MORPHOLOGICAL CHARACTERISTICS OF CHANGES IN LIMB SKELETAL MUSCLE TISSUE DURING EXPERIMENTAL POSTISCHEMIC RECIRCULATION

Academician V. S. Savel'ev,* G. A. Chekareva,
O. D. Mishnev, and O. A. Bogdanov

UDC 617.57/.58-005.4-036.8-07:616.747/.748

KEY WORDS: ischemia of the limbs; postischemic recirculation; muscle fibers; cytophotometry; tissue stereologic analysis.

In a previous communication the writers gave data on changes in skeletal muscles in the ischemic period of acute arterial occlusion of the limbs, and the microscopic picture and functional morphology of the ischemic skeletal muscles 2 h after restoration of the blood flow to the limbs were studied. Early postischemic recirculation is undoubtedly the most reliable test of viability of ischemic tissue structures [1, 2, 4].

Timely restoration of the blood flow enables normalization of metabolism in ischemic tissues [6], but delay leads to failure of adaptation [3, 6].

On restoration of the blood flow in the limbs under experimental conditions it is possible to correct morphological data on the reversibility and irreversibility of skeletal muscle damage, and the criteria of viability of the muscles during ischemia. This enables structural changes in the skeletal muscles of patients with acute occlusion of the main limb arteries to be estimated more correctly, an essential factor when critical periods of ischemia are established and, on a more general level, for the improvement of the surgical care of such patients.

The present investigation was devoted to a study of these problems.

EXPERIMENTAL METHOD

Experiments were carried out on 69 mongrel dogs of both sexes weighing 13-18 kg, in which the blood flow was restored for 2 h by the method of I. I. Zatevakhin et al. (1976), after occlusion of the terminal portion of the aorta for 3, 6, 9, and 12 h. For the morphological investigation of the leg muscles a combination of histologic, histochemical, enzyme-histologic, and quantitative methods was used, similar to that adopted for the study of the ischemic period.

EXPERIMENTAL RESULTS

Restoration of the blood flow in the limbs after 3 h of ischemia led to changes in the relative volumes of the undamaged and injured muscle fibers and the endematous stroma compared with their values in the corresponding ischemic period. According to the results of tissue stereologic analysis they were 56, 12, and 28%, respectively, which showed objectively an increase in the volume of edema by 4% after recirculation for 2 h. The qualitative characteristics of the lesions in the postischemic period were largely unchanged. Just as after 3 h of ischemia, they were characterized by limited contractures of the contraction nodes type, but larger lesions also were encountered (Fig. 1a). Enhancement of the cross-striation of the muscle fibers was more marked in degree, and increased anisotropy of the A-disks of the muscle fibers, with localized areas of approximation and fusion of the disks together could be seen under the polarization microscope (Fig. 1b). The results of qualitative histochemical analysis showed that during 2 h of the postischemic period the normal glycogen and myoglobin concentrations in the muscle fibers are not restored. Meanwhile the data of quantitative enzyme-

*Academy of Medical Sciences of the USSR.

No. 3 Department of Surgical Diseases, and Department of Pathological Anatomy, Faculty of Internal Medicine, N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 9, pp. 351-355, September, 1985. Original article submitted July 9, 1984.

 0007-4888/85/0009-1266$09.50

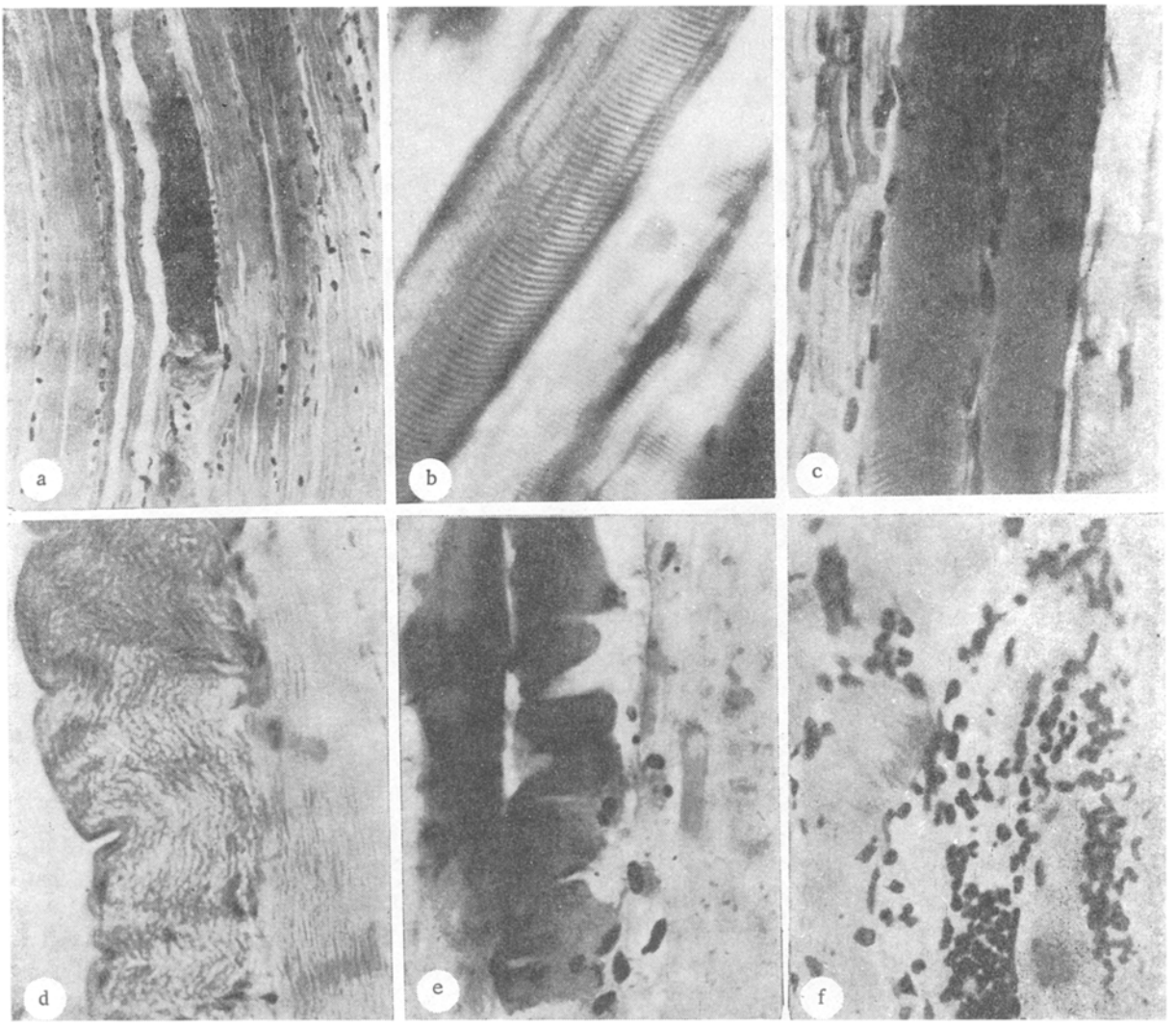

Fig. 1. Microscopic changes in skeletal limb muscles after 2 h of postischemic recirculation: a) contractural damage to muscle fiber, recirculation after 3 h of ischemia. Regaud's stain, 100 ×; b) enhancement of anisotropy of A-disks of muscle fibers with localized areas of their approximation and fusion with each other; contractural damage in top left corner. Recirculation after 3 h of ischemia, polarized light, staining with hematoxylin and eosin, 256 ×; c) marked fucsinophilia of damaged muscle fibers. Recirculation after 6 h of ischemia. Lie's stain, 256; d) colliquation necrosis of muscle fiber. Recirculation after 6 h of ischemia. Hematoxylin and eosin, 256 ×; e) plasmorrhagia and fragmentation of muscle fibers. PAS reaction after preliminary treatment of sections with amylase. Recirculation after 6 h of ischemia, 256 ×; f) dense cellular infiltration of damaged muscle fibers. Recirculation after 9 h of ischemia. Hematoxylin and eosin, 256 ×.

histologic investigation indicate an important and significant improvement in the metabolic situation in the skeletal muscles compared with that in the ischemic period. Although activity of enzymes of the Krebs' cycle, of diaphorases, and of enzymes of protein and lipid catabolism did not reach the control values, it was on average 10-20% higher than after 3 h of ischemia. Restoration of aerobic metabolism was accompanied by a decrease in LDH activity (by 6% compared with the control). Energy utilization was improved, as shown by an increase of 4% in ATPase activity compared with that in the ischemic period.

The experiments thus showed objectively that restoration of the blood flow in the limbs after 3 h of ischemia promotes normalization of the structural and metabolic changes in the skeletal muscles.

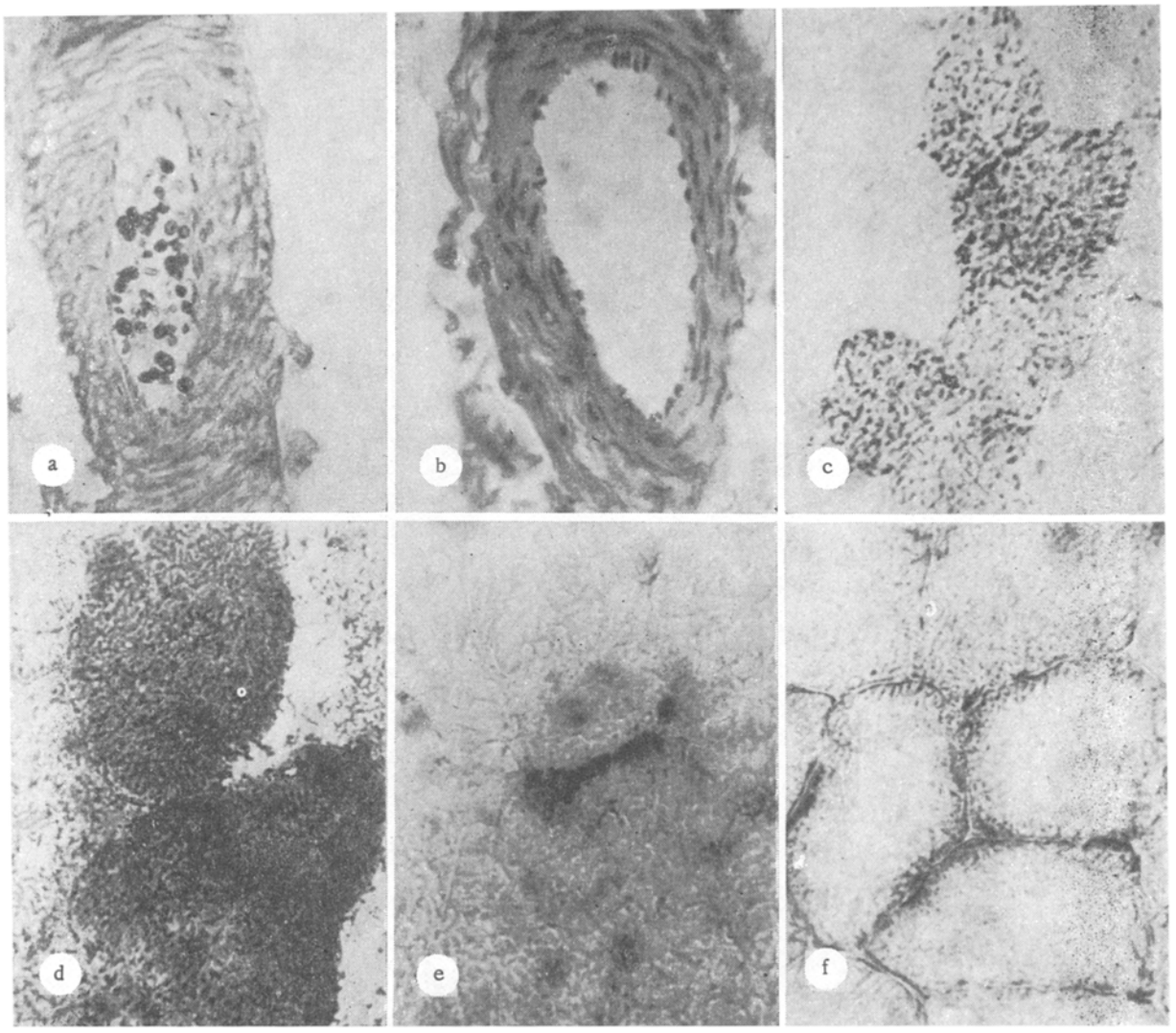

Fig. 2. Microscopic and enzyme-histologic changes in skeletal muscles of limbs after 2 h of postischemic recirculation: a) edema of arteriolar wall, vacuolation of smooth-muscle cells, recirculation after 6 h of ischemia. Goldner's trichrome stain. 256 ×; b) segmental necrosis of arteriolar walls. Recirculation after 12 h of ischemia. Hematoxylin and eosin, 256 ×; c) lipophanerosis of damaged muscle fibers. Recirculation after 6 h of ischemia, Sudan Black, 200 ×; d) Sharp increase in acid phosphatase activity in damaged muscle fibers. Recirculation after 6 h of ischemia. Burstone's azo-coupling method, 256 ×; e) marked decrease in alkaline phosphatase activity in capillary endothelium. Recirculation after 9 h of ischemia. Standard azo-coupling method, 256 ×; f) Sharp decrease in LDH activity. Recirculation after 12 h of ischemia, 256 ×.

Early postischemic recirculation for 2 h after ischemia lasting 6 h led to a considerable increase in the volumes of damaged muscle fibers (by 18% compared with the control and by 5% compared with the ischemic period) and of edema (by 9 and 7%, respectively). Lesions of the skeletal muscle fibers were more severe, the number of foci of contractural damage of the contraction node and band type was increased, and muscle fibers with marked and extensive fucsinophilia when stained by Lie's method were found (Fig. 2c). Examination of the preparations in polarized light demonstrated the trend of the contractural lesions, and areas of continuous anisotropic conglomerates and fragmentation, and muscle fibers in a state of myocytolysis. Areas of myocytolysis during this period could also be found when the preparations were examined in ordinary light. Characteristics of myocytolysis include paler staining of the sarcoplasm, swelling of fibers, destruction of myofibrils, and lysis of nuclei (Fig. 1d). Fragmentation of the damaged muscle fibers was observed, and staining by the PAS reaction after preliminary treatment of the sections with amylase revealed plasmorrhagia of the fibers (Fig. 1e).

Changes in the walls of the small blood vessels, with edema and vacuolation of the cytoplasm of the smooth-muscle cells, were noted (Fig. 2a). On histochemical investigation the glycogen and myoglobin concentrations in the muscle fibers were even lower than in the ischemic period. Signs of lipophanerosis were more marked (Fig. 2c). Activity of dehydrogenases of the Krebs' cycle, of catabolism of lipids and proteins, and also activity of diaphorases were on average 20% lower than before restoration of the blood flow, and almost 80% lower than the control level. LDH activity was considerably reduced (by 25% compared with the ischemic period). ATPase activity was reduced on average by 18% compared with that in the ischemic period; typing of red and white muscle fibers on the basis of their ATPase activity under these circumstances became very difficult. Acid phosphatase activity was considerably increased (Fig. 2d). These results confirm our hypothesis of a possible collapse of compensatory mechanisms with the development of irreversible changes in the muscle fibers after ischemia for 6 h.

Restoration of the blood flow for 2 h in the limbs after ischemia for 9 and 12 h led to protressive intensification of the pathological changes in the muscle fibers. Tissue stereologic analysis revealed the following relative volumes of intact and damaged muscle fibers and of edematous stroma: during recirculation after 9 h of ischemia — 26, 37, and 36%, respectively, during recirculation after 12 h of ischemia — 22, 42, and 36%. Examination of the preparations in polarized light showed predominance of irreversible over reversible lesions. Focal infiltration of dying muscle fibers by neutrophils and macrophages was observed (Fig. 1f). Damage to the walls of small blood vessels, amounting in some cases to segmental necrosis, was more severe (Fig. 2b). Quantitative enzyme-histochemical investigation at these times of the experiment revealed an even greater decrease in activity of all the dehydrogenases studied (Fig. 2f). Acid phosphatase activity in the damaged muscle fibers remained high. Alkaline phosphatase activity in endotheliocytes of vessels of the microcirculatory bed was significantly lower than in the ischemic period (Fig. 2e).

The use of an experimental model of acute arterial occlusion of the limb thus confirmed the effectiveness of restoration of the blood flow after 3 h of ischemia. Restoration of the blood flow after 6 h of ischemia is accompanied by considerable structural and metabolic disturbances in the skeletal muscle fibers. These disturbances are even more marked in the case of recirculation after 9 and 12 h of ischemia. The data are objective evidence that the so-called critical time of ischemia of the skeletal muscles is less than 6 h, and this must be taken into account when aid is given to patients with acute occlusion of the main limb arteries.

Morphological criteria of the reversibility or irreversibility of ischemic damage to skeletal muscle fibers and their viability cannot be reduced to individual signs. A combined functional and morphological investigation is necessary, with quantitative assessment of the structural and metabolic changes discovered.

LITERATURE CITED

1. Yu. M. Lopukhin and É. M. Kogan, Criteria of Viability of Organs and Tissues before Transplantation [in Russian], Moscow (1975).
2. O. D. Mishnev et al., in: Proceedings of the 6th All-Union Congress of Morbid Anatomists [in Russian], Moscow (1977), p. 161.
3. L. O. Dahlbäck, Scand. J. Plast. Reconst. Surg., Suppl. 7 (1970).
4. R. B. Jennings et al., Am. J. Pathol., 81, 179 (1975).
5. G. Makitie and J. H. Terövöinen, Acta Neuropathol. (Berlin), 37, 101 (1978).
6. S. Santavirta et al., Res. Exp. Med., 174, 245 (1979).