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CHANGES IN ULTRASTRUCTURE AND ACTOMYOSIN COMPLEX OF CARDIOMYOCYTES IN EXPERIMENTAL HYPERGRAVITATION

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Centripetal acceleration acting on the living organism during ordinary flights and during exploitation of space gives rise to a number of changes in cardiac activity [5]. Morphological and physicochemical changes arising under these circumstances in cardiomyocytes have not been adequately studied [4, 7]. Accordingly, the aim of the investigation described below was to compare the ultrastructure of cardiomyocytes (CMC) with some parameters of contractile function, physicochemical properties, and protein composition of the actomyosin complex of the heart muscle after gravitational overloading and subsequent rest.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats, male and female, weighing 180-200 g. Hypergravitation was produced by the method described previously [8]. The animals were subjected to acceleration of +5*g* for 15 days, for 25-30 min each day. The rats were killed by decapitation under ether anesthesia next day (Group 1) and 30 days (Group 2) after the last spin. The control consisted of 25 intact animals (Group 3). Pieces of ventricular myocardium for electron-microscopic study were taken from the ventricles of three animals of Group 1 and four animals of Group 2, fixed in buffered OsO_4 solution, dehydrated, and embedded in a mixture of Epon and Araldite. Ultrathin sections, after double staining, were studied in the BS-500 electron microscope. Parameters of contraction of an isolated strip of myocardium (ISM) of the left ventricle of 20 rats from each group were studied as in [3]. Components of the actomyosin complex were identified by electrophoresis in 10% PAG-SDS [12]. To

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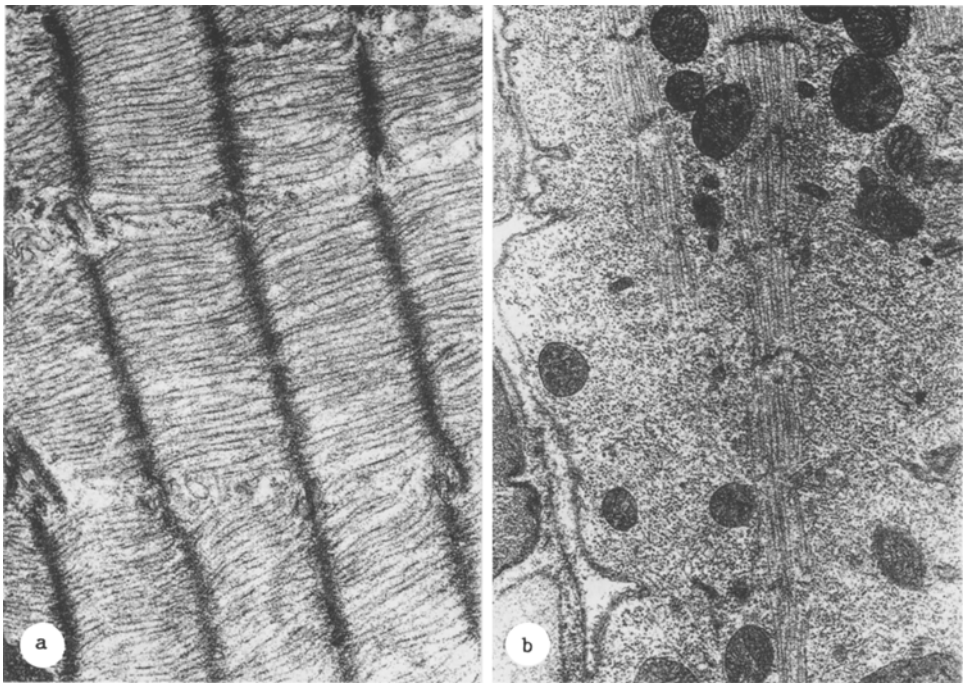

Fig. 1. Signs of hypertrophy in CMC: a) separation of hypertrophy MF into layers. 22,000×; b) de novo formation of contractile structures. 16,300×.

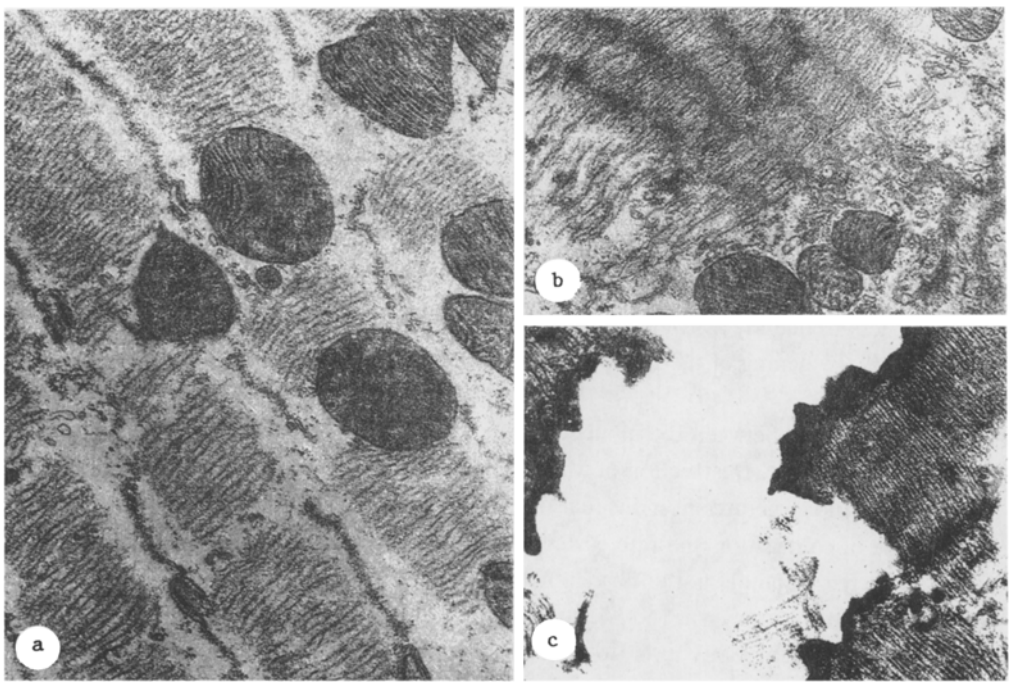

Fig. 2. Destructive changes in CMC. a) Lysis of I disks. 20,870×; b) contractural changes in MF. 20,000×; c) widening of intercellular space. 16,500×.

determine the ratio between the components of the complex quantitatively, the gels after electrophoresis were processed densitometrically. The ATPase activity of the actomyosin complex was studied by a potentiometric method [2]. Altogether 67 animals from Group 1 and 50 from Group 2 took part in the experiments.

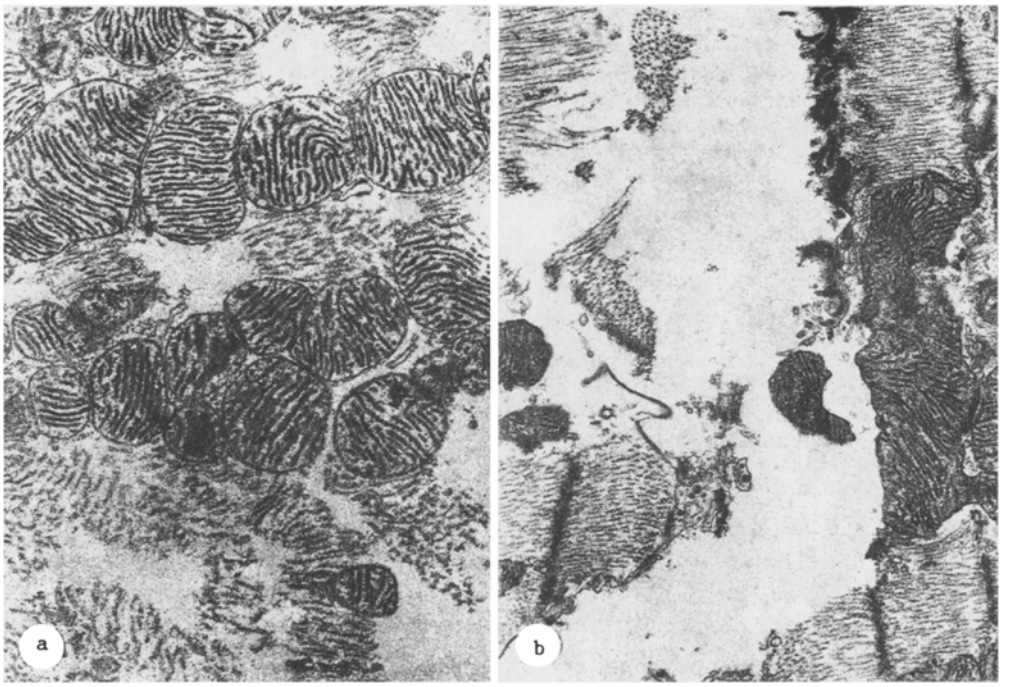

Fig. 3. Destruction of MF during rest: a) disorganization of MF in CMC. 16,700×; b) focal disappearance of contractile structures in CMC. 16,700×.

EXPERIMENTAL RESULTS

Signs of hypertrophy were observed in many ventricular CMC of the rats of Group 1: an increase in diameter of the myofibrils (MF) followed by their separation into layers, de novo formation of contractile structures, with the participation of ribosomes (Fig. 1a, b), hyperplasia of the mitochondria and other cell organoids, activation of the nucleus, and the formation of deep longitudinal invaginations of the sarcolemma (SL), the character of which indicates that the CMC can divide by cleavage. In these cells, in addition, lysosomes (LS) and a well developed Golgi complex could frequently be identified. In a minority of CMC injury to MF was visible, in the form most frequently of lysis of the components of the I disks (Fig. 2a) or of all parts of the sarcomere simultaneously, less frequently as contractures (Fig. 2b). Many cells with lysis of myofibrils were characterized by disturbances of the integrity of SL, widening of the intercellular space (Fig. 2c), and the presence of LS more often than in the intact myocardium.

In the rats of Group 2 the ratio between the numbers of unchanged, hypertrophied, and damaged cells changed toward an increase in the number of the last type. Destruction of MF also was intensified, sometimes with complete disorganization or even disappearance of the contractile structures in individual foci or over the whole territory of the CMC (Fig. 3a, b).

The maximal amplitude of contraction and rate of development of tension and relaxation of ISM in the rats of Groups 1 and 2 showed a tendency to decrease and did not differ significantly from values obtained in Group 3. In Group 1 there was an increase in ATPase activity of natural actomyosin (NAM) to 0.21 ± 0.012, and of desensitized actomyosin (DAM) to 0.256 ± 0.013 $\mu g\ P_i^{-1} \cdot mg^{-1} \cdot sec^{-1}$ (compared with 0.093 ± 0.016 and 0.124 ± 0.125 respectively), and also a decrease in the relative content of heavy chains of myosin and actin, and an irregular increase in the relative content of components of tropomyosin, troponin-I, and myosin light chains 1 and 2 (LC-1 and LC-2). Against the background of these changes, LC-3, usually absent in the control myosin preparations, appeared. Incomplete normalization of the ATPase activity of NAM and DAM and differences in the rate of recovery of the relative quantitative and qualitative parameters of components of the actomyosin complex could be detected in Group 2, but in the course of 30 days the initial level could not be reached (Table 1).

Thus the character of the changes in ultrastructure of MF, and the properties and protein composition of the actomyosin complex under hypergravitation conditions point to the reaching of a definite level of adaptation. Changes noted in actomyosin led to preservation of myocardial function, despite the destruction of part of MF. This took place because the increase in the content of LC-2 and the relative content of troponin-C, arising under these conditions, and combined with the appearance of the

TABLE 1. Content of Components of Native Actomyosin ($M \pm \sigma$)

Component	Group		
	1	2	3
	Content, per cent		
Heavy chains	29,3±0,213*	36,75±0,131	41,66±0,166
α-Actinin	7,42±0,298	7,52±0,200	7,7±0,265
Actin	9,6±0,124*	11,7±0,149	16,0±0,247
Troponin-T	6,55±0,189*	3,3±0,105	3,2±0,105
Tropomyosin	4,9±0,180*	3,5±0,166	3,1±0,137
Troponin-I	3,1±0,111*	3,85±0,164	4,5±0,149
LC-1	6,05±0,251*	2,6±0,152	3,66±0,314
LC-2	13,3±0,133*	4,5±0,149	2,8±0,157
Troponin C	6,5±0,166*	3,7±0,208	3,75±0,155
LC-3	6,56±0,033*	2,22±0,114*	0

Legend. In each group 25 animals were used (n = 10). Significant differences between mean values relative to control group indicated by an asterisk.

isoform LC-3, may increase the affinity of these structures for Ca^{2+} and activity of Ca^{2+}-dependent Mg-ATPase [8], which in turn may lead to inhibition of the relaxation process and to a longer period of stay of MF in the active state.

It will be noted that recovery of the physicochemical properties of the myocardial proteins after 30 days of rest was not accompanied by normalization of CMC ultrastructure. The state of the CMC rather correlates with changes in the actomyosin complex on the 15th day of rest [13]. This delay in the eradication of injuries may be explained on the grounds that degradation and synthesis of contractile proteins, accelerated during a change in the biomechanical conditions of the muscles, precede self-assembly and the organization of ultrastructural components. Damage to the ultrastructures itself was probably connected with the action of proteases, as shown by the increase in the number of LS, and also the similarity of the structural disturbances with those described previously during activation of these enzymes [10]. Protease activity may promote myocardial ischemia, induced by redistribution of the blood during hypergravitation. Potentiation of destructive changes in CMC in the rest period was perhaps due to restoration of activity of neutral proteases after a decline at the end of the adaptation period [1]. Furthermore, lysis of the ultrastructures is an invariable condition of their future renewal [9]. All these observations indicate the multistaged course of regeneration in the myocardium and its wavelike nature, similar to fluctuations of restoration of working capacity of an organ, following the course of a damp sinusoidal curve [6].

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