MORPHOLOGY AND PATHOMORPHOLOGY

Stereological Analysis of Rat Myocardium in Adaptation to High-Latitude Conditions

L. M. Nepomnyashchikh, E. L. Lushnikova, and G. I. Nepomnyashchikh

UDC 611.018.63:612.59

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 10, pp. 432-436, October, 1993
Original article submitted July 23, 1993.

Key Words: *myocardium; adaptation; high latitudes; contrast ecological conditions; stereology*

Transfer to the ecological conditions of the Far North is attended by the development of a particular physiological status, characterized by "stress" of all the morphofunctional systems of the organism [1,2,4,6,7]. This stress manifests itself in metabolic, neuroendocrine, and psychoemotional shifts, which are sometimes considered as pathological states, but in fact represent the implementation of the adaptive program of the species [3]. In this connection it is important to clarify the underpinnings of the new adaptive program and, in particular, the morphogenetic shaping under conditions of high latitudes.

Morphofunctional changes of the cardiovascular system are detected immediately upon venturing beyond the polar regions and determine to a great extent the possibilities of the organism's adaptive reorganization. There is a relationship between the functional state of the cardiovascular system on the seasons of the year and the length of time spent in the Far North [1], but the morphogenesis of adaptive-compensatory reactions of the myocardium under these conditions has remained practically unstudied.

The aim of the present investigation was to perform a qualitative and quantitative assessment of the tissue reorganization of the myocardium in animals transferred to high latitudes.

MATERIALS AND METHODS

The experiments were carried out on 109 male Wistar rats with an initial weight of 180-200 g. Before the experiments they were synchronized to a standard light regimen (12h light : 12h dark) during 1 month. Then some of them were transported by air to Alykel', a locality situated at the 69th parallel in the Far North. Control animals were transported by air at the same altitude and speed along the route Novosibirsk - Omsk - Novosibirsk (to assess flight stress). Intact Wistar rats of the same age served as an additional control. The experimental and control animals were kept in the vivarium under uniform conditions and were sacrificed synchronously after 1, 3, 7, 12, 22, 27, 31, and 37 days.

Paraffin slides were stained with hematoxylin and eosin and the Pearls reaction was performed; staining after van Gieson and the PAS reaction

Institute of Regional Pathology and Pathological Morphology, Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk. (Presented by V. P. Kaznacheev, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0010-1299$12.50

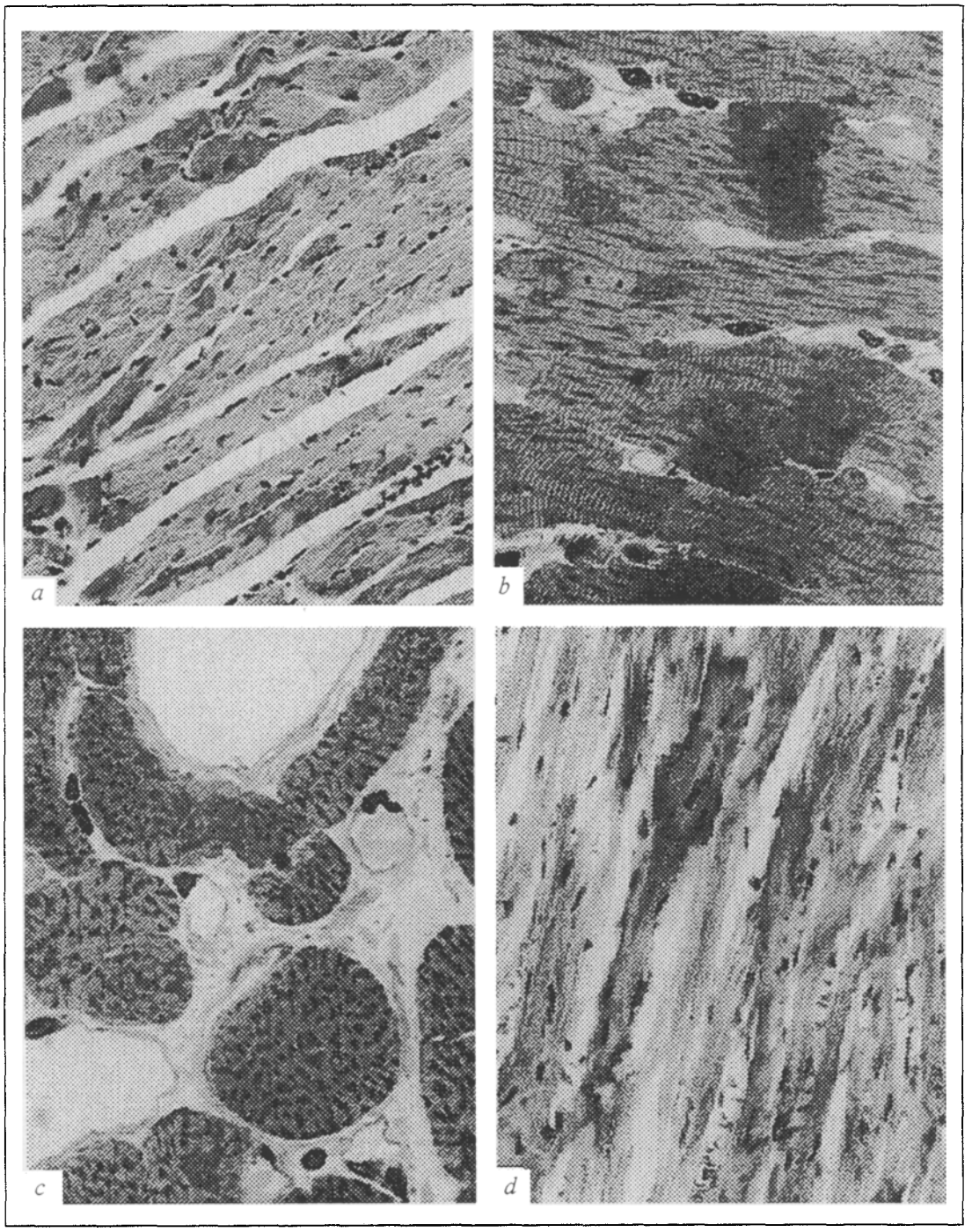

Fig. 1. Morphological changes in rat myocardium after flight in middle latitudes. *a*) eosinophilia of individual cardiomyocytes and moderate edema of intermuscular connective tissue one day after transference; *b*) contracture damage to cardiomyocytes 12 days after flight (semithin section); *c*) lymphostasis and edema of myocardial stroma 27 days after transference (semithin section); *d*) contracture and lytic changes in cardiomyocytes after 37 days of experiment. Hematoxylin–eosin staining (*a*); azure II (*b*, *c*); PAS–reaction (*d*). Magnification: *a*) ×250; *b*) ×800; *c*) ×1000; *d*) ×312.

were also used. The state of the myofibril apparatus was assessed in polarized light using a Docuval microscope. Simultaneously, samples of left-ventricle myocardium were fixed in 4% paraformaldehyde with postfixation in a 1% solution of osmium tetroxide. After dehydration, the myocardial samples were embedded in Epon-Araldite. The blocks were cut into semithin sections (1 μ) stained with Azure II.

The stereological analysis of the tissue [5] was performed on the semithin sections with the use of a multipurpose test system. The volume and

TABLE 1. Results of Tissue Stereological Analysis of Myocardium of Rats Undergoing Flight in Middle Latitudes ($M \pm m$)

Index	Control	Time after transference		
		1 day	12 days	27 days
Volume density, mm^3/cm^3:				
cardiomyocytes	839.3±15.0	851.0±7.9	858.8±10.0	843.4±11.5
cardiomyocyte nuclei	8.7±1.6	11.4±1.7	11.7±1.8	9.0±1.0
capillaries	43.2±3.7	40.9±4.2	28.3±5.4*	36.6±1.2
endothelial cells	19.2±1.3	14.0±1.9	12.6±3.6	16.3±2.8
connective tissue cells	13.9±0.9	8.1±1.5*	6.2±0.7**	9.2±1.3*
connective tissue fibers and matrix	75.7±8.5	74.6±4.9	82.4±11.0	85.5±10.5
Surface density, m^2/cm^3:				
cardiomyocytes	0.1013±0.0068	0.1038±0.0123	0.1030±0.0100	0.0772±0.0017*
cardiomyocyte nuclei	0.0066±0.0004	0.0086±0.0011	0.0093±0.0028	0.0050±0.0002**
capillaries	0.0361±0.0031	0.0296±0.0022	0.0230±0.0037*	0.0264±0.0002*
connective tissue cells	0.0182±0.0020	0.0072±0.0014*	0.0064±0.0004**	0.0071±0.0014*
Surface–volume ratio, m^2/cm^3:				
cardiomyocytes	0.121±0.006	0.120±0.014	0.119±0.012	0.091±0.003**
cardiomyocyte nuclei	0.762±0.062	0.777±0.143	0.779±0.116	0.569±0.048*
capillaries	0.836±0.084	0.745±0.122	0.822±0.019	0.724±0.030
connective tissue cells	1.308±0.169	0.883±0.078	1.056±0.065	0.769±0.092*
capillaries to the cardiomyocytes	0.043±0.005	0.034±0.002	0.026±0.005*	0.031±0.0005*
Volume ratio (number):				
capillaries to cardiomyocytes	0.051±0.001	0.048±0.005	0.033±0.006*	0.043±0.001**
stroma to parenchyma	0.179±0.025	0.160±0.012	0.149±0.013	0.173±0.017

Note. Here and in Table 2 asterisks signify reliability of differences. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

surface density of the main structural components of the myocardium were assessed. The secondary stereological parameters were also calculated: surface-volume and volume ratios of the structures. The quantitative data obtained were processed statistically. The mean values were compared using the Student test.

RESULTS

The analysis of morphometric parameters of rat heart showed that the heart weight was significantly increased (by 24%) toward the 37th day in animals under high-latitude conditions. The increase in heart weight was attended by an increase (by 40%) of the body weight, which helped preserve the relative weight of the heart. The animals transferred to middle latitude exhibited no significant change of heart weight during the whole experiment.

Microscopic changes of rat myocardium after flight in the middle latitudes were insignificant during the first three weeks, but progressively increased toward the end of the experiment and were manifested in disorders of blood and lymph circulation and contracture damage to cardiomyocytes (Fig. 1).

There was a different time course of myocardial changes in rats transferred to high latitudes. One day after the flight pronounced disorders of the circulation, edema of interstitial connective tissue, and lymphostasis were noted. In the same period a pronounced mosaic pattern of damage of cardiomyocytes, related both to eosinophilia of muscle segments and to the presence of cardiomyocytes with features of lysis (Fig. 2, *a*, *b*), was registered. The myocardial edema subsided 3 days later, but the other features of circulatory failure, mainly cardiomyocyte contracture, persisted. These morphofunctional changes were preserved till the 27th day of the experiment.

The edema of interstitial connective tissue in the myocardium intensified from the 27th to the 37th day of the experiment (Fig. 2, *c*); a pronounced plethora of blood vessels and lymphostasis were noted. The number of cardiomyocytes with signs of sarcoplasm lysis was significantly increased, but cardiomyocytes with contracture damage of myofibrils were simultaneously present. There were atrophic and necrobiotic changes of some cardiomyocytes surrounded by clusters of mononuclears. Myoelastofibrosis of intramural arteries developed in the same period (Fig. 2, *d*), testifying to the developmet of stable arterial hypertension.

The tissue stereological analysis of the myocardium of rats moved to the middle latitudes revealed the absence of significant changes of volume den-

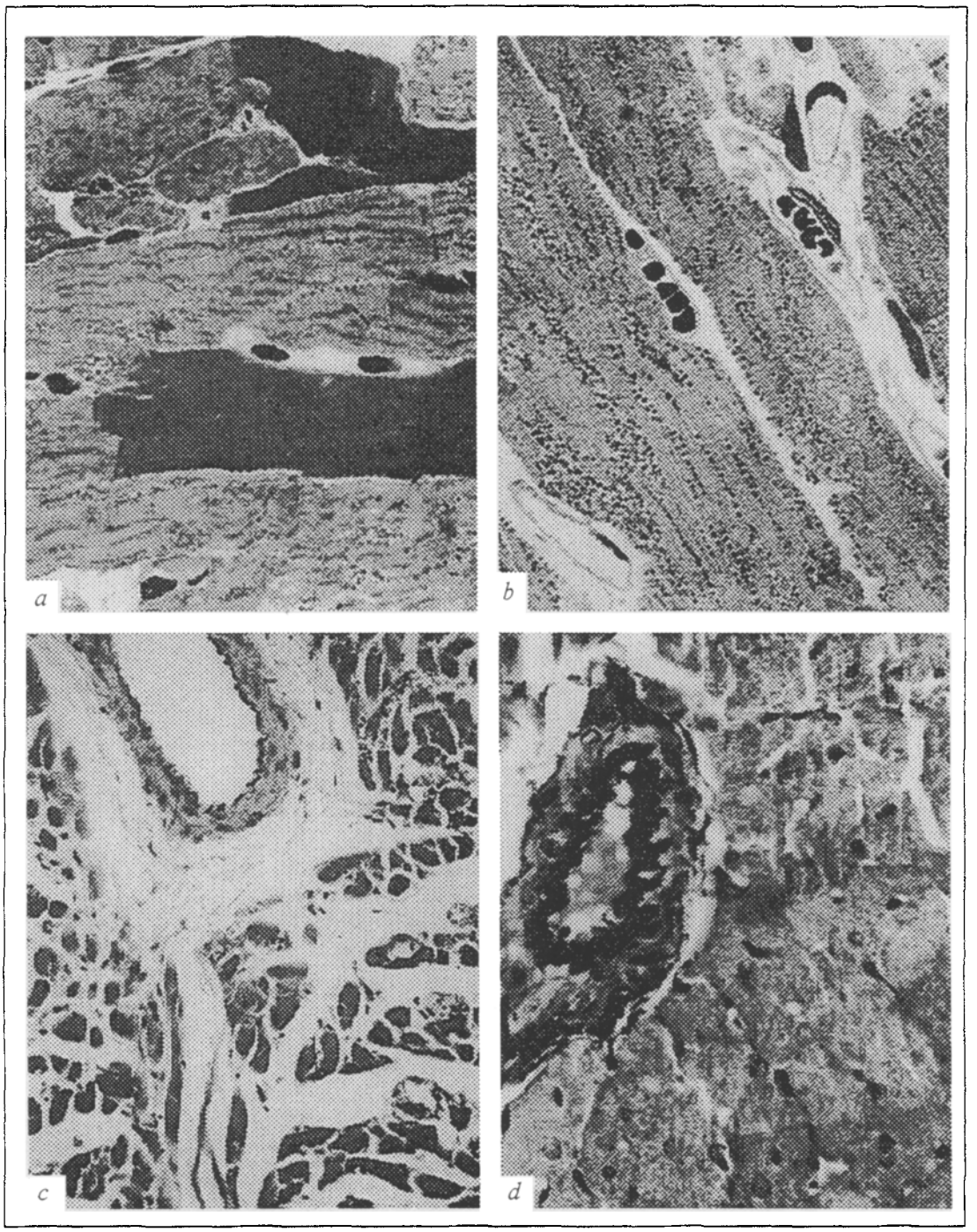

Fig. 2. Morphological changes in rat myocardium after transference to high latitudes. *a*) contracture damage to cardiomyocytes one day after transference; *b*) thinning and lysis of sarcoplasm in cardiomyocytes one day after transference; *c*) marked edema of perivascular and intermuscular connective tissue 37 days after transference; *d*) myoelastofibrosis of intramural arteries on 31st day of experiment. Staining with azure II (*a*, *b*); hematoxylin and eosin (*c*); after van Gieson (*d*). Magnification: *a*, *b*) ×1000; *c*) ×200; *d*) ×500.

sity of cardiomyocytes and of their nuclei, although in the first 12 days after transference this index was increased, especially for the cardiomyocyte nuclei (by 34%) (Table 1). At the same time the surface density and surface-volume ratio of cardiomyocytes and their nuclei were significantly decreased (by 24% and 25% respectively) by the 27th day, attesting to an increase of their size.

The quantitative indexes of the capillaries changed to a greater degree. For example, 12 days after transference the volume density of the capillaries significantly decreased by 34%, and the sur-

TABLE 2. Results of Tissue Stereological Analysis of Myocardium of Rats Transferred to High Latitudes ($M \pm m$)

Index	Control	Time after transference		
		1 day	12 days	27 days
Volume density, mm^3/cm^3:				
cardiomyocytes	839.3±15.0	832.2±15.5	891.7±14.8	859.4±16.2
cardiomyocyte nuclei	8.7±1.6	13.2±3.9	12.8±1.0	11.6±1.5
capillaries	43.2±3.7	41.6±3.9	28.7±1.2*	41.8±3.4
endothelial cells	19.2±1.3	14.0±2.2	12.1±1.2*	14.6±2.2
connective tissue cells	13.9±0.9	8.4±0.4**	7.6±1.1*	8.2±0.6*
connective tissue fibers and matrix	75.7±8.5	90.6±18.0	47.1±15.6	64.4±12.3
Surface density, m^2/cm^3:				
cardiomyocytes	0.1013±0.0068	0.0953±0.0037	0.0782±0.0027*	0.0828±0.0008*
cardiomyocyte nuclei	0.0066±0.0004	0.0088±0.0022	0.0081±0.0003*	0.0081±0.0009
capillaries	0.0361±0.0031	0.0284±0.0018	0.0213±0.0019*	0.0268±0.0007*
connective tissue cells	0.0182±0.0020	0.0082±0.0006**	0.0062±0.0007**	0.0071±0.0006**
Surface–volume ratio, m^2/cm^3:				
cardiomyocytes	0.121±0.006	0.113±0.003	0.087±0.004**	0.095±0.002*
cardiomyocyte nuclei	0.762±0.062	0.684±0.028	0.640±0.043	0.700±0.023
capillaries	0.836±0.084	0.689±0.038	0.742±0.046	0.648±0.036
connective tissue cells	1.308±0.169	0.984±0.046	0.826±0.032	0.865±0.025
capillaries to cardiomyocytes	0.043±0.005	0.034±0.002	0.024±0.002*	0.031±0.001
Volume ratio, number:				
capillaries to cardiomyocytes	0.051±0.001	0.049±0.004	0.032±0.001***	0.048±0.005
stroma to parenchyma	0.179±0.025	0.184±0.024	0.106±0.018	0.148±0.023

face density by 36% (Table 1). Toward the 27th day these indexes were 15% and 27% lower in comparison with the control. The pronounced decrease of the volume and surface parameters of the capillaries caused a reliable decrease of the volume and surface-volume ratio of capillaries to cardiomyocytes, which was most significant on the 12th day after transference (by 35% and 40%, respectively). On the 27th day these indexes were decreased to a lesser degree, namely by 16% and 28%.

The quantitative characteristics of the connective tissue cells markedly changed. The volume and surface density of these structures were significantly decreased in all periods of the experiment but most of all on the 12th day (by 55% and 64% compared to 41% and 60% and to 34% and 61%, respectively, on the 1st and on the 27th day). In general, the parenchymatous-stromal ratios did not significantly change throughout the experiment, although a decrease (by 18%) of the ratio between the stroma and parenchyma volumes on the 12th day after transference is to be noted.

The dynamics of stereological indexes of the main structural components of the myocardium of the rats subjected to transference to high latitudes was similar in many respects to that for a flight in the middle latitudes.

The volume density of the cardiomyocytes practically did not change during the whole experiment, while their surface density was significantly decreased on the 12th and 27th day of flight to the high latitudes (by 23% and 18%, respectively) (Table 2). This caused a significant drop of the surface-volume ratio of cardiomyocytes in these periods by 28% and 21% respectively, which testified to hypertrophy of the cardiomyocytes. An increase of volume and surface density was noted in cardiomyocyte nuclei and was most pronounced one day after the flight.

The changes of volume and surface density of the capillaries were most pronounced on the 12th day after transference. These indexes decreased in this period by 34% and 41%, respectively, while 1 day and 27 days after flight the volume and surface density of the capillaries were decreased by 4% and 26%, respectively. The marked decrease of capillary volume and surface density on the 12th day led to a significant decrease of the volume and surface-volume ratio of capillaries to cardiomyocytes (by 37% and 44%, respectively) (Table 2).

Under conditions of high latitudes, just as after transference in the middle latitudes, the volume and surface density of the connective tissue cells were markedly decreased. These changes were most pronounced on the 12th day of the experiment (by 45% and 66%, respectively). The surface-volume ratio of these structures did not reliably change throughout the experiment. The volume ratio of stroma to parenchyma was not significantly changed during the whole experiment,

although a tendency toward a decrease of this index was noted on the 12th day after transference to high latitudes.

The investigation enabled us to conclude that the dynamics of the stereological parameters of the main structural components of the myocardium for transference to high and middle latitudes is stereotypic. Most pronounced in both series were the changes of the volume and surface density of the capillaries, which reliably decreased toward the 12th day of the experiment. The lowering of these indexes determined a significant decrease of the volume and surface-volume ratio of capillaries to cardiomyocytes in this period and was more pronounced in rats situated in the high latitudes. The similar structural-spatial reorganization of the myocardium for different types of transferences testifies to the common mechanisms of this reorganization. Stress is probably the leading stimulus which determines the nature of tissue reorganization of the myocardium. However, the various degree of morphofunctional changes in the same periods in different experimental series attested to the existence of other factors (notably heliogeophysical) affecting the adaptive reorganization of the myocardium in the high latitudes [4].

The study was funded by the Russian Foundation for basic research (project code 93-04-7353).

REFERENCES

1. A. P. Avtsyn, A. A. Zhavoronkov, A. G. Marachev, *et al., Human Pathology in the North* [in Russian], Moscow (1985).
2. V. P. Kaznacheev, *Modern Aspects of Adaptation* [in Russian], Novosibirsk (1980).
3. V. P. Kaznacheev, *Byull. Sib. Otdel. Acad. Med. Nauk SSSR*, № 6, 19-26 (1987).
4. *Mechanisms of Human Adaptation under High-Latitude Conditions* [in Russian], Ed. V. P. Kaznacheev, Leningrad (1980).
5. L. M. Nepomnyashchikh, E. L. Lushnikova, L. V. Kolesnikova, *et al, Morphometric and Stereological Analysis of the Myocardium: Tissue and Ultrastructural Organization: Procedural Guidelines* [in Russian], Novosibirsk (1984).
6. L. M. Nepomnyashchikh, *Morphogenesis of the Main General Pathological Processes in the Heart* [in Russian], Novosibirsk (1991).
7. L. M. Nepomnyashchikh, L. V. Kolesnikova, and E. B. Leitan, *Physiology and Pathology of Human Adaptation in the Far North* [in Russian], Novosibirsk (1977), pp. 49-51.