FOCAL DEAGGREGATION OF MYOFIBRILS IN ACUTE METABOLIC LESIONS OF THE SOMATIC MUSCLES

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Target electron-microscopic and polarization-optical investigations were made of the skeletal muscles of rats and rabbits after injection of toxic substances and experimental acute ischemia of the limbs. Besides contraction injuries, two types of focal deaggregation of the myofibrils are described. The first arises on a basis of relaxation and is manifested as total deaggregation and lysis of the myofibrils; in the second form lysis arises in the partially contracted fiber and begins in the I-discs, leading to diastasis of the A-discs. Foci of the first type are characteristic mainly of white fibers, those of the second type of red fibers.

KEY WORDS: toxic substances; ischemia of the limbs; deaggregation of myofibrils; red and white fibers.

The most characteristic response of fibers of somatic and heart muscle to injury is contracture [1, 2, 7]. Meanwhile, acute injuries of another type have been described in the myocardium – "intracellular myocytolysis," connected with deaggregation of the myofibrils [4, 6]. At the light-optical level, and with the use of polarized light, foci of disappearance of the myofibrils have also been found in the somatic muscles of albino rats after injection of emetine into the animals [8]. Such changes are found less frequently and they are less marked during ordinary histological investigation than contracture injuries leading to coagulation necrosis, and for that reason they have received little study.

The object of this investigation was to detect foci of deaggregation of myofibrils arising in somatic muscles during various experimental procedures and to describe the morphological picture of this process.

EXPERIMENTAL METHOD

Experiments were carried out on 48 male chinchilla rabbits weighing 1.5-3 kg, and 96 male Wistar albino rats weighing 150-300 g. Injury was caused: in the rats by subcutaneous injection of dimethylparaphenylenediamine in a dose of 2.5 mg/100 g body weight, intravenous injection of papain in a dose of 30-50 mg/100 g body weight, and intraperitoneal injection of cobalt chloride in a dose of 1 mg/100 g body weight; in rabbits by intravenous injection of papain in the same doses as in rats, and by intravenous injection of diphtheria toxin in a dose of 1-1.5 MLD/kg body weight. In 7 rats the femoral artery was ligated and in 27 animals a rubber tourniquet was applied to the thigh. The rats were killed with chloroform and the rabbits by a blow on the back of the head. The animals were killed at various times from 15 min to 5 days after the beginning of the experiment or removal of the tourniquet. Healthy animals and the intact limbs of animals to which a tourniquet was applied served as the control. The diaphragm and the leg muscles (gastrocnemius and soleus) were studied. Material was fixed with cold 4% paraformaldehyde solution in 0.1 M phosphate buffer, pH 7.4, containing 5% sucrose. For electron-microscopic investigation, after fixation for 2 h in formaldehyde pieces measuring 0.5-1 mm were excised and post fixed in cold 1% osmium tetroxide solution [9, 12]. For light microscopy the material was embedded in paraffin wax, for electron microscopy in styrene–methacrylate [11] in the modifications [10, 13] with certain changes adopted in the writers' lab-

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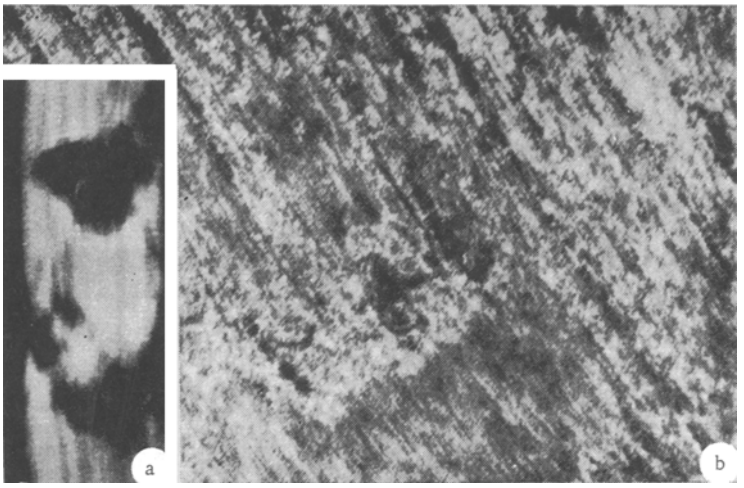

Fig. 1. Focal deaggregation of myofibrils in a white fiber: a) in polarized light (objective 25, ocular 10); b) electromicrograph showing total deaggregation of myofibrils in a focus of injury (26,000×).

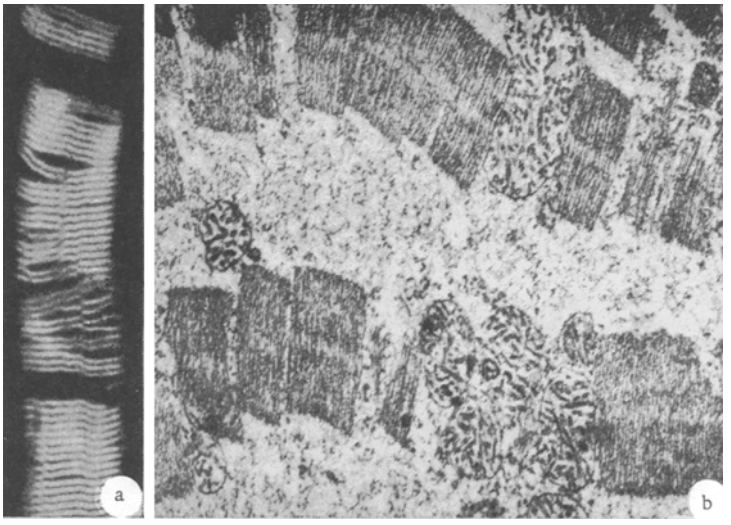

Fig. 2. Focal deaggregation of myofibrils in a red fiber: a) in polarized light (630×); b) electromicrograph showing deaggregation of I-discs (26,000×).

oratory. Paraffin sections were stained by the ordinary histological method and by the PAS reaction. Ultrathin sections were cut on the Tesla-490A ultratome and stained with uranyl acetate and lead citrate. The material was examined in ordinary light, in polarized light, and in the phase contrast system. The investigations in the electron microscope were carried out by the target method [3]: sections 3 μ in thickness were first cut from each block intended for electron-microscopic investigation and stained with toluidine blue; they were then examined in the light microscope in ordinary and polarized light. The ultrastructural investigations were carried out with the Tesla BS-513 electron microscope, with an accelerating voltage of 80 kV.

EXPERIMENTAL RESULTS

Besides focal contracture injuries [5], two types of foci of deaggregation of the myofibrils were found in the material studied. The first type occurred chiefly in white fibers. It consisted of foci of injury isotropic in polarized light. They had clearly defined, sharp borders, often with the appearance of a broken line. They varied in size: some were as large as the height of one sarcomere in both the longitudinal and the transverse direction, but often they covered the whole diameter of the fiber and were up to 20 sarcomeres

in length. The dimensions of the foci along the long axis were only rarely greater than the transverse diameter of the fiber. Longitudinal structures – sometimes weakly anisotropic – remained in the isotropic focus but the cross striation had disappeared. Within the injured area the myofibrils had lost both their mutually parallel arrangement and their rectilinear direction along the long axis of the fiber. The myofibrils in the focus of injury appeared slack (Fig. 1a). In ordinary light and in unstained sections the isotropic segments were less deeply stained, although often this feature was so inconspicuous that the foci of injury of this type were observed only in polarized light.

Examination in the electron microscope showed that the Z-bands had disappeared, the protofibrils were no longer arranged strictly transversely, and deaggregation and disorganization of the thick and thin myofilaments were observed, with the result that individual sarcomeres could not be made out (Fig. 1b). The process ended with fragmentation and lysis of the myofibrils.

The second type of focal deaggregation of the myofibrils was observed in the red muscle fibers. Areas of injury were mainly of considerable length in the long axis of the fiber, sometimes occupying the whole area of a fiber caught in the section. In the initial stages of the process the pattern of crossed striation was intact, and the A-discs showed increased anisotropism in polarized light and well-marked diastasis, so that the distances between them were greater than the ordinary heights of the I-discs (Fig. 2a). Individual groups of sarcomeres appeared to be scattered in the focus of injury. In ordinary light diastasis of the A-discs also was found, so that they were more deeply stained, whereas in areas corresponding to the I-discs no dye could be seen.

Electron microscopy revealed disappearance of the Z-bands and complete deaggregation of the thin myofilaments; where the I-discs should be there were numerous shapeless fragments which subsequently underwent complete absorption. The thick myofilaments preserved their typical structure as well as the mutual arrangement of their A-discs, which were in a state of diastasis, especially marked along the long axis of the fiber (Fig. 2b). Later the thick myofilaments joined in the process, accompanied by lysis of the affected area of the fiber. This type of focal deaggregation of the myofibrils predominated in ischemias of the limbs. The first type was found less frequently, and it occurred chiefly in general disturbances of metabolism caused by the parenteral injection of toxic substances. In focal deaggregation of the myofibrils the reaction both of the blood leukocytes and of the connective tissue cells was completely absent. The PAS reaction was negative in the foci of injury, evidence of absence of plasma seepage.

These observations suggest that the changes described in the somatic muscle fibers constitute a special type of injury that develops on a basis of relaxation of the myofibrils and ends with their lysis. Whereas during contracture injury strong connections appear between the thin and thick myofilaments of the myofibrils, preventing their subsequent lysis [7], in the types of injury now described phenomena of the opposite kind occurred. The isotropic form of deaggregation of the myofibrils evidently arises in fibers in a state of relaxation, as a result of which the myofibrils undergo total deaggregation.

In the second form of injuries described here, some contraction of the myofibrils evidently takes place initially, so that the A-discs become more resistant to autolysis and there is a corresponding increase in their anisotropy, whereas lysis develops initially in the zone of the I-discs.

These types of lytic injuries of muscle fibers described above have features in common with the intracellular myocytolysis observed in the myocardium [7] and with the changes in the myofibrils during autolysis of muscle tissue. Activation of the autolytic processes, which are always present to a slight degree in the living fiber [14], takes place in all probability through a disturbance of oxidative processes and it is not associated with the liberation of hydrolases from the lysosomes, for lysosomes are very rarely found in normal muscle fibers.

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