MORPHOLOGY AND PATHOMORPHOLOGY

Stereological Analysis of Myocardial Tissue Organization in Long-Term Adaptation to High Altitude

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The majority of investigations of the influence of high altitude on the cardiovascular system contain contradictory data about the structural and functional changes occurring in the myocardium in response to short-term high-altitude adaptation [3,4,9-11]. The long-term impact of the complex of high-altitude factors is usually evaluated in persons and animals habitually dwelling at high altitudes, who have developed mechanisms of adaptating to these conditions and who exhibit significant differences in the structure and function of the vital systems [1,5]. For example, biochemical and functional changes have been discovered in the tissues of native mountain-dwellers. These changes facilitate oxygen transport to the tissues during chronic hypoxia, often without the blood pressure rising or even under conditions of moderate hypotension [6].

When the inhabitants of plains are moved to a high altitude and stay there for a long time, they develop adaptive reactions, the main feature of which is a rise of the blood pressure, at first in the lesser and then in the greater circulation. This leads to structural and functional changes not only of the right but also of the left ventricle of the heart. The structural and functional state of the left ventricle myocardium is a factor which determines the adaptive possibilities of the organism. A study of the stereology of the tissue reorganization in the left ventricle myocardium was thus the aim of this investigation.

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MATERIALS AND METHODS

The influence of high altitude was studied in male Wistar rats brought to a laboratory at an elevation of 3200 m above sea level (Tien Shan, Tuya-Ashu pass). The animals were held at room temperature and were fed a standart diet with water ad libitum. Several animals died during the exposure; rats after 5 and 10 months of adaptation (18 and 10 animals, respectively) were used in the experiments. Wistar rats of the same age but living on the plain were used as a control group.

The heart muscle was fixed in 10% neutral formalin solution for light microscopy. The paraffin preparations were stained with hematoxylin and eosin, after van Gieson; the PAS reaction was also performed. For semithin sections, specimens of heart muscle were fixed in 4% paraformaldehyde solution, postfixed in 1% osmium tetroxide solution, and embedded in araldite after dehydration. Semithin sections obtained on a Tesla microtome

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were stained with Azure II and were used in the stereological tissue analysis.

The stereological analysis was performed with the use of a multipurpose test system [7]. The volume and surface density of the main myocardial structures were evaluated. The secondary stereological parameters (surface-volume and volume ratio of structures) were calculated on the basis of the primary parameters. The statistical analysis included finding the mean values, the errors means and their comparison with the use of the Student t test.

RESULTS

The animals living at the high altitude showed a steady weight loss (to 88% of the initial level by

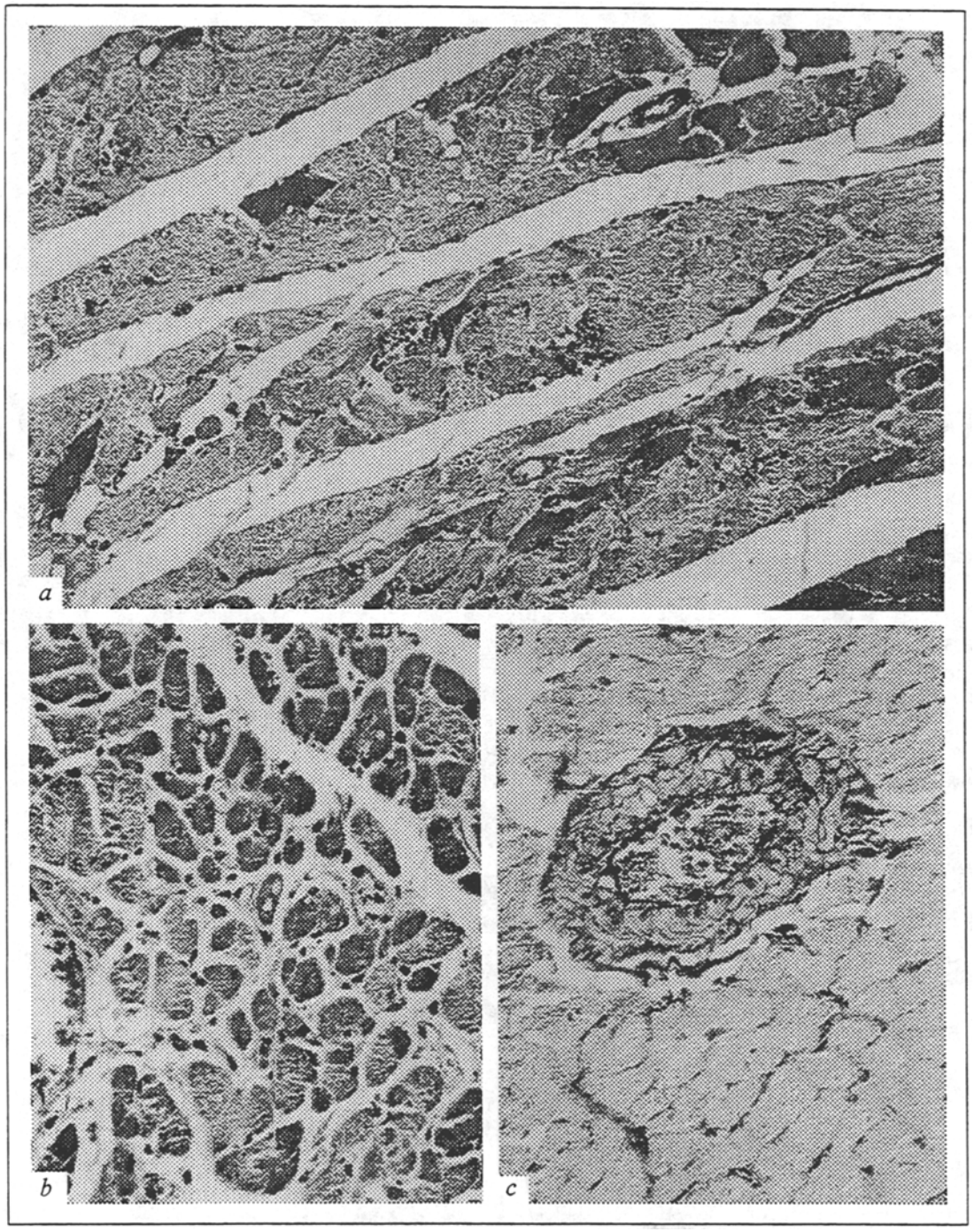

Fig. 1. Morphological changes of rat myocardium after 5 months at high altitude (×250). *a*) significant mosaic changes of cardiomyocytes due to presence of eosinophilic muscle segments and cells with lytic alterations; *b*) atrophic muscle fibers; *c*) thickening of middle layer and narrowing of lumen of intramural artery. Staining: *a*) PAS reaction; *b*, *c*) hematoxylin and eosin.

TABLE 1. Results of Tissue Stereological Analysis of Myocardium in Rats Exposed to High Altitude ($M \pm m$)

Index	Plain		High altitude	
	age 7 months	age 11 months	during 5 months	during 10 months
Volume density, mm^3/cm^3:				
cardiomyocytes	839.3±15.0	833.4±16.3	826.8±4.5	839.2±7.7
cardiomyocyte nuclei	8.7±1.6	9.6±1.1	11.1±1.2	8.8±1.6
capillaries	43.2±3.7	44.8±4.2	30.2±1.8*	34.4±2.2
endothelial cells	19.2±1.3	17.3±2.8	12.8±1.8*	14.7±1.1
connective tissue cells	13.9±0.9	11.4±2.8	6.7±1.0**	6.7±0.7
fibers and ground substance of connective tissue	75.7±8.5	83.5±7.4	112.4±2.6**	96.2±8.6
Surface density, m^2/cm^3:				
cardiomyocytes	0.1013±0.0068	0.0951±0.0023	0.0787±0.0058*	0.0747±0.0078
cardiomyocyte nuclei	0.0066±0.0004	0.0071±0.0007	0.0078±0.0005	0.0057±0.0011
capillaries	0.0361±0.0031	0.0316±0.0036	0.0245±0.0020*	0.0221±0.0027*
connective tissue cells	0.0182±0.0020	0.0106±0.0026	0.0048±0.0004***	0.0044±0.0001
Surface–volume ratio, m^2/cm^3:				
cardiomyocytes	0.121±0.006	0.114±0.005	0.094±0.007	0.088±0.010
cardiomyocyte nuclei	0.762±0.062	0.744±0.034	0.730±0.062	0.641±0.044
capillaries	0.836±0.084	0.767±0.043	0.822±0.086	0.646±0.096
connective tissue cells	1.308±0.169	0.957±0.132	0.723±0.080**	0.664±0.036
capillaries to cardiomyocytes	0.043±0.005	0.038±0.005	0.029±0.003*	0.026±0.003
Volume ratio, number:				
capillaries to cardiomyocytes	0.051±0.001	0.050±0.007	0.036±0.002**	0.041±0.002
stroma to parenchyma	0.179±0.025	0.187±0.021	0.194±0.006	0.179±0.011

Note. Asterisks denote reliability of differences in comparison with age–matched control: * $p<0.05$; ** $p<0.01$; *** $p<0.001$

the end of the experiment). Systolic pressure in the pulmonary artery after 10 months was twice as high as in the control group [2,8]. Diastolic pressure did not increase significantly, this attesting to a systolic type of hypertension and to an increased rigidity of the vascular bed of the lungs.

Changes of the left and right ventricle myocardium after 5 months at a high altitude presented as a mosaic of cardiomyocyte lesions (Fig. 1, *a*) and disturbances of the circulation. A moderate myocardial hypertrophy was seen. The total number of eosinophilic muscle segments increased in all the myocardial layers, but especially in the subendocardial and subepicardial layers, where I,II, and III degree contractural changes of myofibrils were found in polarized light. Simultaneously, cardiomyocytes with lysis were noted in the middle layer. In such cells, sarcoplasmic clearing, myofibrillar-bundle disorganization and thinning, and focal lysis of the organelles in the perinuclear and subsarcolemmal zones could be seen. Often there were atrophic fibers among the hypertrophic fibers (Fig. 1, *b*), especially in the left ventricle.

Circulatory disturbances were manifested as venostasis, hemorrhages (sometimes extensive), intramural edema, and plasmorrhages. Intramural arteries were primarily in a state of spasm. Several animals developed a significant thickening of the middle artery layer (Fig. 1, *c*), which was caused by hypertrohpy of smooth-muscle cells and led to narrowing of the lumen. Moderate perivascular and interstitial sclerosis was seen. Changes of the tinctorial and architectonic properties of erythrocytes took place - basophilia, enlargement, flattening, and uneven margins.

After 10 months at the high altitude the hypertrophy of the left and right ventricles increased. Eosinophilic muscle segments were observed in the myocardium of both ventricles. Lytic processes of different degrees took place in the majority of cardiomyocytes (Fig. 2, *a*), proving that intracellular regenerative processes had broken down. Atrophic changes of some cardiomyocytes were among the disorder manifestations. Accumulations of mononuclears often formed around such zones (Fig. 2, *b*).

Circulatory disturbances were still present after 10 months at the high altitude - focal hemorrhages and plasmorrhages, especially in zones of atrophically changed cardiomyocytes. In comparison with the earlier investigation, the edema of the connective tissue between fibers was less pronounced. The intramural arteries were in a state of spasm or secondary paresis. The artery walls were thickened due to hypertrohpy and hyperplasia of smooth-muscle cells (Fig. 2, *c*). Several ani-

mals showed the formation of an elastic membrane in the venous sinuses of the subepicardial layer. Hyperelastosis of the epicardium was also observed, along with the appearance of thick collagen bundles in it.

According to the data of stereological analysis, the volume density of the left and right ventricle cardiomyocytes did not change significantly after 5 months of living at a high altitude, but the surface density reliably decreased, indicating hypertrophy of cardiomyocytes (Table 1). The animals living at high altitude showed an increase of the volume (28%) and surface (18%) densities of cardiomyocyte nuclei. As a result of these changes, the surface-volume ratio decreased, but this index did not change for the nuclei.

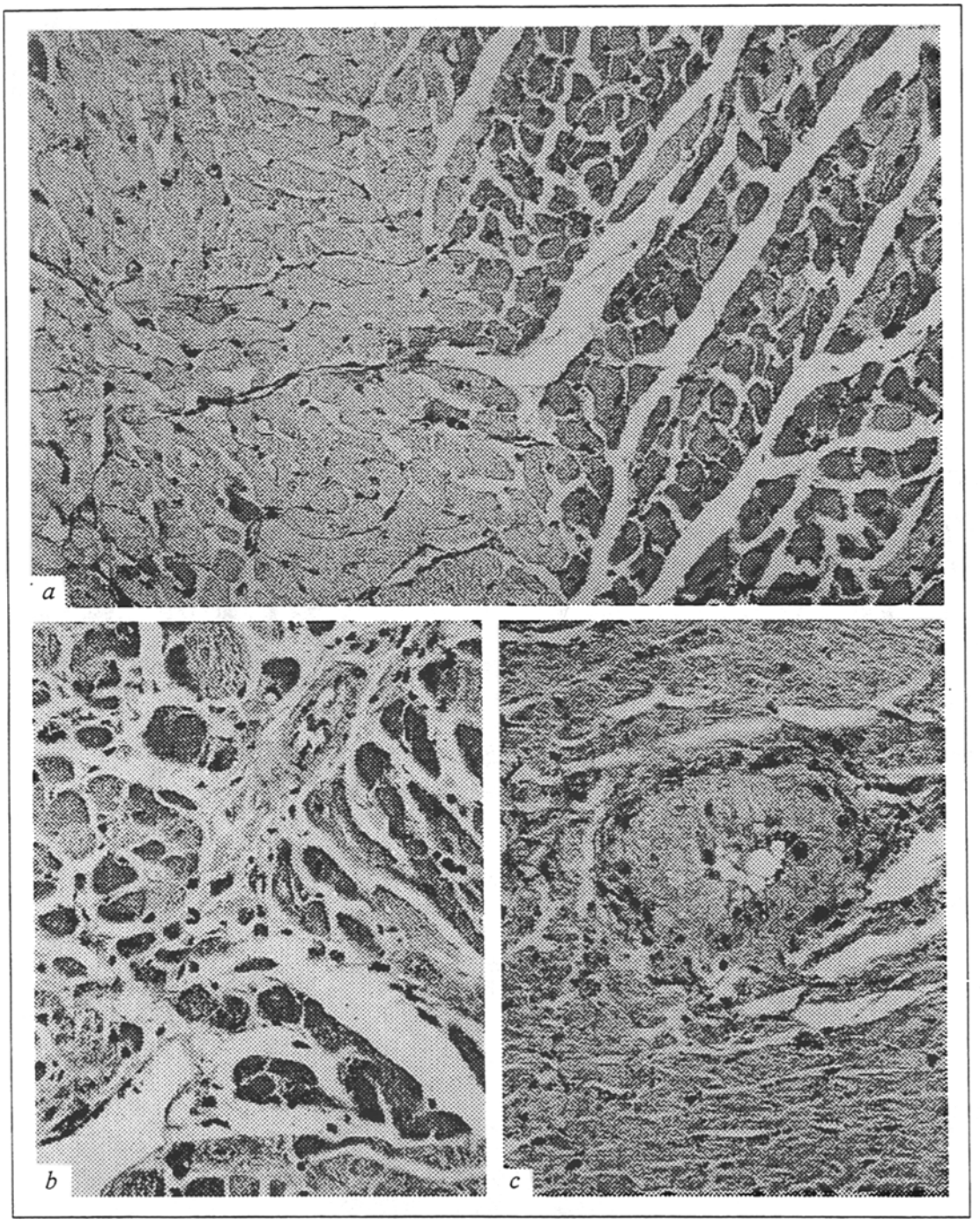

Fig. 2. Morphological changes of rat myocardium after 10 months at high altitude (×250). *a*) thinning and lysis of sarcoplasm in majority of cardiomyocytes; *b*) atrophic cardiomyocytes and accumulation of mononuclears around them; *c*) hypertrophy of smooth-muscle cells and thickening of middle layer of intramural artery. Staining: *a,b*) PAS reaction; *c*) hematoxylin and eosin.

The most significant quantitative changes took place in the capillaries and endothelial cells. The volume density of capillaries decreased by 30% and the surface density by 32%. The same indexes for endothelial cells decreased by 33% and 73%, respectively. The quantitative changes of the capillaries led to altered interrelations with the cardiomyocytes. After 5 months at the high altitude a reliable decrease of the volume (29%) and surface-volume (33%) capillary-cardiomyocyte ratio was recorded.

This period of the experiment was also characterized by a reliable decrease of the volume (52%) and surface (73%) densities of the connective-tissue cells and by an increase of the volume density (48%) of the ground substance and fibers. Such connective- tissue changes brought about an increase (8%) of the stroma-parenchyma volume ratio.

Ten months of living at the high altitude did not bring about significant changes in the volume density of cardiomyocytes and their nuclei in comparison with the control animals living on the plain. Nevertheless, a slight decrease of the surface density of these structures was noted and it led to a decrease of the surface-volume ratio of cardiomyocytes and their nuclei (Table 1).

Just as during the preceding period after 10 months there was a decrease in the volume (23%) and surface (30%) densities of the capillaries. For endothelial cells these indexes dropped 15 and 73% as compared with control animals of the same age. Such quantitative changes of the capillaries caused a decrease of the volume and surface-volume ratio of the capillaries and cardiomyocytes, but the decrease was smaller then earlier.

After 10 months at high altitude the volume and surface density of connective-tissue cells decreased and the volume density of the connective-tissue ground substance and fibers increased slightly. It is important to note that at this time the volume ratio between stroma and parenchyma was virtually the same in the experimental and control groups.

Thus, the tissue reorganization of the left ventricle myocardium after a long-term (10 months) exposure to high altitude manifests itself primarily in a decrease of the volume and surface densities of the capillaries and endothelial cells; it causes a decrease of the volume and surface-volume ratio between the capillaries and cardiomyocytes, which is most expressed in the 5th month of the experiment. Simultaneously, cardiomyocyte heterogenity is recorded, due to the presence of cells with contractures and lytic changes. The increased number of atrophic cardiomyocytes, especially in the left ventricle, attests to progressive decompensation of the myocardium.

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