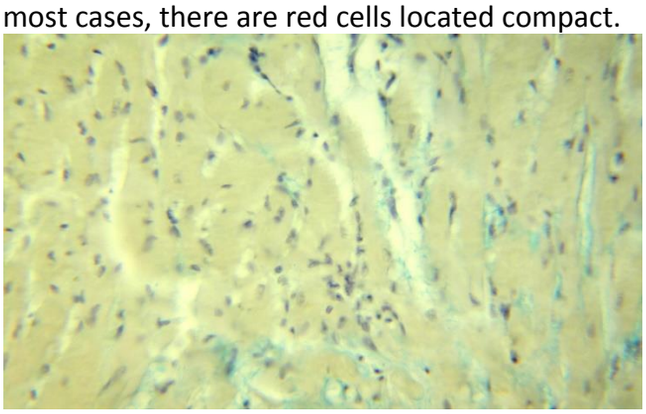

Fig. 1. Mucosal myocardial edema of a rat under the conditions of iodine deficiency. Staining: Alcian blue according to Stidman. Magnification: ×400

Tiny arteries with swelling and vacuolation of the endothelial cell cytoplasm, which bulges slightly into the lumen of the vessels. The nuclei are slightly hyperchromic, irregularly rounded. Other endothelial cells that do not undergo edematous phenomena, spindle-shaped, elongated along the wall of the nucleus. In the middle membrane of small arteries in the cytoplasm of smooth myocytes, there are small vacuoles in the form of small rounded lumenized areas (Fig. 2). The nuclei of such smooth myocytes are slightly hyperchromic, of irregular, ovoid form. Around the individual tiny arteries of the myocardium there are cellular infiltrates in a small amount, mainly represented by macrophages and lymphocytes. In the lumen of such small arteries, moderate amount of red blood cells is found. Small veins of the myocardium in the lumen contain somewhere compactly located erythrocytes. Endothelial cells are with clear spindle-shaped homogeneous nuclei elongated along the walls of vessels, insignificant amount of cytoplasm without signs of vacuolation, clasmatosis. The presence of focal vacuolization of

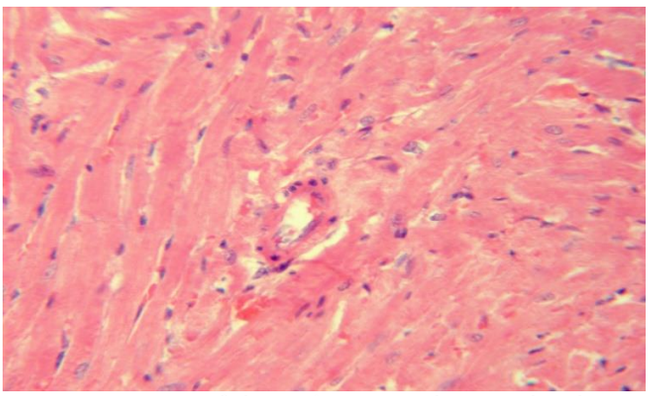

Fig. 2. Artery of the rat's myocardium under the conditions of iodine deficiency. Staining: hematoxylin and eosin. Magnification: ×400

these cells is determined in the middle membrane in the thickness of smooth myocytes.

More significant changes in the structural organization of the myocardium were found in rats that were on a high-calorie diet. In particular, a significant decrease in the thickness of the nucleus was observed (22%, $p<0.001$), somewhere uneven staining of cellular cytoplasm through enlightenment in some of them (see Table 1). In such cells, the cross-striation is veiled and slightly blurred in their contours. In some areas there is the fragmentation of parenchymal cells of the heart. Cardiomyocytes in this case are represented fragmented, the borders of cells are veiled. During staining according to Shabadash, it was determined that a small part of the cardiomyocytes in the cytoplasm contains a reduced amount of glycogen (Fig. 3). Focally are traced small, of round form, transparent, in a small number of vacuoles in the cytoplasm of parenchymal cells of the heart that do not displace the nuclei of cardiomyocytes and do not violate the integrity of cytomembrane. The nuclei of cardiomyocytes are of the ovoid form, located centrally with fine-grained chromatin. Their perimeter and area are smaller than in intact animals at 25 and 36%, respectively ($p<0.05$). The thickness of the parenchymal cells of the heart under the conditions of obesity is less at 22% ($p<0.05$) relative to the control data. These morphological changes may reflect biochemical changes in the organism in obesity, in particular, be due to the reduced allocation of adiponectin and leptin increased excretion. Under such conditions the loose connective tissue between the cardiomyocytes is expanded (parenchymatous-interstitial index is lower than that of intact animals at 74%, $p<0.001$), sometimes it contains a small amount of macrophages, lymphocytes, fibroblasts (see Table 1). The extended and full-blood hemocapillaries are visualized in the interstitium, around individual ones – there are single freely located leukocytes. A small amount of leukocytes is observed in the lumen of individual capillaries along with erythrocytes. Endothelial nuclei are elongated, homogeneous. In the small arteries the solitary focal lumenized areas of the cytoplasm of cells of the inner lining are visualized due to the presence of small transparent vacuoles, accompanied by a slight protrusion of the cytoplasm to the capillary lumen (Fig. 4). The nuclei of such endothelial cells retain an elongated character. Around the solitary small arteries there is a slight growth of connective tissue fibers (Fig. 5).

As a result of the comparative analysis, in obese animals, the decrease of the thickness of cardiomyocytes (34%, $p<0.05$), perimeter and area of nuclei (at 29 and 39%, respectively, $p<0.001$, $p<0.05$) regarding the analogous indeces in rats that were on the iodine-deficient diet. Such data emphasize the important role of obesity in

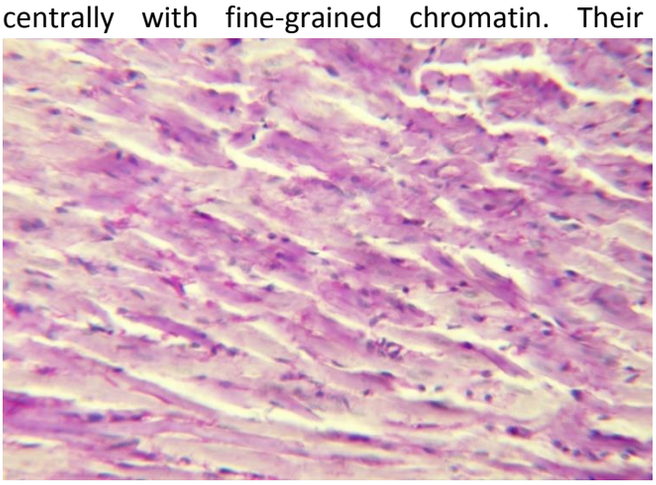

Fig. 3. Glycogen in cytoplasm of rat's cardiomyocytes under obesity conditions. Staining: according to Shabadash. Magnification: ×400.

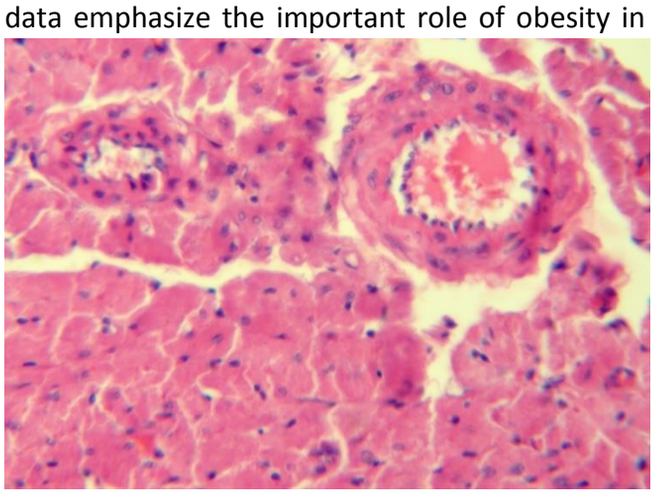

Fig. 4. Arteries of the rat's myocardium in obese conditions. Staining: hematoxylin and eosin. Magnification: ×400

the development of cardiac pathology, which can potentiate in the regions of goiter endemic areas.

Conclusions. Iodine deficiency and high calorie diet accompanied by the

development of structural changes of the myocardium, which significantly increases cardiovascular risk under such conditions.

Prospects for further studies. Relevant topics for further study can be metabolic processes in the myocardium, the dynamics and reversibility of the revealed structural changes.

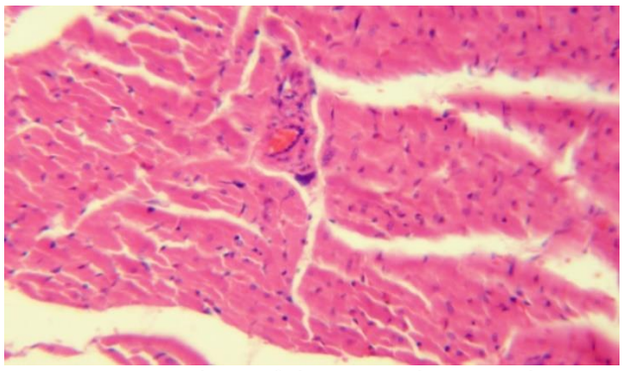

Fig. 5. Myocardium of the rat in obese conditions. Staining: hematoxylin and eosin. Magnification: ×400

Table 1

Changes in the morphometric indices of rats' myocardium against the background of iodine deficiency and obesity (M±m)

Animal groups	Thickness of cardiomyocyte, µm	Perimeter of the nucleus, µm	Area of the nucleus, µm^2	Parenchyma-interstitial index, relative units
Control group (n=20)	14.96±1.19	40.55±3.31	88.93±11.24	45.15±2.11
1st research group (Iodine deficiency, n=20)	17.57±2.47	42.61±1.97	93.69±11.12	10.74±1.03$^{\#}$ $P_{к-1}$<0.001
2nd research group (Obesity, n=20)	11.65±1.05* p_{1-2}<0.05	30.15±3.50* p_{1-2}<0.01	57.05±10.11* p_{1-2}<0.05	11.90±1.15$^{\#}$

*Note. Reliable difference regarding control * - p<0.05, # - p_{1-2} <0.001; p with Arabic numerals – significant changes between indices in experimental groups.*

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