bances described above develop as early as in the first few hours after injection of endotoxin; consequently, hepatic failure formed long before the appearance of its clinical manifestations.

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MORPHOLOGY OF TISSUE COMPONENTS OF LAYERS OF THE RAT MYOCARDIUM IN THE EARLY STAGES OF MYOCARDIAL HYPERTROPHY

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An elegant hypothesis has now been put forward to explain the dynamics of functional and structural changes in the myocardium during the development of adaptive reactions [4, 5]. However, most investigations have not taken account of local differences in the structure of the myocardium, despite data in the literature on structural and metabolic differences between its layers, and also differences in their resistance to experimental procedures [1, 7, 9-11].

This paper describes a morphometric study of different layers of the left ventricular myocardium of Wistar rats at the beginning of the stable stage of myocardium hypertrophy [4], caused by narrowing by 50% the lumen of the abdominal aorta, in order to identify differences in the response of tissue components of the subendocardial, subepicardial, and intermediate layers to changes in the intensity of cardiac function. Isolation of the layers and zones for the investigation was described previously [6].

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TABLE 1. Morphologic Parameters of Tissue Components of Different Layers of Left Ventricular Myocardium of Rats during Formation of Hypertrophy (M ± m)

Parameter	Subendocardial layer			Intramural layer			Subepicardial layer		
	normal	construction of aorta	change, %	normal	construction of aorta	change, %	normal	construction of aorta	change, %
V(m)	0.820±0.007	0.710±0.007	−13.4	0.720±0.008	0.760±0.008*	+5.6	0.699±0.009	0.790±0.008	+12.9
S(m)	48.56±0.85	38.6±2.9	−20.5	48.68±0.87	40.15±3.24	−17.5	58.62±1.18	35.20±2.40	−40.0
V(c)	0.063±0.004	0.077±0.005	+22.2	0.132±0.007	0.073±0.006	−44.7	0.169±0.007	0.143±0.008	−15.4
S(c)	9.84±0.28	18.05±1.38	+83.4	19.70±0.31	10.60±0.90	−46.2	27.30±0.50	18.00±1.42	−34.0
V(ct)	0.173±0.008	0.283±0.008	+63.6	0.273±0.009	0.237±0.008	−13.2	0.302±0.010	0.212±0.005	−30.0
D(m)	12.70±0.02	12.04±0.43*	−5.2	11.88±0.02	13.80±0.41	+16.2	9.28±0.02	10.30±0.40	+11.0
S(m)/V(m)	54.90±0.67	47.07±0.35	−14.3	62.60±0.38	52.83±0.19	−15.6	79.80±0.29	44.56±0.55	−44.2
S(m)/S(c)	4.93±0.05	2.14±0.02	−56.6	2.48±0.06	3.79±0.09	+53.8	2.15±0.10	2.00±0.07*	−7.0
V(m)/V(c)	12.95±0.50	10.65±0.36	−17.8	5.76±0.37	10.41±0.28	+80.7	4.13±0.25	5.53±0.17	+33.9
V(c)/V(ct)	0.408±0.025	0.270±0.021	−33.8	0.509±0.051	0.310±0.027	−39.1	0.570±0.031	0.680±0.019	+19.3

Legend. *$p > 0.05$, in all other cases $p < 0.05$.

EXPERIMENTAL METHOD

Experiments were carried out on six male Wistar rats weighing 180-200 g. Under pentobarbital anesthesia (0.05 mg/kg) laparotomy was performed, the abdominal aorta isolated (the segment between the origin of the renal arteries), its diameter was measured with calipers, and by using calibrated probes, a ligature was applied, reducing the lumen of the aorta by 50%. The wound was closed in layers. Ten days later, under pentobarbital anesthesia, thoracotomy was performed. The heart was perfused with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 10 min and then removed. Thin disks 0.5-1.0 mm thick were excised from the middle third of the plane perpendicular to the axis of the left ventricle. The disks were postfixed with 1% osmium tetroxide solution in phosphate buffer, dehydrated, and embedded in Epon. Blocks were cut from the disks and used to obtain semithin sections with transverse orientation of the cardiomyocytes. The sections were stained with toluidine blue and measured under the MBI-3 microscope with magnification of 1350 times, using a universal multipurpose ocular morphometric grid and an ocular-micrometer [2].

The following parameters were determined: the relative volume of the myocytes V(m); the-relative volume of the connective tissue V(ct); the relative volume of the capillaries V(c); the surface area of the myocytes S(m); the surface area of the capillaries S(c); the surface area of the cardiomyocytes expressed per unit volume of the cardiomyocytes S(m)/V(m); the surface area of a cardiomyocyte expressed per unit surface area of a capillary S(m)/S(c); the volume of the cardiomyocytes expressed relative to the volume of the capillaries V(m)/V(c); the volume of the capillaries as a ratio of the volume of the connective tissue V(c)/V(ct). The mean diameter of the cardiomyocytes D(m) also was calculated. For the calculations and statistical analysis, by Student's t test, and distinguishing primary and secondary objects [3], an Amstrad PC 1640 computer was used.

EXPERIMENTAL RESULTS

In a previous study [6] the myocardium was divided into four different layers: subendocardial, subepicardial, and outer and inner intramural. Since differences between the two last layers were not very great, they were combined into one layer in the present investigation, namely intramural. The results of the measurements and calculations are given in Table 1. They demonstrate marked zonality of changes in the quantitative parameters of the tissue components for the various layers of the myocardium on the 10th day of development of cardiac hypertrophy. In the subendocardial layer a decrease in the relative volume of the myocytes and their surface area (13.4% and 20.5% respectively) and an increase in the relative volume of the connective tissue (63.6%) were observed, evidently due to interstitial tissue edema which was present. The mean diameter of the cardiomyocytes was virtually unchanged (differences not significant). Attention is drawn to the marked increase in volume (22.2%) and surface area of the capillary bed (83.4%), indicating an increase in the regional blood flow. Changes in these parameters led to an improvement in the relative surface area of the myocytes and capillaries, maintaining metabolism in the cardiomyocytes [1, 8]. In the intramural and subepicardial layers the relative volume of the myocytes was increased, but to a different degree (12.9% and 5.6% respectively). Changes in the surface area of the myocytes may indicate an increase in size of the cells. Evidence in support of this view was given by an increase in the mean diameter of the cardiomyocytes in the intramural (16%) and subepicardial (11%) layers. The relative volume of the

connective tissue in these layers was significantly reduced, but again to different degrees: intramural layer 13.2%, subepicardial layer 30%. Changes in the surface area and volume of the blood capillaries in the subepicardial and intramural layers were identical in character: both decreased, but to different degrees. They were more marked in the intramural layer. Comparison of these parameters for all the layers leads to the conclusion that the blood flow in the myocardium at the period of formation of hypertrophy studied in these experiments undergoes redistribution, a conclusion in accordance with data in the literature obtained by the labeled microspheres method [12]. Differences in the direction of the changes of the relative surface area of myocytes and capillaries are noteworthy. This parameter had a tendency to decrease in the intramural layer (differences not significant), but increased significantly in the subepicardial layer, possible evidence of improved conditions for metabolism in the intramural layer.

A study of the morphometric characteristics of tissue components in different layers of the hypertrophied myocardium thus revealed differences in morphometric parameters of the muscular, connective-tissue, and vascular components of the heart. These variations are evidence of differences in reactivity of the layers of the myocardium to an increased load and to different rates of their adequate structural changes, and in turn, these findings may be connected with definite specialization of these myocardial layers in the cyclic activity of the heart.

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