Quantitative Ultrastructural Analysis of Rat Cardiomyocytes after a Prolonged Stay in the Mountains

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UDC 616.127-018.1-02:612.275.1]-76.4-092.9

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 117, № 6, pp. 661-665, June, 1994
Original article submitted March 15, 1994

Disturbances of intracellular regeneration in cardiomyocytes (CMC) are demonstrated in rats kept for a prolonged period in the mountains. These disturbances cause focal destruction and lysis of organelles. The spatial intracellular reorganization of CMC under these conditions is characterized by an increased volume and surface density of myofibrils and decreased volume density of mitochondria, agranular sarcoplasmic reticulum, and T-system.

Key Words: *cardiomyocytes; ultrastructure; intracellular reorganization; high altitudes; stereology*

Living at high altitudes causes cardiac hypertrophy (predominantly of the right ventricle) in all mammalian species [1-3,10]. Such alterations in cardiac structure and function are considered to be adaptive and evolutionary, having developed in response to a rise of the blood pressure in the pulmonary circulation [5,9]. Different types of myocardial reorganization have been observed after different periods of hypobaric hypoxia. After a short-term hypobaric hypoxia, hypertrophy of cardiomyocytes is accompanied by a corresponding increase in the capacity of the microcirculatory bed, the density of capillaries in this case either increasing or remaining unchanged [6,11,12]. The imbalance between the increase in the size of CMC and capillaries is manifested in decreased volume and surface-volume ratios of capillaries to CMC [4]. Changes in the intracellular organization of CMC under different regimes of hypobaric hypoxia have been less studied, which hampers the development of prognostic criteria for the morphofunctional state of the myocardium under these conditions.

After a short-term hypobaric hypoxia an increase in the mass of myofibrils [10,13,14] predominates, i.e., the type of structural changes characteristic of hypertrophied cardiac myocytes. The nature and direction of intracellular organization of CMC caused by a long-term hypobaric hypoxia have not been investigated; they were thus the topic of the present study.

MATERIALS AND METHODS

Male Wistar rats were used. They were kept at an altitude of 3200 m above sea level (Tyan-Shan' Mountains, Tuya-Ashu pass) [4,5] at room temperature on the standard diet and water ad libitum. Intracellular reorganization of cardiomyocytes was studied after a 5- or 10-month stay in the mountains (18 and 10 rats, respectively). Male Wistar rats of the same age kept in the valley were used as the control.

For the electron microscopy studies specimens of the left-ventricle myocardium were fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide, dehydrated, and embedded in araldite. Ultrathin

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0007-4888/94/0006-0669$12.50

Fig. 1. Ultrastructural changes occurring in rat CMC after a 5-month stay in the mountains. *a*) lytic changes in I-disks of myofibrillar bundles, destruction and lysis of organelles in the perinuclear zone; the products of autophagy of CMC ultrastructures are seen in the capillary lumen; *b*) compact arrangement of organelles in an atrophied CMC. ×5000.

sections were cut on an LKB III microtome, contrasted with uranyl acetate and lead citrate, and studied in a JEM 100B electron microscope.

Stereological analysis of the intracellular reorganization of CMC was performed with the use of a multipurpose test system of short segments [7]. The volume and surface density of the major CMC organelles (myofibrils, mitochondria, agranular sarcoplasmic reticulum, and T-system) were evaluated. The secondary stereological parameters (surface-volume and volume ratios of the organelles) were calculated from the primary parameters. Statistical

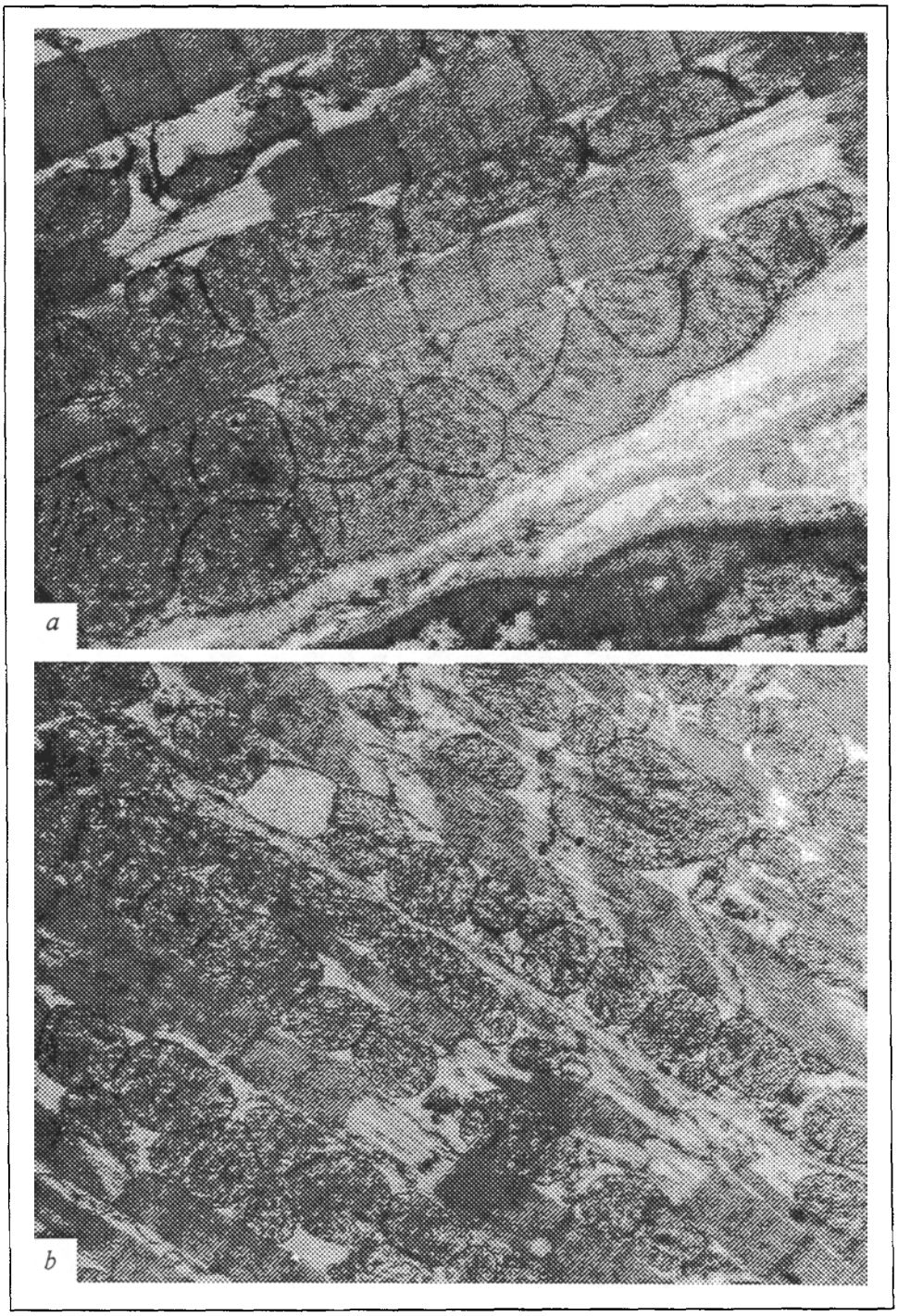

Fig. 2. Ultrastructural changes occurring in rat CMC after a 10-month stay in the mountains. *a*) focal lysis of sarcomeres in myofibrillar bundles, ×6000; *b*) preferential lysis of I-disks of myofibrillar bundles; lytic changes of the matrix and destruction of some mitochondria, ×5000.

analysis included the calculation of the means and their comparison using Student's *t* test.

RESULTS

A 5-month stay in the mountains induced considerable changes in the CMC ultrastructure. Lytic damage to intracellular ultrastructures (predominantly to myofibrils) prevailed in numerous cells. Myofibrillar bundles looked disintegrated due to partial lysis of microfilaments. Overcontraction and overtension of some sarcomeres along the myofibrillar bundles and preferential lysis of the thin (actin) myofilaments (clearing and destruction of I-

TABLE 1. Results of Stereological Analysis of the Intracellular Organization of Rat CMC during Adaptation to Life in the Mountains ($M \pm m$)

Parameter	Valley		Mountains	
	age 7 months	age 11 months	during 5 months	during 10 months
Volume density, mm^3/cm^3:				
myofibrils	488.6±12.7	517.3±13.4	530.3±12.8*	553.0±26.2
mitochondria	329.4±15.8	305.8±16.5	315.9±5.7	297.7±14.1
SPR	20.3±2.5	20.7±1.6	21.0±1.9	14.4±1.2*
T-system	19.0±1.3	18.6±1.0	13.5±0.9**	13.4±1.1*
sarcoplasm	142.6±15.5	137.5±14.3	119.3±14.0	121.5±18.6
Surface density m^2/cm^3:				
myofibrils	1.4698±0.1330	1.488±0.087	1.9719±0.0719**	2.0372±0.1002*
mitochondria	1.4817±0.0410	1.415±0.101	1.7748±0.0475***	1.8004±0.0308**
SPR	0.3821±0.0509	0.367±0.045	0.3703±0.0656	0.2446±0.0356
T-system	0.2629±0.0314	0.258±0.036	0.1644±0.0187*	0.1630±0.0104*
Surface-volume ratio, m^2/cm^3:				
myofibrils	3.03±0.31	2.9±0.2	3.72±0.14	3.69±0.16*
mitochondria	4.58±0.56	4.6±0.3	5.62±0.17	6.08±0.31*
SPR	18.92±1.19	17.7±1.8	17.30±1.64	16.89±1.87
T-system	13.41±1.43	13.9±0.9	12.02±0.78	12.22±0.55
Volume density, number of:				
mitochondria to myofibrils	0.676±0.037	0.591±0.039	0.597±0.020	0.544±0.043
SPR to myofibrils	0.042±0.006	0.040±0.004	0.040±0.003	0.026±0.003*
T-system to myofibrils	0.039±0.005	0.036±0.003	0.025±0.002*	0.024±0.002*
mitochondria, SPR, and T-system to myofibrils	0.0757±0.036	0.667±0.030	0.662±0.020*	0.595±0.046

Note. Asterisks indicate values statistically different from the control (animals of the same age kept in the valley) at $p<0.05$ (one asterisk), $p<0.01$ (two asterisks), and $p<0.001$ (three asterisks).

disks) occurred in some CMC (Fig. 1, *a*). Mitochondrial ultrastructure was more stable; however, occasional mitochondria with focal destruction of cristae were seen. Signs of partial necrosis and destruction of organelles were noted in the perinuclear zone; as a result, this zone often looked "empty". Generally, in atrophied cells (Fig. 1, *b*) the organelles were compactly arranged, and the nuclei were localized in the subsarcolemmal zone. Polysomes were seen in the sarcoplasm of the CMC whose ultrastructure was close to that of normal cells.

After a 10-month stay in the mountains the nature of the ultrastructural changes was not altered; however, the lytic processes were intensified. In some CMC myofibrils were lysed considerably, most frequently in the I-disk zone (Fig. 2, *a*, *b*), so that the myofibrils looked broken. Partial myocytolysis was observed (usually near the I-disks) in some CMC; in these areas myofibrils were lysed completely, and only occasional mitochondria were seen in the sarcoplasm. Generally speaking, in all the cells with pronounced myofibrillar lysis the mitochondria were freely arranged without forming strands. Myelinlike structures were present in intercellular spaces and capillary lumens throughout the experimental period, these structures probably resulting from the destruction and autophagy of CMC organelles.

The spatial reorganization of rat CMC after a 5-month stay in the mountains was manifested in a significant increase in volume and surface density of myofibrils (8.5 and 34%, respectively) (Table 1). The volume density of mitochondria remained practically unchanged compared with the control (animals kept in the valley), but the surface density of mitochondria increased significantly (20%), which led a to significant increase (23%) in the surface-volume ratio of these organelles. During this period, the volume and surface density of the T-system decreased (29 and 37%, respectively).

The analysis of the volume ratios of the major CMC organelles and myofibrils revealed a decrease in these parameters for mitochondria (12%) and the T-system (35%). The total volume ratio of mitochondria, sarcoplasmic reticulum (SPR), and T-system to myofibrils was lowered by 12.5%.

After a 10-month stay in the mountains the general nature of the changes in the spatial organization of CMC was preserved. In comparison with the control animals, the volume and surface

densities of myofibrils were increased (7 and 37%, respectively), as well as their surface-volume ratio (27%). The volume density of mitochondria decreased slightly, whereas their surface density increased significantly (27%), which led to an increase (32%) in the surface-volume ratio of these organelles.

The volume density of SPR and the T-system was significantly decreased (30 and 28%, respectively). The surface density of these ultrastructure decreased by 33 and 37%, respectively; as a result, their surface-volume ratio remained practically unchanged.

The analysis of the relationships between the major CMC organelles revealed a significant decrease in the volume ratio of SPR and the T-system to myofibrils (35 and 33%, respectively) and a decrease in the total volume ratio of the major organelles to myofibrils (11%).

Thus, a long stay in the mountains induced pronounced changes in the intracellular regeneration of rat CMC, which resulted in destruction and lysis of the sarcoplasmic reticulum and organelles with subsequent atrophy of cardiomyocytes. The intracellular spatial reorganization of CMC under these conditions was characterized by an increase in the volume and surface densities of myofibrils and a decrease in the volume density of mitochondria, SPR, and the T-system, which led to a decrease in the total volume ratio of these organelles to myofibrils. A similar intracellular reorganization of cardiomyocytes was found in reduced functional activity of the heart. [8]. Disproportional changes in the volume density of myofibrils were documented after a short-term hypobaric hypoxia [13,14]; however, the volume density of SPR and the T-system remained unchanged. A long-term high-altitude hypoxia reduces the synthesis of all structural proteins in CMC, particularly of those forming the sarcolemma and SPR membranes.

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