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ULTRASTRUCTURAL CHANGES IN STRIATED MUSCLES UNDER THE INFLUENCE OF SPACE FLIGHT FACTORS

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KEY WORDS: soleus, gastrocnemius, and diaphragm muscles; muscle fibers; atrophy; ultrastructure.

Human sojourn in space on a space ship is accompanied by functional disturbances in the muscular system, which are based on changes in its structural organization and metabolism [1, 2]. The character and severity of the changes in the muscles are variable and depend on their functional role [3, 5, 6], and for that reason the influence of factors arising during a 7-day space flight on muscle can best be studied in relation to the structural response of different types of muscles.

EXPERIMENTAL METHOD

Pieces of the soleus, gastrocnemius, and diaphragm muscles of six rats, kept on board the "Kosmos-1667" biosatellite for 7 days, and seven rats of the animal house control, were taken for ultrastructural analysis (all the rats were of the Wistar strain, SPF colony). The animals were killed by decapitation 4-8 h after returning to earth.

Material was prefixed in 4% formaldehyde, buffered to pH 7.4 with acetate-Veronal buffer, with the addition of iso-osmotic sucrose; postfixation was carried out in 1% OsO_4 solution and embedding in Araldite. Sections were stained by Reynolds' method and examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

Changes affecting virtually all its structural elements — myofibrils, nuclei, mitochondria, sarcoplasmic reticulum — were discovered in the muscle fibers of the soleus muscle. The most widespread type of change was atrophy of the myofibrils and muscle fibers. Thinning of the myofibrils and widening of the spaces between them were observed, with the formation of slits, vacuoles, and cavities (Fig. 1a). Fibers with focal destruction of the sarcomeres, a zigzag arrangement of the material of the Z-lines (Fig. 1b), and sometimes complete destruction of myofilaments with the formation of homogeneous pale finely granular fields, in which the nuclei, small dense mitochondria, fragments of membranes, and myelin-like formations were chaotically arranged (Fig. 1c). In some muscle fibers there was in increased number of fat droplets and subsarcolemmal and intermyofibrillar rows of glycogen granules.

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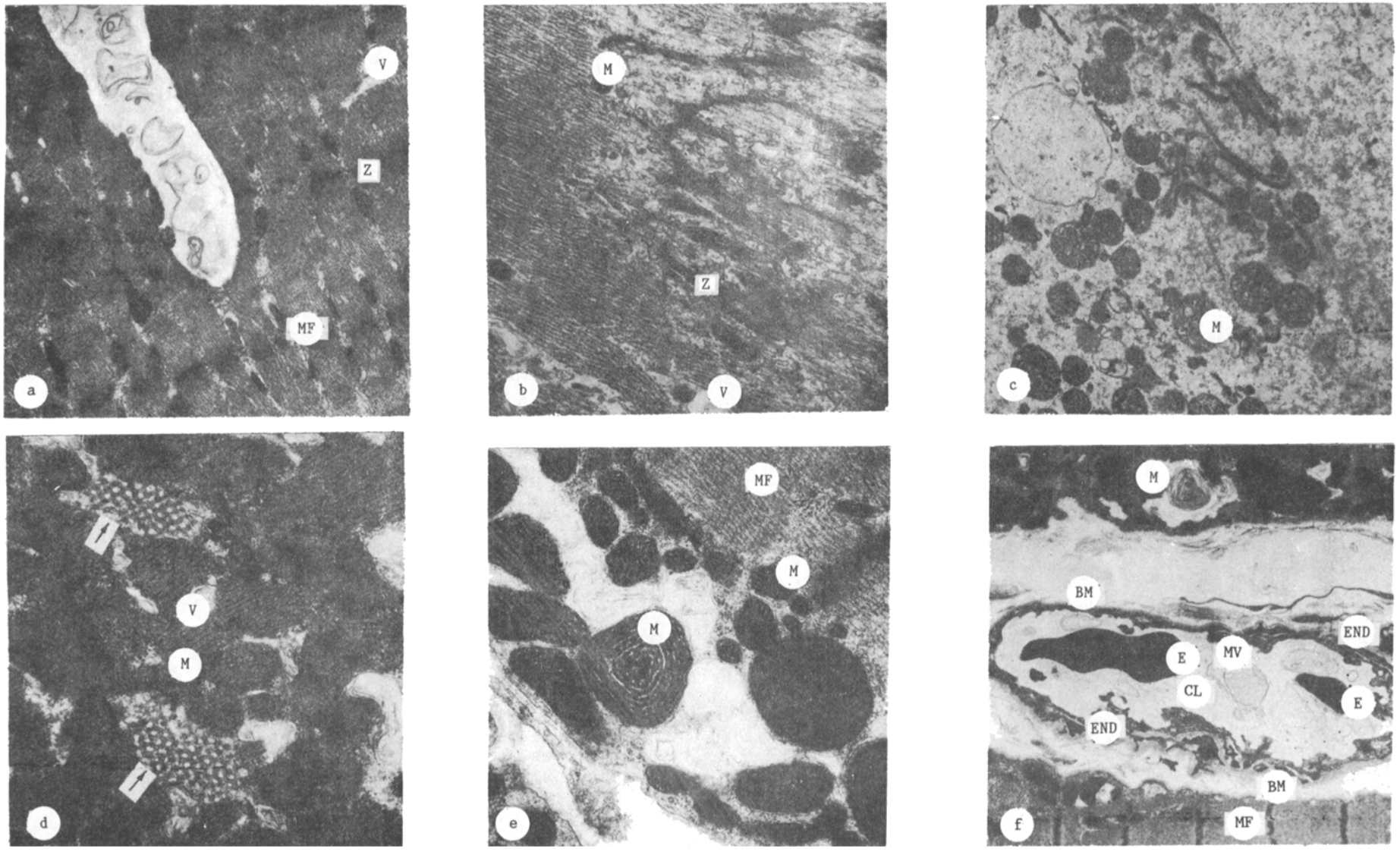

Fig. 1. Change in soleus muscle of rats taking part in a 7-day space flight. a) Destruction of myofibrils with formation of cavities filled with fragments of membranes. 20,000×; b) Local disturbance of structural organization of sarcomeres, zigzag arrangement of Z-lines. 32,000 ×; c) Disintegration of myofilaments and formation of pale zones filled with homogeneous finely granular contents, with chaotic arrangement of mitochnondria, vacuoles, and membrane fragments in them. 20,000 ×; d) Hyperplasia of elements of sarcotubular system and formation of honeycombed structures. 40,000 ×; Fig. 1 (continued) e) Subsarcolemmal accumulations of a typical giant mitochondria. 32,000 ×; f) Compressed erythrocytes visible in capillary lumen, together with cell ghosts and microvesicles. Endotheliocytes are electron-dense, with many micropinocytotic vesicles, microvilli, and in some places, plasmatosis. Basement membrane widened, loose in structure, many collagen fibrilis present, death of processes of pericytes. 17,000 ×. M) Mitochondria, MF) myofibrils, V) vacuoles, BM) basement membrane, MV) microvilli, CL) capillary lumen, END) endotheliocytes, E) erythrocytes.

The mitochondrial population was heterogeneous. Besides normal mitochondria there were many giant forms, with an unusual structural organization due to the numerous densely packed cristae. In some mitochondria swelling of the matrix and fragmentation of the cristae were observed (Fig. 1e). Hyperplasia of elements of the sarcotubular system was found, with the formation of vacuoles and of honeycombed structures (Fig. 1d). Muscle fibers with central nuclei were seen. In many fibers segregation of nucleo-sarcoplasmic regions and an increase in the number of satellite cells at different stages of differentiation were found.

The state of the microcirculatory bed is evidence of considerable disturbances of the microhemodynamics. The arterioles were contracted and the capillaries and venules dilated. Compressed erythrocytes, microvesicles with a single-layered membrane, and remains of blood plasma were visible in the capillary lumen. The basement membrane of the capillaries was widened and loose in texture, and in some areas it was split. Death of processes of pericytes was observed and the number of collagen fibrils was increased. The endotheliocytes of all microvessels were electron-dense and active and contained large numbers of micropinocytotic vesicles; numerous microvilli, veils, and marginal folds were present (Fig. 1f). Many mast cells, partially degranulated, and active fibroblasts and macrophages could be identified in the ground substance.

Changes were found in virtually all structural elements also in the muscle fibers of the gastocnemius muscle. Their extent of spread was much less than in the soleus muscle and was more local in character. Atrophy of individual myofibrils was observed, in some places they had completely disappeared and were replaced by finely granular contents with inclusions consisting of membrane fragments, vacuoles, myelin-like structures, and autophagosomes. Fibers with disturbance of the structural organization of individual sarcomeres and of material of the Z-lines, and with widening of elements of the sarcotubular system, were discovered. Migration of nuclei from the periphery into the center could be observed. Segregation of nucleo-sarcoplasmic regions and an increase in the number of satellite cells also were noted.

Slight atrophy of the myofibrils was seen in the diaphragm muscle fibers. "Combed" myofibrils and fibrils with central nuclei, local disturbances of the structural organization of individual sarcomeres, chains of lipid drops, and also subsarcolemmal accumulations of large, electron-dense mitochondria, with complex organization of their cristae, were found. Changes in microvessels of the gastrocnemius and diaphragm muscles were similar to the changes described above in the soleus muscle, but they were less marked and more limited in character.

Ultrastructural investigation thus revealed destructive-atrophic changes of varied severity in the muscles of rats exposed to the influence of factors of a 7-day space flight. As regards the degree and character of their extent, the soleus muscle occupies first place, followed by the gastrocnemius and diaphragm muscles in that order, reflecting their functional differences.

Conditions of weightlessness completely abolish the load on the soleus muscle and lead to the development of widespread atrophic changes in it [3]. This process is aggravated by manifestations of hypoxia, as shown by the state of the microvessels (the contracted arterioles, dilated capillaries and venules, death of the pericytes and an increase in the number of collagen fibers). The influence on blood vessels of the way in which the animals were killed, namely decapitation, may possibly have some influence on the formation of changes such as increased density of the endothelium and its activation.

A damaging factor for muscles is the considerable restructuring of their synaptic apparatus [4]. A fall in the level of neuromuscular interaction leads to disturbance of trophic influences and to the development of denervational changes in the muscle fibers, as is shown by migration of the nuclei from the periphery into the center and by hyperplasia of elements of the sarcotubular system. The discovery of lipid drops, glycogen, and electron-dense giant mitochondria point to reorganization of metabolism in the muscle fibers. Meanwhile segregation of the nucleo-sarcoplasmic regions and the increase in the number of satellite cells are evidence of parallel activation of repair processes. Morphological features of atrophy are significantly less marked in the gastrocnemius and diaphragm muscles. These changes can be attributed to loss of function of the muscles during weightlessness.

Thus even a comparatively short stay of animals on a biosatellite is reflected in the morphological and functional state of the muscles, and corresponds to the degree to which they participate in the antigravity functions on earth. Signs of regeneration which were found are evidence that these structural and functional disturbances are reversible.

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AGE CHANGES IN NUMBER AND INTENSITY OF FLUORESCENCE OF SMALL INTENSIVELY FLUORESCENT CELLS OF RAT AUTONOMIC GANGLIA

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The general principles of the postnatal development of the extra-adrenal chromaffin tissue in rodents are considered to be well established. It has been shown that reduction of the chromaffin tissue with age has many exceptions, characterizing both its species-specificity and its differences in different organs [4]. The tissue of paraganglia is preserved and actually increased in volume many times in Fisher 344 rats between the ages of 3 and 33 months [7], whereas the organ of Zuckerkandl is destroyed during the first week of postnatal development [6]. Small intensively fluorescent (SIF) cells contained in autonomic ganglia are evidently the earliest adrenergic structures to be differentiated. In rat ontogeny concentrations of SIF cells in the superior cervical sympathetic ganglion are observed from the 13th day of embryonic development [8] and the number of SIF cells in ganglia of the rat fetal heart reaches a maximum at the 29th day of pregnancy [9]. After birth the number of SIF cells in the superior cervical ganglion increases until the 23rd day of life, and thereafter undergoes very little change [5]. In rats of the long-living Fisher 344 line no marked changes in the number of SIF cells in the superior cervical sympathetic ganglion are observed with age, whereas in the great pelvic ganglion their number increases [7]. A more detailed study of the SIF-cell pool of the ganglia at successive stages of postnatal ontogeny is therefore interesting.

The aim of this investigation was to analyze the number of SIF cells and their content of paraform-induced fluorophores in the lumbar ganglia (LG) of the sympathetic trunk and in the great pelvic ganglion (GPG) of rats at the times of formation of the sympathetic and parasympathetic innervation and in later life.

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