MORPHOLOGICAL CHANGE OF MYOCARDIUM IN HYPOTHYROIDISM

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ABSTRACT

Introduction. The work covers data on the most common forms of functional disorders of the thyroid gland – hypothyroidism and a laboratory research on myocardium, performed on baby rat’s hearts to study dystrophic changes observed in a state of hypothyroidism. The aim of the research is to reveal and analyze dystrophic changes observed in a state of hypothyroidism, which developed as a result of a long-term persistent deficiency of thyroid hormones or decreasing their biological effect on the cellular level. Material and methods. The object of the study was the hearts of 50 white outbred rats of the following age groups: 3, 7, 14, 21, 30 days. Animals were divided into 3 groups. After every experimental week, the hormone level from the rat caudate vein was determined. The control and experimental groups of animals were kept in the same vivarium conditions. At the end of the experiment, the baby rats of the experimental and control groups were killed under anesthesia, the heart was kept in 10% neutral formalin, followed by piping in alcohols, pouring in paraffin and preparing histological sections. Sections of 8-10 microns thick were prepared from
paraffin blocks. Microsections were stained with hematoxylin and eosin, van Gieson. **Results and discussion.** The study process of growth dynamics of the wall thickness of both the left and right ventricles of baby rats’ heart in hypothyroidism state, depending on different parts of the heart, showed that the wall thickness of all departments was less than the control indices. Comparing the thicknesses of wall of the right and left ventricles of the baby rats of the experimental group with the control group, a significant lag in myocardial indices was revealed. Those changes were most pronounced in 14-day-old baby rats, manifested by perivascular and interstitial lymphohistiocytic infiltrate. **Conclusion.** The intensity and prevalence of morphological changes are less pronounced, destructive changes in the myocardium are not detected. The use of antioxidants in analysis of experimental hypothyroidism on laboratory animals has a protective effect and prevents the development of severe destructive changes in the myocardium.

**Key words:** heart, myocardium, hypothyroidism, myocytolysis, tocopherol acetate.

**Introduction.** One of the most common forms of functional disorders of the thyroid gland is hypothyroidism, which develops as a result of a long-term persistent deficiency of thyroid hormones or decreasing their biological effect on the cellular level. Hypothyroidism may not be detected for a long time. This is due to the gradual, inconspicuous start of the process, satisfactory well-being of patients in mild to moderate degrees of illness, erased symptoms, regarded as overwork, depression, pregnancy.

Increasing of thyroid hormones causes systemic changes in the body. Thyroid hormones regulate energy metabolism in the cells of organs, and their deficiency is manifested in a decrease in tissue oxygen consumption, a decrease in energy expenditure and the processing of energy substrates [7,8,12]. With hypothyroidism, the synthesis of various energy-dependent cellular enzymes necessary for the normal functioning of the cell is disrupted. In the case of advanced hypothyroidism, mucinous (mucous) edema occurs - myxedema, most pronounced in connective tissue. Myxedema develops as a result of excessive accumulation of glycosaminoglycans in the tissues, which, having increased hydrophilicity, retain water.

The most frequent and serious complication of both thyrotoxicosis and hypothyroidism is lesion of the cardiovascular system, so this problem is not only endocrinological, but also cardiological [5,6,10]. Heart pathology with thyroid dysfunction is often the leading disease in the clinic and often leads to disability, and in severe cases, to death in this category of patients. The clinical picture shows a variety of complex cardiac arrhythmias, arterial hypertension, dishormonal cardiomyopathy with the development of chronic heart failure [1,2]. Meanwhile, data on the pathogenetic mechanisms of the development of “thyrotoxic” and “hypothyroid” hearts are scarce and contradictory [3,4]. An analysis of recent data shows that cardiovascular pathology remains the main cause of death in all developed countries [13,14]. The heart is most often exposed to the pathological
effects of endogenous and exogenous stimuli, as a result of which not only a violation of its function occurs, but also various pathological processes develop throughout the body [9,11]. It is known that heart dysfunction is a very complex, poorly predictable and difficult to recognize based on clinical symptoms, medical and physiological process. Lesion of any organ, particularly a heart, naturally begins with the lesion of blood vessels and connective tissue stroma of the organ. In the subsequent lesions, the actual muscle tissue of the heart is exposed. Therefore, with heart lesions, the main quantitative and qualitative changes occur both in the stroma - vascular, and in the muscle structures of the heart. Structural changes are necessarily accompanied by clinical symptoms in the form of impaired cardiac activity. The aim of our work was to identify structural and morphological changes in the walls of various parts of baby rats’ heart in a state of hypothyroidism and the use of the antioxidant tocopherol acetate.

Material and methods. The object of the study was the hearts of 50 white outbred rats of the following age groups: 3, 7, 14, 21, 30 days. Animals were divided into 3 groups. The first group of animals consisted of female rats receiving mercazolil at a dose of 5mg per 100g of body weight for 30 days, then for the month before pregnancy, a maintenance dose of mercazolil was used at a rate of 2.5mg per 100g. After pregnancy and during feeding, rats were injected with mercazolil in a maintenance dose of 2.5mg per 100g through a probe. As a solvent for mercazolil 1% starch paste was used. The second group of the research consisted of female rats who were injected with mercazolil the same days of the experiment as in the first group, but after pregnancy they received tocopherol acetate at a dose of 0.5mg per 100g of weight together with mercazolil. The third group included female baby rats from the mother of the control group, which, after birth, were daily fed on an empty stomach, depending on the term, in the amount of 1 ml distilled water. After each experimental week, the hormone level from the rat caudate vein was determined.

The control and experimental groups of animals were kept in the same vivarium conditions. At the end of the research, the baby rats of the experimental and control groups were killed under ether anesthesia. After that, the heart was taken from animals, fixed in 10% neutral formalin, followed by piping in alcohols, pouring in paraffin and preparing histological sections. Sections of 8-10 microns thick were prepared from paraffin blocks. Microsections were stained with hematoxylin and eosin, van Gieson.

Results and discussion. The ventricular endocardium of the control group of animals consisted of longitudinally directed bundles of collagen fibers. A histological section reveals places where longitudinally lying bundles of collagen fibers interwoven with each other. Bundles of collagen fibers located closer to the ventricular myocardium are intertwined with bundles of collagen fibers from connective tissue layers located between bundles of cardiomyocytes of the myocardial inner layer (Fig. 1). The bundles of elastic fibers of the ventricular endocardium lie loosely compared to bundles of collagen fibers. In bundles of fibers adjacent to the myocardium of the ventricles, the density of arrangement
increases, the direction of which changes from longitudinal to oblique, and are interwoven with bundles of connective tissue located between bundles of cardiomyocytes of the inner layer of the myocardium. Reticular fibers in the endocardium of the ventricles are located close to each other.

Upon further study of the slice, the cardiomyocytes of the outer layer of the myocardium are directed longitudinally, in the middle layer circular directed beams are detected; the inner layer contains weakly obliquely oriented bundles of cardiomyocytes. The inner bundles of fibers as they approach the endocardium acquire a more oblique direction and pass into the papillary muscles. A study of the direction of the bundles of ventricular myocardial fibers showed that the circularly directed layer does not always have a clear orientation. The bundles of fibers of the middle layer on the lower wall of the ventricles are directed obliquely and deviate towards the endocardium. Between the layers of ventricular myocardial cardiomyocytes, the border is weakly expressed. They fit snugly together. The inner layer of the myocardium consists of parallel located bundles of cardiomyocytes running in parallel with the endocardium. The outer layer of the myocardium has a loose structure in which cardiomyocytes are located in different directions (Fig. 2).

In the middle layer of the myocardium of the left ventricle of the heart, bundles of cardiomyocytes are located perpendicular to the inner layer. In the center of cardiomyocytes, 1-2 oval-shaped nuclei are determined. The nucleus of cardiomyocytes is located in the center of the cell, while myofibrils are located on the periphery. In the right ventricle, bundles of cardiomyocytes in the layers of the myocardium are similar to the left ventricular myocardium. But unlike the left ventricle in the myocardium of the right ventricle, the thickness of the circular layer of cardiomyocytes is 2-3 times thinner than the thickness of the longitudinal layers of cardiomyocytes.

In the myocardium of the heart ventricles, depending on the site, bundles of collagen fibers have different directions. At the top of the heart, the bundles of collagen fibers are directed obliquely, while part of the bundles of collagen fibers changes direction from oblique to longitudinal. In the inner layer of the myocardium, bundles of collagen fibers lie in the longitudinal direction, separating bundles of cardiomyocytes from each other. In the middle layer of the myocardium, collagen fibers form bundles having a circular direction. When studying the outer layer of the myocardium, bundles of collagen fibers lie obliquely between bundles of cardiomyocytes. In the inner layer, the reticular fibers lie longitudinally and in the region of the apex of the heart they are intertwined with the reticular fibers from the outer layer of the myocardium of the heart. In the middle layer of the myocardium, the reticular fibers are located in a circular direction between the bundles of cardiomyocytes. Reticular fibers around bundles of cardiomyocytes form a network of various sizes and shapes (Fig. 3.). The direction of the bundles of connective fibers depends on the direction of the cardiomyocytes.
Figure 1. The wall of the left ventricle of the heart of 14 days old rat in the control group. Collagenic fibers of the endocardium. Coloring: according to van Gieson. Vis.: approx. 10, vol. 40.

Figure 2. The side wall of the right ventricle of 21 days old rate of the control group. Coloring: hematoxylin and eosin. Vis.: approx. 10, vol. 20.

The interventricular septum consists of two longitudinal and one circular layer. The longitudinal layers on the left and on the right are from the corresponding longitudinal layers of both ventricles, and the middle circular layer is formed due to the circular layer of the left ventricle of the heart. The internal diameter of the arteriole ranges from 9.5 to 15.2 microns and an average of 11.7 ± 0.6 microns. The inner diameter of the venules ranges from 15.7 to 20.5 microns. Myocardial sinusoids are elongated, oval or irregular in shape. The thickness of the inner diameter of the capillaries is from 5.7 to 11.4 microns, an average of 9.3 ± 0.6. Myocardial blood vessels are direct along the bundles of cardiomyocytes (Fig. 4). Around the cardiomyocytes and blood vessels are bundles of collagen and elastic fibers. The fibrous structure of the connective tissue of cardiomyocytes connecting with the blood vessels captures the capillaries around the muscle fibers.

In the epicardium of the ventricles of the heart, bundles of collagen and elastic fibers lie longitudinally, and have a higher density than bundles of collagen and elastic fibers of the endocardium. In the epicardium of the ventricles, the reticular fibers are located longitudinally.
Figure 3. The front wall of the left ventricle of the heart of 14 days old rats in the control group. 1. Increased content of endocardial reticular fibers. 2. Reticular myocardial fibers. Coloring: imputation according to Foot in the modification of Yurina. Vis.: approx. 10, vol. 40.

Figure 4. Vessels of the posterior wall of the left ventricle of 21 days old rats in the control group of animals of the subepicardial layer. Coloring: hematoxylin and eosin. Vis.: approx. 10, vol. 40.

Indicators of the thickness of the lateral wall layers of the ventricles in the rat heart, in microns

<table>
<thead>
<tr>
<th>Days</th>
<th>Ventricles</th>
<th>Control</th>
<th>1st group of research</th>
<th>2nd group of research</th>
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<td>3</td>
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<td>1149±33</td>
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<td>470±13***</td>
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<td>R, n=8</td>
<td>610±18,1**</td>
<td>402±51,1**</td>
<td>540±14,9**</td>
</tr>
</tbody>
</table>

The histological picture of 3-day-old rats undergoing hypothyroidism did not reveal significant differences compared with the control group. Cardiomyocytes had an oblong shape, formed muscle fibers, in the center of the fiber an oval-shaped nucleus was determined and myofibrils were clearly differentiated. In the subepicardial zone of the myocardium, dilated and full-blooded veins were detected with signs of redistribution of blood by the presence of clotted red blood cells, and in the myocardial stroma, beginning edema was observed. The endocardium is also somewhat thickened and uneven, in some places foci of depression and deepening in the form of cracks and vessels of Tebesia are visible. Endothelial cells are somewhat hypertrophied and hyperchromic, sometimes with foci of enlightenment in the basement membrane.

A morphological study of 7-days-old baby rat’s heart in a state of hypothyroidism, with minor changes in the form of expansion of visible vessels (Fig. 5). An increase in the permeability of microvessels and vessels of the venous link was accompanied by the release of the liquid part of the blood through the vessel wall to the surrounding connective tissue (Fig. 6). Due to inflammatory infiltrate, the vascular wall thickens and the lumen narrows. These discirculatory disorders lead to perivascular edema, loosening of the intramuscular space. At the
same time, cardiomyocytes are loosened with the development of dystrophy and vacuolization in their cytoplasm. Myofibrils have a granular appearance, of different thickness and density. During this period, the epicardium of the heart is significantly thickened due to the proliferation of young connective tissue cells and swelling of fibrous structures. The endocardium is also swollen, in places forms uneven thickenings from proliferative endothelial cells.

Figure 5. Anemia of the pancreatic vessels of the heart and blood supply. Hematoxylin and eosin staining. Vis.: vol. 20, approx. 10.

Figure 6. Pancreatic tissue of 7 days old baby rats with blood and vasodilation. Painting according to Van Gieson, Vis.: vol. 10, approx. 10.

At the age of 14 days, destructive and inflammatory phenomena joined the above-described discirculatory and dystrophic changes. In the myocardial stroma, increased edema was noted mainly in the perivenular and pericapillary spaces. Collagen fibers were swollen, loosened, in some places there is a separation of collagen bundles, edema of the main substance of the connective tissue with the initial signs of surface disorganization. Connective tissue cells were also swollen; their nuclei slightly increased in size. Vascular disorders were common, endothelial cells in the vessels, acquired a rounded shape. In the cytoplasm of cardiomyocytes, small vacuoles were found filled with a clear cytoplasmic fluid, i.e. hydropic dystrophy developed. Intracellular edema was focal in nature, along with dystrophically altered cardiomyocytes, unaffected cells were found. In the myocardium of the left ventricle of the heart, around the vessels and in places in the stroma, inflammatory foci consisting of lymphohistiocytic cells appeared (Fig. 7). In this case, cardiomyocytes were loosened with the development of dystrophy and vacuolization in their cytoplasm. Myofibrils had a granular appearance, different thickness and density. The endocardium was also swollen, in places formed an uneven thickening of proliferative endothelial cells. At that time, the epicardium of the heart was significantly thickened due to the proliferation of...
young connective tissue cells and edema of fibrous structures. A histochemical study revealed a significant decrease in the number of collagen structures in the composition of the epicardium, instead of them, the content of SHIK-positive substance increases, especially in the walls of blood vessels. Endothelial cells were somewhat hypertrophied, hyperchromic, sometimes with foci of enlightenment in the basement membrane.

The study process of growth dynamics of the wall thickness of both the left and right ventricles of the heart of baby rats in hypothyroidism state, depending on different parts of the heart, showed that the wall thickness of all departments was less than control indicators. Comparing the thicknesses of wall of the right and left ventricles of the animals of the experimental group with the control group, a significant lag in myocardial indices was revealed. Those changes were most pronounced in 14 days old baby rats. The thickness of the left ventricular myocardium was less than that of the control group by 29%. The thickness of the right ventricle compared with the control group was less by 17% (Table 1).

On the 21st day, vascular disorders persisted in the myocardium: pronounced plethora of veins, stasis, numerous perivascular hemorrhages of diapedetic nature and increase in edema intensity was noted. Edematous fluid was located between the muscle fibers, as if pushing them apart. Edema of not only connective tissue cells, but also their processes was revealed. The edema processes of neighboring fibroblasts were in contact with each other and formed syncytium in the myocardial stroma, numerous small infiltrates from lymphocytes, histiocytes and fibroblasts. In cardiomyocytes, signs of protein hydropic dystrophy with the development of intracellular edema were observed; numerous foci of plasmolysis throughout the myocardium were determined. Plots of plasmolysis (intracellular myocytolysis) looked like optically empty spaces. With intracellular myocytolysis, dissolution of myofibrils in certain sections of the fiber along its length was noted. In adjacent areas, myofibrils were identified. By the method of silvering according to Foote, the formation of reticular fibers in the form of coarse clumps and intermittent thick dark brown fibrous structures was determined. A histochemical study using the Van Gieson method revealed loosening and reduction in the number of collagen structures, especially in the epicardium, and increase in the content of SHIK-positive substance. Elastic fibers of the endocardium and intramuscular stroma were in a state of loosening and rupture due to edema and lesion of the connective tissue.

The study process of the dynamics of growth of wall thickness of both the left and right ventricles of the heart of baby rats in a state of hypothyroidism with the use of tocopherol acetate depending on different parts of the heart showed that the wall thickness of all departments was less than by the control indices. Comparing the wall thicknesses of the right and left ventricles of the animals of the experimental group with the control, a significant lag in myocardial indices was determined. These changes were most pronounced in 21 days old baby rats. The thickness of the left ventricular myocardium was less than that of the control group.
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After 30 days, interstitial edema intensified and spread to the entire myocardium, reaching the greatest value in the perivenular spaces. Due to edema, swelling of collagen fibers, their delamination and shedding were observed. The main substance was swollen and collapsed, signs of connective tissue lesion appeared (Fig. 8). When stained with toluidine blue, the phenomenon of metachromasia was observed. Dystrophic changes in the myocardium acquired a diffuse character, increased resorption of the cytoplasm, intracellular edema was noted. The foci of plasmolysis were numerous and larger than on the 21st day. Intracellular myocytolysis extended to the entire myocardium; total lesion of cardiomyocytes was noted. In the focus of myocytolysis, most myofibrils were absent, single fibrils were detectable. At the same time, marked loosening of the subendocardial and intramural layers of the heart wall due to edema and dissociation of cardiomyocytes was noted.

The study process of the growth dynamics of thickness of wall of both left and right ventricles of the heart of baby rats at different stages of postnatal ontogenesis depending on the parts of the heart walls showed that by the 30th day of the current research, comparing to 3 days old; thickening occurred in average in all parts of the heart 1.7 times, only in the interventricular septum - 1.5 times. Those morphometric data were confirmed by microscopic rearrangements occurring in the dynamics of postnatal ontogenesis (Table 1).

The subepicardial layer of the myocardium remained dense, where cardiomyocytes formed large muscle bundles. Inflammatory infiltrate and sclerosis were defined around the vessels. When staining according to the Weigert method, it was noted that in the walls of the arteries the inner elastic membrane was thickened, winding, sometimes homogenized. In the muscle layer and in the surrounding connective tissue, elastic fibers were fibrous and lysed.

**Figure 7.** LV wall of a 14-day-old experimental group baby rat. Lymphohistiocytic infiltration around the vessels. Hematoxylin and eosin staining. Vis.: vol.20, approx. 10

**Figure 8.** Myocardial tissue with slight sclerosis of the capillaries. The staining of the vessel wall is densely red. Coloring according to Van Gieson, Vis.: vol. 40, approx. 10
In cardiomyocytes with small foci of myocytolysis, the nuclei were preserved. In cardiomyocytes with large foci of myocytolysis, the nuclei were destroyed with the development of collicational necrosis. Remains of fibers in the form of tubules surrounded by a sarcolemma were found in foci of collication necrosis; the contents were not stained. Sarcolemma was thickened and well stained with picrofuxin. The described tubules were collapsed, around them there were excessive deposits of glucosaminglycans.

In some areas, counter-abnormalities and small foci of coagulation were determined, fragmentation of muscle fibers was observed in some places. However, collision processes dominated in the myocardium.

Since metabolic imbalance primarily affects the energy supply system of cells, the therapy should be aimed at increasing energy generation and increasing myocardial resistance to hypoxia. The second experimental group included 26 rats, which were daily injected with mercazolyl and antioxidant α-tocopherol.

In 3 days old baby rats receiving hypothyroidism and tocopherol acetate, the normal histological picture was determined in the rat myocardium, pathohistological changes in the myocardium were not identified.

In 7 days old rats vascular disorders in the form of plethora and expansion of veins, stasis, plasmorrhagia were noted (Fig. 5). Vascular disorders persisted, there was increase in plasmorrhagia, plasma impregnation and fibrinoid edema of the arteries walls.

After 14 days from the research start, against the background of tocopherol using in the myocardial stroma, a small edema was found mainly around the veins. In individual cardiomyocytes, intracellular edema was identified in the form of accumulations in the cytoplasm of vacuoles filled with tissue fluid.

Intracellular edema was focal in nature. In the myocardial stroma, vascular disorders persisted in the form of plethora, stasis and venous stasis. When staining with toluidine blue, metachromasia was not found, fibrinoid edema of the artery walls was observed.

After 21 days, edema was determined in some places in the myocardial stroma, which was focal in nature and had a lower intensity than in the first experimental group. In areas of edema, collagen fibers were swollen and homogenized. There was a superficial disorganization of connective tissue with the accumulation of glycosaminoglycans. On histological preparations, mucoid edema was observed (Fig. 9).

In places in the cytoplasm of cardiomyocytes, hydropic dystrophy was observed, which was focal in nature. The transverse striation of muscle fibers was preserved, in some areas myofibrils were swollen. Vascular disorders became less intense.

After 30 days, against the background of daily use of the antioxidant tocopherol, partial relief of vascular disorders was noted. In the myocardial stroma, edema developed, which extended to almost the entire myocardium, however, the intensity of the edema was less pronounced compared with the experimental group I. In the connective tissue of the myocardial stroma, swelling and decomposition of
the main substance, accumulation of glucosaminglycans, edema, homogenization and partial decomposition of collagen fibers with the formation of a fibrinoid were observed. At this time, for the first time in the stroma, small focal infiltrates from lymphocytes, histiocytes, and fibroblasts were detected (Fig. 10).

**Figure 9.** The cytoplasm of some cardiomyocytes is unevenly colored, sarcolemma is clearly defined. In the sarcoplasm, transverse bands are visible. Hematoxylin and eosin staining. Vis.: vol. 20, approx. 10

**Figure 10.** Wall of the right heart core of a 30-day-old baby rat with hypothyroidism. Myocardial hypercellularity is preserved. Hematoxylin and eosin staining. Vis.: vol. 20, approx. 10

At the indicated times, a picture of intracellular edema was observed in the myocardium and for the first time against the background of tocopherol use, a little foci of plasmolysis (myocytolysis) appeared. Those foci were optically empty portions of the cytoplasm with complete preservation of the plasmolemma. Compared with the experimental group I, the processes of myocytolysis were focal in nature.

Thus, with the daily use of the antioxidant a-tocopherol in baby rats of the experimental group II, no histological changes in the myocardium were identified in the first 7 days. The first signs of myocardial lesion (beginning of periventricular edema of single cardiomyocytes) appeared on the 14th day, that was, 7 days later than in the 1st experimental group. An expanded picture of a change in the myocardium such as diffuse intracellular edema, myocytolysis was determined on the 28th day, then later it was compared with the 1st experimental group. Destructive processes with total lesion of the myocardial fibers in the 2nd experimental group of baby rats were not identified. Regenerative processes with the replacement of damaged myocardial cells with connective tissue proceeded more intensively in comparison with the 1st experimental group I. The above-mentioned data prove that a-tocopherol has a protective effect on the myocardium in conditions of hypothyroidism.
Conclusion
1. In case of transient experimental hypothyroidism, the application of mercazolil on baby rats in the ventricular myocardium resulted to dystrophic, destructive and atrophic changes in cardiomyocytes, diffuse edema and stromal fibrosis. The first signs of hypothyroidism were identified on 7-14 days; detailed picture developed on the 21st day.
2. When using an antioxidant in the myocardium of experimental animals, signs of hypothyroidism were revealed on the 21st day, and detailed picture on the 30th day.
3. The intensity and prevalence of morphological changes were less pronounced, destructive changes in the myocardium were not determined. The use of antioxidants in experimental hypothyroidism on laboratory baby rats has a protective effect and prevents the development of severe destructive changes in the myocardium.

References