EXPERIMENTAL LIGHT-OPTICAL AND ELECTRON-MICROSCOPIC STUDY OF CHANGES INDUCED BY SEA WATER AND THYMOGEN IN STRIATED MUSCLE TISSUE

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With the recently increasing number of disasters at sea, accompanied by exposure of human victims to sea water (SW) at a temperature above 0°C, a comprehensive study of this problem is required. Cooling in SW has particular features of its own, manifested as a disturbance of water and electrolyte metabolism, and accompanied by high mortality among the victims [2]. Attempts have been made [3] to increase resistance to hypothermia by the use of thymus preparations. Experimental limb cooling in SW leads to death of animals [1]. Changes taking place in striated muscle tissue (SMT) under these circumstances have so far received little study.

The aim of this investigation was to study changes in SMT of the hind limbs and the survival rate of rabbits exposed to the action of SW and receiving thymogen.

EXPERIMENTAL METHOD

Experiments were carried out on two groups of animals (100 male and 100 female rabbits weighing 2-2.5 kg), whose hind limbs were cooled in SW at a temperature of 4-8°C for 30 min and 3, 6, 12, and 24 h (20 rabbits at each time). Immediately before the beginning of cooling the experimental animals were given a single intramuscular injection of 300 μg thymogen. The percentage of rabbits surviving after cooling was determined. Material for light-optical (paraffin sections, hematoxylin and eosin) and ultrastructural (fixation by Caulfield's method, embedding in Epon 812, JEM 100CX electron microscope) investigation was taken immediately after 30 min and 3 h of cooling, and 1 and 7 days thereafter.

EXPERIMENTAL RESULTS

SMT in the experimental animals 30 min after the beginning of cooling was in a state of strong contraction, more marked in the superficial layers. The epimysium with the subjacent muscle tissue was strongly folded and the perimysium narrowed. The pattern of cross striation in some fibers was greatly intensified, whereas others, in a state of contraction, became folded, as could be seen in the sections by the wavy appearance (Fig. 1a). The endomysium was narrower than in the control and the basement membrane condensed. As a result of spasm the lumen of the arteries was constricted and some veins were collapsed. In neighboring parallel myofibrils the sarcomeres were shifted relative to one another. The A bands and M-lines were indistinct (Fig. 1b). In some sarcomeres the M-line was separated by an additional pale band. Perinuclear concentration of chromatin appeared in the nuclei of the monocytes and the perinuclear space was narrowed. Besides disorganization of the sarcomeres, destruction of the sarcolemma, spasm of the capillaries, and destruction of the endothelial membranes also were observed (Fig. 1c).

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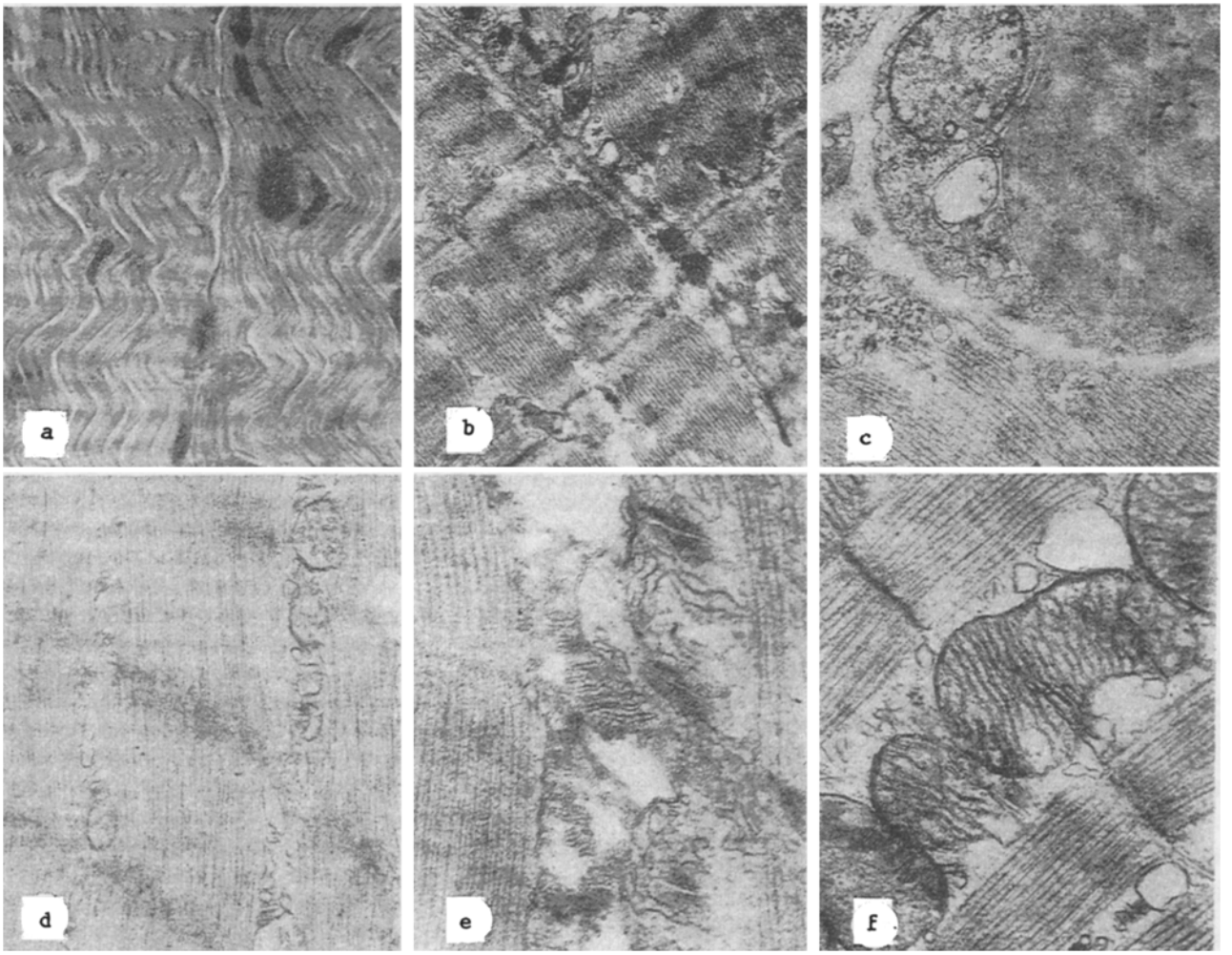

Fig. 1. Changes in SMT after a single cooling of limbs 30 min (a-c), 3 h (d), and 7 days (e) in sea water: a) marked folding of muscle fibers (hematoxylin and eosin 900×); b) narrowing of endomysium and sarcoplasmic reticulum, indistinct lines and bands in sarcomeres (10,000×); c) destruction of sarcolemma and endothelial membrane (13,000×); d) absence of bands and lines in sarcomeres (6600×); e) diorganization of cristae, swelling of mitochondria (20,000×); f) mitochondria 1 day after injection of thymogen and cooling (20,000×).

Fibers of the subcutaneous SMT 3 h after the beginning of cooling were gathered into bundles, tightly packed together. In some areas the endomysium was unevenly widened. Edema of the perimycium was seen. The cross striation in individual muscle fibers was very distinct, whereas in others it could hardly be distinguished. Some muscle fibers were in a state of contraction and formed folds. Some fibers could be seen to be ruptured. The arteries were in spasm and the endotheliocytes protruded into the lumen. The veins were collapsed. Aggregates of blood cells appeared in some blood vessels. Small hemorrhages could be seen between the muscle fibers.

The boundaries of the sarcomeres in the myofibrils were indistinct, the Z-lines blurred, and the typical bands and lines could not always be seen (Fig. 1d). Contrast of the myofilaments was weak. In some cases a narrow I-band could be detected. Some of the sarcomeres contained destructively changed myofilaments. Most mitochondria were swollen, with a pale matrix. Numerous invaginations of the karyolemma could be seen in the nuclei of the monocytes. Folding appeared in the endothelium of the capillaries and micropinocytotic vesicles were distributed predominantly outside the wall.

After 1 day edema of the endomysium was abundant, and hemorrhages were seen in SMT. The arteries were in spasm and the veins overfilled with blood. Individual leukocytes could be seen by the walls. Staining revealed hypochromia of some muscle fibers. The stain was distributed diffusely, evidently indicating the presence of dystrophic processes in SMT.

The volume of the sarcoplasm was increased and the intervals between the myofibrils widened. The compactness of the myofibrils and their parallel arrangement in the sarcomeres were disturbed. In the peripheral part of the muscle fibers the sarcomeres in neighboring parallel myofibrils were displaced. The electron density of Z-lines was increased. Some muscle fibers were in a state of strong contraction. The sarcolemma formed folds. The sarcomeres were shortened and the I-bands could hardly be seen. The H-zone was indistinct In other muscle fibers the sarcolemma was loose in texture and the sarcomeres widened, and their typical structure modified. Most mitochondria were of the dark type.

Staining of the muscle fibers after 7 days was hypochromic, and they were separated by narrow strands of endomysium. Cross striation was ill-defined and fragmented in places, and sometimes was almost completely invisible, The nuclei of the myocytes were hypochromic and contained only solitary clumps of chromatin. The volume of the sarcoplasmic reticulum was reduced. The Z-lines had very weak electron density. Typical lines and bands were almost invisible In the myocytes the perinuclear space was narrowed and the integrity of the karyolemma disturbed. Most mitochondria in the sarcoplasm were long (Fig. 1e), irregular in shape, with the integrity of the outer and inner membranes often disturbed; numerous pale areas were present in the matrix, and the cristae were short and few in number.

Cooling the limbs in SW reduced the survival rate in cases of cooling for 12 and 24 h, down to 70 and 5% respectively. After administration of thymogen the survival rate was increased to 80%, but only after cooling for 12 h.

Normalization of the mitochondrial ultrastructure was observed 1 day after injection of thymogen (Fig. 1f): long cristae were predominant in the mitochondria, the integrity of their membranes was undisturbed, and single small pale areas were found in the matrix.

The results of this investigation show that cooling the hind limbs in SW at a temperature of 4-8°C once has a damaging action on SMT and is accompanied by circulatory disturbances. These observations agree with data in the literature on the effect of circulatory disturbances in the state of the tissues [4]. Disturbances of this kind in arterioles, venules, and capillaries are accompanied [7] by a change in vascular permeability. These processes progressed, and subsequently (after 3 h to 1 day) hemorrhages and stasis of leukocytes near the inner aspect of the vessel wall, an indicator of microcirculatory failure [5], could be observed in the muscle tissue [5]. At all times of observation ultrastructural disturbances were found, in the form of disorganization of myofibrils, disappearance of typical bands and lines and atrophy of the contractile apparatus. Changes in the mitochondria were manifested as edema, swelling, disorganization of the matrix, and shortening of the cristae. After 7 days, in some cases complete disappearance of cross striation of the muscle fibers could be seen in the muscle tissue, and this was confirmed by an ultrastructural study of the myofibrils. Changes of this kind could be due not only to the action of the cold factor, but also to the damaging action of metabolites, accumulating in the cytoplasm during cooling, described in the literature [6].

In the case of cooling of the limbs after preliminary injection of thymogen, normalization of the structure of the mitochondria was noted 1 day after cooling. This fact may perhaps be linked with the pharmacologic basis of thymogen (Glu-Trp). It can be tentatively suggested that the hypoxia which develops during hypothermia inhibits activity of tryptophan hydroxylase, thereby disturbing metabolism of tryptophan, which is a biological fuel in the tricarboxylic acid cycle after hydrolysis to acetyl-CoA. Prophylactic administration of thymogen evidently creates additional energy reserves, increases resistance to hypothermia, and manifests itself as an increase in survival rate of the animals.

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