Effect of Weightlessness on the Ultrastructure of Rat Striated Muscles

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The ultrastructure of rat striated muscles fixed on the 13th day of space flight was studied. It was found that adaptive atrophic and degenerative changes occur in parallel with atypical cellular and intracellular regeneration. The muscles are not completely restored after a 14-day readaptation on the Earth.

Key Words: *ultrastructure; skeletal muscle; weightlessness; atrophy; regeneration*

Ultrastructural studies have shown that even a 7-day space flight affects skeletal muscles [2,4,6]. It should be remembered that ultrastructural changes in the muscle are caused by at least two factors: weightlessness and gravitational overload. Changes caused by gravitation develop rapidly and aggravate structural shifts caused by weightlessness, thus masking them and hampering data interpretation.

In the present study we investigated the ultrastructure of rat skeletal muscles collected and fixed during a 14-day flight of American biomedical laboratory SLS-2, immediately after landing, and after a 14-day readaptation on the Earth. This allowed us to assess the effects of weightlessness and gravitation-induced changes.

MATERIALS AND METHODS

The ultrastructure of *m. soleus* and the inner head of *m. gastrocnemius* was studied. Sprague Dowly rats (body weight 260 g) were decapitated on the 13th day of space flight (group 1) and 5 h and 14 days after landing (groups 2 and 3). Intact rats (control) were killed on the same days. Rats from the same batch served as basal control; they were killed on day of SLS-2 launch. Each group consisted of 5 animals.

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In all experiments, muscle specimens were prefixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.0) with 2.5% sucrose, rinsed in the buffer, postfixed with 1% OsO_4 in the same buffer, dehydrated in ascending alcohols, and embedded in Araldite. Semithin sections were stained with Toluidine Blue and viewed under a light microscope to choose the proper area for preparations of ultrathin sections. After contrasting with uranyl acetate and lead citrate, ultrathin sections were examined in a JEM-100S electron microscope.

RESULTS

Electron microscopic investigation showed that under conditions of weightlessness (group 1) atrophic processes develop in rat skeletal muscles. Muscular fibers (MF) are in the state of adaptive relaxation (Fig. 1, *a*), which is characterized by clearly outlined sarcomeres, light cytoplasm with decreased number of organelles, large elongated nuclei with finely divided chromatin and one or two nucleoli, and the presence of glycogen and lipid granules. The population of mitochondria is heterogeneous: it contains giant abnormal mitochondria with the signs of destruction. Changes typical of atrophy, such as thinning and lysis of myofibrils, pyknosis of the nuclei, disintegration of sarcomeres, and widening of the sarcotubular reticulum components, were observed in some MF. The loss of sarcoplasmic and myofibrillar proteins led

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to a decrease in the MF diameter and considerable widening of the extracellular space, which contained degranulated mast cells and fibroblasts varying in size, shape, and functional state. The fibroblasts produced collagen (Fig. 1, *b*). Local coagulative-necrotic and denervation changes occurred in some MF (Fig. 1, *c*).

Gravitation load (group 2) aggravated destructive changes in atrophied MF, which was accompanied by homogenization and fragmentation of some MF. These processes induced an intense macrophagal reaction.

At the same time, the signs of intracellular regeneration were observed in groups 1 and 2 (Fig. 2, *a*) in line with the presence of individual myoblasts and immature muscular tubes (Fig. 2, *b*, *c*). However, even at the initial stages the regeneration was atypical.

Reparative processes predominated in group 3 rats (14-day readaptation). Reparative regeneration of the mitochondria and myofibrils (hyperplasia), accumulation of ribosomes and polyribosomes, proliferation of the rough endoplasmic reticulum, and activation of nuclei were observed practically in all MF (Fig. 3, *a*, *b*). However, the presence of degenerative-atrophic changes indicated that these MF were not completely restored. Widened interstitial spaces were filled with the connective tissue components. The satellite cells, nuclear-sarcoplasmic areas were at different stages of differentiation into myoblasts and muscle tubules (Fig. 3, *c*).

In groups 1 and 2, the signs of atrophy were less pronounced in *m. gastrocnemius* than in *m. soleus*. Muscle fibers with almost normal structure predominated; the signs of atrophy — a decrease in the fiber diameter and widened extracellular space filled with collagen fibrils, fibroblasts, and degranulated mast cells — were observed in some of them. The presence of MF with impaired organization of sarcomeres, streaming of Z line, and migration of nuclei from periphery to center indicate that atrophy of MF is accompanied by their denervation.

The above-mentioned structural changes are aggravated after landing (group 2), being accompanied by the formation of necroses and fragmentation of some MF. After 14 days on the Earth (group 3), atrophic changes persisted in some MF. Activation of regeneration at the cellular and subcellular levels resulted in practically complete restoration of MF structure.

Degenerative and necrotic changes occurring in the capillary endotheliocytes indicate that the permeability of capillary wall is impaired. Under the conditions of weightlessness, most capillaries had thinned electron-dense endothelium that formed twisted microvilli and marginal crinkles. Some capillaries were sclerotized. After a 14-day readaptation, the capillary structure was practically normal and newly-formed capillaries appeared.

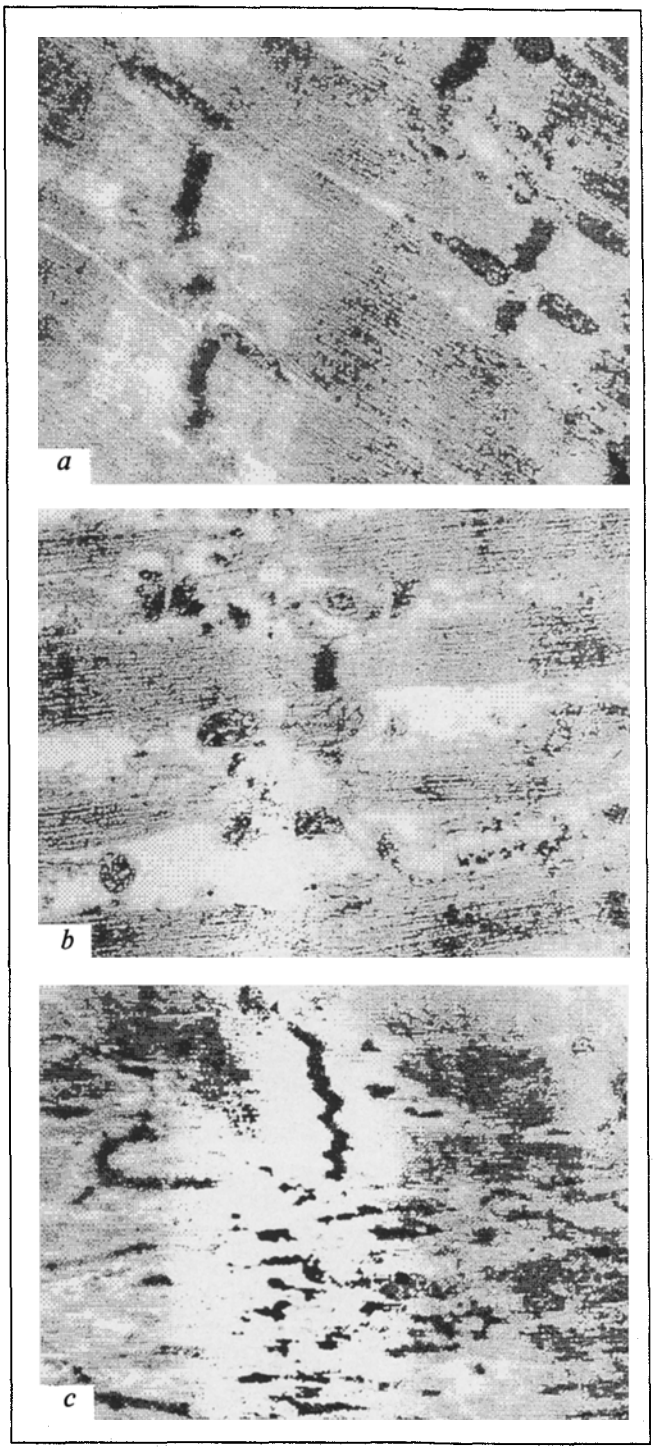

Fig. 1. Longitudinal sections of *m. soleus* fibers fixed under conditions of weightlessness (group 1). *a*) typical state of preserved muscle fibers with almost normal structure, ×30,000; *b*) atrophy of muscle fiber: lysis of myofibrils, decreased number of organelles, and widened extracellular space, ×30,000; *c*) fragment of muscle fiber with the signs of denervation: disorganization of the structure of sarcomeres and Z line, ×36,000.

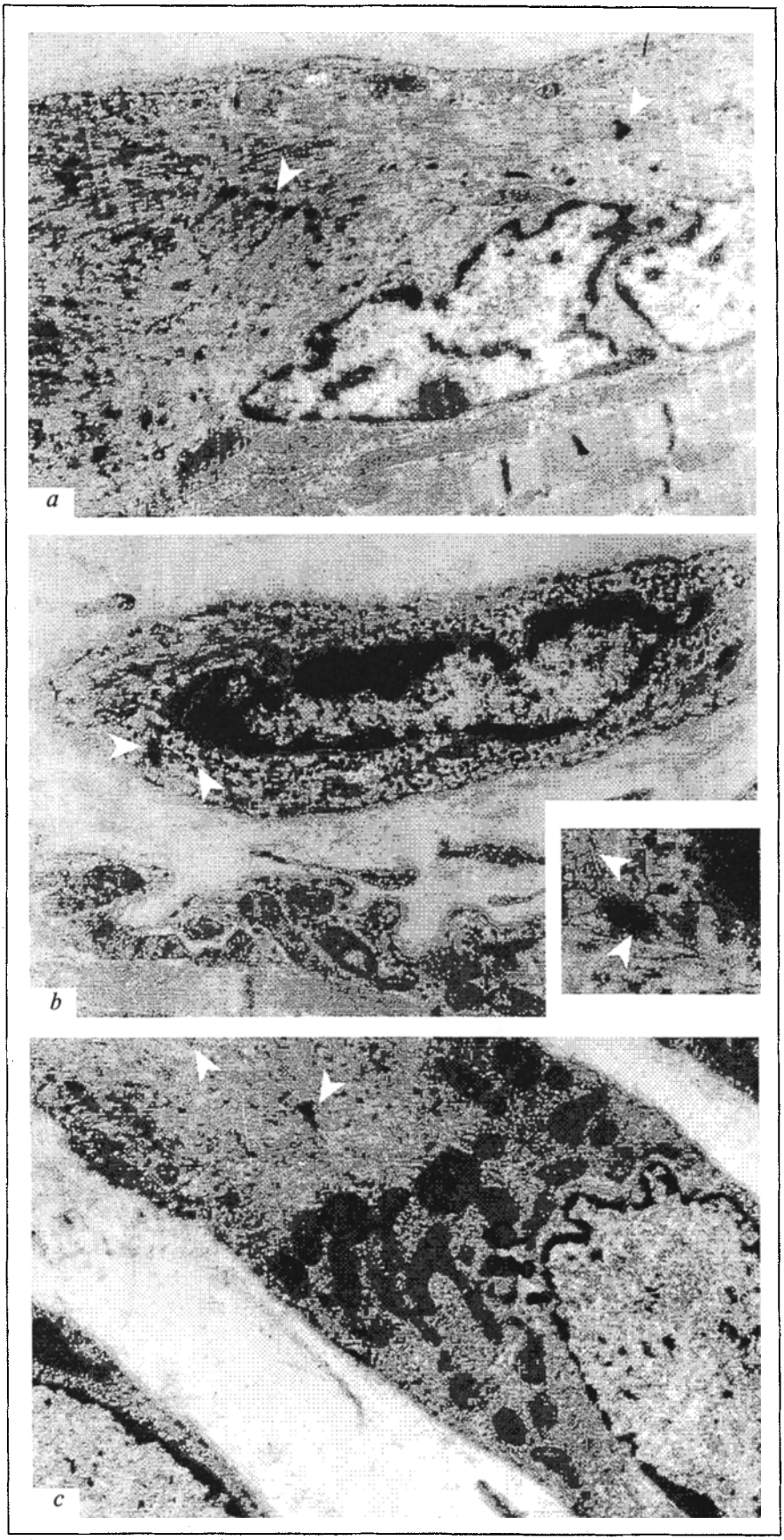

Fig. 2. Intracellular and cellular regeneration of muscle fibers in *m. soleus*. *a*) group 1 (weightlessness); local intracellular regeneration: subsarcolemmal accumulation of chaotically oriented myofibrils and Z line substance indicating atypical sarcomeregenesis (arrows), ×12,000; *b*) group 2: primary muscle tube in the extracellular space with large nucleus in the center, mitochondria, myofibrils, Z-line compound (arrows), and numerous ribosomes are seen in the cytoplasm, ×12,000; insert: fragment of a muscle tubule, myofibrils and Z-line compound (arrows) are indicative of sarcomeregenesis, ×38,000; *c*) fragment of muscle tubule; nucleus, ribosomes, and mitochondria are seen. Chaotically oriented myofibrils and electron-dense Z line-like structures indicate an atypical sarcomeregenesis (arrows), while lipofuchsin granules point to degenerative changes, ×12,000.

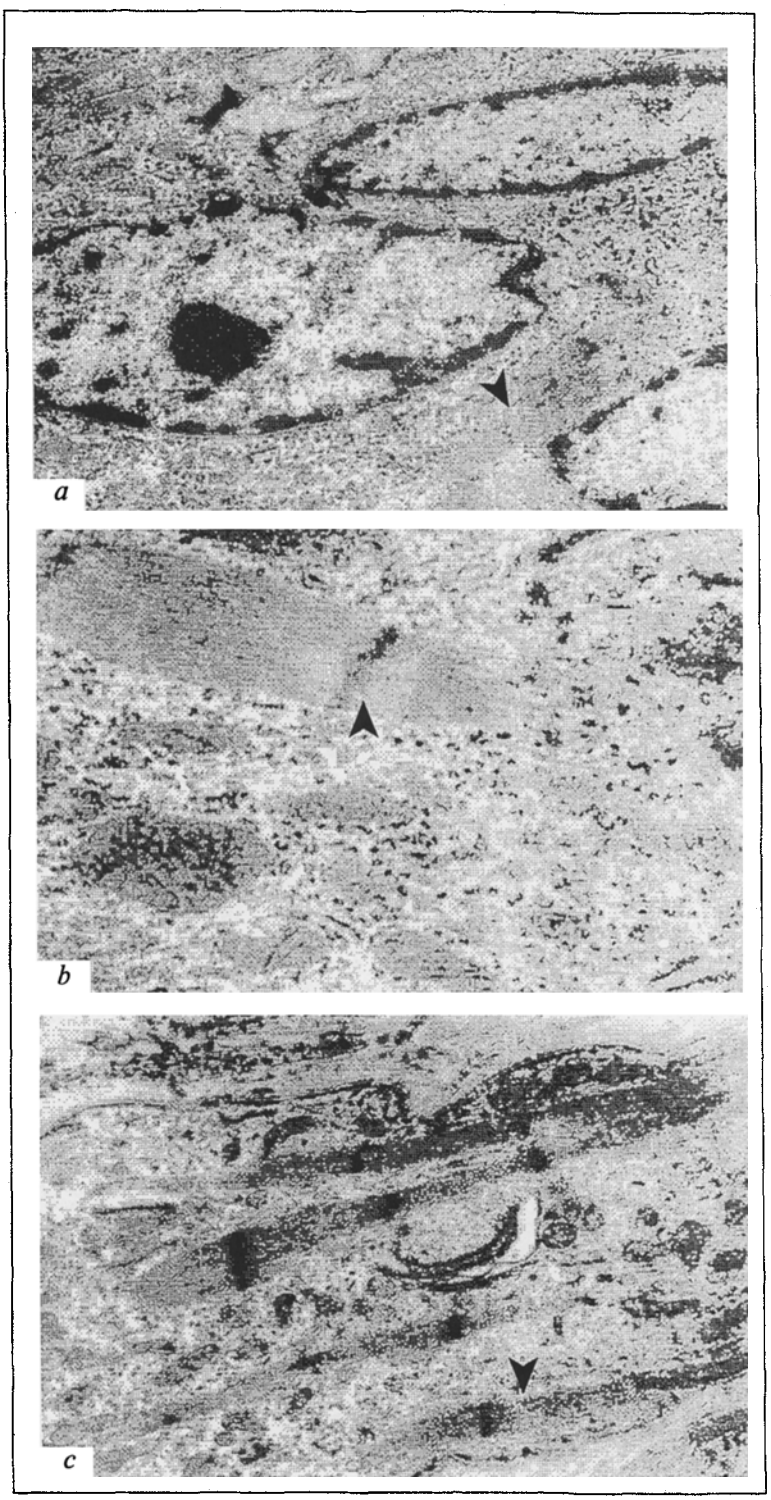

Fig. 3. Regenerative changes in the *m. soleus* fibers 14 days after landing (group 3). *a*, *b*) fragments of muscle fibers with active intracellular regeneration: activation of nuclei and increase in their number, accumulations of ribosomes, mitochondria, and polysomes in the cytoplasm are seen; free myofilaments form sarcomeres (arrow), ×21,000; *c*) fragment of muscle tubule with forming sarcomeres (arrow); the cytoplasm is filled with ribosomes, polysomes, and mitochondria, ×21,000.

Structural and functional organization of MF in group 1 rats corresponds to physiological requirements of weightlessness. Changes occurring in the *m. gastrocnemius* are similar to those in *m. soleus*, although less pronounced [3-5]. This may account for the fact that under conditions of weightlessness regenerative processes in *m. gastrocnemius* are extremely weak in comparison with those occurring in *m. soleus*. Regeneration is triggered after a certain level of damage had been reached; in the case of *m. soleus*, this levels is reached by the 13th day, after the muscle had adapted to weightlessness. Subcellular and cellular mechanisms of regeneration start operating against the background of stable morphology

[1]. It should be remembered that this muscle performs the "antigravitational" function.

In contrast to *m. soleus*, only the initial stages of regeneration (separation of satellite cells and segregation of the nuclear-sarcoplasm area) have been observed in *m. gastrocnemius* without subsequent development and signs of intracellular regeneration.

It should be stressed that muscular reparation starts under conditions of weightlessness against the background of atrophy, i.e., destructive and regenerative processes occur in parallel [6]. Atrophy of the skeletal muscle caused by hypokinesia triggers an abortive cellular regeneration manifesting itself as separation of satellite cells and nuclear-sarcoplasmic areas followed by their differentiation into myoblasts and muscle tubules. Obviously, such a regeneration cannot compensate the loss of MF; presumably, the formed muscle tubules start degrading.

Structural changes in the muscles are aggravated (in the *m. soleus* to a greater extent) by overload during landing and gravitation stress. The presence of sclerotized areas in the interstitium indicates indirectly that some MF have died.

During the adaptation period on the Earth, muscular structure and function are restored predominantly as a result of intracellular regeneration. The mass of the nuclei and cytoplasm in MF increase, which coincides with hypertrophy and hyperplasia of ribosomes and polysomes. Continued segregation of the satellite cells may indicate that these cells are involved not only in cellular but also in intracellular regeneration of MF.

Thus, weightlessness has no effect on the initial stages of genetically-determined regeneration. However, atypical sarcomeregenesis, microcirculatory and denervation changes hamper the prognosis of regeneration [7]. The data obtained on the 14th day of readaptation suggest that the muscle structure is not restored completely, although the attained level of regeneration could be quite sufficient.

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