ULTRASTRUCTURAL CHARACTERISTICS OF THE MYOCARDIUM IN RHEUMATIC DISEASES

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The myocardium from the auricle of the left atrium and the left ventricle of the heart in 22 patients undergoing operations for rheumatic defects was studied by ordinary and histochemical electron-microscopic methods. Marked changes were found in succinate dehydrogenase, adenosinetriphosphatase, phosphorylase, and acid phosphatase activity accompanied by changes in the mitochondria, the sarcomeres, and other ultrastructural elements of the muscle cells.

Analysis of the enzyme activity of the intracellular structures at whose level specificity of structure and function is first observed [3] is an important criterion of the unity of structure and function of the tissues as a whole. From this point of view electron-microscopic and electron-histochemical studies of the myocardium affected by various rheumatic lesions is of considerable interest.

EXPERIMENTAL METHOD

The myocardium of 22 patients aged 14-25 years, undergoing operations for mitral stenosis (6), combined mitral defects (9), and combined mitral and aortic defects (7) was investigated. The patients were admitted to the hospital with decompensated defects (stages II-III in A. N. Bakulev's terminology). At the time of operation they were all in a state of relative compensation of their cardiac function.

Morphological analysis of the tissues of the auricle revealed signs of disorganization of the connective-tissue fibers of the endocardium and myocardium, cellular proliferation, infiltration with lymphocytes and histiocytes (11 patients), and also typical granulomatosis (6 patients). Five patients showed marked sclerosis of the interstitial tissue. Three had a combined mitral defect while the other two had a combined mitral and aortic defect. Pieces from the auricle of the left atrium and the anterior wall of the left ventricle for electron-microscopic study were fixed in 1% buffered osmic acid solution, dehydrated in alcohols, and embedded in butylmethylmethacrylate (4:1). Activity of adenosinetriphosphatase [10], succinate dehydrogenase [7], phosphorylase [6], and acid phosphatase by Gomori's method [4] was studied simultaneously in the blocks of tissue. Incubation was carried out in a Warburg apparatus at 37°C in small flasks connected by a special device to the manometric stands. Sections cut on the LKB-880 ultratome were negatively stained with lead hydroxide by Reynolds' method or with uranyl acetate and examined in the UÉMV-100 microscope.

EXPERIMENTAL RESULTS

The investigation revealed characteristic changes in the ultrastructural components of the myocardial muscle cells. The mitochondria were rich in succinate dehydrogenase, which was found along the membrane and on the cristae (Fig. 1). Mitochondrial adenosinetriphosphatase was localized at these same places. The intensity of the enzyme reactions varied in the different organelles. Activity of the enzymes in the cristae in the numerous swollen mitochondria was reduced or absent, and remained only in the

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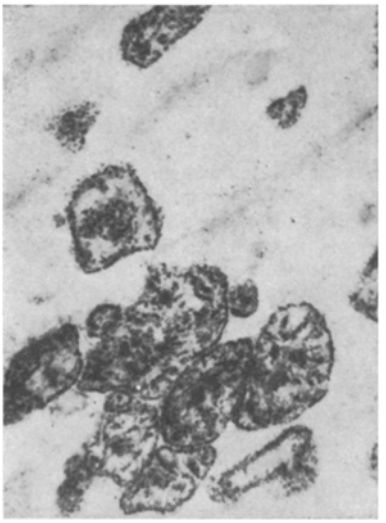

Fig. 1. Succinate dehydrogenase in mitochondria, 9125 ×.

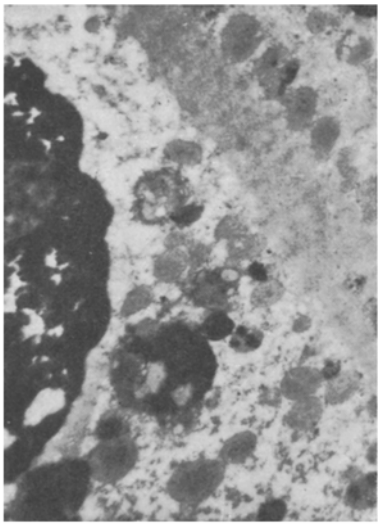

Fig. 2. Different degrees of acid phosphatase activity in lysosomal elements (L_1, L_2, L_3, L_4), 13,200 ×. *

membrane. Foci of disintegration of the mitochondria were regularly seen. In the myocardium of the left ventricle of patients with combined mitral and aortic defects, mitochondria were few in number in the perinuclear zone of many of the cells. They were polymorphic, and their succinate dehydrogenase and adenosinetriphosphatase activity varied considerably.

Lysosomes were numerous in the muscle cells and were found in the perinuclear zone, between the myofibrils, and beneath the sarcolemma. The last of these positions was more characteristic of the myocardium of the auricles. Primary lysosomes were surrounded by a single membrane and contained a homogeneous or microgranular electron-dense material reacting strongly for acid phosphatase. The secondary forms were much more numerous than the primary and consisted of conglomerations of osmiophilic bodies and lipid inclusions. Acid phosphatase activity in these structures varied from a well-marked reaction in some to only traces or no reaction whatever in others (Fig. 2). There was a definite correlation between the number of lysosomes and the intensity of myocardial function depending on the type of lesion. In mitral stenosis there were many more lysosomal elements in the muscle cells of the atria than in the myocardium of the left ventricle. In lesions accompanied by hypertrophy of the left ventricle (combined mitral or combined mitral and aortic defects) there were many tens of lysosomes in its individual muscle cells.

The sarcolemma and intercalary disks showed a high adenosinetriphosphatase concentration (Fig. 3). Signs of edema – separation of the sarcolemma from the sarcoplasm in arcades, widening of the sarcoplasmic reticulum – were found in the muscle cells. Rupture of the cell membrane and liberation of the populations of mitochondria, lysosomes, and other ultrastructural elements into the surrounding space could occur. In combined mitral and aortic defects frequently the intercalary disks were frequently widely separated. Under these conditions the adenosinetriphosphatase of the disk membranes was distributed irregularly, its activity in some places being considerably inhibited.

In the myofibrils there were zones of sarcomeral contraction alternating with those of sarcomeral elongation. In the contracture band, striation had faded. Here the sediment indicating the localization of phosphorylase showed dispersion without any structural orientation. Sarcomeral extension was accompanied by diffusion of the enzyme whose activity had decreased. In many myofibrils we saw areas of fine-granular disintegration. The muscle cell nuclei were rich in acid phosphatase, phosphorylase, and adenosinetriphosphatase. In cases of chromatin margination the enzymes displayed their activity at the site of nucleoplasmic concentration. We also noticed numerous lipid granules lacking specific localization.

In lesions of the heart profound changes thus take place in the membranous structures and contractile elements in the myocardial muscle cells. During swelling and loss of the matrix the protein content in the mitochondria is reduced [8, 12]. These changes, as was pointed out above, are accompanied by inhibition

*As in Russian original.

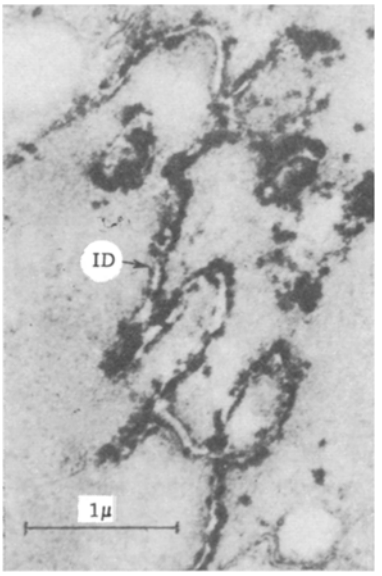

Fig. 3. Adenosinetriphosphatase in membranes of intercalary disk (ID) of a muscle cell, 30,000 ×.

of succinate dehydrogenase phosphatase and adenosinetriphosphatase activity especially in the cristae, evidence of a decrease in the intensity of the energy metabolism. During functional stress of the myocardium of the left ventricle (in combined mitral and aortic defects) the damage of the mitochondria is more severe and affects the perinuclear zone of the cell. This state of affairs may influence metabolic processes in the nuclei, as a result of which their ability to synthesize RNA and protein is limited.

Activation of the lysosomal elements in the myocardial muscle cells, observed by many investigators in rheumatic fever [1, 2] is particularly interesting. The present investigation revealed not merely an increase in the number of lysosomes, especially of the secondary forms, but also differences in the level of functional activity (as shown by the intensity of the reaction for acid phosphatase) in different structures. An increase in the number of lysosomes in the tissues of the auricle is found constantly whether the disease pursues an active or a latent course. However, it must be emphasized that, regardless of the phase of the process, accumulation of lysosomes is observed in the myocardium of the left ventricle in combined defects (combined mitral, mitral and aortic) which are known to involve functional stress and definite structural changes in the heart. It must therefore be supposed that the increased intensity of the lysosomal reaction is largely associated with the need for removing products of intermediate metabolism and imperfect structural components of the organelles, arising in the intensively functioning myocardium in the presence of a defect, from the cell.

Characteristic changes also were found in the sarcomeres, which showed excessive contraction and stretching. The accompanying disturbance of phosphorylase activity indicates profound changes in the anaerobic part of the energy formation cycle. These processes are accompanied by marked changes in the sarcolemma, the intercalary disks, and the endoplasmic reticulum. The ruptures observed in these membranes unquestionably disturb the connection between the sarcolemma and contractile proteins in the same cell [9] and between individual muscle cells of the myocardium [5, 11], necessary for normal function.

When assessing the functional capacity of the myocardium in pneumatic heart disease consideration must therefore also be paid as far as possible to all the qualitative changes at the level of the ultrastructural components of the muscle cells.

LITERATURE CITED

1. N. N. Kleimenova, Electron-Microscopic Investigation of Changes in the Auricles of the Heart in Rheumatic Fever. Author's Abstract of Candidate's Dissertation, Moscow (1971).
2. P. Ya. Mul'diyarov, Vopr., No. 3, 14 (1969).
3. D. S. Sarkisov and B. V. Vtyurin, Electron-Microscopic Analysis of Increased Tolerance of the Heart [in Russian], Moscow (1969).
4. M. Burstone, Enzyme Histochemistry [Russian translation], Moscow (1965).
5. R. A. Bullon, Rev. Clin. Esp., 78, 4 (1960).
6. A. Dorn, Acta Histochem. (Jena), 21, 406 (1965).
7. S. Kerpel-Fronius and F. Hagos, J. Histochemie, 14, 343 (1968).
8. B. Kleitke, W. Schulze, and A. Wollenberger, Acta Biol. Med. Germ., 17, 343 (1966).
9. W. G. Nayler, Am. Heart J., 65, 404 (1963).
10. W. Schulze and A. Wollenberger, Dtsch. Akad. Wiss. Berlin, 3, 34 (1963).
11. N. Thomas and L. Sherf, Am. J. Cardiol., 22, 389 (1968).
12. J. Wedell, H. Merker, and D. Neubert, Arch. Path. Anat., 338, 355 (1964).