MORPHOLOGY AND PATHOMORPHOLOGY

Intracellular Reorganization of Rat Cardiomyocytes Following Transfer to High Latitudes

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UDC 616.127-018.1-02:612.275.1]-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 5, pp. 538-542, May, 1994
Original article submitted January 2, 1994

It is shown that as soon as the 1st day after transfer to high latitudes, the regenerative processes are suppressed in some cardiomyocytes, this manifesting itself in lytic changes of the sarcoplasm and organelles in the perinuclear zone. After flight in the middle latitudes morphological signs of disturbances in the processes of intracellular regeneration are observed only toward the end of the experiment. Intracellular spatial reorganization of cardiomyocytes for transfer to high latitudes and for flight in the middle latitudes has a stereotypic pattern and primarily manifests itself in an increase of the volume density of myofibrils and a decrease of this parameter for mitochondria.

Key Words: *cardiomyocytes; intracellular organization; high latitudes; stereology*

Transfer to high latitudes is attended by marked changes in the activity of different morphophysiological systems [1-3,5], which is reflected in structural rearrangements in the tissues and cells making up these systems. The cardiovascular system is among the first to be involved in the adaptive reorganization of the organism [1] and largely affects this process. Tissue reorganization in the myocardium has a wavelike pattern [7] and is underpinned by the development of the general adaptive syndrome, as well as by specific ecological factors of the Far North, notably heliogeophysical factors [2]. Prediction and assessment of the reliability of the adaptive-compensatory responses of the myocardium depend not only on the pattern and tendency of tissue reorganization, but also on the dynamics of intracellular rearrangements in cardiomyocytes, which has scarcely been studied for the conditions obtaining in high latitudes.

In this study we performed a quantitative analysis of intracellular spatial reorganization of rat cardiomyocytes for transfer to high latitudes.

MATERIALS AND METHODS

The experiments were carried out on 109 male Wistar rats transported by air to Alykel', a locality situated at the 69th parallel [7]. Control animals were transported by air in the middle latitudes under the same conditions of flight (for the assessment of flight-induced stress). Intact animals of the same age kept in the vivarium served as an additional control. Tissue samples for electron-microscopic examination were synchronously taken from experimental and control animals on days 1, 12, 27, and 37.

For electron microscopy tissue samples of the left ventricle myocardium were fixed in 4% paraformaldehyde and postfixed in 1% osmium tetrox-

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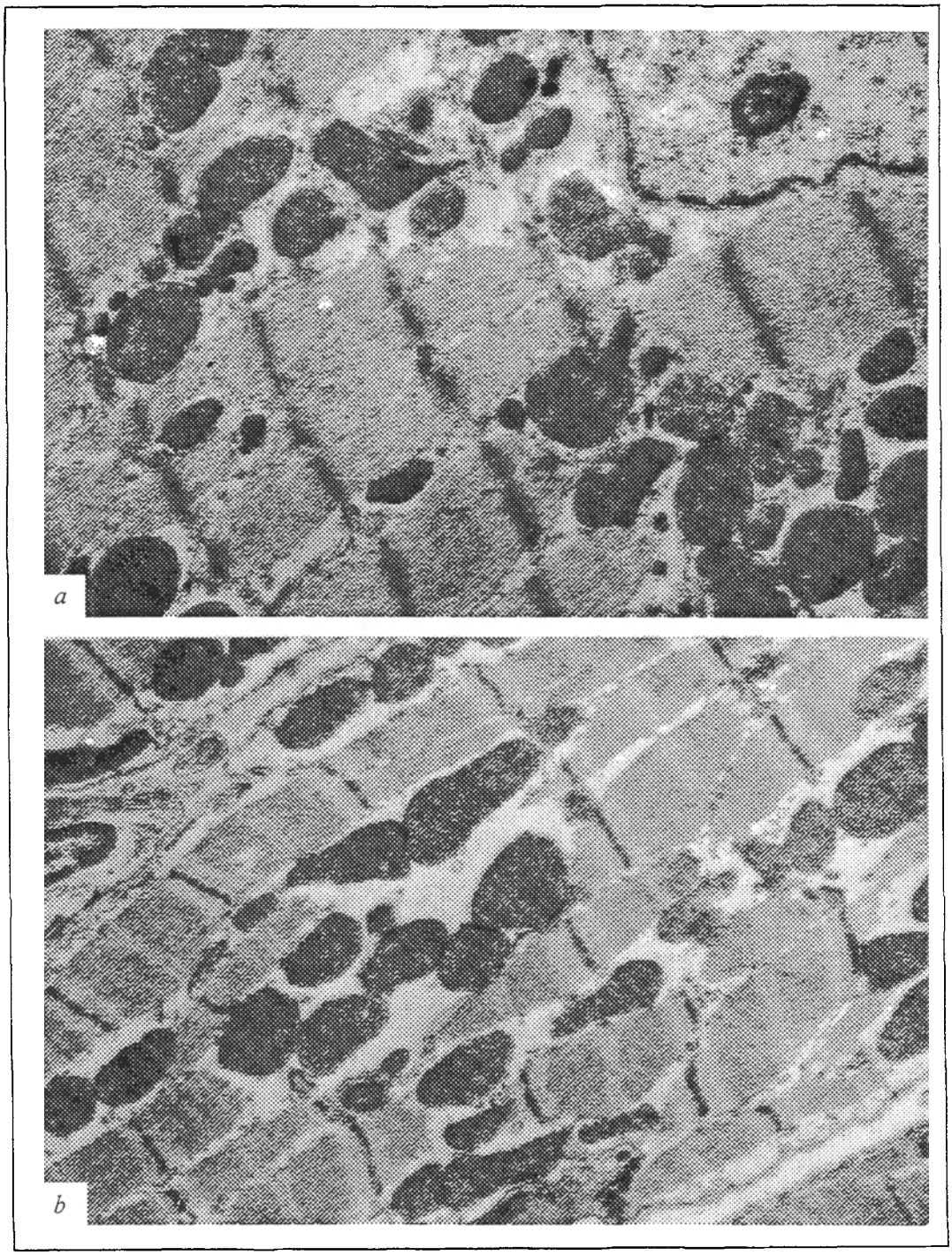

Fig. 1. Ultrastructure of rat cardiomyocytes 12 days after transfer to high latitudes. ×5000. *a*) lysis of sarcoplasm and reduced number of organelles in perinuclear zone; *b*) lytic changes of sarcoplasm and myofibril bundles in atrophied cardiomyocyte.

ide. After dehydration and impregnation the tissue was embedded in Epon-Araldite. Ultrathin sections were obtained on an LKB III ultratome, contrasted with uranyl acetate and lead citrate, and examined under a JEM-100B electron microscope. Stereological analysis of the intracellular structure of cardiomyocytes was performed using negatives (final magnification 18,000; initial magnification 5000). The volume and surface density of myofibrils, mitochondria, sarcoplasmic reticulum, and the T system, as well as the volume density of the sarcoplasmic matrix were assessed using a multipurpose test system [4]. The secondary stereological parameters (the surface-volume and volume ratios of structures) were quantified on the basis of these results. Statistical analysis of the data involved cal-

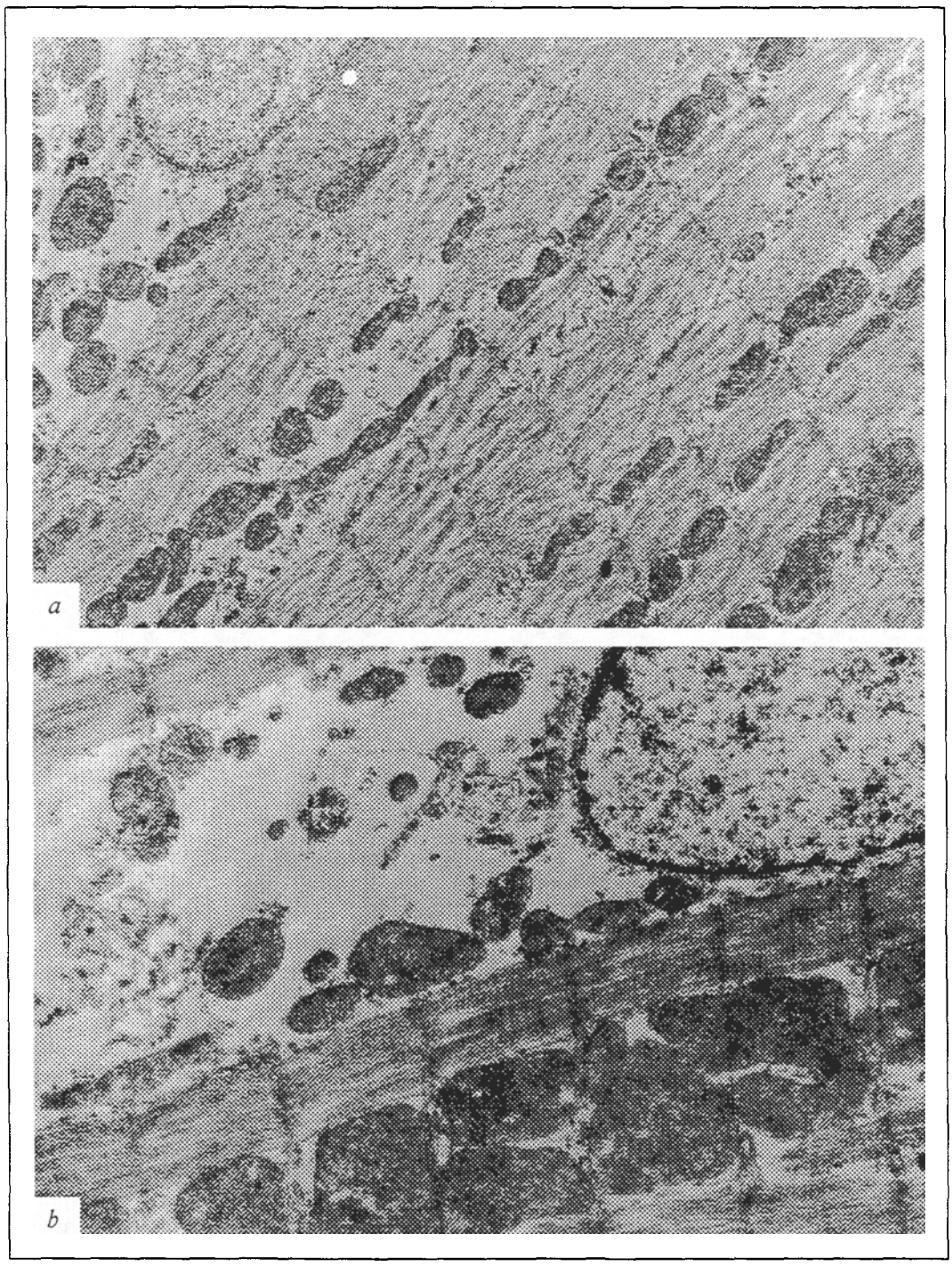

Fig. 2. Ultrastructure of rat cardiomyocytes 27 days after transfer to high latitudes. ×5000. *a*) separation and lysis of myofilaments in myofibril bundles and pronounced lysis of sarcoplasmic matrix; *b*) lysis of sarcoplasm and destruction of organelles in perinuclear zone.

culation of the means and the error of the means and comparison of the means using Student's test.

RESULTS

During the first 3 weeks after flight in the middle latitudes, the ultrastructure of rat cardiomyocytes was virtually unchanged. Mitochondria and myofibrils were close-packed in longitudinal strands; contracture of myofibrils was noted in some cells. On day 27 after the trip small foci of lysis of the sarcoplasm and myofibrils as well as "fraying" of myofibrils were observed in cardiomyocytes. These changes were preserved till the end of the experiment.

Transfer to high latitudes caused marked changes in the ultrastructure of cardiomyocytes as soon

TABLE 1. Results of Stereological Analysis of Intracellular Structure of Rat Cardiomyocytes for Flight in the Middle Latitudes ($M \pm m$)

Parameter	Control	Time after transfer		
		day 1	day 12	day 27
Volume density, mm^3/cm^3:				
myofibrils	516.1±10.2	531.1±22.1	589.2±11.5*	588.8±9.4*
mitochondria	346.1±23.6	323.6±10.4	258.8±10.9*	267.0±9.7*
ASR	14.9±1.5	18.7±3.5	15.2±1.2	16.7±2.2
T system	13.7±1.7	14.1±3.2	11.2±0.6	12.9±1.4
sarcoplasm	109.2±10.1	112.5±10.6	125.6±2.3	114.6±5.8
Surface density, mm^2/cm^3:				
myofibrils	1.2484±0.0418	2.1148±0.2241	2.0724±0.0321***	1.7770±0.0447
mitochondria	1.2966±0.0678	2.3365±0.0544**	2.0677±0.0236***	1.8490±0.0797*
ASR	0.2719±0.0098	0.2534±0.0533	0.2293±0.0302	0.2212±0.0429
T system	0.1489±0.0138	0.1661±0.0410	0.1310±0.0100	0.1445±0.0125
Surface/volume ratio, m^2/cm^3:				
myofibrils	2.42±0.13	4.03±0.58	3.52±0.02**	3.02±0.06*
mitochondria	3.75±0.06	7.22±0.11***	8.02±0.38**	6.92±0.06***
ASR	18.36±1.19	13.41±0.86*	15.00±0.98	13.17±0.76*
T system	10.91±0.35	11.66±0.08	11.70±0.25	11.34±0.72
Volume ratio (number) between:				
mitochondria and myofibrils	0.672±0.059	0.613±0.044	0.440±0.027*	0.455±0.022*
ASR and myofibrils	0.028±0.003	0.036±0.008	0.025±0.001	0.028±0.003
T system and myofibrils	0.026±0.003	0.027±0.007	0.019±0.001*	0.022±0.002
(mitochondria + ASR + T system) and myofibrils	0.727±0.054	0.676±0.057	0.485±0.025*	0.504±0.018*

Note. Here and in Table 2: one, two, and three asterisks denote reliable differences for $p<0.05$, $p<0.01$, and $p<0.001$, respectively. ASR: agranular sarcoplasmic reticulum.

as on day 1. The crucial factors of intracellular reorganization of cardiomyocytes were reduced biosynthesis and increased lysis of ultrastructures. Clearing of the sarcoplasmic matrix, fraying and thinning of myofibril bundles, and foci of lysis of myofilaments were noted. Lytic changes of the sarcoplasm were most pronounced in the perinuclear and subsarcolemmal zones (Fig. 1, *a*, *b*). An enlargement of vesicles of the agranular sarcoplasmic reticulum was observed. The cardiomyocyte nuclei contained mainly euchromatin; as a rule, nucleoli were fragmented. At this time, immature forms of fibroblasts appeared in the interstitial connective tissue. The capillaries and intercellular spaces were filled with a floccular substance.

Such changes were preserved throughout the course of the experiment and were even more pronounced by its end: on days 27-37. At this time, pronounced lysis of myofibril bundles and sarcoplasmic matrix were noted in some cardiomyocytes (Fig. 2, *a*). It should be mentioned that at all times the mitochondria seemed to be the most stable organelles, although their number in the cells markedly declined, notably in the perinuclear zone (Fig. 2, *b*). During this period polysomes were noted in the sarcoplasm of cardiomyocytes, specifically, in the foci of lysis of myofilaments, which was indicative of enhanced processes of intracellular regeneration. Despite this fact, the number of atrophied cardiomyocytes increased by the end of the experiment.

Stereological analysis of the intracellular structure of rat cardiomyocytes one day after flight in the middle latitudes failed to reveal reliable changes in the volume density of the major cell compartments (Table 1). The surface density of myofibrils increased by 69% and of mitochondria by 80% ($p<0.01$), which led to an increase of their surface/volume ratio (by 66 and 92%, respectively). A reliable decrease (by 27%) of the surface/volume ratio was recorded for the sarcoplasmic reticulum. The volume ratios between the major sarcoplasmic organelles and myofibrils did not change markedly.

The most pronounced changes in the stereological parameters of cardiomyocytes were observed on day 12 after the trip. At this time the volume and surface density of myofibrils reliably increased (by 14 and 66%, respectively). The more marked increase of the surface density of myofibrils caused a reliable increase of their surface/volume ratio (by 45%). The volume density of mitochondria dropped 25% and their surface density rose 59% in parallel, resulting in a twofold increase of the surface/volume ratio of these organelles.

Opposite changes of the volume density of myofibrils and mitochondria caused a reliable decrease (by 34%) in the volume ratio between mitochondria and myofibrils. The volume ratio between the T system and myofibrils also reliably dropped (27%) at this time. These changes caused a reliable decrease (by 33%) in the total volume ratio between the major organelles of cardiomyocytes and myofibrils.

Twenty-seven days later the pattern and tendency of changes in the quantitative ratios between cardiomyocyte structures were preserved. The volume and surface density of myofibrils remained increased (by 14 and 42%, respectively). The volume density of mitochondria was reliably lower (by 23%), and their surface density and surface/volume ratio were reliably higher (by 43 and 85%, respectively). As during the preceding period, the volume ratio between mitochondria and myofibrils and the total volume ratio between the major sarcoplasmic organelles and myofibrils were reliably reduced (by 32 and 31%, respectively).

After transfer of the animals to high latitudes pronounced changes in the cardiomyocyte architectonics were observed as soon as on day 1. At this time the volume and surface density of myofibrils reliably increased (9 and 48%, respectively) (Table 2). The volume density of mitochondria dropped 27% ($p<0.05$), and their surface density and the surface/volume ratio reliably rose (44 and 98%, respectively). An increase in the volume density of the sarcoplasmic reticulum (by 28%) and sarcoplasmic matrix (by 38%) was also noted. After just one day a reliable decrease was discovered in the volume ratio between mitochondria and myofibrils and in the total volume ratio between the major organelles and myofibrils (by 33 and 31%, respectively).

Later, the dynamics of quantitative changes in the major sarcoplasmic organelles of cardiomyocytes was preserved. On day 27 the volume and surface density of myofibrils were 10 and 42%, respectively, higher. The volume density of mitochondria was reliably lower (by 27%), and their surface density and the surface/volume ratio were increased (by 51 and 107%, respectively). At this time a reliable increase (by 46%) of the volume density of the agranular sarcoplasmic reticulum and a reliable decrease in its surface/volume ratio (by 27%) were noted.

Analysis of the volume ratio of the major organelles of cardiomyocytes revealed a reliable reduction of this parameter for mitochondria and myofibrils (by 34%), as well as a decrease in the total volume ratio between the major sarcoplasmic organelles and myofibrils (by 30%).

Thus, intracellular spatial reorganization of cardiomyocytes for transfer to high latitudes and in the middle latitudes had a stereotypic pattern, but

TABLE 2. Results of Stereological Analysis of Intracellular Structure of Rat Cardiomyocytes for Transfer to High Latitudes ($M \pm m$)

Parameter	Control	Time after transfer		
		day 1	day 12	day 27
Volume density, mm^3/cm^3:				
myofibrils	516.1±10.2	564.9±9.9*	562.6±11.1	570.5±10.6*
mitochondria	346.1±23.6	252.1±7.3*	270.8±7.4*	252.9±8.2*
ASR	14.9±1.5	19.0±1.8	19.9±0.6*	21.7±1.4*
T system	13.7±1.7	13.4±1.5	17.0±0.9	16.0±1.8
sarcoplasm	109.2±10.1	150.6±9.5	129.7±3.1	138.9±10.8
Surface density, mm^2/cm^3:				
myofibrils	1.2484±0.0418	1.8487±0.0377**	1.8744±0.1257*	1.7665±0.1531
mitochondria	1.2966±0.0678	1.8719±0.0541**	1.9728±0.0561**	1.9528±0.1083*
ASR	0.2719±0.0098	0.2492±0.0171	0.2512±0.0343	0.2878±0.0178
T system	0.1489±0.0138	0.1516±0.0181	0.1884±0.0108	0.1773±0.0112
Surface/volume ratio, m^2/cm^3:				
myofibrils	2.42±0.13	3.27±0.01**	3.34±0.26	3.09±0.20
mitochondria	3.75±0.06	7.44±0.28**	7.29±0.20***	7.76±0.66*
ASR	18.36±1.19	13.29±1.21	12.57±1.48	13.45±1.02*
T system	10.91±0.35	11.27±0.32	11.18±1.14	11.48±1.43
Volume ratio (number) between:				
mitochondria and myofibrils	0.672±0.059	0.447±0.018*	0.482±0.023*	0.446±0.030*
ASR and myofibrills	0.026±0.003	0.024±0.003	0.030±0.002	0.029±0.002
T system and myofibrils	0.028±0.003	0.033±0.003	0.035±0.002	0.038±0.004
(Mitochondria + ASR + T system) and myofibrils	0.727±0.054	0.504±0.025*	0.548±0.025*	0.512±0.035*

under conditions of high latitudes the changes were more pronounced and started to manifest themselves from the first day of the experiment. Marked differences were observed in the ultrastructure of cardiomyocytes. After flight in the middle latitudes, morphological signs of disturbances in intracellular regeneration manifested themselves only toward the end of the experiment. In the high latitudes marked suppression of regenerative processes in some cardiomyocytes was observed in animals as soon as on the first day of the experiment, attesting to the development of plastic insufficiency of cardiomyocytes [6,8]. The described complex of ultrastructural changes in cardiomyocytes under conditions of high latitudes probably reflects peculiarities of space-time organization of cellular regenerative processes under heliogeophysical conditions of the Far North.

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

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