FOCAL LESIONS IN SOMATIC MUSCLES PRODUCED BY DIMETHYLPARAPHENYLENEDIAMINE

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UDC 616.74-031.84-092.9.001.57

Metabolic injuries to skeletal muscles induced in albino rats by subcutaneous injection of dimethylparaphenylenediamine were investigated by histological, histochemical, polarization-microscopic, and immunotopographic methods. The development of focal necrotic changes in the muscles was associated with contractures of the myofibrils. The foci of injury in the red muscle fibers form "bands" and are accompanied by rapidly developing and intensive imbibition of plasma. In white fibers the foci are nodular or medal-like in shape, and their plasma imbibition develops more slowly and less intensively. Differences in the shape of the foci are connected with functional differences between red and white muscle fibers.

Focal degenerative changes in somatic muscles have been known for a long time and have frequently been described since the time of Zenker [16], but until recently there was no convenient experimental model of the injuries produced in skeletal muscles by metabolic disturbances. To study degenerative changes in muscle fibers, local procedures such as incisions in the muscles [4], ligation of vessels [5, 13], or the local application of chemicals or heat [9, 14, 15] have been used in most investigations. Recently papers have been published in which lesions produced in somatic muscles by parenteral injection of chemical compounds have been described [11, 12, 13].

In the search for a model reproducing metabolic lesions in somatic muscles the writer has tested the parenteral injection of the papain, cobalt chloride, and dimethylparaphenylenediamine (DPD) and has chosen the last of these substances because it yielded the most constant and clearly defined changes in the muscles. The mechanism of action of DPD is evidently connected with a disturbance of oxido-reduction in the tissues and of capillary permeability [10]. Unlike Jagmin and Gareau [12], the writer gave only a single injection of the compound, so that the process could be studied dynamically.

EXPERIMENTAL METHOD

Experiments were carried out on 102 albino rats weighing 150-300 g, 10 of which were controls. The animals received a single subcutaneous injection of DPD hydrochloride in 1% aqueous solution in a dose of 25 mg/100 g body weight.

The rats were killed at intervals from 15 min to 43 days from the beginning of the experiment. The gastrocnemius and soleus muscles and the diaphragm were investigated. The muscles fixed in situ in 10% neutral formalin and embedded in paraffin wax. For the immunotopographic and histochemical reactions, pieces of tissue were frozen with liquid nitrogen and sections cut in a cryostat. The sections were stained with hematoxylin–eosin, by Van Gieson's and Hale's methods, with toluidine blue, impregnated with silver by Gomori's method; the PAS reaction was performed with and without an amylase control, proteins were detected by mercuric chloride and bromphenol blue, tryptophan by Adams' method, acid phosphatase by Gomori's method, and succinate dehydrogenase by Burstone's method. To investigate plasma imbibition

Laboratory of Pathomorphology, Institute of Cytotology and Genetics, Siberian Division, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 5, pp. 116-119, May, 1973. Original article submitted October 2, 1972.

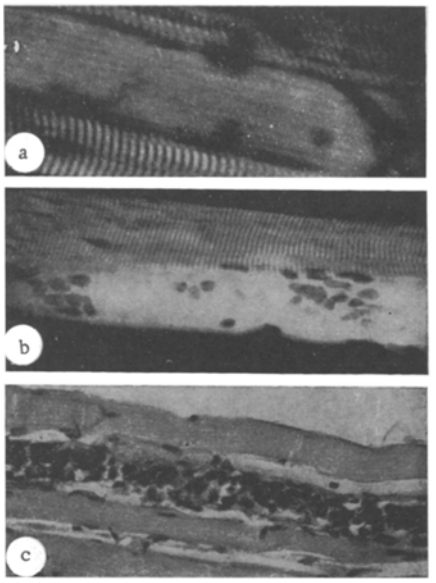

Fig. 1

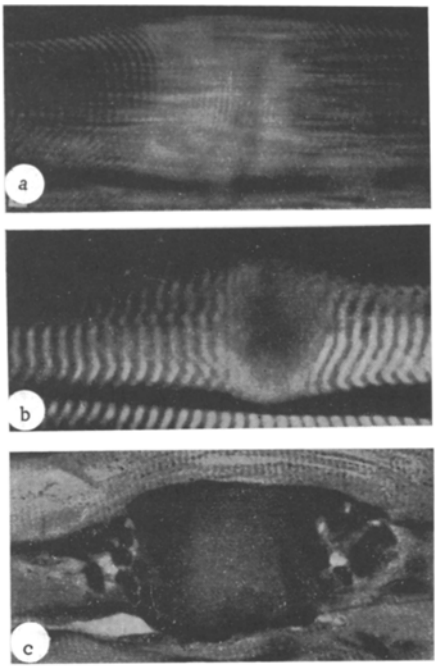

Fig. 2

Fig. 1. Contractural lesions of the first type (bands) in a muscle fiber: a) approximation of A disks (polarized light, 800 ×); b) fusion of A disks (polarized light, 500 ×); c) granular degeneration with cellular infiltration (PAS reaction, hematoxylin, orange; 250 ×).

Fig. 2. Contractural lesions of the second type (medal) in a muscle fiber: a) approximation of A disks (polarized light; 1000 ×); b) nodule of contraction (polarized light; 630 ×); c) fragmentation of injured area of fiber, cellular infiltration (PAS reaction, hematoxylin, orange; 630 ×).

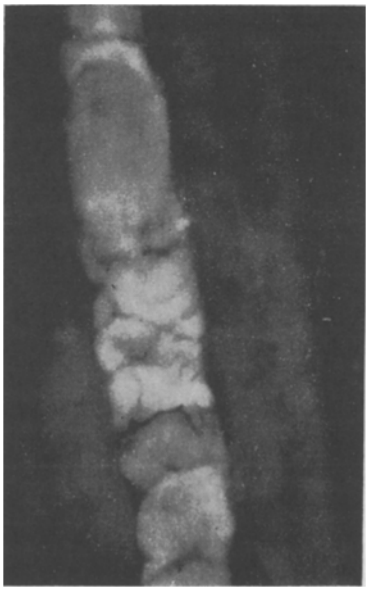

Fig. 3. Plasma imbibition in focus of contractural injury of the first type in a muscle fiber. Treatment with luminescent antiplasma serum by Coons' method. Luminescence, 500 ×.

the Coons' method of labeled antibodies was used in the writer's previous modification [7]. Polarization, phase-contrast, and luminescence microscopy were used in the investigations.

EXPERIMENTAL RESULTS

Lesions of the somatic muscles were found 6 h after injection of DPD. Degenerative changes began with the appearnace of foci covering a small area of the muscle fiber where anisotropy of the A disks appeared in polarized light. Later the A disks came closer together (Figs. 1a, 2a) and in the extreme degree of this process the cross striation was lost and continuous anisotrophy of the affected area developed (Figs. 1b, 2b). In ordinary light the foci at this stage appeared homogeneous and wax-like. These changes corresponded to the contracture type of lesion described in the myocardium [8] and somatic muscles [3]. The next stage was fragmentation of the affected area, accompanied by a decrease in the intensity of anisotrophy. Parallel with fragmentation, disintegration of the myofibrils took place with the formation of deeply stained masses, eventually breaking down into tiny granules. Cellular infiltration, predominantly by macrophages, appeared. With their aid, resorption of the necrotic masses took place, after which the blood and connective tissue cells disappeared from the focus of injury, and it was left as an empty tube of sarco-

lemma or as a collapsed band of sarcolemma. Anisotropic granules persisted in the focus for a very long time, often with preservation of the longitudinal, and sometimes the transverse striation also. The processes described were not synchronized in their course in different foci, and in the period from the 1st to the 7th day various stages of the process could be seen simultaneously in the sections.

A positive PAS reaction, not prevented by treatment with amylase, was usually observed in the first stage of the degenerative changes. After treatment with labeled antiplasma serum, a more or less intense luminescence was found in the same areas (Fig. 3). Tests for protein, especially tryptophan, in the injured areas were strongly positive; in the stage of cellular infiltration acid phosphatase activity appeared. The results of the reaction for succinate dehydrogenase agreed with those obtained by other workers [12].

Two types of foci of contractural injury were observed in the muscles examined. In the first type the lesion appeared over a wide area along the length of the fibers, the contracture usually was not sharply demarcated from the poles, fragmentation began later, and the fragmented fragment had the appearance of a band (Fig. 1c). Cellular infiltration usually took place equally intensively from the poles and from the borders between the necrotic fiber and sarcolemma. Anisotropy of the granules disappeared more rapidly. The PAS reaction was strong and appeared in the initial stage of contracture. Luminescence of the foci treated with labeled antiplasma serum was intense.

The second type of contractural lesion consisted of a small focus. The initial stage was a contracture affecting a small number of sarcomeres (20-30), and the contraction nodule had clear boundaries with the poles. The fiber at this place was swollen (Fig. 2b). Fragmentation began early, and the fragment appeared like a medal (Fig. 2c). Cellular infiltration took place from the poles. The anistropic granules persisted for a long time. A positive PAS reaction appeared later, and it was weaker at the beginning of necrobiosis. Luminescence of the foci treated with labeled antiplasma serum was rather weaker.

In the experiments on isolated muscle fibers the appearance and spread of the lesions in the muscle fiber were related to their functional state at the time of injury and with the character of spread of the contraction wave [1, 2, 4]. The results of the present experiments agree with these conclusions and show that different types of foci of injury are connected with different types of muscle fibers. Band-shaped foci are characteristic of fibers of the first type (red), in which contraction develops relatively slowly and lasts a long time. Fibers of the second type (white) are characterized by rapid and brief contraction, and the foci resemble medals in appearance. The large number of myofibrils in the white fibers and their small content of sarcoplasm led to rapid fragmentation of the affected area and delayed plasma imbibition, which in this case evidently takes place by diffusion through the sarcolemma and not through the T system, the tubules of which are open after injury [6].

Parenteral administration of dimethylparaphenylenediamine thus provides an experimental model of focal degenerative and necrobiotic changes in skeletal muscles similar to the changes observed in myopathies, and it can be used to study the dynamics of their development. The model merits further study.

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