Ultrastructure of Skeletal Muscle Capillaries under Conditions of Space Mission

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Capillaries of the rat forepaw skeletal muscles were examined on day 14 of space mission and on days 1 and 14 after landing. Ultrastructural studies revealed apoptosis caused by muscle fiber atrophy and necrobiotic changes eventuating in coagulation or monocellular necrosis of endothelial cells. Formation of capillaries was detected, which can be regarded as an adaptive reaction to injuries caused by space mission factors.

Key Words: *ultrastructure; capillaries; endothelium; skeletal muscle; space mission*

The hydrostatic blood pressure sharply decreased and the volume of circulating fluid is redistributed under conditions of weightlessness [3,4,8], which is essential for such an important component of the cardiovascular system as the capillaries. Muscle fiber atrophy under conditions of weightlessness is associated with a decrease in the number of capillaries per muscle fiber [4]. On the other hand, ultrastructural criteria for evaluating the capillary status under conditions of space mission are poorly studied [5]. These studies became the aim of our work.

MATERIALS AND METHODS

We examined *m. extensor hallucis longus* from Sprague-Dawley rat. The animals flew into space on board the SLS-2 satellite. Material for ultrastructural analysis was collected and fixed on day 14 of mission and on days 1 and 4 after return to the Earth. The animals were sacrificed by decapitation.

Muscle fragments were fixed in cold phormol/sucrose solution and then in 1% osmium tetroxide (OsO_4) and embedded in araldite. Ultrathin sections were examined under a JEM-100B electron microscope.

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RESULTS

Ultrastructural study showed that an appreciable part of capillaries underwent structural changes under conditions of weightlessness and after landing; these changes indicate disorders in microcirculation, permeability, and transcapillary metabolism. Similar morphological changes were detected in the blood and lymph capillary, arteriolar and venular endothelium.

Successive stages of transformation of the endothelial luminal surface were detected on day 14 of space mission: marginal folds, microvilli, bay-like depressions with subsequent sequestration of cell fragments and villi into the lumen and formation of microemboli (Fig. 1, *a*, *c*, *d*). The initial stages of cell surface configuration were not paralleled by impairment of the cellular and intracellular membrane integrity (Fig. 1, *a*). Signs of irreversible injuries (cytoplasm osmiophilia and homogenization, nuclear chromatin condensation, karyopyknosis and karyorrhexis (Fig. 1, *b*, *d*)) were seen in an appreciable part of capillaries. Destructive degenerative changes in vessels indicated ultrastructural death of the cells, characteristic of apoptosis [1], in many cases: degradation of capillary wall into fragments surrounded by plasma membranes (apoptotic bodies, Fig. 1, *c*; 3, *b*).

Muscle fibers are relaxed under conditions of weightlessness; morphological features of replace-

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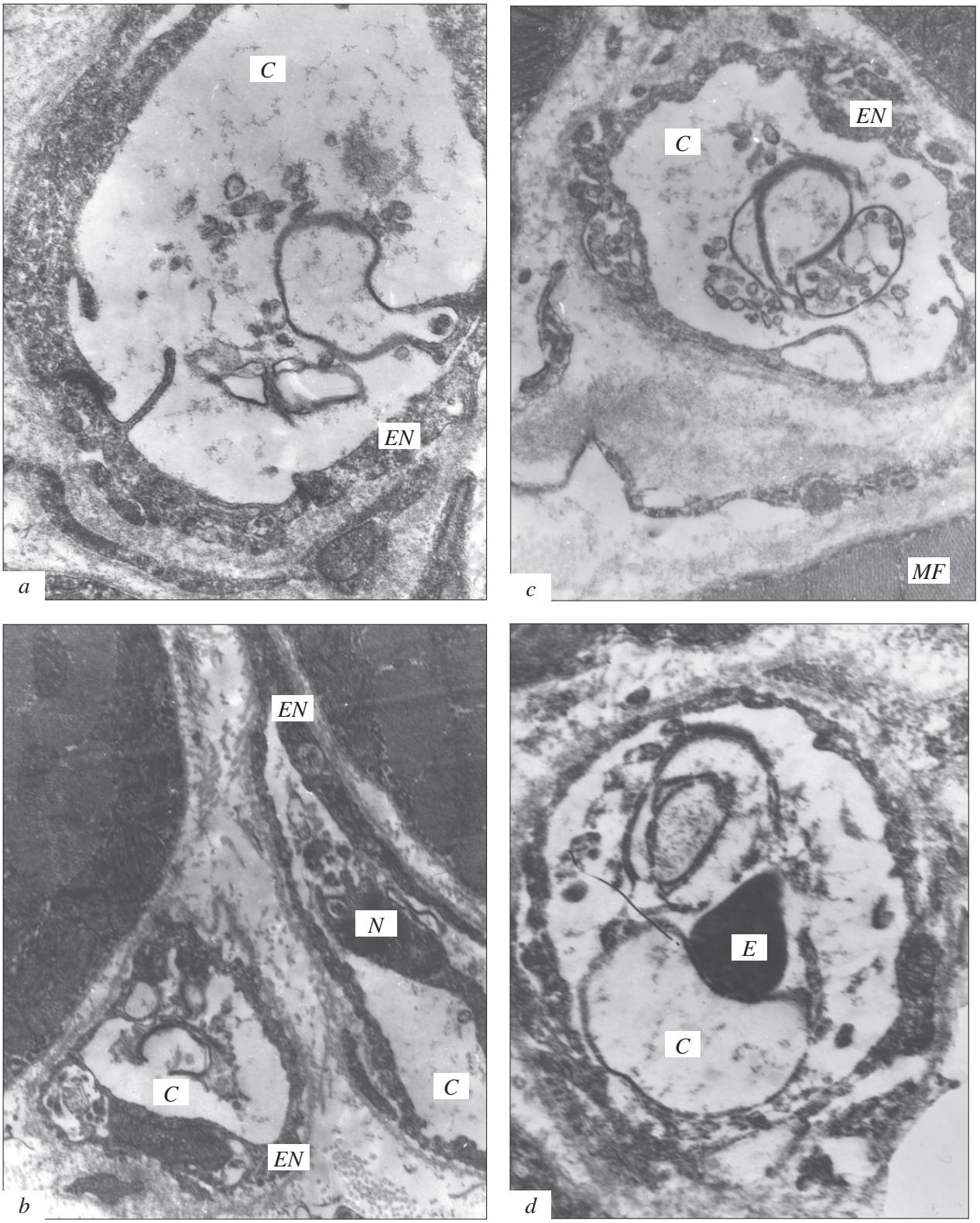

Fig. 1. Destructive degenerative changes in capillary wall under conditions of space mission. *a*) initial stages of destructive changes in endotheliocytes, ×29,000; *b*) irreversible destructive necrobiotic changes (fragments of villi, membranous structures in vascular lumen), ×14,000; *c*) endotheliocyte apoptosis in capillaries, 10,000; *d*) small-granular degradation of endothelium, fragmented cytolysis of capillary wall (fragments of cells and villi in the lumen), ×10,000. Here and in Fig. 2: EN: endothelium; N: nucleus; MF: muscle fiber; E: erythrocyte; C: capillary.

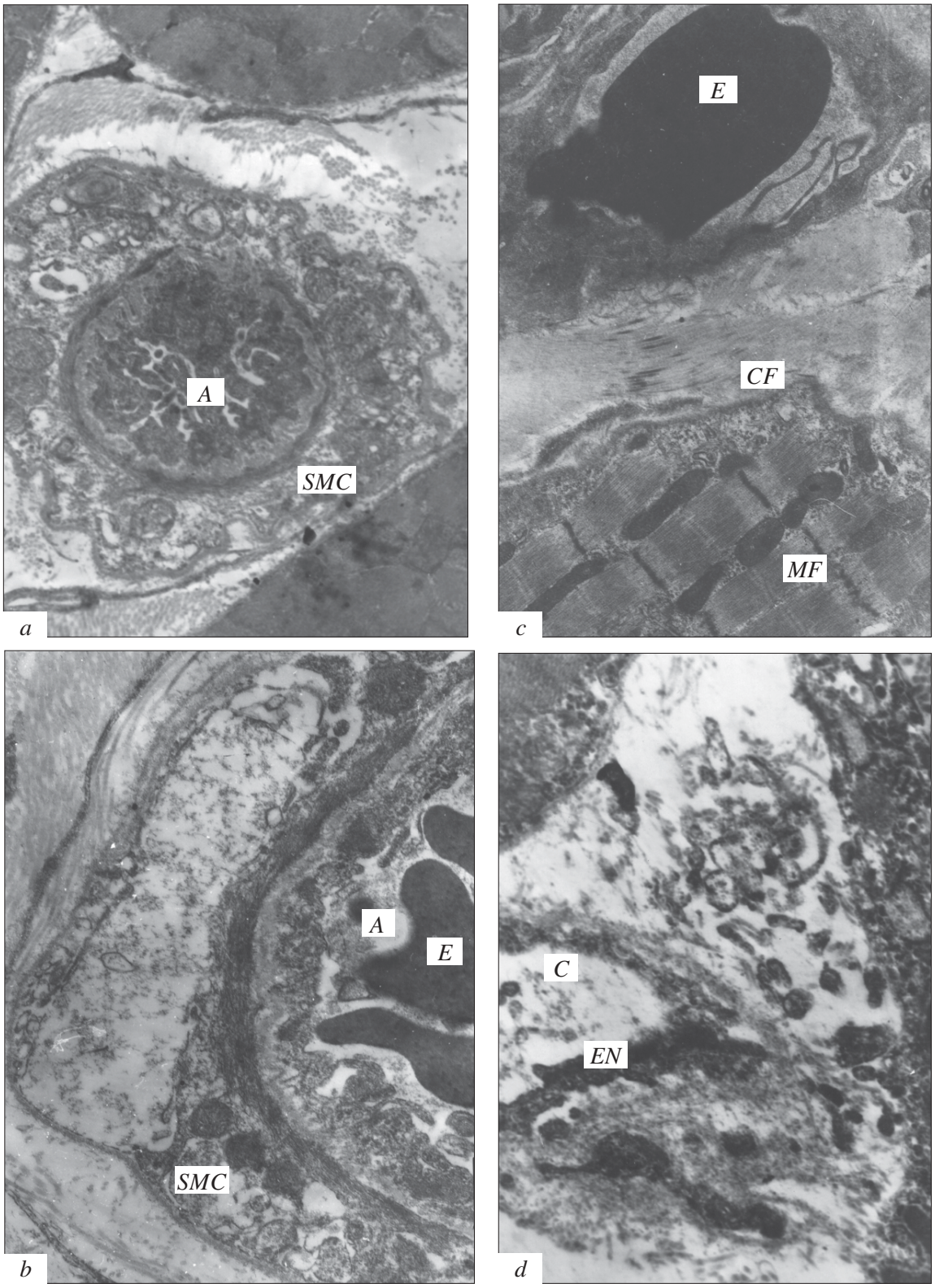

Fig. 2. Destructive degenerative changes in arterioles and capillaries under conditions *of* space mission. *a*) vacuolation of arteriolar smooth muscle cells (SMC), ×7200; *b*) destruction of SMC contractile system (fragments of cells, hemolytic erythrocytes, plasma protein precipitate in arteriolar lumen), ×14,000; *c*) monocellular colliquation necrosis of endotheliocytes, perivascular sclerosis, 7200; *d*) "swamping" of interstitium, ×14,000. Here and in Fig. 3: CF: collagen fibers; A: arteriolar lumen.

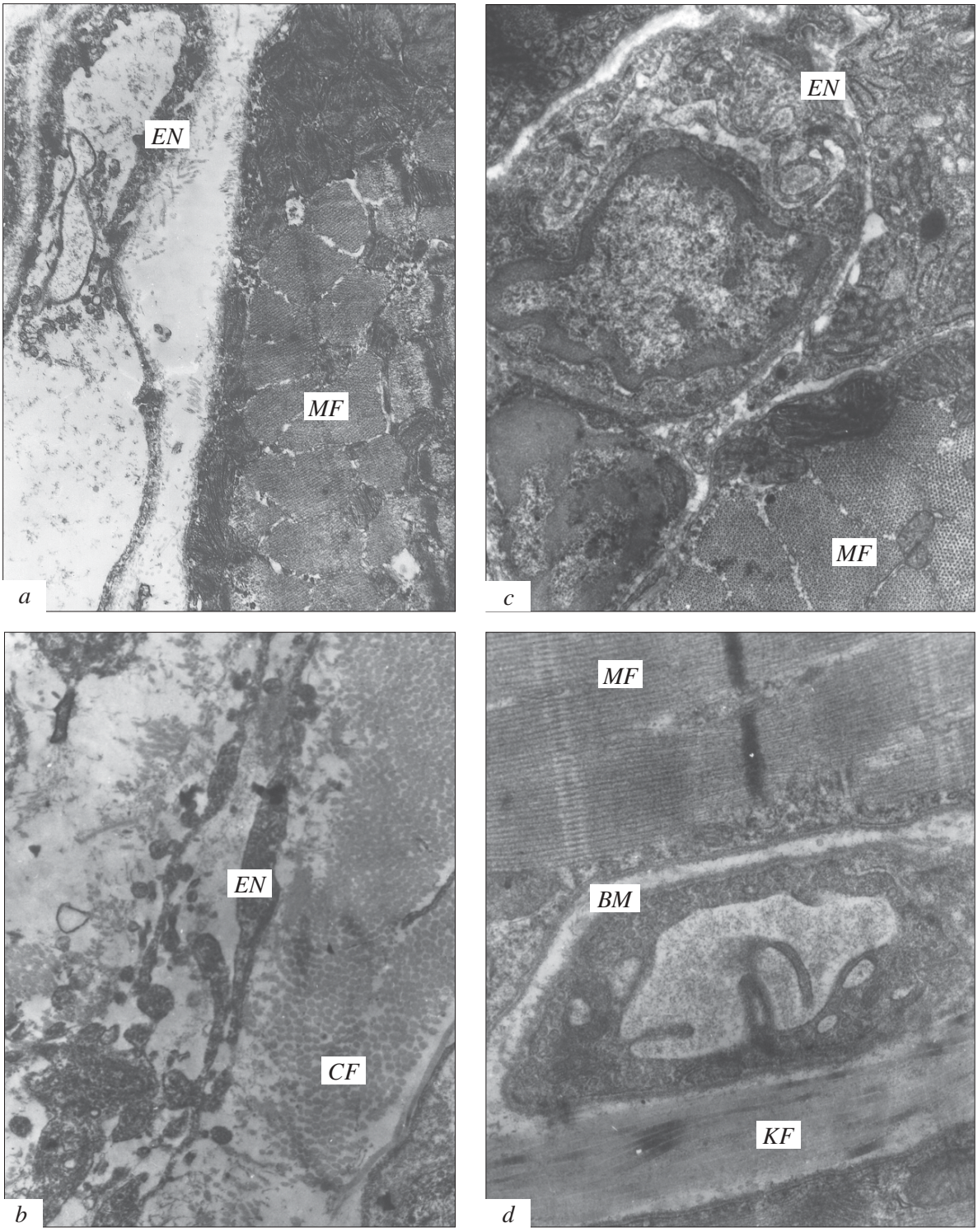

Fig. 3. Destructive degenerative changes and neoformation of lymph capillaries under conditions of space mission. *a*) venular compartment of capillary bed (vascular wall thinned, basement membrane fragmented; membrane complexes and fragments of villi in the lumen), ×10,500; *b*) lymph capillary, lumpy degradation of capillary wall, perivascular sclerosis, ×10,500; *c*) new capillary, ×16,000; *d*) "mature" capillary. Transport micropinocytous system of endotheliocytes is formed and presented by luminal, free, basal vesicles (plasma protein precipitate and villous fragments in the lumen), ×14,600. BM: basement membrane.

ment sclerosis are clearly seen at the site of destroyed muscle fibers (Fig. 2, *c*; 3, *d*). Endotheliocyte apoptosis is most likely a result of decrease in the number of muscle fibers. In many cases the destructive necrobiotic changes in endotheliocytes eventuate in coagulation monocellular necrosis

(Fig. 1, *d*; 2, *d*) according to the classifications [7]. Morphological signs of increased permeability of cell membranes were detected. Endotheliocytes underwent plasma impregnation, diffuse cytolysis, and destruction of intracellular organelles. The contours of the inner and outer membranes of endotheliocytes were blurred; this process eventuated in colliquation necrosis (Fig. 2, *c*). Perivascular collagen fibers in the interstitium underwent plasma impregnation and lost characteristic striation; amyloid fibrils were detected. "Swamping" of the interstitium was noted; fragments of cells, collagen fibrils, floccular material were seen in it (Fig. 1, *c*; 2, *c*). Arterioles were constricted; presumably this was a reaction to decapitation. Pronounced vacuolar degeneration and disintegration of the smooth-muscle cell contractile system were seen (Fig. 2, *a*). The inner elastic membrane was locally destroyed; fragments of cells, villi, and erythrocytes were seen in the lumens (Fig. 2, *b*, *c*). Plasma accumulation was seen subendothelially in some arterioles. These signs are morphological manifestations of intracellular edema and impairment of endotheliocyte permeability.

The venous compartment of the capillary bed and lymph capillaries underwent similar changes, leading to lumpy degradation of the vascular wall by the apoptosis type (Fig. 3, *a*, *b*). The lumens of some capillaries were obliterated with collagen fibrils. These changes in the venous and lymph compartments of the capillary network indicate disorders in their draining function. The ultrastructural changes in the blood and lymph capillaries were augmented by interstitial edema, which prolonged circulatory hypoxia in the muscle, thus promoting the arteriolar endotheliocyte and smooth muscle cell ischemia. Intracellular membrane complexes, cytoplasm vacuolation, and lumpy degradation were ultrastructural manifestations of this process.

Despite destructive degenerative changes in many capillaries, new capillaries were detected in the muscle (Fig. 3, *c*). In some capillaries morphological signs of high functional activity of endotheliocytes were observed: the cytoplasm was filled with transport micropinocytotic vesicles, ribosomes. Microvilli protruding into the lumen also contained solitary vesicles. Basement membranes in vessels were clearly contoured (Fig. 3, *d*).

The direction and morphological signs of ultrastructural changes in capillaries persisted during gravity readaptation on the Earth. This means that destructive degenerative processes were not arrested on day 14 after landing. It seems that recovery of ultrastructure of the capillaries and muscle tissue during readaptation requires longer time.

Another type of ultrastructural changes in capillaries can be explained by physical factors (acceleration, weightlessness), which presumably modulate cell membrane permeability and eventuate in coagulation or monocellular necrosis of endotheliocytes. The type of these changes can be conditionally qualified as microangiopathy, which is characterized by peculiar morphological features under conditions of space mission, distinguishing it from posttraumatic, diabetic, dyslipoproteinemic, and other chronic diseases. Its main specific feature is the absence of alternation of death and regeneration processes of capillary endothelial lining. As a result of these processes the vascular wall becomes multilamellar, with pyknotic fragments of "old" endothelial cells discernible in it [2].

Capillary injury is realized within very short terms under conditions of space mission and readaptation to the Earth gravity. Presumably, one of the causes of capillary damage is lack of training in the animals, which, in contrast to aeronauts, are not trained before and during the mission. Structural bases of adaptation include the "adaptation structural reserve of cell organelles" essential for overcoming the overload [6]. It seems that these processes cannot fully form during short period.

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