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AGE-DEPENDING PECULIARITIES OF ULTRASTRUCTURAL MYOCARDIUM CHANGES CAUSED BY EXPERIMENTAL HYPERURICEMIA AND ITS COMBINATION WITH HYPERCHOLESTEROLEMIA

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Summary

Ultrastructural myocardial changes are the first signs that let characterizing morphological features of various pathological processes morphogenesis, including metabolic cardiomyopathy. It was defined that in animals of pubertal period hyperuricemia and its combination with hypercholesterolemia led to myocardial hyperfunction, which manifested mainly by hyperplasia of different cellular ultrastructures. Among adult animals in modelled metabolic deviations could be noted degenerative myocardial changes that appeared as partial or total organelles destruction.

Key words: myocardium, hyperuricemia, ultrastructure, experiment.

Introduction. The problem of various metabolic deviations in the whole body that manifest themselves into such nosological units as metabolic syndrome, atherosclerosis, diabetes mellitus, gout and others is quite sufficient nowadays, which is determined by facts: quarter of adults all around the world have metabolic syndrome, people with metabolic syndrome have twice higher risk of sudden death, tree times higher risk of heart attack or stroke, five times higher

expectancy of diabetes mellitus manifestation, up to 80 % of them die from cardio-vascular diseases [5, 7].

As about heart lesion caused by gout and, in particular, specified by the syndrome of hyperuricemia, worldwide are registering the increasing number of such patients [6, 8, 10]. Among factors with the strongest impact on gout morbidity rate are age, sex and race that define the level of sex hormones, which influence on purine metabolism and different genetic determinants [1, 2]. While surveying literature related to this topic it was found out that structural myocardium subcellular reorganization due to the increased level of uric acid in blood, as well as due to hyperuricemia combined with hypercholesterolemia, is not studied well enough and doesn't have clarified age-depending features of these processes.

Aim of the research. To determine the specific features of ultrastructural myocardial changes due to the influence of increased levels of uric acid and cholesterol in blood among animals in the course of experiment.

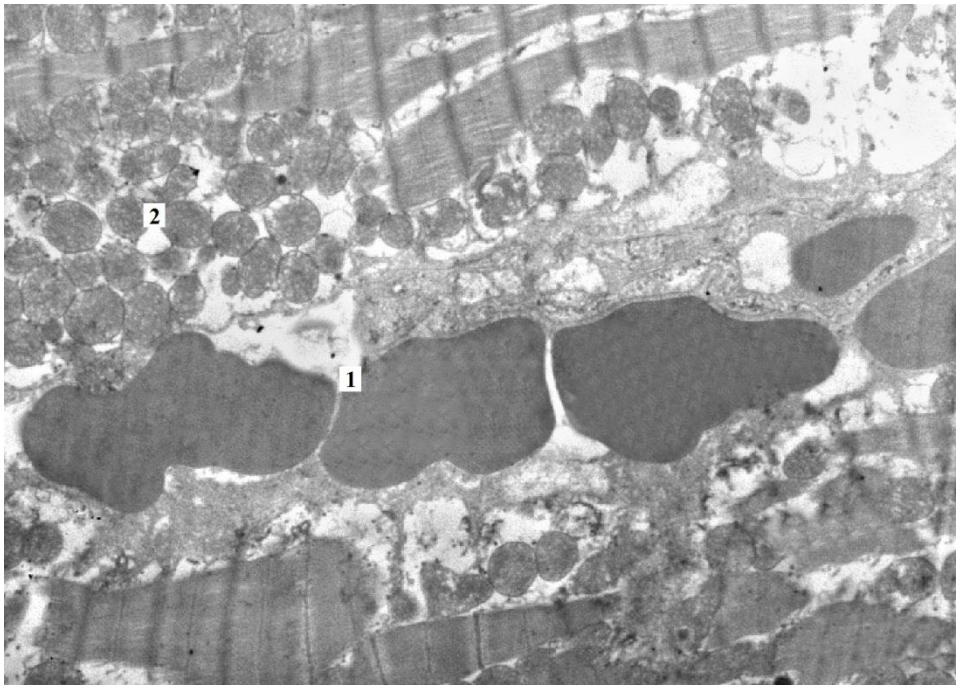
Materials and methods. The study of pubertal and adult white male rats' hearts was conducted; there were experimentally modelled hyperuricemia, hypercholesterolemia and their combination (6 animals in each group in the age of 3 and 12 months). Control group was in line with experimental and the animals were on a standard vivarium diet. Experimental hyperuricemia was modelled according to O.Synyachenko methodic in our modification [9] by the way of 30 days lasting high in protein diet. The fodder included mix of black pudding with autolyzed yeast extract, molybdenum and inosine. Hypercholesterolemia was modelled by the classical methodic of Anichkov lasting 30 days. For combined metabolic deviations both models were used simultaneously. Protocol of experiment, animals keeping, making experimental models and their withdrawal out of experiment were conducted according to the principles of bioethics, rules of GLP, likewise it met the standards of "The European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasburg,1985), "General ethic principles of conducting experiments on animals" (First National Bioethics Congress, Kyiv, 2001) and requirements of bioethics commission of SHEE "Ternopil I.Ya.Horbachevsky State Medical University" (protocol № 24 from August, 27 2014). The levels of uric acid and general cholesterol were measured according to general biochemical method. Analysis of the results made in the department of systemic statistical research SHEE "Ternopil I.Ya.Horbachevsky State Medical University" in the software package "Statsoft Statistica". Submicroscopic myocardial structure was studied at electron microscope PEM – 125 K with different magnifications. For making histological micropreparations myocardial tissue of left ventricle was fixed in 2,5-3 % solution of glutaraldehyde for 30 minutes, 1 % osmium tetroxide with farther dehydration in

alcohols and acetone following adopted procedure. After dehydration pieces of myocardium were covered with mixture of epoxide resin. Ultrathin microscopic sections were received at the ultramicrotome, contrasted by uranyl acetate staining and citras plumbi according to the methodic of Reynolds.

Results and their discussion. The blood measured levels of uric acid among young experimental animals with modelled hyperuricemia substantially exceeded control up to 87,58 % ($p \leq 0,01$) in rats with hyperuricemia and up to 124,45 % ($p \leq 0,01$) – with hyperuricemia and hypercholesterolemia. This rates among adult animals increased to 82,74 % ($p \leq 0,01$) and 116,8 % ($p \leq 0,01$) accordingly. In hypercholesterol model in both age groups significant rise of uric acid concentration was not noticed. General cholesterol concentrations increased dramatically at hypercholesterol model and combination of metabolic deviations among animals of pubertal age twice as many ($p \leq 0,01$) and 2,5 ($p \leq 0,01$) times, while among adult animals the indexes increased into 2,2 ($p \leq 0,01$) and 2,3($p \leq 0,01$) times accordingly. Within hyperuric model in both age groups significant increase of general cholesterol was not registered.

Myocardial submicroscopical changes in consequence of metabolic deviations are characterized as nonspecific that can be evidenced by processes of muscular cells hypertrophy and dystrophy, as well as proliferation of the elements of connective tissue.

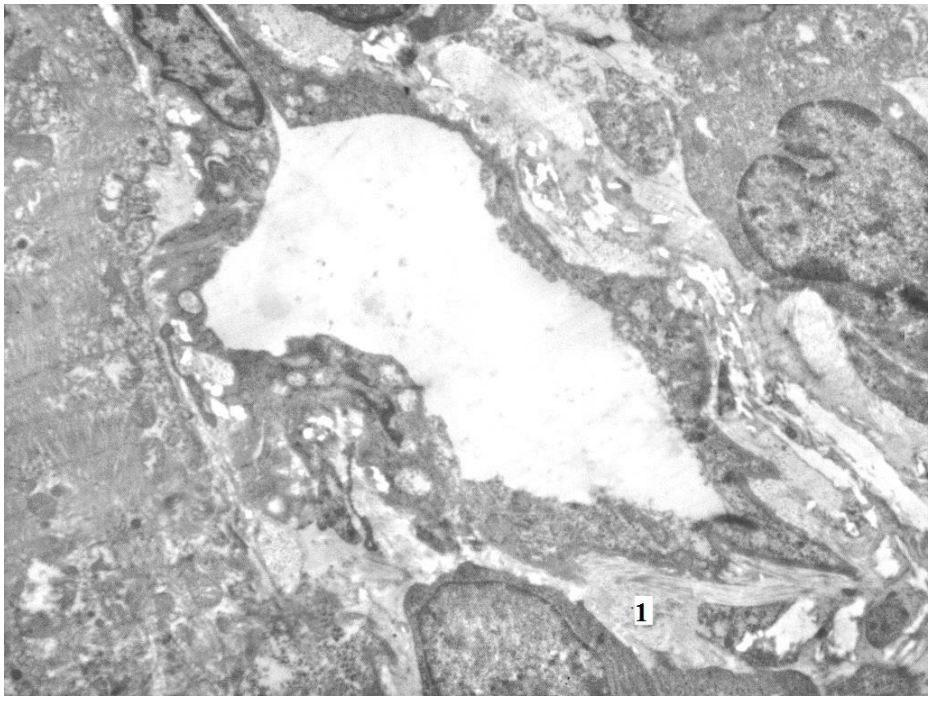
While modelling hyperuricemia there were marked contractive elements hypertrophy, appearance of huge mitochondria with thick solidly packed cristae in myocardium. Increasing number of connective tissue was observed, here and there with areas of cardiomyocytes' destruction. Intensification of myocardial function caused significant mitochondrial sarcoplasmic swelling. Besides, intra- and outer cellular edema was noticed, lots of mitochondria with arched arrangement of cristae, cardiomyocytes nuclei borders were not clear with numerous kariotheca invaginations. Myofibrils kept their typical sarcomere arrangement, however somewhere thickened. Strong perivascular edema could also be noted. Inside vessel lumens – blood forming cells located as chain – sludge phenomenon. Endotheliocytes are slightly edematous, with translucent cytoplasm, containing lots of pinocytotic elements. Inside extended interstitial lumen – leukocytes which confirm proinflammatory activity of uric acid [3].



Picture 1 – Sludge phenomenon (1) and conglomerates of mitochondria (2) under submicroscopic investigation of young rat's heart with hyperuricemia. Magnification ×15000.

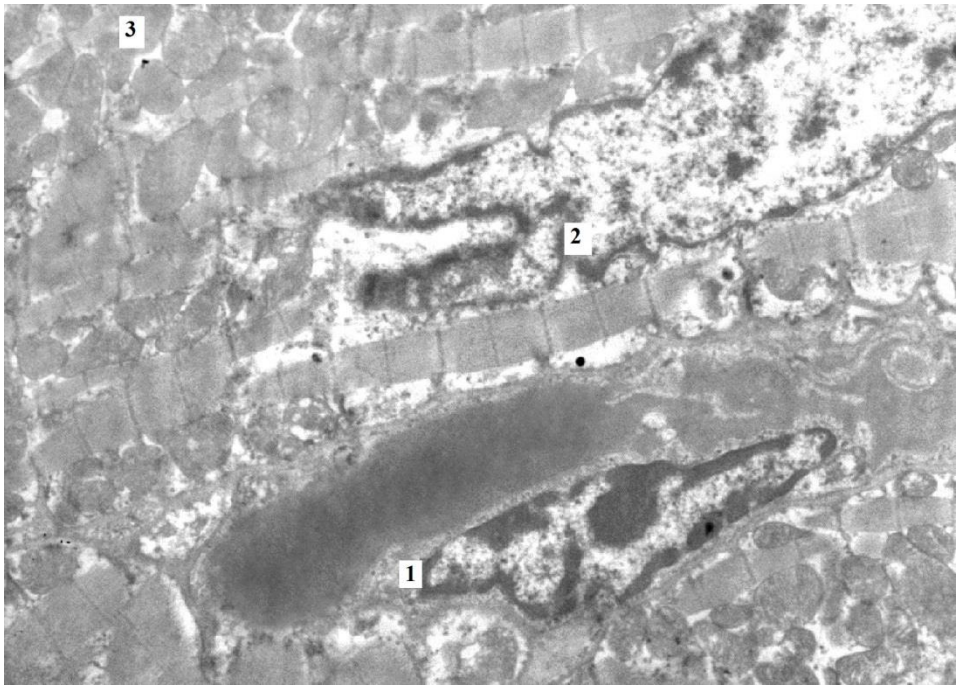
Among adult rats hyperuricemia modelizing manifested with more significant destructive processes along with increasing of cells energetic ability. Myofibrils were in the state of contraction, partially with disrupts of myofilaments and areas of homogenization, conglomerations of mitochondria among them. The total number of mitochondria was less in comparison with young animals. Their cristae were deformed, some of them ruined and these spaces were homogenized. Homogenization of matrix varied from little extent to the size of whole mitochondria. Somewhere there were noted edematous matrixes-less mitochondria with damaged outer membrane.

Among animals of this group we observed perivascular myocardial sclerosis as result of thickened layers of connective tissue. Seldom thickened fibrils of collagen were observed in stroma as well.



Picture 2 – Perivascular location of thick collagen fibrils (1) on the electron microphotography of adult rat's heart with hyperuricemia. Magnification ×10000.

Changes resulting from hypercholesterol diet among animals of both age groups manifested by development of capillary and parenchyma dystrophy. That was evinced by dystrophic infractions of membrane components of organelles, cardiomyocytes and endotheliocytes. The following changes are typical for hypoxic conditions [4, 11] with prior lesion of microcirculatory system and failure of proper capillary and cellular permeability, development of fatty and protein myocardial parenchyma dystrophy. Cardiomyocytes matrixes were translucent and with expanded perinuclear spaces. Mitochondria were destructively changed; their matrixes included rather small homogeneous dense lipid inclusions. Such inclusions were also noticed in sarcoplasm, mainly around mitochondria. The special features of these mitochondria were dark matrixes and thin meandering cristae. Completely homogenized organelles without organelles were often observed as well. There were noted numerous invaginations of cardiomyocytes nuclei karyotheca, hyperplasia of channel system. Myofibrils were in the state of contraction, with partial lysis. The fact is, that there were much more areas of destruction among adult animals. Endotheliocytes formed rare narrow cytoplasmic offshoots. Their nuclei were oval with invaginations, chromatin unevenly divided with little brim margination.

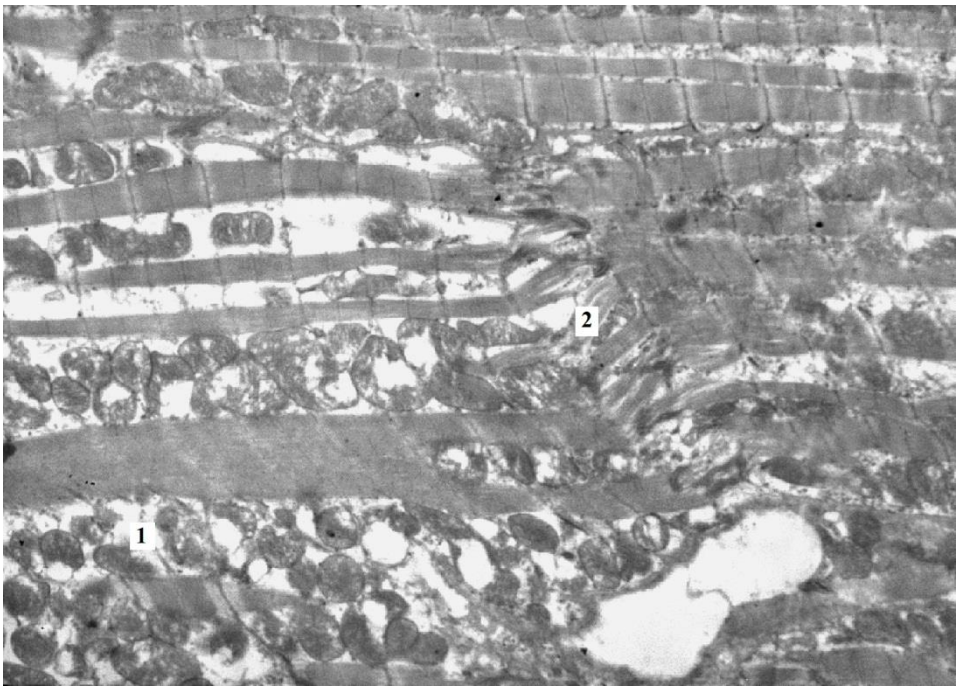


Picture 3 – Endotheliocyte with marked micropinocytosis (1), invagination of cardiomyocyte karyotheca (2), mitochondrial hyperplasia (3) on submicroscopic investigation of adult rat's heart with hypercholesterolemia. Magnification ×15000.

The following changes are caused by endothelial laying deformation as a result of endothelium cell-tropic effect of cholesterol and both inner- and out vessels factors. In particular, the first group includes the veer of blood rheological features – sludge phenomenon, and the second – interstitial edema, stroma proliferation and blood vessels compression by injured cardiomyocytes.

In combined metabolic experimental model cardiomiopathy in animals revealed itself as an overbalance of destructive changes rather than compensatory and adaptive mechanisms. Histotoxic effects of uric acid and cholesterol varied broadly among young and adult animals. Ultrastructural changes were more expressed and recorded among adult animals. They mostly characterized reduction of myocardium contracting ability. Significant numerous mitochondrial destructions with abrupt decrease of cristae and partial ruining of sarcotubular system were noted also. Myofibrils were relaxed or fairly contracted, somewhere with zones of over contraction and seats of myofilaments disorganization and disruptions, small areas of homogenization. Cardiomyocytes nuclei had their borders invaginated and chromatin margination. There could be observed intracellular lipid insertions, mostly located round mitochondria. Blood vessel lumens were either narrowed or absolutely blocked by edematous

endotheliocytes; cytoplasmic offshoots of endotheliocytes were goffered, with slightly marked pinocytosis.



Picture 4 – Mitochondrial destruction (1), infringement of myofibrils orientation and their filament organization failure (2) on the electron microphotography of adult rat's heart on a combined diet. Magnification ×12000.

The state of collagen filaments also draws attention - in addition to their considerable thickening and increased producing rate among animals with hyperuricemia and hypercholesterolemia, the stromal layers were considerably disorganized. In some areas they were set perpendicularly, more like interweaved into myocardium. Possibly, such areas were formed as a result of partial necrobiotic changes under the influence of metabolic deviations with the aim of safekeeping of undivided myocardium functioning.

Conclusions:

1. Hyperuricemia is followed by alterative infractions in the structure of myocardium, including all its components and noted at all levels of heart organization.

2. The level of expression and character of these changes depend on animal age. Among young rats myocardial lesion has compensatory and adoptive character, whilst in adult animals – degenerative and destructive.

3. Due to the combination of hyperuricemia and hypercholesterolemia, the character of myocardial lesion is more progressive and attaches features of both – cholesterol and hyperuric - types of lesion.

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