MORPHOLOGICAL CHANGES IN SKELETAL MUSCLE TISSUE IN EXPERIMENTAL ACUTE ISCHEMIA OF THE LIMBS

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Modern methods of reparative surgery of the blood vessels enable the patency of the main arteries of the lower limbs to be restored comparatively quickly after acute occlusion. However, operative revascularization is not always effective, ischemic changes in the skeletal muscles are progressive, and under these conditions amputation of the affected limb cannot be avoided [3-6, 8, 15].

Development and implementation of timely and effective correction of acute ischemia of the limbs would undoubtedly be aided by knowledge of the dynamics of the structural and metabolic changes taking place in skeletal muscles during acute occlusion of the main arteries. Data in the literature on this problem do not deal with the problem as a whole, but only with certain of its aspects, and as a rule, they do not give an objective and quantitative evaluation of the morphological changes discovered [1, 7, 10, 12, 14].

The aim of this investigation was a combined quantitative morphological analysis of changes in the skeletal muscles during acute arterial occlusion of the limbs.

EXPERIMENTAL METHOD

Skeletal muscles from the leg of 36 dogs weighing 13-18 kg, with experimental acute occlusion of the terminal part of the aorta [2], were studied. The duration of ischemia was 0 (control), 3, 6, 9, and 12 h. Sections through the leg muscles, after embedding in paraffin wax, were stained with hematoxylin and eosin, by Van Gieson's Regaud's, and Lie's methods, and by the trichrome method of Goldner [9, 11]. Microscopic preparations were studied in ordinary and polarized light. Tissue stereologic analysis of volumes of intact and injured muscle fibers and the edematous stroma was carried out on sections stained by Regaud's method in 10 fields of vision on the "Struktura" apparatus (Central Design Bureau, Academy of Medical Sciences of the USSR). The following histochemical methods were used: the diaminobenzidine method for myoglobin [13], staining with Sudan Black B and Oil Red for phospholipids and neutral fats, the PAS reaction with amylase control to detect glycogen and neutral glycosaminoglycane. The enzyme–histological investigation of the soleus muscle was undertaken on frozen section 10 μ thick. Activity of the following enzymes was determined by the usual methods [11]: succinate (SDH), isocitrate (ICDH), malate (MDH), α-glycerophosphate (α-GPDH), lactate (LDH), glucose-6-phosphate (G-6-PDH), glutamate (GDH), and β-hydroxybutyrate (β-HBDH) dehydrogenases; NAD- and NADP-diaphorases, alkaline and acid phosphatases (AlP and AcP respectively), myosin ATPase, and phosphorylase. Changes in SDH, LDH, β-HBDH, NAD, NADP, and ATPase were estimated quantitative on an MIF-7 integrating photometric microscope. In one transverse section 66 areas of red and white muscle fibers (RMF and WMF respectively) were measured photometrically. All numerical data were subjected to statistical analysis. The significance of differences between the values for the parameters was determined by Student's test.

EXPERIMENTAL RESULTS

After ischemia for 3 h damage to the muscle fibers was found. Tissue analysis showed changes in the ratio of the relative frequency of areas of intact and injured fibers and stroma compared with the control. In the control group, for instance, the ratio was 67:6:22%, and after ischemia for 3 h it was 60:13:24%. The focal and more widespread increase in cross-striation of the muscle fibers will be noted (Fig. 1a), and in polarized light this was revealed as more intensive fluorescence of the A-disks. The time course of contractural injuries to the muscle fibers could be discerned: localized areas of approximation of the A-disks, which merged

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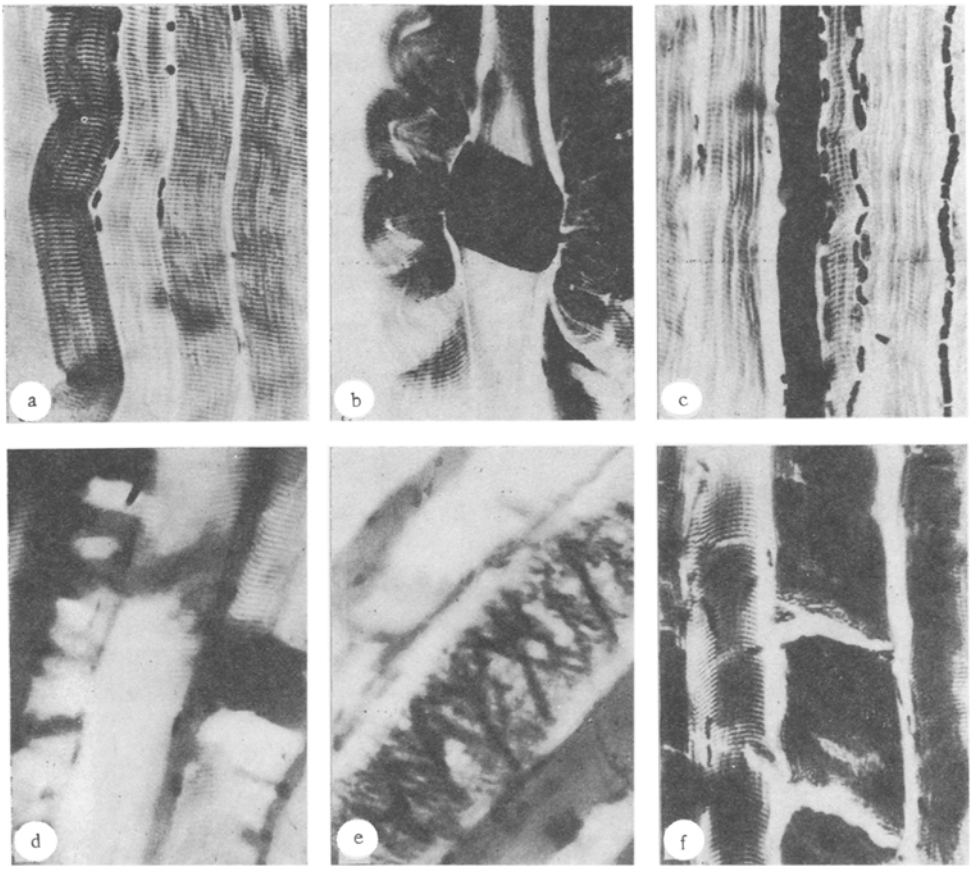

Fig. 1. Structural changes in skeletal muscle in ischemia: a) increased cross-striation of muscle fibers (ischemia for 3 h; 200 ×); b) contractural changes in muscle fibers, lesion of "contraction node" type in center (ischemia for 3 h, 256 ×); c) lesion of muscle fiber of "contraction band" type (ischemia for 6 h, 200 ×); d) muscle fiber with merging of A-disks in center of field, muscle fibers with circumscribed foci of myocytolysis, and with loss of anisotropic structures alongside it (ischemia for 6 h, 256 ×); e) contractures and fragmentation of muscle fibers (ischemia for 9 h, 256 ×); f) discoid necrosis of muscle fiber (ischemia for 12 h, 256 ×). a, b, c, f) Stained by Regaud's method, d, e) stained with hematoxylin and eosin, polarized light.

with one another. Contractural injuries of the "contraction node" type were clearly identified in sections stained by Regaud's method (Fig. 1b). The results of the qualitative histochemical investigation showed a decrease in the glycogen reserves and myoglobin content. The results of the quantitative enzyme–histochemical investigation revealed changes in metabolism of the skeletal muscles and their adaptation to the conditions of acute ischemia. With a significant (on average by 20-30%) decrease in activity of enzyme of the Krebs cycle and of protein and lipid catabolism, as well as of diaphorases, the reserve pathway for production of high-energy compounds (anaerobic glycolysis) became activated, as was confirmed by an increase of 21% in LDH activity in RMF and of 15% in WMF. An indirect morphological sign of structural adaptation of the muscle fibers during ischemia lasting 3 h was their clear differentiation into different types, i.e., identification of RMF and WMF, the relative percentages of which agreed with the control (46% of RMF and 54% of WMF).

Significant structural and metabolic changes in the skeletal muscles were found after ischemia for 6 h, i.e., what many investigators consider to be the critical period [10, 12, 14]. The results of tissue stereologic analysis reflect progression of the lesion and edema of the skeletal muscles. The relative frequency of areas of intact and injured fibers and of edema was 56:19:24%. The qualitative character of the lesions also was changed: Besides constructural lesions of the "contraction node" type, longer "contraction bands" began to appear (Fig. 1c), and were characterized by more intense staining, homogenization, a waxy appearance and disappearance of transverse structures. Examination of the sections in polarized light revealed, besides contractural lesions, signs of myocytolysis, typified by disappearance of the anisotropic structures and the formation of circumscribed areas of isotropic material (Fig. 1d). Reversibility of the lesion in certain muscle fibers was indicated by the diffuse PAS-positive staining of their sarcoplasm, which was not removed by

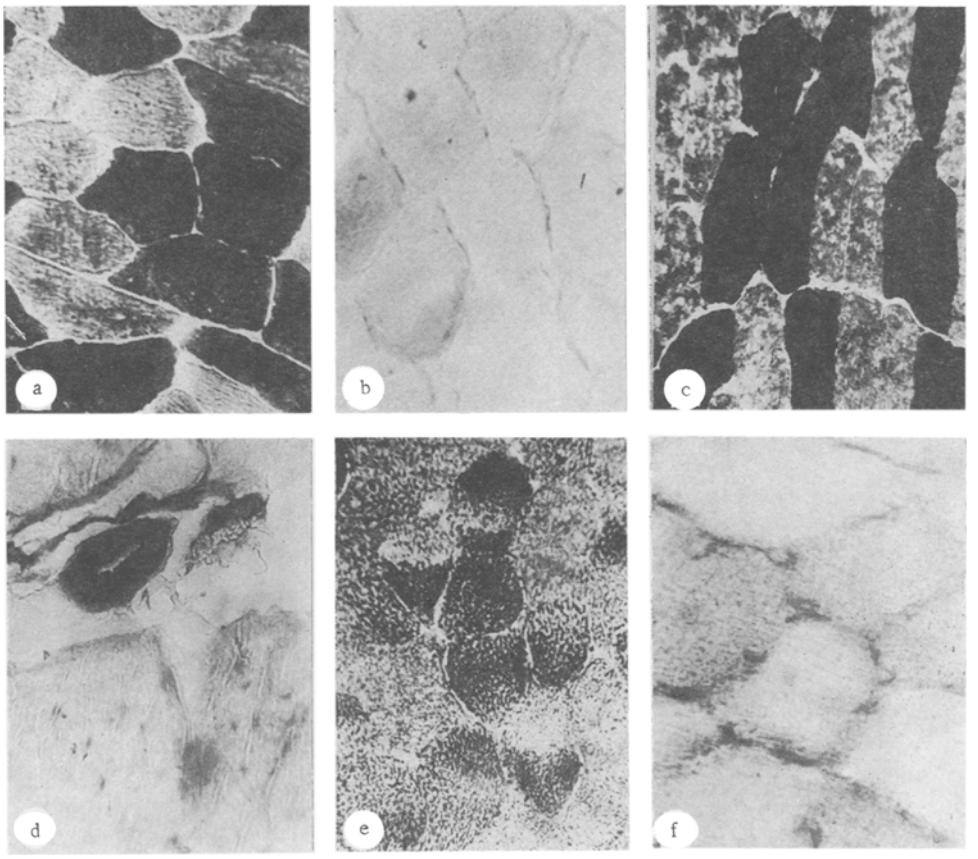

Fig. 2. Changes in enzyme activity in skeletal muscle fibers during acute ischemia (256 ×). a) Phosphorylase activity in control much higher in WMF than in RMF; b) sharp decrease in phosphorylase activity during ischemia for 6 h, different types of muscle fibers not distinguishable (Takeushi's method); c) ATPase activity in control is much higher in WMF than in RMF; d) sharp fall in ATPase activity during ischemia for 9 h, different types of muscle fibers cannot be identified, ATPase activity in wall of arteriole still preserved (Padykula's method); e) marked increase in AcP activity during ischemia for 9 h (Burstone's method); f) fall in NADP-diaphorase activity after ischemia for 12 h (Hess' method).

preliminary treatment with amylase. The glycogen and myoglobin concentrations in the sarcoplasm fell sharply, and at the same time, there was an increase in the concentrations of phospholipids and neutral fats, evidence of the commencing destabilization of the intracellular structures. Quantitative enzyme histochemical investigation revealed a greater decrease in activity of enzymes of the Krebs cycle and of protein and lipid catabolism (on average by 45-60%) compared with the control. Anaerobic glycolysis in the skeletal muscle after ischemia of the limbs for 6 h is no longer effective, as shown by the decrease of 5% in LDH activity in RMF and by 15% in WMF compared with the control. ATPase activity was 68% lower in RMF and 63% lower in WMF. Because of the sharp fall in phosphorylase activity in WMF (Fig. 2a, b) the muscle fibers could no longer be correctly typed in sections stained by Goldner's trichrome method after ischemia for 6 h. Progression of the changes in the ischemic muscle fibers and disturbance of their structural and metabolic features of heterogeneity were accompanied by increased activity of a lysosomal enzyme, namely AcP. On the whole, the functional morphology of the skeletal muscle after ischemia for 6 h suggests the possibility of collapse of the intracellular mechanisms of homeostasis and the beginning of development of irreversible changes.

Considerable changes were found in the ischemic skeletal muscles, as regards both extent and severity of injury, 9 and 12 h after acute occlusion of the terminal part of the aorta. The relative frequency of areas of intact and injured muscle fibers and of edema was 38:29:28% after 9 h of ischemia and 36:35: 29%, respectively, after 12 h of ischemia. Polarized light microscopy revealed that contractural lesions predominated over myocytolysis and mixed forms of lesions, resembling primary fragmentation of the muscles, were frequently observed and were characterized by a simultaneous combination of contractures and myocytolysis in the same

muscle fiber (Fig. 1e). A characteristic feature was fragmentation of the damaged areas, going on to the development of a picture of discoid necrosis (Fig. 1f). Progression of the changes was regularly accompanied not only by increasingly severe edema, but also by the appearance of small groups of cells consisting of neutrophilic granulocytes and macrophages. Glycogen and myoglobin granules could not be determined at these times in the sarcoplasm of the muscle fibers, and signs of fat phanerosis were observed. Quantitative enzyme histochemical investigation at these times of the experiment revealed a sharp fall in activity of all dehydrogenases studied. In particular, SDH activity in RMF after ischemia for 9 h was reduced by 80% and in WMF by 76%; LDH activity was reduced by 67 and 58%, respectively, compared with the control. After ischemia for 12 h, dehydrogenase activity was reduced even more (Fig. 2f). It was no longer possible to distinguish between the types of muscle fibers on the basis of their ATPase activity. Total ATPase activity of RMF and WMF after ischemia for 9 h was 89% lower than in the control (Fig. 2c, d), and after 12 h of ischemia it was 91% lower. ATPase activity was still preserved, however, in the vessel walls. Meanwhile, AlP activity, concerned in transport of metabolites, was reduced in the endotheliocytes of blood vessels belonging to the microcirculatory system. There was a progressive increase in AcP activity in the muscle fibers, especially in areas of injury (Fig. 2e).

It is, thus, evident that during a period of ischemia lasting 6 h or more widespread metabolic disturbances and structural changes take place in the skeletal muscles. Quantitative analysis revealed substantial disturbances of metabolism in skeletal muscle after 6 h of ischemia, while the volume of affected muscle fibers remains relatively small. These observations must be taken into account when optimal times of restoration of the blood flow in the limbs are determined.

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