MORPHOLOGY AND PATHOMORPHOLOGY

Structural Features of Acute Focal Metabolic Injuries to Somatic Muscle Fibers Caused by Dimethylparaphenylenediamine

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 127, No. 6, pp. 700-705, June, 1999
Original article submitted May 11, 1999

The pathological picture of an acute disorder of cell metabolism caused by dimethylparaphenylenediamine consists of typical focal reactions of striated muscles to injury: myofibril contractures and intracellular myocytolysis. Analysis of the pathological process demonstrates the time course of contracture injuries as a succession of overcontracture (first-third degree contractures) and necrobiotic phases (fourth degree contracture), followed by lump degradation and macrophagal resorption. Two polar types of contractures are clearly differentiated by the size, shape, and structural and metabolic characteristics of target fibers: "ribbons " and "medallions". Disappearance of Z strips, disaggregation, disorientation, and fragmentation of myofibrils are typical of intracellular myocytolysis. The observed changes form the morphological basis for acute disease of striated muscles.

Key Words: *metabolic injuries; somatic muscles; polarization microscopy*

The histological picture of pronounced clinical manifestations of somatic muscle disease reflects, as a rule, the interactions between destructive and compensatory-adaptive processes running under heterogeneous structural and metabolic conditions at all levels. The mechanism underlying pathological changes can be elucidated by extrapolation of experimental data characterizing the morphological manifestations during a myopathological process.

General mechanisms underlying metabolic injuries to muscle fibers include primary and secondary disorders of energy metabolism, including those caused by blocking of mitochondrial enzyme systems [10, 12-15].

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We analyzed the structural features and evolution of acute focal dystrophic injuries to the skeletal muscles caused by dimethylparaphenylenediamine, a blocker of respiratory enzymes and redox processes in the cell [9].

MATERIALS AND METHODS

Acute metabolic injuries to somatic muscle fibers were induced by a single subcutaneous injection of dimethylparaphenylenediamine in a dose of 2.5 mg/100 g [5,9]. Thirty-nine male Wistar rats weighing 150-300 g were used, 14 of then were controls. The animals were sacrificed 6-8 h, 1, 3, and 5 days postinjection.

For making the crural muscle preparation in the "strain at rest" state [5], the left hind limb was amputated at the level of the hip joint after decapitation, the skin was rapidly separated, and the whole limb was fixed in cold (4°C) 4% paraformaldehyde in Mil-

0007-4888/99/0006-0640$22.00

lonig's phosphate buffer (pH 7.4). The diaphragm was fixed with the costal ring for 24 h. Fragments of crural muscles and diaphragm were dissected from macrospecimens for further processing.

Tissue samples for light microscopy were fixed in 12% neutral formalin. Histological preparations were stained with hematoxylin-eosin in combination with Perls' test to detect iron ions; according to Van Gieson-method with post staining of elastic fibers with Weihert's resorcin-fuscin; with colloid iron-periodic acid Schiff hematoxylin; and periodic acid Schiff (PAS) test was performed.

For electron microscopy, the fragments were fixed in 4% paraformaldehyde and 1% osmium tetroxide, and after standard treatment embedded in Epon-Araldite. Semithin sections were stained with 1% Azur II, PAS test was carried out, and the preparations were examined under a Docuval light microscope. Semithin sections contrasted with uranyl acetate and lead citrate were examined under a JEM-1010 electron microscope.

For examining the fibrillar system of muscle cells, stained and non-stained paraffin sections with longitudinally oriented muscle fibers were examined in polarized light [1].

RESULTS

The study of focal degenerative changes in the myocardium and somatic muscles showed that acute metabolic injuries to cardiomyocytes and muscle fibers were paralleled by changes in the myofibril polarization properties at the earliest stages [2,5,6]. Moreover, examination under polarized light helped to select the sites for electron microscopic examination.

Polarization and spot electron microscopy showed the evolution of structural manifestations of the myopathological process from the first signs of alteration to a complex variegated picture with gradual accumulation of reparative and residual changes. Typical focal reactions of somatic muscles to injury underlie the qualitative and quantitative variety of the morphological picture: contractures of muscle fibers of different severity and focal disaggregation of myofibrils (intracellular myocytolysis).

Analysis of myofibril anisotropy helped us to reconstruct the time course of contracture injuries and single out 4 degrees of contractures corresponding to successive phases in the development of overcontractions [5]. However the type of changes and their evolution were not determined solely by the type of structural reaction (contracture or lysis).

The study of contracture injuries in somatic muscles showed 2 polar types of focal changes in muscle fibers, which differed by shape, size, and other structural and metabolic features. The first type were "ribbons", or foci involving an extensive site of a fiber along its longitudinal axis and its entire cross-section area. The ribbons usually had the same cross-section area as the fiber and looked like its continuation, because the interface between the injury and the intact site of the fiber was vague. Another type was "medallions" with a short length along the fiber; sometimes such a focus involved 20-30 sarcomers and looked like a "contraction node" (Fig. 1, *a*; 2). Medallions were convex, sometimes deformed the adjacent fibers, and looked like a well outlined foreign body. Ribbons were seen mainly in the white muscle fibers, while medallions in red fibers [5].

Apart from their shape and size, "ribbons" and "medallions" differed by the pattern and course of the process.

Early after injection of the damaging agent (after 6-8 h), all stages of contractures were observed in the *red fibers (type 1)*: increased anisotropy of A disks (first-degree contractures) in sites with increased eosinophilia, approximation of the disks (second-degree contractures), and their fusion into an anisotropic conglomerate (third-degree contractures). In some foci high density of the contracture material was paralleled by a drop of anisotropy (fourth-degree contractures). Isotropic sites looked (in common light) like amorphous fragments, sometimes with the initial signs of lumpy degradation.

Examination in polarized light 1 day after the start of the experiment showed lumpy degradation of myofibrils. The majority of lumps were characterized by isotropy, rarely by weakly anisotropic longitudinal or (still more rarely) transverse streaks. In common light, large homogenous waxy lumps and moderate cell infiltration were observed (Fig. 1, *b*). Infiltration represented mainly by macrophages and blood polynuclears was noted in the damaged site or along the borders of the focus of injury near the poles and the sarcolemma interface on both sides of the sarcolemma.

Three days after the start of the experiment, myofibrillar structures completely disappeared from some foci. Many "ribbons" were filled with infiltrating cells, mainly macrophages, and were isotropic in polarized light. After 5 days, the number of macrophages in "ribbons" decreased and clear borders of infiltration disappeared.

In the *white muscle fibers (type 2)* mainly fourth-degree contractures were observed during the first hours. Changes following the overcontraction of the fibers (formation of amorphous matrix, lumpy degradation, cell infiltration, and resorption of necrotic debris) were delayed: lumpy degradation of a considerable number of "medallions" was clearly seen

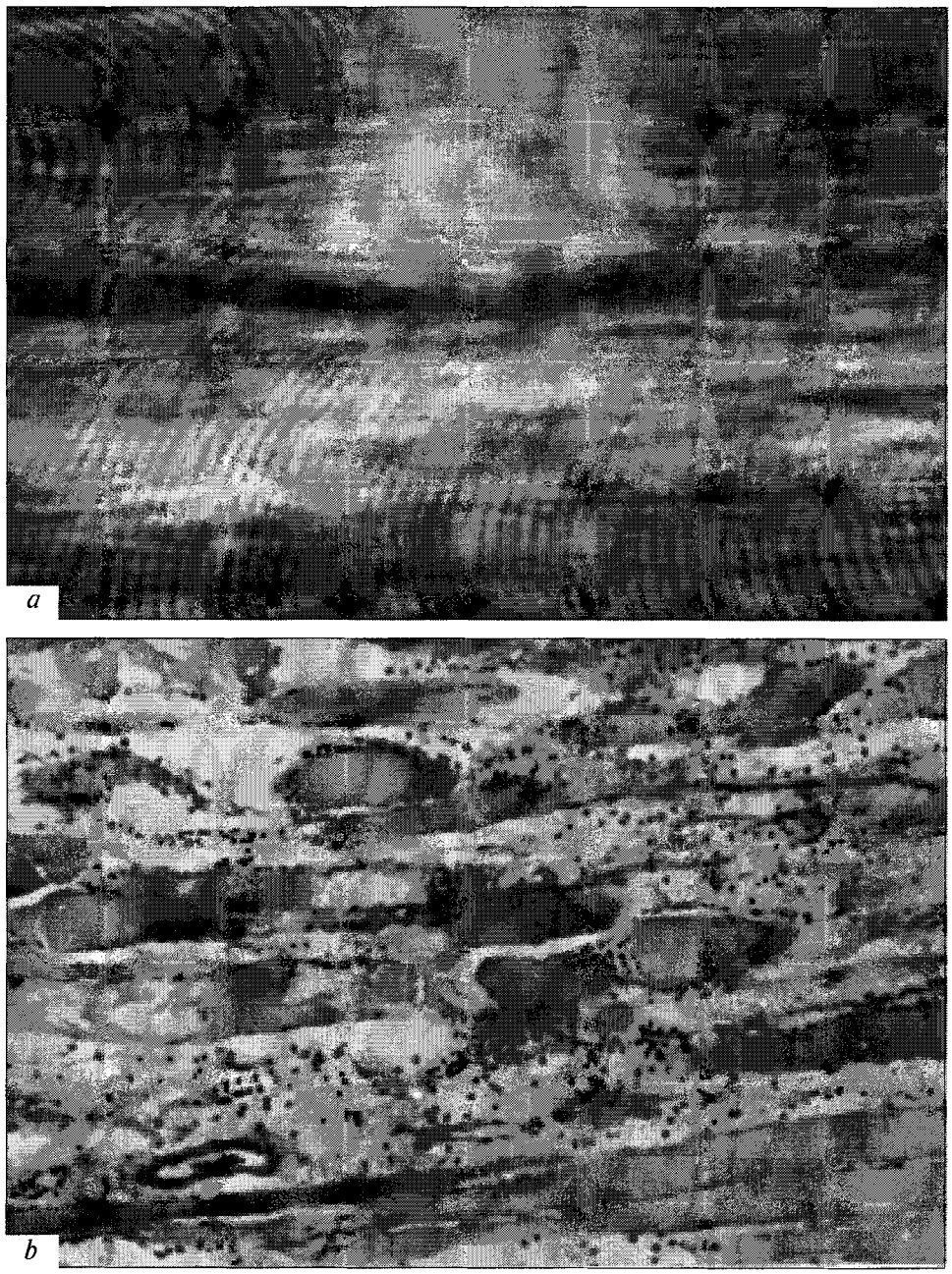

Fig. 1. Acute focal injury to rat gastrocnemius muscle induced by dimethylparaphenylenediamine. *a*) contracture nodes ("medallions"): myofibril contractures of the second-third degree 6 h after the start of experiment. Photography in polarized light, ×1200; *b*) necrobiotic changes in muscle fibers, intense mononuclear infiltration 1 day after the start of experiment. Hematoxylin-eosin staining, ×600.

only on day 3, cell infiltration accumulated near the poles and, to a lesser extent, in the focus. Even after 5 days there were some PAS-positive lumps in the cell infiltrates, sometimes with weakly anisotropic longitudinal streaks.

Therefore, analysis of the pathological process shows the time course of contracture injuries as a succession of overcontractions and necrobiotic phases followed by lumpy degradation and macrophagal resorption. One of the main differences in the pattern and time course of the process for different types of contracture foci is that contractures (first-third degree) in a "medallion" develop much more rapidly, while the necrobiotic phase is prolonged, probably due to decrement-free conduction of excitation (injury) and strong contraction (and hence, a higher density of contracture foci), characteristic of type 2 fibers.

Electron microscopy showed similar changes in the myofibrillar system in both the "ribbons" and "medallions". On the one hand, contracture streaks appeared at the level of Z disks and A disks approached each other, which was followed by disappearance of

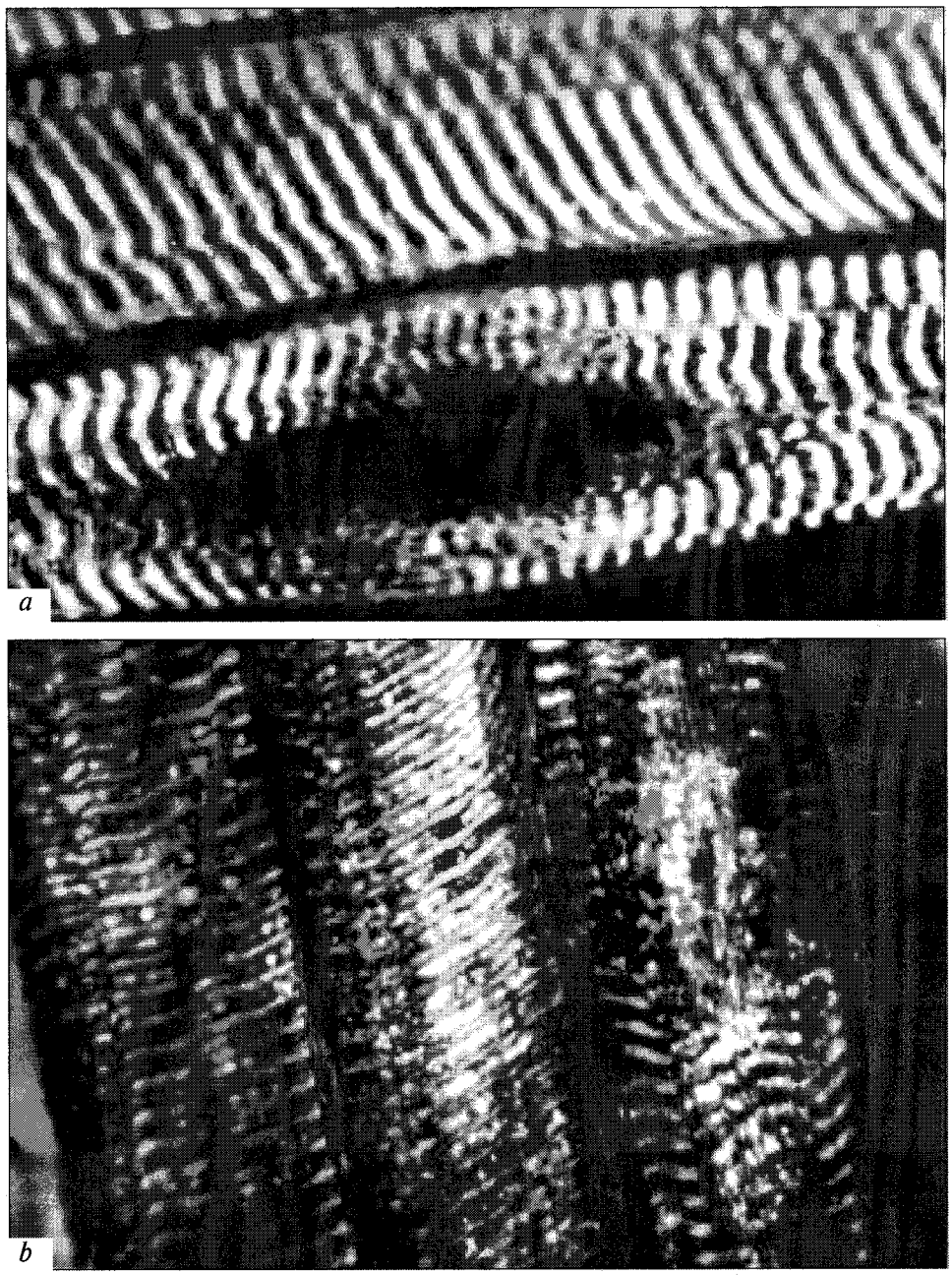

Fig. 2. Focus of myofibril alteration (intracellular myocytolysis) in rat gastrocnemius muscle 6 h after injection of dimethylparapherylenediamine. Photography in polarized light. *a*) ×1200; *b*) ×1200, the preparation turned by 90°.

Z disks; the myofilament arrangement became completely chaotic (with sites of overcontractures), and on the other hand, the height of I disks increased and H zone leveled and disappeared because of extension, which was followed by destruction of Z disks and dissociation of the myosin and actin filaments (overstretched zones). The differences consisted only in the degree and relative location of these sites: more pronounced alterations with clear-cut interface and the absence of intermediate stages were characteristic of "medallions". During the final stage of contracture injuries, the foci looked as agglomerates of disaggregated myofilaments.

Intracellular myocytolysis foci in this model were much rarer and at the early stages (1 day) looked in polarized light as "empty" irregular isotropic sites. Ultrastructurally, these zones contained no Z disks, were characterized by chaotic transverse protofibril arrangement, disaggregation and disorientation of thick and thin myofilaments, so that no individual sarcomers were detected. A characteristic sign of these foci was little number of mitochondria or their complete

absence. The process eventuated in fragmentation and lysis of myofibrils.

Therefore, our findings and published data [3,4,7, 10-13] indicate that the described changes correspond to typical structural reactions forming the morphological basis of acute diseases of human and animal striated muscles.

The diversity of the general picture is explained by the fact that the final structural reaction is determined by accidental combination of many circumstances (function of a certain site of muscle fiber at the moment of exposure and quantitative parameters of the damaging factor) and by general structural and metabolic characteristics of muscle fibers. Apart from polar types which are singled out by different classifications, there are numerous intermediate forms which can be transformed one into another in the course of adaptation [8], which indicates a possibility of a great diversity of structural variants of universal reactions.

In this connection, of special interest are the data on the regularities and mechanisms of injuries to skeletal muscles, obtained in animal experiments; being extrapolated to similar processes in humans disease, these data will help to distinguish the chief elements in the morphological picture and its dynamic interpretation.

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