STEREOLOGIC ANALYSIS OF STRUCTURES OF THE PARENCHYMA AND STROMA OF HYPERTROPHIED MYOCARDIUM IN ACUTE ARTERIAL HYPERTENSION

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The development of hypertrophy of the heart is a complex general pathological process in which both parenchymatous and stromal cells are involved [3-5, 16]. An understanding of the mechanisms of interaction of these two tissue components will determine success in the prediction of hypertrophic states of the myocardium of different genesis [10]. On the practical level research into the morphogenesis of cardiac hypertrophy in experimental arterial hypertension is very important [7, 9, 11].

The aim of this investigation was to study, by quantitative morphological methods (morphometry and stereology), the structural and functional changes taking place in the parenchyma and stroma of the hypertrophied myocardium in experimental renal arterial hypertension. Arterial hypertension was induced in 35 male Wistar rats with an initial body weight of 20.9-20.3 g. Initially, under open ether anesthesia, the diameter of a segment of the aorta located between the renal arteries was measured in the animals. A spiral coil with 2 to 2.5 turns was made from titanium wire 0.1 mm in diameter. This coil was applied to the previously mobilized segment of the aorta, to reduce its diameter by two thirds, and this led to a disturbance of the circulation in the left renal artery. The ischemia thus produced provides a model of renovascular hypertension.

The systolic arterial pressure was measured by means of a transducer with rubber cuff, applied to the animals's tail. The signal from the transducer was recorded on a Mingograph-34 apparatus (Elema-Schonander, Sweden). Intact normotensive rats of the same line and the same age were used as the control.

The animals were decapitated 5, 12, 15, 20, and 35 days after the operation. After the heart had stopped beating in a cold chamber it was weighed to determine absolute and relative weight, and for primary fixation it was immersed in 4% paraformaldehyde, pH 7.4, in 0.1 M phosphate buffer.

To prepare paraffin sections, samples from different parts of the myocardium were postfixed in 10% neutral formalin, and the standard formula was used to prepare tissues for histological study. Sections were stained with hematoxylin and eosin and by Van Gieson's method, with counterstaining of elastic fibers with resorcin–fuchsin and by the alcian blue–PAS-hematoxylin method. To prepare Epon–Araldite sections, specimens from the left papillary muscles were postfixed in 2% OsO_4, and semithin sections 1 μm thick were stained with azure II.

The light-optical investigation of sections of myocardial tissue was carried out by means of the "Docuval" universal biological photomicroscope. Stained and unstained dewaxed sections were examined in direct and polarized light.

A quantitative morphological analysis of parenchymatous–stromal interrelations in the myocardium was carried out on the 5th, 12th, and 35th days. Using the MOV-1-15 ocular micrometer, the mean diameter of the cardiomyocytes for each group of animals was determined in

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TABLE 1. Quantitative Morphological Characteristics of Parenchymatous–Stromal Interrelations of the Myocardium in Wistar Rats with Arterial Hypertension (M ± m).

Parameter	Control	Time after operation, days		
		9	12	35
Morphometric investigations				
Body weight, g	205,0±25,0	212,3±7,9	216,7±8,8	250,0±11,5
Absolute weight of heart, mg	970,0±70,0	1083,2±52,4	1230,0±30,6*	1356,7±56,7*
Relative weight of heart, mg/g body weight	4,76±0,24	5,12±0,21	5,97±0,23*	5,47±0,48
Diameter of cardiomyocytes, μm	15,9±0,2	16,5±0,4	24,5±0,7***	24,4±0,5***
Stereologic investigations				
Relative volume (V^t_{Vi}), mm^3/m^3				
of cardiomyocytes	839,3±15,0	827,6±16,5	821,4±17,8	831,4±11,2
of cardiomyocyte nuclei	8,7±1,6	10,7±2,3	11,0±2,2	9,4±1,0
of capillaries	43,2±3,7	40,9±4,3	37,3±1,3	33,2±6,5
of endothelial cells	19,2±1,3	18,8±2,0	19,8±1,2	18,3±1,9
of connective-tissue cells	13,9±0,9	13,2±0,7	14,0±0,3	8,7±1,1***
of ground substance and connective tissue fibers	75,7±8,5	88,8±9,3	96,5±8,7***	99,0±9,6***
Relative surface area ($S^{ti}_{V_i}$), m^2/cm^3				
of cardiomyocytes	0 1013±0,0068	0,0878±0,0056	0,0666±0,0082*	0,0678±0,0061*
of cardiomyocyte nuclei	0,0066±0,0004	0,0067±0,0005	0,0064±0,0004	0,0058±0,0009
of capillaries	0,0361±0,0031	0,0323±0,0027	0,0275±0,0021	0,0262±0,0041
of connective-tissue cells	0,0182±0,0020	0,0188±0,0058	0,0206±0,0064	0,0121±0,0006*
Surface-to-volume ratio (Sv_i/Vv_i), m^2/cm^3				
of cardiomyocytes	0,121±0,007	0,106±0,008	0,081±0,009*	0,082±0,008*
of cardiomyocyte nuclei	0,762±0,062	0,627±0,083	0,629±0,142	0,616±0,066
of capillaries	0,836±0,084	0,790±0,078	0,743±0,083	0,823±0,109
of connective-tissue cells	1,309±0,169	1,426±0,102	1,471±0,085	1,419±0,129
Ratio of bulk density of stroma to bulk density of parenchyma ($V_{V_{st}}/V_{V_{cmc}}$)	0,179±0,025	0,193±0,021	0,202±0,026	0,190±0,017
Ratio of bulk density of capillaries to bulk density of cardiomyocytes ($V_{V_{cap}}/V_{V_{cmc}}$)	0,051±0,001	0,049±0,005	0,045±0,002*	0,040±0,008
Ratio of surface density of capillaries to bulk density of cardiomyocytes ($S_{V_{cap}}/V_{V_{cmc}}$), m^2/cm^3	0,043±0,005	0,039±0,005	0,032±0,003	0,031±0,005

Legend: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

semithin sections. In longitudinal sections through the left papillary muscles, under a magnification of 1000, the relative volume and relative surface area of the parenchymatous (cardiomyocytes and their nuclei) and stromal structures were estimated by stereologic methods [1, 5, 8]. Capillaries, endothelial cells, connective-tissue cells (without any attempt at their differentiation), and total ground substance plus connective-tissue fibers were identified in the stroma. On the basis of these primary data, the surface-to-volume ratio of the tissue structures, the ratio of relative volume of some structures to the relative volume of others, and surface-to-volume ratio of some structures compared with others were calculated. The results were subjected to statistical analysis by Student's test. The structural-metabolic state of the myocardial tissue was subjected to information analysis [13] in the course of its hypertrophy.

EXPERIMENTAL RESULTS

Elevation of the systolic arterial pressure (from 108 ± 55 mm Hg in the control to 205 32 mm Hg on the 35th day after the operation) caused hypertrophy of the heart in the experimental animals, as shown by an increase in the absolute and relative weight of the heart and in the diameter of the cardiomyocytes (Table 1).

In the earliest stages after the operation (5th day) edema of the myocardial tissue was observed, and this caused an increase in the total relative volume of ground substance and connective-tissue fibers. There was a parallel decrease in the bulk density of the capillaries (Table 1). Changes such as these in the relative volume of the interstitial tissue in the early stages of development of cardiac hypertrophy, due to tissue edema, have been described in stenosis of the subdiaphragmatic portion of the aorta [14], and they reflect

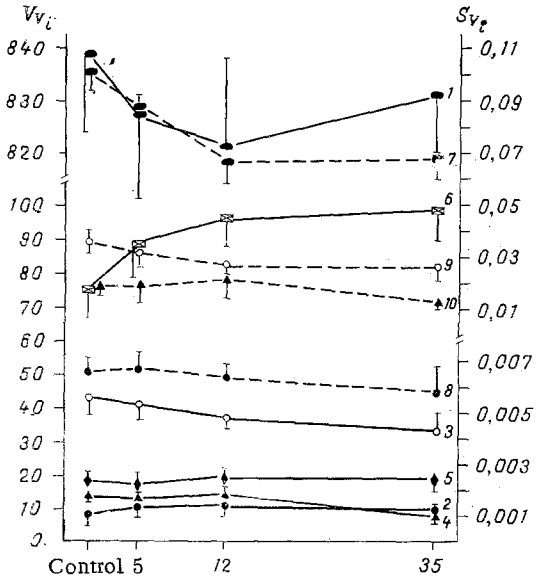

Fig. 1

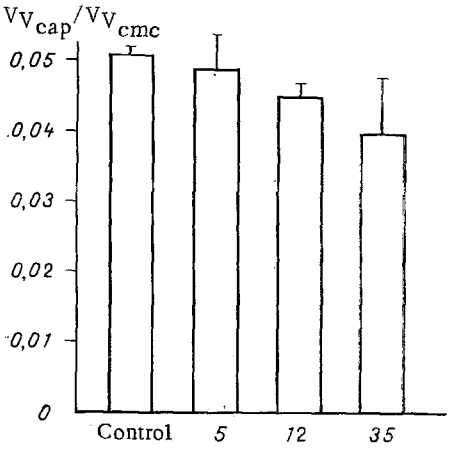

Fig. 2

Fig. 1. Results of measurement of primary stereologic parameters of myocardial tissue structures in Wistar rats with arterial hypertension. Abscissa, time from beginning of experiment (in days); ordinate: on left — bulk density (in mm^3/cm^3), on right — surface density (in m^2/cm^3); 1, 7) cardiomyocytes, 2, 8) cardiomyocyte nuclei, 3, 9) capillaries, 4, 10) connective-tissue cells, 5) endothelial cells, 6) ground substance and connective-tissue fibers.

Fig. 2. Ratio of bulk density of capillaries to bulk density of cardiomyocytes in Wistar rats with arterial hypertension. Abscissa, time after beginning of experiment (in days); ordinate, volume-to-volume ratio of tissue structures (number).

the response of the tissue to the operation. In intramural arteries signs of spasm were observed: The elastic membranes were twisted into coils, the smooth-muscle cells were in a state of contraction, and nuclei of the endotheliocytes were located close together and projected visibly into the lumen of the vessels.

At these same times uneven staining of the cardiomyocytes with eosin and picric acid was observed. The bulk and surface density of the parenchymatous cells was reduced (Fig. 1). The relative volume of the cardiomyocyte nuclei, on the other hand, was increased by 23%, whereas their surface density was virtually unchanged. This led to a decrease in the surface-to-volume ratio of these structures. The changes noted in the cardiomyocyte nuclei are evidence that their hypertrophy takes place as early as during the first day after the increased load on the heart.

By the 12th day after the operation the mosaic character of staining of the cardiomyocytes with acid dyes was intensified, and they showed uneven contraction. The cardiomyocytes at this time were definitely hypertrophied (their diameter was increased by 54%) and the bulk density of their nuclei was increased by 26%. Hypertrophy of the cardiomyocytes at this and subsequent times was accompanied by a significant decrease in their surface density and their surface-to-volume ratio, as the writers have also demonstrated in cardiac hypertrophy in old age [4]. A very mild degree of edema still persisted, as shown by the enlarged intercellular spaces. However, a polarization microscopic investigation showed that the increase in bulk density of the noncellular component of the interstitital connective tissue was due mainly to accumulation of bundles of collagen fibers.

Hyperplasia and hypertrophy of the smooth-muscle cells were observed in the middle layer of the walls of the intramural arteries, the elastic membranes were thickened, and were often irregularly coiled. Moderate intervascular and perivascular sclerosis developed. Changes in the capillaries were manifested as a marked decrease in their bulk and surface density (by 14 and 24% respectively). Delay in growth of the capillaries also was observed

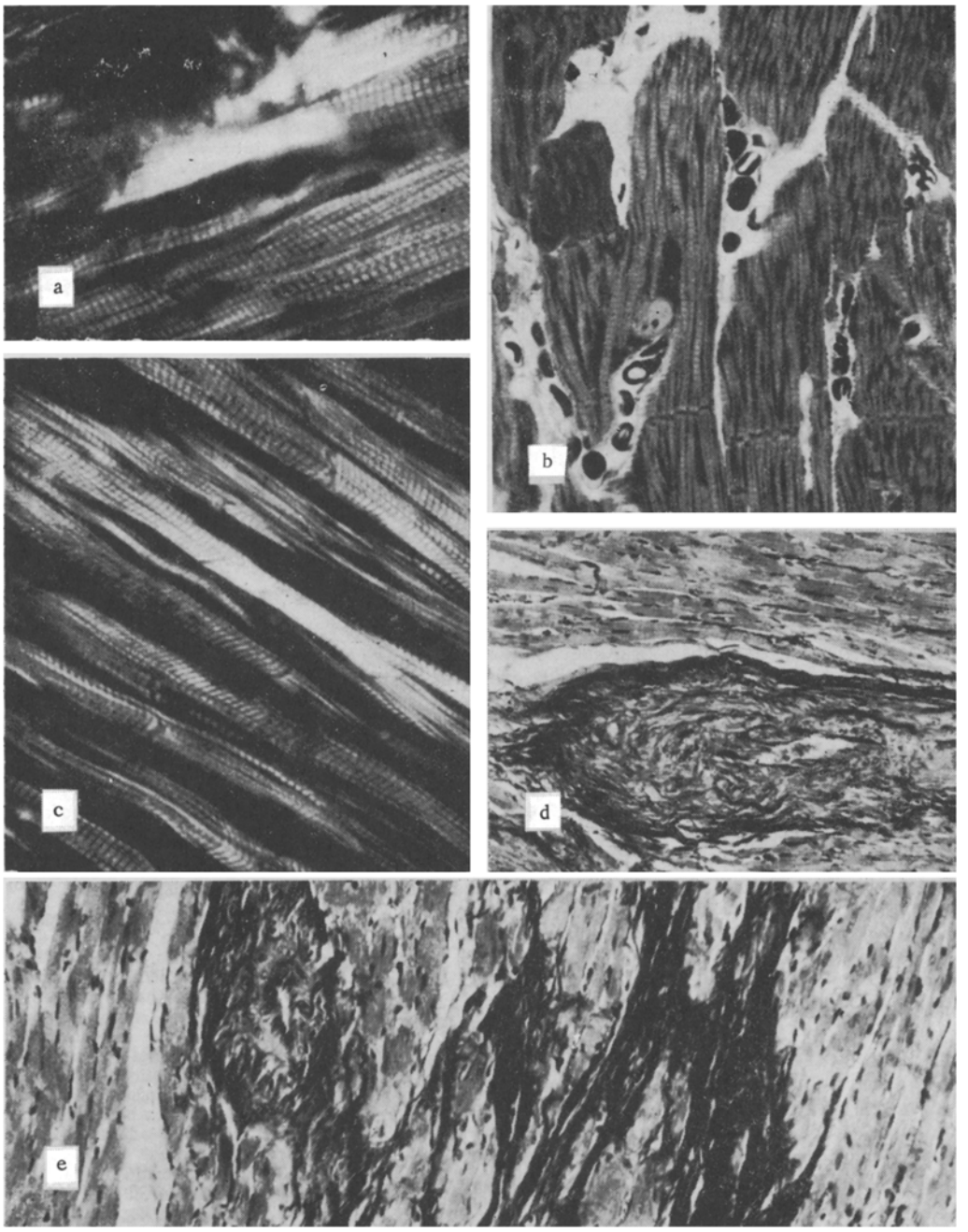

Fig. 3. Morphological changes in myocardium of Wistar rats with arterial hypertension 35 days after disturbance of renal circulation. a) Contractures of myofibrils in damaged cardiomyocytes. Photograph of unstained preparation in polarized light. 1250 ×. b) Hypertrophied muscle fibers. Semithin section, stained with azure II. 1250 ×. c) Solitary atrophied muscle fibers among hypertrophied fibers. Hematoxylin and eosin, photograph in polarized light. 1250 ×. d) Thickening of wall of intramural artery on account of hyperplasia and hypertrophy of smooth-muscle cells with development of myoelastofibrosis, leading to narrowing of lumen. Stained by Van Gieson's method with resorcin–fuchsin. 400 ×. e) Perivascular and interstitial sclerosis of myocardium. Stained by Van Gieson's method. 600 ×.

relative to the increase in size of the heart muscle cells, as shown by a decrease in the volume-to-volume and surface-to-volume ratio of capillaries to cardiomyocytes (Fig. 2). These quantitative parameters were chosen [6] as representative, for they reflect structural and functional relations between the two most important structural units of the tissue microregion [2].

TABLE 2. Information Parameters of Stereologic Investigation of Myocardium of Wistar Rats with Arterial Hypertension

Period of experiment, days	Test object	Entropy, binary units	Relative entropy	Excess, %
Control	Parenchyma and stroma	0,934	0,361	63,8
	Stroma	1,709	0,855	14,5
5	Parenchyma and stroma	0,967	0,374	62,6
	Stroma	1,633	0,817	18,3
12	Parenchyma and stroma	0,998	0,386	61,4
	Stroma	1,595	0,798	20,2
35	Parenchyma and stroma	0,953	0,368	63,1
	Stroma	1,467	0,733	26,7

The number of cardiomyocytes showing injury of contracture type, which was clearly visible in myocardial tissue examined in polarized light (Fig. 3a), was increased in the rat myocardium 35 days after constriction of the aorta (Fig. 3a). Among the hypertrophied muscle fibers (Fig. 3b) some which were atrophied were occasionally seen (Fig. 3c), evidence of the mobility of the compensatory-adaptive reactions developing in the heart. However, the mean diameter of the cardiomyocytes remained increased. The bulk and surface density and the surface-to-volume ratios of cardiomyocytes and their nuclei were reduced.

Significant changes also took place in the intramural arteries. Their walls were considerably thickened (as a result of hyperplasia and hypertrophy of the smooth-muscle cells) with the development of microelastofibrosis; the lumen of the vessels was sharply contracted (Fig. 3d). Marked perivascular sclerosis was observed, and spread to adjacent interstitial tissue (Fig. 3e).

At the tissue microregion level a further decrease was observed in the bulk and surface density of the microcirculation, although the relative volume of the endothelial cells showed a very small decrease. This led to a further decrease in the volume-to-volume and surface-to-volume ratios of the capillaries to the cardiomyocytes (Table 1). The relative volume and relative surface area of the connective-tissue cells were significantly reduced by (by 37 and 34% respectively), whereas the bulk density of the ground substance and of the interstitial connective-tissue fibers was significantly increased by 31%.

The total relative volume of the cells, fibers, and ground substance of connective tissue showed a marked increase on the 12th and 35th days after disturbance of the renal circulation (from 89.6 ± 9.2 to 110.5 ± 8.9 and 107.7 ± 10.0 mm^3/cm^3). This result is in agreement with data obtained by other workers who showed an increase in the collagen content in the second week after development of cardiac hypertrophy as a result of stenosis of the ascending and descending aortas [15, 17]. However, other investigators found that on the 8th day after constriction of the subdiaphragmatic part of the aorta the relative volume of the connective-tissue cells and of the extracellular space amounted to 9.4%, just as in the control; in other words, there was proportionate growth of the parenchymatous and stromal components [14]. According to our own data, the increase in volume of the stromal cells precedes hypertrophy of the cardiomyocytes, as will be clear from the increase in the ratio of bulk density of the stroma to relative volume of parenchyma (Table 1).

Information analysis revealed an increase in entropy and relative entropy and a decrease in excess of the myocardial tissue in the course of this hypertrophy (Table 2). The greatest value of entropy and the smallest value of excess were found on the 12th day after creation of the model of renovasal hypertension, evidence of the greater indeterminacy of the system (myocardial tissue) at this period. Under these circumstances the entropy of the parenchyma did not change significantly whereas entropy and relative entropy of the stroma decreased successively in the course of development of cardiac hypertrophy (Table 2), indicating the orderliness of the structural metabolic processes in the stroma. During the development of a general pathological process such as myocardial hypertrophy under conditions of experimental arterial hypertension, the parenchyma and stroma were thus found to differ in informative capacity.

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