form of small conglomerates, distributed throughout the nucleus. The increase in the quantity of condensed chromatin is proportional to the decrease in transcription activity of the nucleus [7]. Concentrations of ICG with indistinct separate granules, an increased number of PCG, collected into "bunches" and unconnected with condensed chromatin, partial segregation of the nucleus, and also the appearance of RNP-positively stained granules, which are never observed in the nuclei of normochromic neurons, also point to changes in RNA and protein synthesis. Similar granules were discovered by Swanson and co-workers [9] in nuclei of neurons attacked by herpes simplex virus.

Thus two groups of nerve cells with signs of hyperchromia and vacuolation are found in the sensomotor cortex of rats exposed to anoxic anoxia: cells with irreversible degenerative changes and cells with no signs of irreversible degeneration, but with changes in their DNA-RNA-protein synthesizing apparatus.

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STEREOHISTOLOGIC ANALYSIS OF THE MYOCARDIUM OF HOMOIOTHERMIC ANIMALS DURING COOLING

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The cardiovascular system, which plays an important role in adaptation of the organism to cold stress [1], is in a state of functional strain when exposed to this factor, and this frequently leads to the development of irreversible pathological changes in the myocardium [9, 13-16]. To understand the mechanisms of formation of compensatory-adaptive reactions in the myocardium during hypothermia, quantitative morphologic investigations are very important [6, 10]. However, the time course of quantitative structural changes in the muscular and connective tissue of the myocardium during exposure to low temperatures has not been fully investigated [5].

The aim of this investigation was a stereologic study of parenchymatous-stromal interrelations in the myocardium of rats subjected to long-term hypothermia.

EXPERIMENTAL METHOD

Experiments were carried out on 26 male Wistar rats aged 4 months and weighing initially 200-230 g. The animals were subjected to continuous hypothermia (except when feeding) in a

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TABLE 1. Results of Morphometric Investigation of the Heart of Rats Exposed to Hypothermia (M ± m)

Parameter	Control	Duration of hypothermia		
		4 h	8 days	16 days
Body weight, g	213,3±8,8	230,0±17,6	210,0±20,2	123,3±8,8*
Weight of heart, mg	933,3±44,1	980,7±58,3	979,3±54,5	800,0±0,1
Relative weight of heart, mg/g body weight	4,37±0,08	4,28±0,16	4,74±0,48	6,55±0,45*

Legend. *p < 0.01.

thermal chamber at −7°C. The investigastion was conducted 4h, and 8 and 16 days after the beginning of the experiment. Five rats of the same age and strain, kept under ordinary animal house conditions, served as the control. The animals were decapitated and the heart quickly removed and transferred into a cold chamber until it stopped beating. Paraffin sections were obtained after fixation of the heart in a 10% solution of neutral formalin, and stained with hematoxylin and eosin, together with Perls' reaction, by Van Gieson's method with counterstaining of elastic structures with resorcin-fuchsin, and by the PAS reaction. To obtain semithin sections, pieces of myocardium from the wall of the left ventricle were fixed in 1% OsO_4 solution and embedded in a mixture of Epon and Areldite. Semithin sections, strained with azure II, were used for the tissue stereologic analysis [7].

EXPERIMENTAL RESULTS

Under the conditions of hypothermia chosen (−7°C) the animals developed an incompensated endocrine-metabolic reaction, indicating that they were subjected to severe stress [12]. Throughout the experiment the rectal temperature of the animals did not fall, evidence of absence of decompensation of temperature homeostasis. Hypothermia for 16 days caused a significant decrease in the animals' body weight (Table 1), mainly due to depletion of their fat reserves. Since the absolute weight of the heart was virtually unchanged during this period, a significant increase took place in the relative weight of the heart.

Keeping the animals for 4 h at a temperature of −7°C led to marked disturbances of the hemodynamics: venous congestion and acute focal hemorrhages, most marked in the subepicardial and middle zones of the myocardium of both ventricles. The combined drainage function of the venous and lymphatic systems was reduced in the stroma of the myocardium, with lymphostasis and the development of massive edema of the interstitial connective tissue (Fig. 1a). Changes in the parenchymatous cells were mainly connected with contractural injuries to the cardiomyocytes (mainly in the left ventricle), as reflected in their eosinophilia and increased

TABLE 2. Results of Tissue Stereologic Analysis of Myocardium of Hypothermic Rats (M ± m)

Parameter	Control	Duration of hypothermia		
		4 h	8 days	16 days
Relative volume, mm^3/cm^3:				
of cardiomyocytes	835,7±7,5	809,8±11,8	838,1±8,0	808,0±13,1
of cardiomyocyte nuclei	10,6±0,2	9,8±1,4	10,5±1,5	11,3±1,0
of capillaries	48,8±5,6	24,5±2,6*	39,6±5,5	40,3±3,8
of endothelial cells	19,4±2,1	16,1±1,5	13,3±1,1	24,5±1,7
of connective tissue cells	12,0±1,4	14,7±1,4	12,0±2,0	15,8±1,8
of fibers and ground substance of connective tissue	73,5±1,4	125,2±11,6*	87,9±14,0	100,1±6,9*
Relative surface area, m^2/cm^3:				
of cardiomyocytes	0,1072±0,0062	0,1209±0,0053	0,1142±0,0054	0,1144±0,0040
of cardiomyocyte nuclei	0,0068±0,0003	0,0076±0,0008	0,0079±0,0005	0,0101±0,0013
of capillaries	0,0358±0,0016	0,0230±0,0020**	0,0313±0,0012	0,0308±0,0004
of connective tissue cells	0,0126±0,0014	0,0142±0,0020	0,0103±0,0006	0,0138±0,0014
Surface-volume ratio, m^2/cm^3:				
of cardiomyocytes	0,128±0,008	0,150±0,009	0,136±0,008	0,142±0,003
of cardiomyocyte nuclei	0,650±0,031	0,791±0,061	0,780±0,097	0,885±0,040**
of capillaries	0,745±0,049	0,949±0,005	0,811±0,081	0,778±0,080
of connective tissue cells	1,056±0,031	0,956±0,065	0,892±0,094	0,880±0,057
of capillaries to cardiomyocytes	0,043±0,002	0,029±0,003*	0,038±0,002	0,038±0,0003
Volume ratio, number:				
of stroma to parenchyma	0,182±0,011	0,220±0,018	0,179±0,013	0,221±0,020
of capillaries to cardiomyocytes	0,058±0,007	0,030±0,003*	0,047±0,006	0,050±0,005

Legend. *p < 0.05, **p < 0.01.

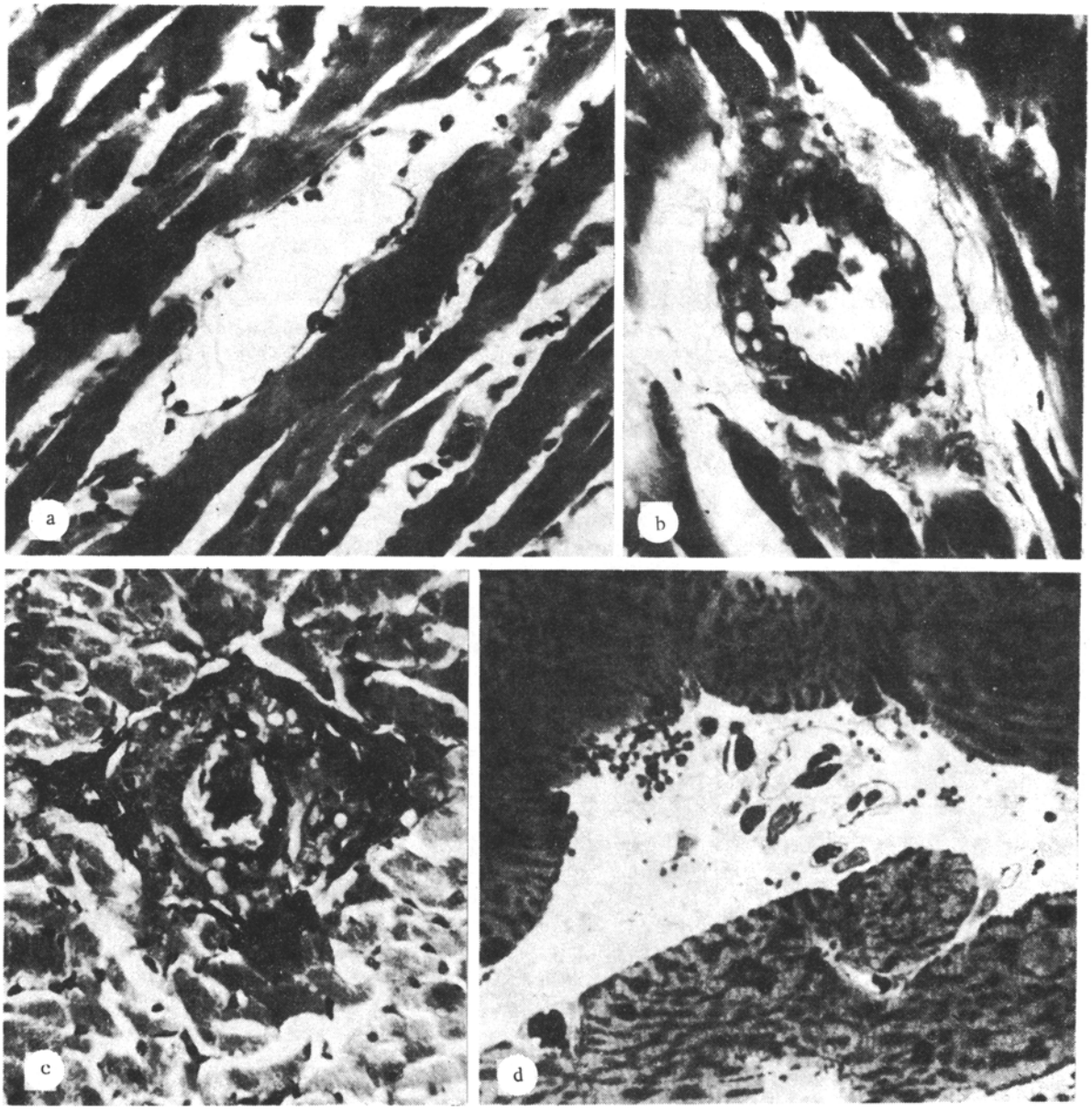

Fig. 1. Myocardium of rats exposed to low temperatures. a) Eosinophilia of muscle segments, lymphostasis, and marked edema of tissue 4 h after exposure to cold; b) spasm of intramural artery, edema of perivascular connective tissue 4 h after exposure to cold; c) thickening of wall of an intramural artery as a result of spasm on 8th day of cooling, coarsening of perivascular connective tissue; d) edema of intermuscular connective tissue on 8th day of cooling. a, b) Stained with hematoxylin and eosin; c) by Van Gieson's method; d) semithin section, stained with azure II. Magnification: a, b, c) 280; d) 700.

anisotropy when examined under polarized light. The nuclei of the cardiomyocytes were euchromic, and contained one or two nucleoli and small masses of heterochromatin.

The intramural arteries were in a state of spasm: the elastic membranes were tightly coiled and the endothelial cells projected appreciably into the lumen of the vessels (Fig. 1b). The arterial walls were edematous. The number of mononuclear cells was increased in the intermuscular zones and, in particular, in the adventitia of the arteries.

Tissue stereologic analysis showed that the bulk and surface density of the cardiomyocytes was virtually unchanged at these times, whereas significant changes were taking place in the stroma of the myocardium. For instance, the bulk (by 50%) and surface (by 36%) density of the capillaries was significantly reduced (Table 2). The bulk density of the

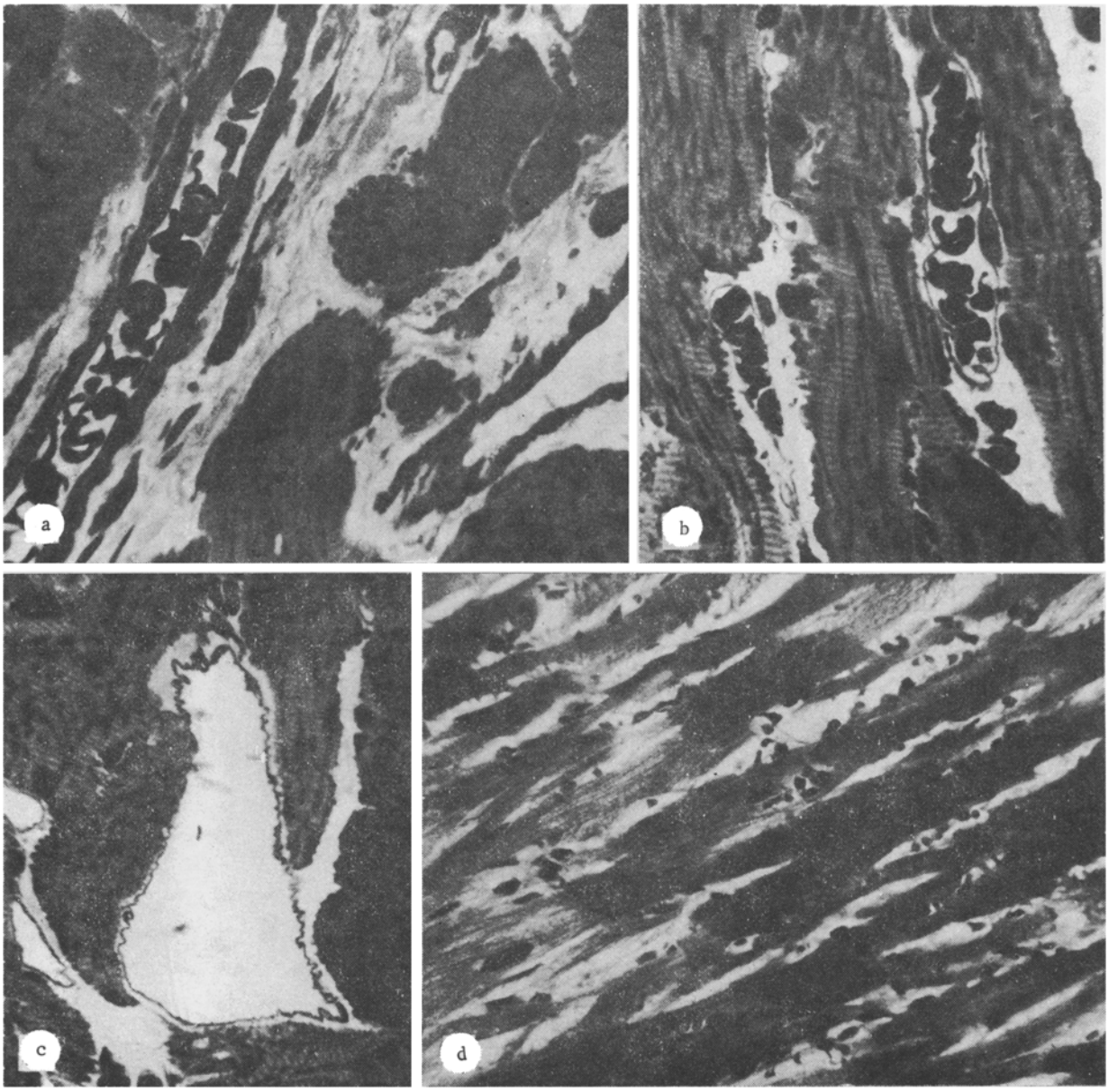

Fig. 2. Myocardium of rats exposed to hypothermia for 16 days. a) Contractural and lytic changes in cardiomyocytes; b) congestion of capillaries and sludging of erythrocytes. c) Dilatation of lymphatics and edema of interstitial connective tissue; d) development of perivascular interstitial sclerosis. a) Stained with hematoxylin and eosin. 280 ×; b, c, d) semithin sections, stained with azure II. 700 ×.

endothelial cells also was reduced. The considerable reduction of the bulk and surface density of the capillaries led to a significant decrease in the volume (by 48%) and surface to volume (by 33%) ratio of the capillaries to the cardiomyocytes. The relative volume of the cells, fibers, and ground substance of the interstitial connective tissue, on the other hand, was significantly increased (by 64%), and this caused basically an increase (by 70%) of the bulk density of the noncellular component of the connective tissue due to edema.

On the 8th day of cold stress an increase in the number of cardiomyocytes with contractural damage was observed in the myocardium of the left and right ventricles (especially in the middle zone of the left ventricular myocardium). Hemodynamic disturbances were preserved: marked venous and capillary congestion and acute hemorrhages. The intramural arteries were in a state of spasm (Fig. 1c) and their walls were edematous. The stroma of the myocardium was edematous (Fig. 1d) and its fibers separated, and moderately infiltrated by mononuclear cells. Small foci of mononuclear infiltration, containing leukocytes, were observed in the

subepicardial and subendocardial zones of the myocardium of the right and left ventricles. Thickened bundles of collagen fibers were observed in the interfibrillary and perivascular connective tissue.

Stereologic analysis of the tissue organization of the myocardium in this period revealed no significant quantitative changes in the parenchymatous and stromal components (Table 2). However, the bulk density of the capillaries and endothelial cells remained below that in the control, while the relative volume of the fibers and ground substance of the connective tissue was increased by 19%.

Eosinophilia of the muscle segments (Fig. 2a) caused by contractural injuries to the cardiomyocytes was preserved in the myocardium of the right and left ventricles of the rats on the 16th day of hypothermia. Contractural changes in the cardiomyocytes were mosaic or focal in character and were most marked in the left ventricle. Besides these injuries, signs of lysis of the myofibrils appeared in some cardiomyocytes. The cytoplasm in these cells was greatly reduced in density and was translucent, but the cross-striation of the myofibrils was partially preserved, i.e., lysis of the myofibrils was incomplete. Under these circumstances, regions of the myocardium with contractural lesions of the cardiomyocytes alternated with zones in which cardiomyocytes with evidence of lysis of the myofibrils predominated.

Disturbances of the hemodynamics and lymphatic circulation were preserved: venous and capillary congestion, spasm of the intramural arteries, and lymphostasis were present (Fig. 2b, c). Edema of the stroma was reduced, but sclerotic processes were intensified: thickening of bundles of collagen fibers took place in the intermuscular layers of connective tissue (Fig. 2d). Diffuse infiltration of the stroma by mononuclear cells, among which lymphocytes predominated, was observed. Coarsening of the perivascular connective tissue and concentrations of mononuclears in the adventitia of the arteries were observed.

According to the data of stereologic analysis, the bulk density of the cardiomyocytes and of their nuclei on the 16th day of cold stress showed no significant change (Table 2). However, the surface-volume ratio of the nuclei of the cardiomyocytes was significantly increased (by 36%), due to a marked increase (by 48.5%) in their surface density. The bulk and surface density of the capillaries virtually reached the control values. The bulk density of the endothelial cells increased, evidently on account of their proliferation. The relative volume of the interstitial connective tissue was significantly increased, mainly through the accumulation of fibrous structures.

The ratio of the volume of the capillaries to that of the cardiomyocytes was not significantly changed, whereas their surface-volume ratio remained decreased (by 12%).

In the later stages compensatory and adaptive reactions developed, aimed at restoring the relative volumes of the capillaries and cardiomyocytes and normalizing metabolism. These reactions included an increase in the bulk and surface density of the endothelial cells and capillaries, observed toward the end of the experiment. Nevertheless, as a result of a general reduction in the supply of plastic materials for the myocardium, due to the increase of thermogenesis, besides contractural changes, processes of lysis also were observed in some of the cardiomyocytes. These changes in the parenchymatous cells were accompanied by intensification of desmoplastic reactions in the myocardial stroma. By the end of the experiment the proliferative cellular response was intensified and diffuse sclerosis of the interstitial connective tissue developed.

We know that acute hypothermia, which is a powerful stress-inducing factor, causes excitation of nonspecific sensory-endocrine systems, thereby leading to the development of a general adaptation syndrome and activation of thermoregulatory reactions [3]. Endogenous catecholamines, whose action on target tissues is mediated mainly through β-adrenoreceptors [2], play an important role in the development and maintenance of these reactions. Endogenous catecholamines under these circumstances intensify both contractile and noncontractile thermogenesis, thereby increasing the resistance of the body to cooling. The increase in the catecholamine concentration in the myocardium during cold stress leads to the development of contractural injuries of the cardiomyocytes, just as under the influence of exogenous catecholamines [8].

However, the absence of signs of coagulation necrosis in the myocardium of these animals at all stages of the experiment must be noted. Activation of thermoregulatory reactions and increased heat production requires a larger supply of plastic materials for the organs of thermogenesis (primarily the skeletal musculature, liver, and lungs). Under these circum-

stances the heart remains deficient in plastic materials and its functions undergo "minimization" [4]. This leads to increasing manifestations of plastic insufficiency of the cardiomyocytes, accompanied by intensification of diffuse collagenization of the stroma [11].

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