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Influence of iodine deficiency state and influence of strumogenic products on organs of the cardiovascular system

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Abstract. Currently, iodine deficiency diseases are a global medical and social problem: according to the WHO, various health disorders caused by iodine deficiency rank third among the 38 most common non-infectious diseases. The frequency of damage to the organs of the cardiovascular system and the prevalence of iodine deficiency is a prerequisite for studying the effect of thyroid imbalance on the heart and blood vessels. The goal was to follow the morphofunctional changes in the endocardium and the wall of arteries of different types in the dynamics of iodine deficiency development in postnatal ontogeny. The work was performed on experimental animals of sexually immature and sexually mature age. Achieving a state of iodine deficiency is confirmed by ioduria indicators and a thyroid profile. Both on the 60th day and on the 90th day of the iodine deficiency state, edematous changes occur in the connective tissue components, in the wall of blood vessels, especially in the endothelial layer, in all the examined organs, which was confirmed by light-optical and electron microscopic research methods. Thus, the available data allow us to consider iodine deficiency as a systemic factor that can affect the cardiovascular system due to thyroid dysfunction. The combined intake of current-generating substances can increase this effect, especially if iodine deficiency has already formed.

Keywords: experiment; rats; heart; arteries; metabolic disorders; morphological changes; microscopy; ontogenesis.

INTRODUCTION

The structure of endocrine disease is characterized by a significant spread of thyroid pathology, namely, about 47% is precisely thyroid pathology, which continues to grow [1, 2].

An important reason for the growth of thyroid pathology is a decrease in the functional activity of the thyroid gland due to the influence of a number of environmental factors [3, 4]. Although the morphofunctional state of the thyroid gland is the resulting consequence of the direct or indirect action of various natural, environmental, climatic, social and other factors, the main factor of damage is the imbalance of biometals, the leading place in which belongs to iodine deficiency [5, 6]. Thyroid hormones, the component of which is iodine, regulate energy homeostasis, and even a slight decrease in their level is a risk factor for cardiovascular diseases [7–9]. Under conditions of iodine deficiency, cardiovascular dysfunction develops asymptomatically [10].

Adequate synthesis of thyroid hormones is a critically important component for maintaining the health of the cardiovascular system, since the synthesis of proteins and enzymes necessary for the functioning of the endocardium depends on a sufficient amount of iodine, which emphasizes the need for dietary corrections in case of its deficiency [11].

The territories of Ukraine are characterized by a mosaic combination of biogeochemical localities with reduced iodine content in environmental objects and areas with anthropogenic and man-made pollution of the environment with heavy metal ions, substances

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with thyroid-disruptive and radioactive properties.All these factors have the ability to affect various links of the hypothalamic-pituitary-thyroid axis, which can disrupt the synthesis, secretion, and transportation of thyroid hormones, distort their local effect on target cells, and cause functional and organic changes in the gland with subsequent disorders of the entire organism [12, 13, 14].

Taking into account the prevalence of iodine deficiency and consumption of stromogens and fragmentary data on their effects on the endocardium and arteries of various types, there was a need for their comprehensive study.

The aim of the study: to follow the morphofunctional changes in the endocardium, the wall of arteries of various types in the dynamics of the development of iodine deficiency and iodine deficiency combined with the consumption of stromogens in postnatal ontogenesis.

MATERIALS AND METHODS

The experiment was performed on 78 white outbred male rats (39 animals each of sexually immature (3–5 months) and sexually mature (6–8 months) age). 11 animals of both age groups made up 1 group (control), 14 animals each entered 2 and 3 experimental groups with simulated iodine deficiency and material intake on 60 and 90 days of the experiment, respectively. The 4th and 5th research groups consisted of animals of both age groups, which were simulated combined iodine deficiency with the consumption of stromogens [15]. All manipulations were carried out in strict compliance with the rules of humane treatment of animals.

The collection of material for light-optical and electron microscopic studies was carried out on the 60th and 90th day of the experiment. Tissues of the endocardium, aorta, external carotid, and renal arteries were studied. A morphometric, biochemical study with statistical data processing was also conducted [16].

RESULTS AND DISCUSSION

Thyroid status of sexually immature animals of group 1: TSH (0.10±0.01) μIU/ml ($p<0.01$), Tz (3.63±0.12) nmol/l ($p<0.01$), T4 (75.44±4.01) nmol/l ($p<0.01$); in sexually mature individuals – (0.08±0.00) μIU/ml ($p<0.01$), (2.79±0.15) nmol/l ($p<0.01$), (55.18±2.72) nmol/l ($p<0.01$), respectively. Cholesterol content under age-standard conditions is: in sexually immature rats (1.60±0.05) mmol/l ($p<0.01$), in sexually mature rats – (1.40±0.09) mmol/l ($p<0.01$). Ioduria in sexually immature animals of group 1 is (97.13±5.40) μg/l, in sexually mature animals – (101.06±3.44) μg/l ($p<0.01$).Thyroid status of sexually immature animals of 2 groups: TSH (0.17±0.02) μIU/ml ($p<0.01$), Tz (3.46±0.36) nmol/l ($p<0.01$), T4 (75.49±9.60) nmol/l ($p<0.01$); in sexually mature – (0.12±0.01) μIU/ml ($p<0.01$), (2.97±0.37) nmol/l ($p<0.01$), (67.49±8.25) nmol/l ($p<0.01$), respectively. Cholesterol content on the 60th day of the experiment is: in sexually immature rats (1.63±0.17) mmol/l ($p<0.01$), in sexually mature rats – (1.39±0.12) mmol/l ($p<0.01$). Ioduria in sexually immature animals of group 2 is (2.73±0.29) μg/l, in sexually mature animals – (3.77±0.34) μg/l ($p<0.01$).

In light microscopic examination on the 60th day of iodine deficiency, the endocardium has the appearance of a thin eosinophilic line lining the myocardium, with well-contoured oval-shaped nuclei in the cytoplasm of endothelial cells, in some loci they are rounded. Endotheliocyte nuclei are not visualized in all fields of view, they have different intensity of basophilia. The cytoplasm of endothelial cells is weakly eosinophilic, with indistinct boundaries.

Submicroscopically, general membrane organelles are found in the cytoplasm of endotheliocytes. The lumenal surface of the plasmolemma of cells forms a relief contour, forming numerous protrusions. The amorphous substance of the subendothelial layer has an average electron density. In the muscle-elastic layer, smooth muscle cells are visualized, in the cytoplasm of which mitochondria and endoplasmic reticulum are clearly identified. However, in some areas, loci of optical illumination are visible (Fig. 1). The content of endothelin-1 in the blood of sexually immature animals increased by 2.9 % ($p<0.05$), it did not change in sexually mature animals.

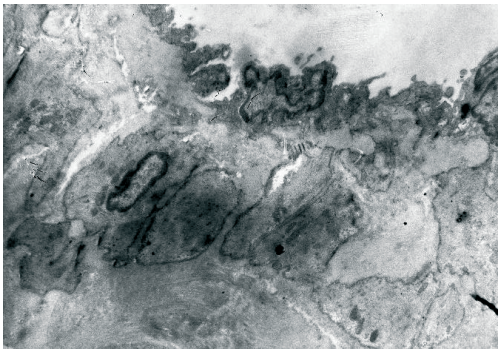

Fig. 1. Structural organization of the endocardium of a sexually mature animal on the 60th day of iodine deficiency. Electronic microphotograph. x4000.

When microscopically studying the wall of the vessels of the arterial bed, it was established that in the arteries of elastic, mixed and muscular types, all layers are identified, however, the perimeter of their lumen is deformed due to the unevenness of the convolutions of the connective tissue framework. The internal elastic membrane forms uneven protrusions along the perimeter of the lumen. The endothelial layer of the intima is illuminated, the nuclei of endotheliocytes, which are mainly located at the apical poles, are not visible in all fields of view. The nuclei of smooth muscle cells of the middle layer are multidirectional, with varying degrees

of basophilia. The adventitia membrane is veiled, has islands of layers.

Submicroscopically, it was established that the endothelial cells lie on the basement membrane, under which the subendothelial layer with varying intensity of electron density is located. A noticeable increase in the size of organelles and multiple protrusions of their lumenal plasmolemma are visualized in the cells. The internal elastic membrane is observed with luminal loci. The sarcoplasm of smooth myocytes is illuminated and has a granular-fibrillar structure. Mitochondria and myofilaments are visualized in it. Membrane organelles are expanded in fibroblasts. Connective tissue fibers are surrounded by an amorphous substance.

According to morphometric analysis, in sexually immature animals, the thickness of the wall of the aorta, external carotid and renal arteries increased by 0.9 % ($p<0.05$), 0.9 % ($p<0.05$) and 0.6 % ($p<0.05$), respectively. In sexually mature animals, this parameter increased by 0.2 % ($p<0.05$), 0.7 % ($p<0.05$) and 1.1 % ($p<0.05$), respectively.

Thyroid status of sexually immature animals of 3 groups: TSH (0.16±0.02) μIU/ml ($p<0.01$), Tz (3.47±0.39) nmol/l ($p<0.01$), T4 (51.31±6.53) nmol/l ($p<0.01$); in sexually mature individuals – (0.23±0.03) μIU/ml ($p<0.01$), (2.43±0.29) nmol/l ($p<0.01$), (54.91±7.05) nmol/l ($p<0.01$), respectively. Cholesterol content on the 90th day of the experiment is: in sexually immature rats (1.66±0.20) mmol/l ($p<0.01$), in sexually mature rats – (1.44±0.16) mmol/l ($p<0.01$). Ioduria in sexually immature animals of group 3 is (1.89±0.24) μg/l, in sexually mature animals – (2.29±0.21) μg/l ($p<0.01$).

On the 90th day of the iodine deficiency experiment, the endocardium is visualized as an unevenly swollen, veil-like line, that covers the myocardium, with an illuminated subendothelial layer (Fig. 2). Endotheliocyte nuclei have varying degrees of basophilia, are located at different cell levels, and are flattened. The cytoplasm of endotheliocytes has a mesh structural organization.

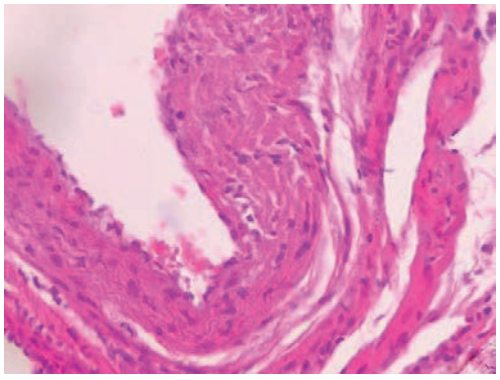

Fig. 2. The structure of the endocardium on the 90th day of iodine deficiency in sexually mature animals. Staining with hematoxylin and eosin. x400.

Under a submicroscopic examination, endothelial cells are characterized by an average electron density with bad differentiated general organelles. A swollen, homogeneous subendothelial layer is revealed in the form of a homogeneous deformed strip. Swollen multidirectional collagen fibers are localized in the muscle-elastic layer surrounded by an illuminated amorphous substance. Noticeable vacuolization of the sarcoplasm of smooth muscle cells and swelling of the fibers of the muscle-elastic layer. The content of endothelin-1 in the blood of sexually immature animals increased by 4.3 % ($p<0.05$), in sexually mature animals it increased by 1.2 % ($p<0.05$).

When studying the walls of arteries of elastic, muscular and mixed types, edematous manifestations are often revealed, flattening of the forms of endotheliocytes is noted, not all cells visualize nuclei (Fig. 3). Adhesion of formed blood elements covering endotheliocytes is observed in the vessel lumen. Endothelial cells are located on a poorly differentiated thin basement membrane. Their lumenal surface has a bad contour around the vessel perimeter. The cytoplasm is diffusely granular with alternating optical illumination.

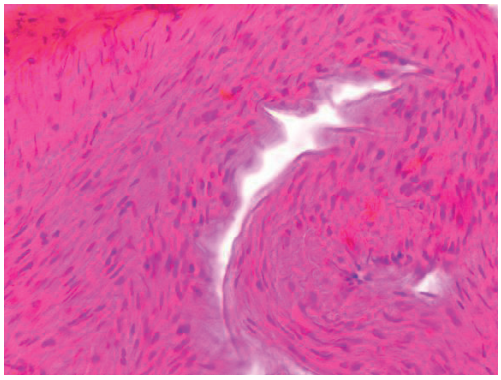

Fig. 3. The structure of the wall of the renal artery on the 90th day of the iodine-deficient state of a sexually mature animal. Staining with hematoxylin and eosin. x400.

Submicroscopically, it is noticeable that the subendothelial layer has a low electron density. The collagen-elastic framework of the middle layer is deformed, swollen, the bundles of elastic fibers are uneven. Elastic and collagen fibers in the middle layer of the arterial wall are swollen and distended. The nuclei of smooth myocytes of the media are multidirectional, stained weakly basophilic. In some fields of vision, the sarcoplasm of cells is illuminated. Adventitia is represented by stratified collagen fibers in the swollen amorphous component of the intercellular substance.

According to morphometric analysis, in sexually immature animals, the thickness of the wall of the aorta, external carotid and renal arteries increased by 1.0 % ($p<0.05$), 1.2 % ($p<0.05$) and 0.8 % ($p<0.05$), respectively. In sexually mature animals, this parameter

increased by 0.6 % (p<0.05), 1.3 % (p<0.05) and 1.7 % (p<0.05), respectively.

Thyroid status of sexually immature animals of 4 groups: TSH (0.18±0.02) μIU/ml (p<0.01), Tz (3.44±0.36) nmol/l (p<0.01), T4 (74.40±7.10) nmol/l (p<0.01); in sexually mature individuals – (0.14±0.01) μIU/ml (p<0.01), (2.39±0.24) nmol/l (p<0.01), (63.60±7.46) nmol/l (p<0.01), respectively. Cholesterol content on the 60th day of the experiment is: in sexually immature rats (1.66±0.19) mmol/l (p<0.01), in sexually mature rats – (1.44±0.15) mmol/l (p<0.01). Ioduria in sexually immature animals of group 2 is (1.42±0.16) μg/l, in sexually mature animals – (1.92±0.21) μg/l (p<0.01).

During light microscopy, the endocardium has the appearance of an uneven line lining the myocardium. Endotheliocyte nuclei are not visualized in all fields of view, they have different intensity of basophilia. Cytoplasm is weakly eosinophilic, with indistinct boundaries.

During ultrastructural examination, membranous organelles of a rounded shape are found in the cytoplasm of endotheliocytes. The lumenal surface forms a relief contour. Subendothelial layer of medium electron density. Locus of optical illumination are visible in the muscle-elastic layer (Fig. 4). The content of endothelin-1 in the blood of sexually immature animals increased by 4.3 % (p<0.05), in sexually mature animals – by 2.4 % (p<0.05).

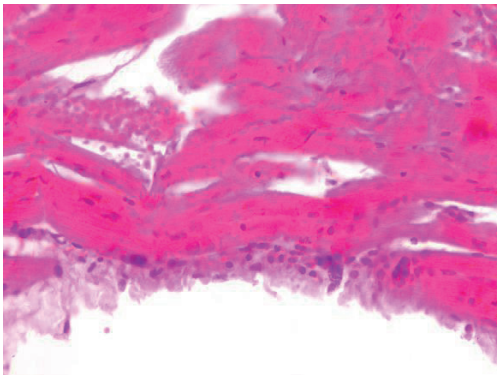

Fig. 4. Endocardium of a sexually mature animal on the 60th day of iodine deficiency with stromogens. Staining with hematoxylin and eosin. x400.

In the wall of arteries of different types, their layers are differentiated. The internal elastic membrane forms uneven protrusions along the perimeter of the lumen. Endotheliocyte nuclei are visualized on their apical poles. The nuclei of smooth muscle cells of the middle layer are basophilic stained. The adventitia has islands of stratification (Fig. 5).

According to the morphometric analysis, in sexually immature animals, the thickness of the wall of the aorta, external carotid and renal arteries increased by 0.9 % (p<0.05), 1.1 % (p<0.05) and 0.8 % (p<0.05), respectively. In sexually mature animals, this parameter increased by 0.3 % (p<0.05), 0.9 % (p<0.05) and 1.4 % (p<0.05), respectively.

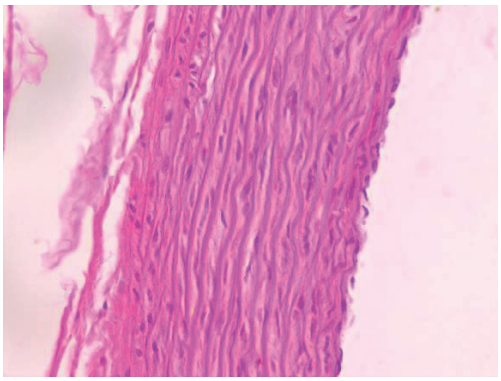

Fig. 5. Aorta of a sexually immature animal on the 60th day of iodine deficiency with stromogens. Staining with hematoxylin and eosin. x400.

Under a submicroscopic examination, endotheliocytes are located on the basement membrane, under which a subendothelial layer with varying intensity of electron density is visible. Mitochondria and myofilaments are visualized in the illuminated sarcoplasm of smooth muscle cells. Membrane organelles are expanded in fibroblasts. Connective tissue fibers are surrounded by an amorphous substance.

Thyroid status of sexually immature animals of 5 groups: TSH (0.14±0.02) μIU/ml (p<0.01), Tz (3.36±0.33) nmol/l (p<0.01), T4 (50.14±5.73) nmol/l (p<0.01); in sexually mature individuals – (0.26±0.02) μIU/ml (p<0.01), (2.06±0.18) nmol/l (p<0.01), (50.88±5.69) nmol/l (p<0.01), respectively. Cholesterol content on the 90th day of the experiment is: in sexually immature rats (1.68±0.15) mmol/l (p<0.01), in sexually mature rats – (1.49±0.14) mmol/l (p<0.01). Ioduria in sexually immature animals of group 3 is (0.92±0.08) μg/l, in sexually mature animals – (1.54±0.14) μg/l (p<0.01).

On the 90th day of the experiment, edematous changes in the endocardium progress. During light microscopic examination, it has the appearance of a veil-like strip covering the myocardium. Flattened, slightly basophilic-stained nuclei of endotheliocytes are visualized in the light pink cytoplasm. The content of endothelin-1 in the blood of sexually immature animals increased by 5.7 % (p<0.05), in sexually mature animals – by 2.4 % (p<0.05).

Submicroscopically, the cytoplasm of endotheliocytes has an average electron density. There are expanded membrane organelles, as well as vacuoles. The subendothelial layer is swollen, homogenized. Noticeable vacuolization of the sarcoplasm of smooth myocytes and swelling of the fibers of the muscle-elastic layer.

Edema changes are also found in the wall of the examined arteries. Endotheliocytes are flattened,

their nuclei are not identified throughout. The collagen-elastic framework of the media is deformed, the bundles of elastic fibers are uneven. Nuclei of smooth myocytes are unidirectional, weakly basophilic. In some fields of vision, the sarcoplasm is illuminated. The adventitia is edematously changed.

According to morphometric analysis, in sexually immature animals, the thickness of the wall of the aorta, external carotid and renal arteries increased by 1.1 % ($p<0.05$), 1.3 % ($p<0.05$) and 1.0 % ($p<0.05$), respectively. In sexually mature animals, this parameter increased by 0.6 % ($p<0.05$), 1.4 % ($p<0.05$) and 1.8 % ($p<0.05$), respectively.

Ultrastructural examination reveals flattened endotheliocytes on a poorly differentiated basement membrane, under which the subendothelial layer is swollen (Fig. 6).

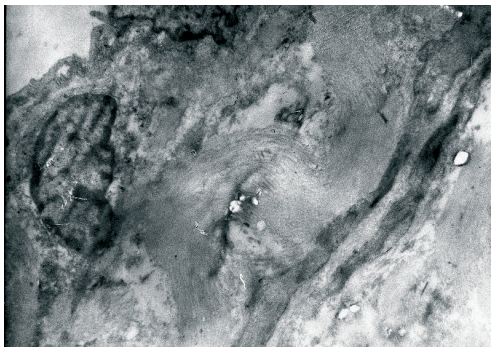

Fig. 6. Ultrastructure of the wall of the carotid artery of a sexually mature animal on the 90th day of iodine deficiency with stromogens. x4800.

The same edematous changes are expressed in the fibers and smooth muscle cells of the arterial wall. Submicroscopically, it is noticeable that the subendothelial layer has a low electron density. The collagen-elastic framework of the middle layer is deformed, swollen, the bundles of elastic fibers are uneven. Elastic and collagen fibers in the middle layer of the arterial wall are swollen and distended. Adventitia is represented by stratified collagen fibers in the swollen amorphous component of the intercellular substance.

Iodine is a key trace element for the synthesis of thyroid hormones, and thyroid hormones directly affect metabolism, vascular tone, myocardial contractility, lipid profile, and overall cardiovascular risk [17, 18, 19]. Iodine deficiency can cause a decrease in the production of thyroxine and triiodothyronine with a compensatory increase in thyroid-stimulating hormone, which creates conditions for the formation of a hypothyroid state or subclinical dysfunction of the thyroid gland [17, 20]. Modern reviews emphasize that even moderate disorders of thyroid status are associated with dyslipidemia, arterial hypertension, endothelial dysfunction, atherosclerotic process and heart failure [18, 19, 21].

Of particular importance is the fact that the relationship between iodine status and the cardiovascular system is not limited to clinically pronounced hypothyroidism. In population studies, the concentration of iodine in urine was considered as a marker of iodine supply and was associated with metabolic disorders, mortality or cardiovascular consequences [22–24]. In particular, U-shaped associations between iodine status and metabolic disturbances suggest that both iodine deficiency and excess may be undesirable for metabolic homeostasis [22]. Iodine status has also been considered as a potential long-term risk factor in US and Spanish adult mortality studies [23, 24].

Mechanistically, the effect of iodine deficiency on the heart can be realized through a decrease in thyroid stimulation of cardiac muscle cells, a change in the expression of genes that regulate myocardial contractility, a violation of lipid metabolism, an increase in systemic vascular resistance, and the development of tissue hypoxia. In hypothyroid conditions, a decrease in cardiac output, diastolic dysfunction, an increase in cholesterol level, and the formation of an unfavorable cardiometabolic profile are described [18, 19, 21]. This is an important basis for the morphological study of the heart, particularly the endocardium, since the endocardium and subendocardial structures are sensitive to changes in hemodynamics, swelling of connective tissue components, microcirculation disorders, and metabolic imbalance.

Another factor that can increase the consequences of iodine deficiency is the intake of current-forming substances with food products. Such products primarily include plants of the Brassicaceae family, soy, millet, cassava and other sources of compounds capable of influencing thyroid metabolism [25–27]. Glucosinolates and their metabolites, in particular thiocyanates and goitrin, can compete with iodide or affect the processes of iodine organization, especially under conditions of insufficient iodine supply [26, 27]. At the same time, a modern systematic review on Brassica vegetables shows that, with adequate iodine intake, the usual dietary use of such products mostly does not have a clinically significant negative effect on the thyroid gland [27].

Experimental data are particularly useful for analyzing the combined effect of iodine deficiency and stromogenic products. In the work of Paśko et al. it was shown that the interaction of iodine and glucosinolates from rutabaga sprouts affects biomarkers of thyroid gland function in male rats [28]. In a subsequent study by the same research group, it was found that animals with models of thyroid damage responded differently to the consumption of Brassica sprouts; under conditions of a hypothyroid state, such products could increase negative changes in certain biochemical indicators [29].

For the endocardium, edematous changes, remodeling of the connective tissue fibers, microcirculation disturbances, and secondary changes related to hemodynamic and metabolic imbalance are potentially important. That is why further morphological, morphometric and ultrastructural study of the endocardium under conditions of isolated iodine deficiency and its combination with stromogenic factors is relevant for experimental cardiomorphology [29, 30].

CONCLUSIONS

Thus, the available data allow us to consider iodine deficiency as a systemic factor that can affect the cardiovascular system due to thyroid dysfunction. The combined intake of current-generating substances can increase this effect, especially if iodine deficiency has already formed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Вплив йододефіцитного стану та струмогенних продуктів на органи серцево-судинної системи

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Анотація. На сьогодні йододефіцитні захворювання становлять глобальну медико-соціальну проблему: згідно з даними ВООЗ, різноманітні порушення здоров'я, зумовлені дефіцитом йоду, посідають третє місце серед 38-ми найпоширеніших неінфекційних захворювань. Частота ураження органів серцево-судинної системи та поширеність йододефіциту є передумовою вивчення впливу тиреоїдного дисбалансу на серце та кровоносні судини. Метою було простежити за морфофункціональними змінами в ендокарді та стінці артерій різного типу в динаміці розвитку йододефіциту в постнатальному онтогенезі. Роботу виконано на експериментальних тваринах статевонезрілого та статевозрілого віку. Досягнення стану йододефіциту підтверджується показниками йодурії та тиреоїдним профілем. Як на 60, так і на 90 доби йододефіцитного стану в усіх досліджених органах виникають набрякові зміни в сполучнотканинних складових, у стінці кровоносних судин, особливо в ендотеліальному шарі, що підтверджено за допомогою світлооптичного та електронно-мікроскопічного методів дослідження. Таким чином, наявні дані дозволяють розглядати йододефіцит як системний фактор, що через тиреоїдну дисфункцію здатний впливати на серцево-судинну систему. Комбіноване надходження струмогенних речовин може посилювати цей вплив, особливо якщо дефіцит йоду вже сформований.

Ключові слова: експеримент; щури; серце; артерії; метаболічні порушення; морфологічні зміни; мікроскопія; онтогенез.