QUANTITATIVE RELATIONS BETWEEN PARENCHYMA AND STROMA OF THE MYOCARDIUM IN RABBITS WITH ATHEROSCLEROTIC CARDIOSCLEROSIS

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UDC 616.132.2-004.6-092.9-07:
616.127-091.8

The relative volume of the muscle fibers, connective-tissue cells, capillaries, and ground substance and the surface area of the muscle fibers and capillaries per unit volume of tissue were measured in the myocardium of animals with diffuse cardiosclerosis accompanying experimental atherosclerosis. A significant decrease was found in the relative volume of the ground substance of the stroma.

KEY WORDS: parenchyma and stroma of the myocardium; experimental atherosclerosis; cardiosclerosis; stereology.

There is no information in the literature on quantitative relations between structures of the myocardium during the development of sclerosis and cicatrization. There are merely statements indicating changes in the ratio between cells of the stroma and parenchyma of the heart muscle in ischemia and necrosis of the myocardium [2].

The object of this investigation was to make a stereological analysis of relations between parenchymatous and stromal structures of the heart muscle in experimental atherosclerotic cardiosclerosis.

EXPERIMENTAL METHOD

Atherosclerotic changes were produced in 70 rabbits by the writers' method [4] based on periodic starvation (endogenous hypercholesteremia) combined with administration of cholesterol to the animals. The atherosclerotic changes in the arteries were assessed by Avtandilov's method [1]. The heart was studied by histological, histochemical, and electron-microscopic methods. Semithin (1 μ) styrene–methacrylate sections through the myocardium of nine experimental animals which had developed diffuse cardiosclerosis, and of six intact animals were used for stereological analysis [1, 3, 5, 9]. The transverse orientation of the muscle fibers was verified [7]. Sections were stained with toluidine blue at 60°C for 10 min. The morphometric investigation was based on the principles developed by Sitte [8] for the stereological study of oriented membrane-surrounded structures. The magnification used for counting was 900×.

The relative volume of the muscle fibers, connective-tissue cells, capillaries, and ground substances of the interstitial connective tissue was measured. An ocular measuring grid (Fig. 1) with a step of 0.66 μ (P_t, the number of test points on the grid, is 225), and the test area of tissue for analysis 10,696 μ^2. Altogether 60,750 points were counted in the experimental and 33,750 in the control series; the relative content of the tissue structures of the myocardium was calculated in per cent.

For example:

$$V_{mf/tis} = \frac{V_{mf}}{V_{tis}} = \frac{P_{mf}}{P_{tis}},$$

Laboratory of Pathological Anatomy, Institute of Clinical and Experimental Medicine, Siberian Division, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 8, pp. 247-250, August, 1977. Original article submitted January 17, 1977.

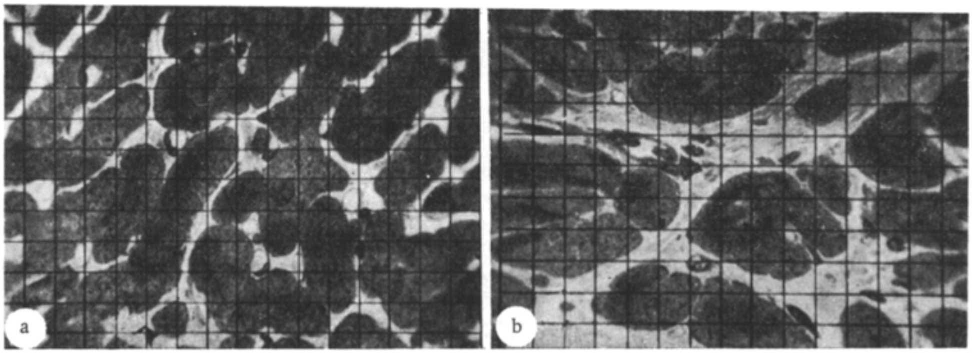

Fig. 1. Semithin (1 μ) transverse sections through myocardium of left ventricle of rabbit heart after superposition of test grid for stereological analysis: a) normal myocardium; b) myocardium of rabbit 120 days after beginning of experiment: diffuse cardiosclerosis. Embedding in styrene–methacrylate, staining with toluidine blue, 756×.

where $V_{mf/tis}$ is the relative volume of the muscle fibers in the tissue; V_{mf} the total volume of muscle fibers; V_{tis} the total volume of myocardial tissue; P_{mf} the number of points lying within the outline of the muscle fibers; and P_{tis} the number of points of the test system falling on the tissue. The relative volume of the remaining myocardial structures was determined in the same way.

The surface area of the muscle fibers and capillaries per unit volume of myocardial tissue was calculated by the formula

$$S_V = \frac{\pi}{2}\frac{C}{P_{tis}\cdot Z},$$

where C is the number of intersections of the boundaries of the outlines of the test structures with the test lines; Z the length of the test line divided by the magnification (2.13/900). In this particular case an ocular test system of short segments (n = 21; P_t = 42) and with a test area of tissue for analysis of 9748 μ^2 was used. Altogether 11,340 points in the experimental series and 6300 in the control were counted.

EXPERIMENTAL RESULTS

Changes in the capillary endothelium (leading to disturbance of the microcirculation), degenerative changes in the myocardiocytes, and increased production of acid glycosaminoglycans by the fibroblasts were observed in the heart muscle 30-60 days from the beginning of the experiments. After 90 days profound disturbances of the structure of the myocardiocytes were observed (atrophied muscle cells with a reduced number of myofibrils and with densely packed intracellular organelles appeared) and progressive development of the collagen stroma took place against the background of the formation of fibrous plaques in the aorta. After 120 days marked atherosclerosis of the aorta, diffuse cardiosclerosis, and evidence of chronic heart failure were found in the animals. Increased heterogeneity of the contractile myocardium could be attributed to the presence of normal, atrophied, and hypertrophied muscle cells.

Cardiosclerosis developed parallel to the increased intensity of the atherosclerotic changes in the aorta. Fibrous plaques were observed in the coronary arteries (the main trunks and large branches) only in the late stages. The character of the sclerotic changes in the myocardium and their severity depended mainly on the local disturbances of the coronary circulation and the state of the parenchymatous structures.

The results of the stereological analysis of the myocardial sections are given in Table 1. They show a significant decrease in the relative volume of muscle fibers in the myocardium of the animals with diffuse cardiosclerosis (73.27 ± 0.79% compared with 76.49 ± 0.83% in the control) together with an increase in the relative volume of ground substance of the interstitial connective tissue of the myocardium (19.39 ± 1.64% compared with 13.65 ± 0.93% in the control; $P < 0.05$). A tendency was observed for the surface area of the muscle fibers to increase (456.61 ± 30.78 cm^2/cm^3 compared with 385.83 ± 28.28 cm^2/cm^3 in the control), possibly on account of a compensatory response to the decrease in their volume. On the basis of these figures a decrease in the relative volume of the capillaries with an increase in surface area of the microcirculatory system can be deduced. These results were confirmed by calculating the relative volumes and the area/volume relationships for structures of the parenchyma and stroma of the myocardium.

TABLE 1. Results of Stereological Investigations of Structures of Myocardial Parenchyma and Stroma of Rabbits with Experimental Atherosclerotic (diffuse) Cardiosclerosis (M ± m)

Time of investigation	Relative volume, %				Surface area, cm^2/cm^3		Relative volume		Area/volume ratios, cm^{-1}	
	$V_{mf/tis}$	$V_{ctc/tis}$	$V_{cap/tis}$	$V_{gs/tis}$	S_{mf}/V_{tis}	S_{cap}/V_{tis}	V_{mf}/V_{cap}	V_{mf}/V_{gs}	S_{mf}/V_{mf}	S_{mf}/V_{cap}
Control (intact rabbits)	76,49±0,83	2,92±0,15	7,02±1,33	13,65±0,93	385,83±28,28	61,83±2,44	12,38±2,06	5,71±0,38	5,05±0,40	10,15±1,90
120 days	73,27±0,79	2,63±0,14	5,57±0,79	19,39±1,64	456,61±30,78	68,48±4,88	15,35±2,21	**4,04±0,40**	**6,24±0,42**	**13,73±1,71**
P	<0,05	>0,05	>0,05	<0,05	>0,05	>0,05	>0,05	<0,05	>0,05	>0,05

Legend. ctc) Connective tissue cells, tis) tissue, mf) muscle fibers, cap) capillaries, gs) ground substance of connective tissue, V) volume, S) surface area of tissue.

The specific function of the parenchymatous structures is closely connected with the state of the connective-tissue stroma. Experimental and pathological investigations show that mechanisms responsible for automatic regulation of the ratio of stroma to parenchyma operate at the tissue level. The relations between parenchyma and stroma of the myocardium during the development of diffuse cardiosclerosis in these experiments can be explained on the basis of Tsellarius' concept [6] of dynamic equilibrium between the desmoplastic and desmolytic processes in the stroma and the leading role of the functional state of the parenchymatous structures in the regulation of these processes.

Changes in the quantitative relations between stromal and parenchymatous structures in the myocardium cannot be considered separately from data on the relative weight of the heart. The measurements made showed some increase in the relative weight of the heart in the experimental animals (3.31 ± 0.2 g/kg compared with 2.88 ± 0.17 g/kg in the control). With this fact in mind, as well as the results of light and electron microscopy, it can be concluded that in the myocardium of animals with diffuse cardiosclerosis associated with experimental atherosclerosis there is an increase in the quantity of both parenchyma and stroma, but that the greatest increase is observed in the content of connective tissue.

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