HISTOSTEREOLOGIC ANALYSIS OF THE PARENCHYMA AND STROMA OF THE ATROPHIED MYOCARDIUM IN STARVATION

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The reduced functional load during starvation or protein deficiency leads to reduced protein synthesis in heart muscle [11-13, 15]. Inhibition of protein metabolism associated with interruption of the supply of structural material with the food causes delay in the renewal of contractile proteins, and the unsynchronized changes in the ultrastructural organization of cardiomyocytes are connected with this [3, 4]. The principal structural manifestation of inhibition of protein synthesis when the functional load is reduced is atrophy of the myocardium [2, 6, 9]. Quantitative morphological investigations of the myocardium during starvation have revealed considerable changes in the architectonics of the cardiomyocytes during their atrophy [3, 7]. However, the character and direction of relations between the parenchyma and connective tissue in heart muscle during starvation have not been adequately studied.

The aim of this investigation was a morphometric and stereologic study of interrelations between parenchymatous and stromal tissues during the development of myocardial atrophy in rats subjected to starvation.

EXPERIMENTAL METHOD

Experiments were carried out on 32 mature male Wistar rats. The animals (eight rats in each group) were placed in individual cages and totally deprived of food for 2, 4 and 8 days, but were allowed water ad lib. Control animals (eight rats) received the standard laboratory diet. At the end of the experiment all the animals were weighed and decapitated. The hearts were quickly removed and placed in a cold chamber until they completely stopped beating, after which the heart and the left ventricle were weighed. Pieces of tissue for light-optical investigation were fixed in 10% neutral formalin solution, and for electron-microscopic study in a 4% solution of paraformaldehyde. The material was postfixed in 1% osmium tetroxide solution, dehydrated, and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on an LKB Ultratome (Sweden) stained with uranyl acetate and lead citrate, and examined in the JEM-100B electron microscope. Histostereologic analysis of the tissue organization of the rat myocardium [5] was carried out on semithin (1 μ) longitudinal sections through the papillary muscle of the left ventricle, stained with azure II, under a magnification of 1000. A Docuval universal biological photomicroscope and ocular grid with test lines ($n = 36$, $P = 72$) were used. The bulk and surface density of the cardiomyocytes and their nuclei and the relative volume of the stroma of the myocardium were determined. The bulk and surface density of the capillaries and interstitial cells and the relative volume of the endotheliocytes were calculated separately in the connective tissue, and these parameters were determined collectively for the fibers and ground substance of the connective tissue. Secondary calculated indices characterizing bulk and surface-bulk interrelations of the main components of the parenchyma and stroma were obtained: the surface to volume ratio of the cardiomyocytes and their nuclei, the capillaries, and connective-tissue cells, the ratio of the bulk density of the stromal components of the myocardium to the relative volume of the cardiomyocytes, and the

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TABLE 1. Results of Morphometric and Stereologic Investigation of the Heart of Wistar Rats during Complete Starvation

Parameter	Control	Duration of starvation, days		
		2	4	8
Morphometric characteristics of the heart and muscle fibers				
Body weight, g	293,3±6,3	271,2±8,2	250,0±3,3**	207,2±7,2**
Absolute weight of the heart, mg	892,5±18,9	860,3±17,0	834,0±15,4	745,0±24,7**
Relative weight of the heart, mg/g body weight	2,99±0,05	3,17±0,06	3,34±0,05*	3,59±0,06**
Absolute weight of the left ventricle, mg	590,0±4,2	570,0±8,3	549,6±11,6	500,9±13,0**
Relative weight of the left ventricle, mg/g body weight	1,98±0,04	2,10±0,04	2,20±0,04*	2,42±0,04**
Diameter of cardiomyocytes, μ	15,90±0,10	15,40±0,10	15,00±0,09*	13,30±0,10***
Stereologic characteristics of tissue organization of myocardium				
Relative volume ($V_{V_i}^t$, mm³/cm³ of:				
Parenchyma	873,2±7,4	855,8±6,1	838,4±5,7*	855,9±4,6
Cytoplasm of cardiomyocytes	859,1±7,7	842,1±7,0	825,1±4,6*	843,7±4,5
Nuclei of cardiomyocytes	14,1±0,5	13,7±1,1	13,3±1,7	12,2±1,2
Stroma	126,8±7,4	144,2±6,1	161,6±5,7*	144,1±4,6
Capillaries	50,7±3,5	53,9±4,0	57,1±4,3	47,5±1,9
Endothelial cells	10,1±1,9	9,8±1,0	9,5±0,9	7,9±1,0
Connective tissue cells	4,2±0,7	6,1±1,0	7,9±1,1	5,9±1,2
Fibers and ground substance	61,8±11,4	74,4±7,2	87,1±4,5	82,8±4,1
Relative surface area ($S_{V_i}^t$, m₂/cm³) of:				
Cardiomyocytes	0,1125±0,0046	0,1167±0,0023	0,1208±0,0031	0,1353±0,009**
Nuclei of cardiomyocytes	0,0085±0,0005	0,0085±0,0005	0,0084±0,0009	0,0076±0,0002
Capillaries	0,0316±0,0019	0,0316±0,0020	0,0316±0,0023	0,0299±0,0024
Connective tissue cells	0,0026±0,0001	0,0046±0,0003	0,0066±0,0008**	0,0050±0,0010*
Surface to volume ratio (S_{V_i}/V_{V_i}, m²/cm³) of:				
Cardiomyocytes	0,131±0,006	0,136±0,004	0,146±0,003	0,160±0,001*
Nuclei of cardiomyocytes	0,604±0,049	0,621±0,050	0,638±0,040	0,640±0,063
Capillaries	0,623±0,005	0,586±0,010	0,554±0,016*	0,642±0,046
Connective tissue cells	0,649±0,065	0,750±0,050	0,850±0,080	0,850±0,071
Ratio of bulk density (V_{V_i}/V_{V_j}, number) of:				
Nuclei to sarcoplasm	0,0165±0,0006	0,0163±0,0010	0,0160±0,0021	0,0144±0,0015
Stroma to parenchyma	0,145±0,009	0,169±0,009	0,193±0,008*	0,168±0,003
Capillaries through cardiomyocytes	0,058±0,004	0,063±0,003	0,068±0,005	0,054±0,002
Connective tissue cells to cardiomyocytes	0,0048±0,0008	0,0071±0,0010	0,0094±0,0014*	0,0069±0,0014
of fibers and of ground substance to cardiomyocytes	0,0709±0,0138	0,0870±0,0060	0,1039±0,0058	0,0978±0,0051
Ratio of surface density of capillaries to bulk density of cardiomyocytes, m²/cm³	0,0361±0,0022	0,0369±0,0021	0,0377±0,0029	0,0350±0,0029
Ratio of surface density (S_{V_i}/S_{V_j}, number) of:				
Capillaries through cardiomyocytes	0,283±0,029	0,271±0,004	0,263±0,022	0,222±0,019

Legend. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared with control.

ratio of the surface density of the capillaries to the bulk density and surface density of the cardiomyocytes. The diameter of the cardiomyocytes was measured in transverse and longitudinal semithin sections through the muscle fibers with the aid of an MOV-1-15× ocular micrometer. The significance of differences between the mean values was determined by Student's test (level of significance $p < 0.05$). Values of entropy, relative entropy, and excess were calculated by information analysis for the myocardial tissue in the course of the experiment.

EXPERIMENTAL RESULTS

Complete starvation of the rats led to a progressive decrease of their body weight and of the absolute weight of the heart by 30.5 and 16.5%, respectively (Table 1). The relative weight of the heart increased in the course of the experiment, due to the more rapid regression of body weight compared with weight of the heart. The diameter of the cardiomyocytes decreased significantly by 5.7% as early as the 4th day of starvation, and a more marked decrease (by 16.4%) was observed on the 8th day. Reduction of the absolute weight of the hearts and of the diameter of the cardiomyocytes in rats after starvation is evidence of the development of myocardial atrophy [6, 7]. Correlation analysis revealed positive correlation between the change in weight of the heart and the diameter of the cardiomyocytes in the presence of myocardial atrophy due to starvation (r = 0.630).

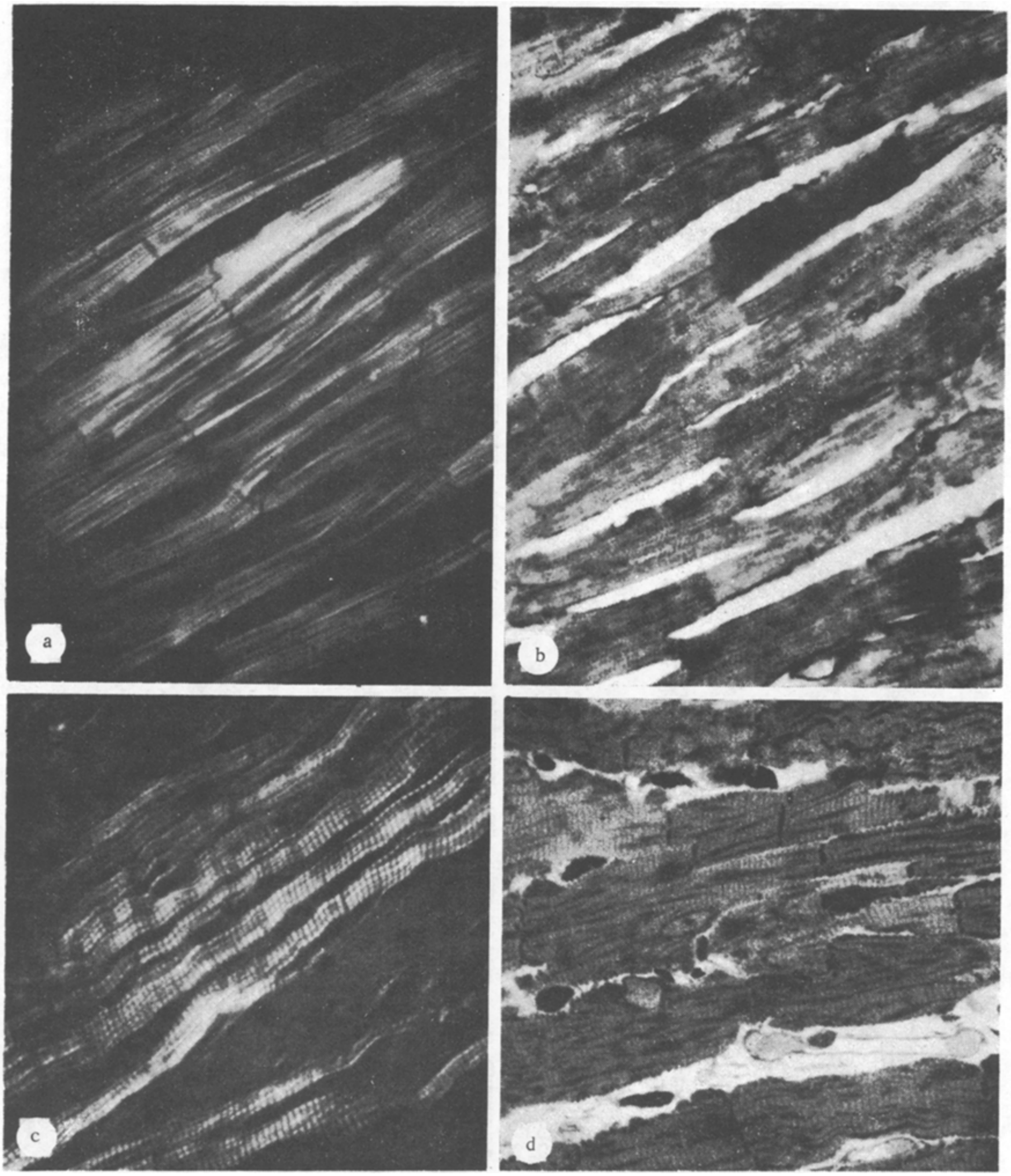

Fig. 1. Tissue changes in rat myocardium during starvation. a) Contractures of myofibrils in cardiomyocytes during starvation for 4 days (photographed in polarized light, 1250 ×); b) the same, PAS reaction; c) reduction in thickness of myocardial muscle fibers during starvation for 8 days (photographed in polarized light, 1200 ×); d) the same, stained with hematoxylin and eosin.

The results of correlation analysis and the results of the light-optical and electron-microscopic investigations showed that the decrease in weight of the heart in the starving animals was caused by a decrease in both the size and the number of muscle cells.

In the early stages of the experiments (2nd-4th day), for instance, against the background of marked signs of circulatory disturbance (hyperemia, edema, small foci of diapedetic hemorrhages), acute structural changes also were found in individual muscle fibers of the contracture type (Fig 1a, b). These processes were evidently due to stress induced by hunger [14]. In the cases of irreversible contracture damage, the muscle cells underwent coagulation necrosis and were resorbed by macrophages. A diffuse proliferative cellular reaction of the stroma was observed under these circumstances.

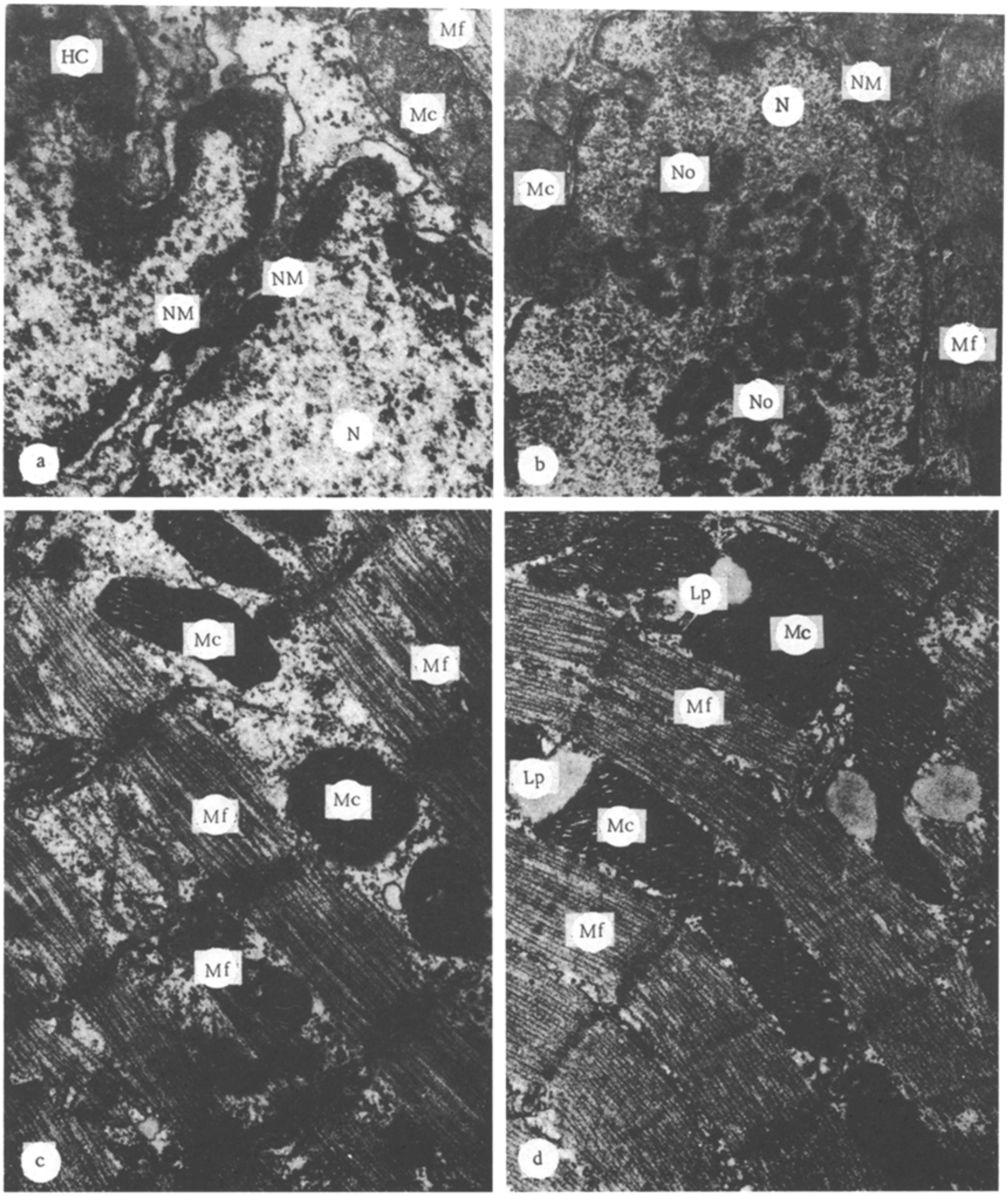

Fig. 2. Ultrastructural changes in cardiomyocytes of rats during starvation for 8 days. a) Increase in heterochromatin content, deep invaginations of nuclear membrane (13,000 ×); b) fragmentation of nucleoli (20,000 ×); c) foci of lysis of myofibrils (9000 ×); d) increase in number of lipid drops in myofibrillary zone (8300 ×). Hc) Heterochromatin; Lp) lipid drop; Mf) myofibril; Mc) mitochondrion; N) nucleus; No) nucleolus; NM) nuclear membrane.

In the later stages of the experiment the hyperemia and edema became a little less marked. The muscle fibers were distinctly reduced in thickness (Fig. 1c, d). At the ultrastructural level signs of a fall in the level of protein synthesis were observed in the atrophied cardiomyocytes [10]: a change in the ratio between fibrillary and granular components of the nucleolus with reduction of the latter (Fig. 2a), segregation and fragmentation of the nucleoli (Fig. 2b), punctate lysis of the myofibrils, translucency of the matrix and destruction of the cristae of the mitochondria (Fig. 2c). In the sarcoplasmm the number of ribosomes was sharply reduced, but the number of glycogen granules and lipid drops increased (Fig. 2d). It can be tentatively suggested that a sharp decline or total cessation of protein synthesis in individual cells may also cause their death [10].

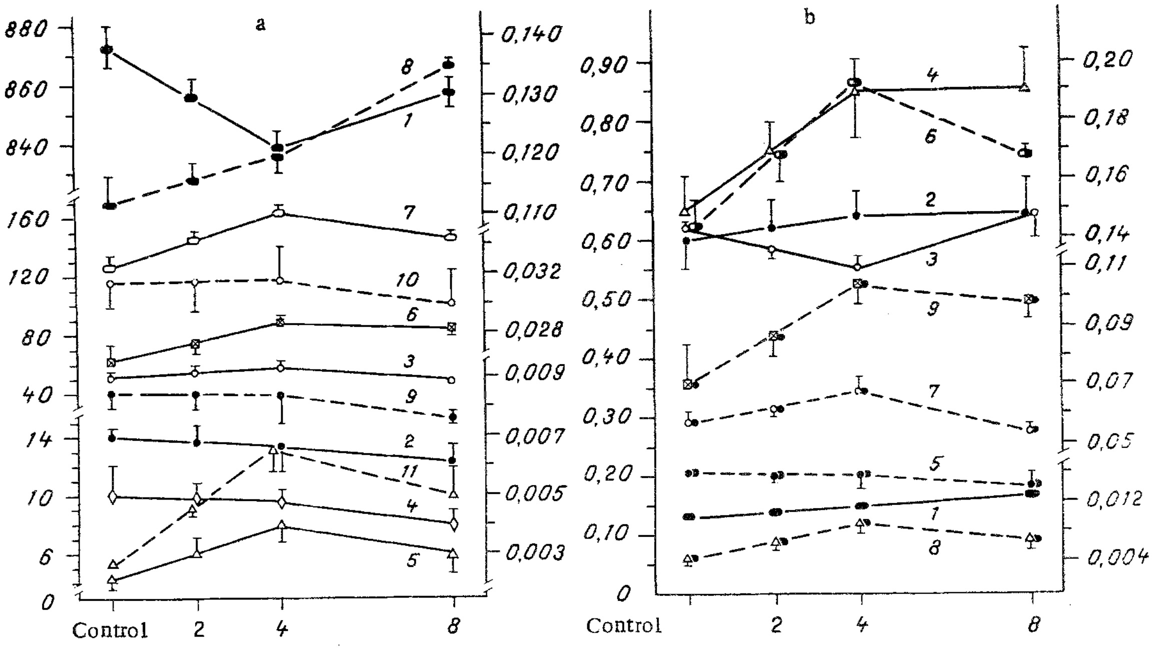

Fig. 3. Results of histostereologic analysis of rat myocardium during starvation. a) Primary stereologic parameters: abscissa, duration of starvation (in days); ordinate: left — bulk density (V_{V_i}, mm^3/cm^3), right — surface density (S_{V_i}, m^2/cm^3). 1, 8) Cardiomyocytes; 2, 9) nuclei of cardiomyocytes; 3, 10) capillaries; 4) endothalial cells; 5, 11) connective-tissue cells; 6) fibers and ground substance of connective tissue; 7) stroma. b) Secondary stereologic parameters: abscissa, duration of starvation (in days); ordinate: left — surface to volume ratio (S_{V_i}/V_{V_i}, m^2/cm^3), right — volume ratio (V_{V_i}/V_{V_i}, number). 1) Cardiomyocytes, 2) nuclei of cardiomyocytes, 3) capillaries 4) connective-tissue cells, 5) nucleus/cytoplasm of cardiomyocytes, 6) stroma/parenchyma, 7) capillaries/cardiomyocytes, 8) connective-tissue cells/cardiomyocytes, 9) fibers and ground substance of connective tissue/cardiomyocytes.

Stereologic analysis revealed definite changes in the tissue organization of the rat myocardium after prolonged starvation (Fig. 3). On the 4th day of starvation the bulk density of the muscle tissue was significantly reduced by 4% ($p < 0.05$), but by the end of the experiment it did not differ significantly from the control (Table 1). The surface density and surface to volume ratio of the cardiomyocytes were unchanged during the first 4 days of the experiment, and were significantly increased after 8 days of starvation (by 20.3 and 22.1%, respectively), due to disparity between the changes in volume and surface area of the muscle cells during myocardial atrophy. The bulk and surface density of the cardiomyocyte nuclei and also the ratio of the volumes of the nuclei and cytoplasm did not change significantly in the course of the experiment, and only a tendency for these parameters to decrease was found on the 8th day of starvation.

The relative volume of the stroma (made up of capillaries, cells, fibers, and connective-tissue ground substance) was increased after 4 days of starvation by 27.4% ($p < 0.05$), and the ratio of the bulk density of the stroma to the relative volume of the parenchyma increased by the same degree (by 33.1%). Toward the end of the experiment the relative volume of the stroma showed a small decrease, to 113.6% of the control level, whereas the volume ratio of stroma and parenchyma under these circumstances was 15.9% higher than initially.

The structural density of the microcirculatory bed showed minimal changes in the course of the experiment. The relative volume of the capillaries increased by 12.6% after 4 days of starvation, but the relative surface area was unchanged, so that the surface to volume ratio was reduced to 0.554 ± 0.016 m^2/cm^3 (0.623 ± 0.005 m^2/cm^3 in the control, $p < 0.05$). After 8 days of starvation the bulk and surface density of the capillaries did not differ significantly from the control.

Important representative parameters characterizing interaction between capillaries and cardiomyocytes include the ratio of the bulk and surface density of the capillaries to the relative volume and relative surface area of the cardiomyocytes [5, 8]. No significant changes

TABLE 2. Information Parameters of Stereologic Investigation of Tissue Organization of the Myocardium in Wistar Rats during Starvation

Time of experiment, days	Test object	Entropy, binary units	Relative entropy	Excess, %
Control	Parenchyma and stroma	0,841	0,325	67,5
	Stroma	1,486	0,743	25,7
2	Parenchyma and stroma	0,910	0,352	65,8
	Stroma	1,477	0,739	26,1
4	Parenchyma and stroma	0,973	0,376	62,4
	Stroma	1,465	0,733	26,7
8	Parenchyma and stroma	0,890	0,344	65,6
	Stroma	1,406	0,703	29,7

were found in the ratio of the bulk and surface density of the capillaries to the relative volume of the cardiomyocytes during 8 days of starvation, but the ratio of the surface densities of the capillaries and cardiomyocytes showed a tendency to diminish. This is evidence of a decrease in the area of "exchange surface" between the cardiomyocytes and the microcirculatory bed [1]. Under these circumstances a small decrease in the relative volume of the endothelial cells also was observed toward the end of the experiment.

The relative volume and surface density of the connective-tissue cells, among which undifferentiated forms of fibroblasts and macrophages predominated, increased after 4 days of starvation by 88.1 and 153.8%, respectively. On the 8th day of starvation these parameters were 5.9 ± 1.2 mm^3/cm^2 and 0.0050 ± 0.0010 m^2/cm^3, respectively, or 40.5 and 92.3% above the control values. The increase in bulk and surface density of the connective-tissue cells was evidently attributable not only to the proliferative cellular reaction, but also to the disproportionate reduction of the bulk density of the parenchymatous and stromal components. These processes were most marked on the 4th day of starvation: the ratio of the relative volume of the connective-tissue cells to the bulk density of the cardiomyocytes increased by 95.8%, but after 8 days of starvation this parameter was 43.8% higher than in the control.

The relative volume of the noncellular components of the connective tissue increased from 61.8 ± 11.4 to 87.1 ± 4.5 mm^3/cm^3 on the 4th day of starvation, and it remained relatively high until the end of the experiment. The ratio of the bulk density of the fibers and ground substance to the relative volume of the cardiomyocytes followed the same time course. The increase in bulk density of the stroma was due mainly to pericapillary edema and it was not accompanied by any increase in the content of collagen fibers.

Information analysis of the stereologic parameters of the tissue organization of the myocardium during its atrophy due to starvation for 8 days revealed the progressive increase in entropy and relative entropy, a reduction of excess in the first 4 days of the experiment, and some decrease in the first two parameters toward the end of the experiment (Table 2). Meanwhile, structural changes in the stroma of the organ led to a successive reduction of entropy and relative entropy and to an increase in excess, evidence of reduction of indeterminacy in the system of this tissue component [5, 8].

The reduction in weight of the organ in the first 4 days was thus due mainly to a preferential decrease in the volume of the muscle cells and it was accompanied by marked changes in parenthymatous-stromal interrelations. Toward the end of the experiment the principal stereologic parameters of the tissue organization of the myocardium did not differ significantly from the control, due to the proportionate reduction in components of the parenchyma and stroma of the atrophied heart [6].

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