Ultrastructure of the Contractile Apparatus in Cardiomyocytes during Regenerative and Plastic Insufficiency of the Myocardium

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Lytic changes in cardiomyocyte myofibrils constituting the morphological basis of contractile insufficiency were found in Wistar rats with regenerative and plastic myocardial insufficiency 3 h after daunomycin administration. Myofibrils became less dense, empty spaces appeared in many sarcomeres, sometimes total lysis of myofilaments within the sarcomere was noted. These changes were most pronounced in the perinuclear zone. Intracellular regeneration of cardiomyocytes was characterized by disorientation of newly formed myofibrils in relation to the long axis of muscle fibers and preserved myofibrils. Progressive inhibition of protein synthesis, lysis of myofibrils, and focal degradation of the sarcoplasm caused apoptotic death of some cardiomyocytes.

Key Words: *anthracycline-induced cardiomyopathy; regenerative and plastic insufficiency; cardiomyocytes; myofibrils; ultrastructure*

Changes in the contractile apparatus of cardiomyocytes (CM) accompanying damages to the myocardium attract much attention due to their considerable diagnostic and prognostic importance [2,9]. Classification of various forms of CM injuries caused by ischemic and metabolic disturbances and characterized by peculiar microscopic picture in polarized light is important for morphological diagnostics of prenecrotic states and early stages of myocardial infarction [2]. The type and severity of damages to the contractile apparatus of CM accompanied by attenuation or suppression of biosynthetic processes determine functional and regenerative capacities of cardiomyocytes and serve as ultrastructural criteria for regenerative and plastic insufficiency of CM. Of particular interest is to compare myofibril damage during regenerative insufficiency with those observed during myocardial alteration.

Here we studied the type and dynamics of ultrastructural changes in the contractile apparatus of CM during regenerative and plastic insufficiency of the myocardium.

MATERIALS AND METHODS

Experiments were performed on 65 male Wistar rats weighing 160-220 g with anthracycline-induced cardiomyopathy. Group 1 rats were decapitated 1-24 h and 1-5 days after single intraperitoneal injection of daunomycin hydrochloride in a cardiotoxic dose of 30 mg/kg. Group 2 rats were repeatedly (3 times with 7-day intervals) administered with daunomycin hydrochloride (10 mg/kg, intraperitoneally) and decapitated 5 days after the last injection. Control animals received intraperitoneal injections of physiological saline in an equivalent volume.

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For electron microscopy, myocardial samples were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and treated by routine methods [8]. Ultrathin slices were contrasted with uranyl acetate and lead citrate and examined under Tesla BS500, JEM 100B, and JEM 1010 electron microscopes (acceleration potential 80 kV).

RESULTS

Anthracycline antibiotics produced most pronounced damages to CM myofibrils. It should be emphasized that lysis of myofilaments (MF) was preceded by changes in CM nuclei and nucleoli, which appeared 1 h after daunomycin administration and manifested in their segregation and fragmentation. These changes persisted over 1 day and were characterized by the formation of ring-shaped nucleoli.

Lysis of MF in myofibril was found 3 h after daunomycin administration and persisted over 2 days of the experiment. Myofibrils became less dense, and narrow spaces appeared between MF of A discs. We revealed loosening of sarcomeres and sometimes total lysis of MF within the sarcomere. Myofilaments looked like thinned bamboo-shaped structures. The distance between adjacent myofibrils increased. Tubules of the T system, free mitochondria, and glycogen granules were found in sites of pronounced myofibril lysis (Fig. 1, *a*).

Thinning or even disappearance of myofibrils was most pronounced in the central zone of CM. Perinuclear spaces not containing myofibrils were enlarged; the number of organelles decreased. Fragmentation of Z lines was accompanied by their shift in relation to each other. Similar fragmentation of Z lines accompanied ischemic and metabolic damages and CM mitosis [6,10].

Pronounced lytic changes in myofibrils, degradation of other cell ultrastructures, and intensive autophagy led to atrophy of CM, apoptotic death and elimination of 30% CM, and diffuse substitutive sclerosis [5,7]. These ultrastructural changes in myofibrils were related to rapid and selective inhibition of expression of genes encoding various myofibrillar proteins, including α-actin, troponin I, myosin light chain 2, and creatine kinase M [13].

Ultrastructural signs of regenerative processes were found 4 days after daunomycin administration. We revealed newly formed myofibril bundles (MFB) and agglomerates of ribosomes and polyribosomes in the peripheral region of thinned myofibrils (Fig. 1, *b*). In some CM, newly formed MFB were positioned at angle to the long axis of cells, extended in a fan-like manner, or had a spiral form (Fig. 2). This abnormal localization of MFB in regenerating CM [4] indicates severe disturbances in regulatory mechanisms and impaired biosynthesis of cytoskeleton proteins, which are responsible for MFB arrangement in sarcomeres. It was shown that progression of genetically determined dilated cardiomyopathy is accompanied by disorganization of sarcomeres, in particular, Z lines [17], due to deficiency of cytoskeleton protein dystrophin [14-16]. Impaired synthesis of cytoskeleton proteins during anthracycline-induced cardiomyopathy probably contributes to disorientation of newly formed myofibrils.

Disorganization of MFB in regenerating CM can be associated with transverse mechanical forces [1] and isometric contractions similar to those in cultured and embryonic CM [11].

Fractional administration of daunomycin produced similar ultrastructural changes, but less pronounced lysis. In the majority of CM, we observed thinning and loosening of MFB (Fig. 3, *a*) and lightening of the sarcoplasm. Apart from lytic damages, individual CM were characterized by ultrastructural signs of intracellular regeneration: ribosomes and polyribosomes appeared in lysed sarcoplasmic regions between preserved MF (Fig. 3, *b*, *c*). Some newly formed myofibrils were disoriented (Fig. 3, *d*).

The dynamics and morphological signs of anthracycline-induced changes in myofibrils differed from those during intracellular myocytolysis [2]. Intracellular myocytolysis proceeds in individual cells and is characterized by burst lytic processes. By the end of day 2, the structure of myofibrils is normalized due to intracellular regeneration.

Diffuse and slow lytic processes in CM myofibrils peaked 1-2 days after daunomycin administration. Thinned myofibrils preserved their ultrastructural organization. The T system of CM, whose tubules and terminal cisternae are visualized in lightened sarcoplasmic matrix, is not involved in these processes. Submicroscopic regions of myofibril lysis contain round-shaped secondary lysosomes. In our experiments, polarization microscopy revealed no changes in transverse striation of CM after myofibril lysis. Therefore, daunomycin-induced damage to CM is not the stage or form of intracellular myocytolysis.

Changes in myofibrils during anthracycline-induced cardiomyopathy correspond to diffuse myolysis after acute functional overload of the heart [12] and regenerative and plastic myocardial insufficiency that accompanies diphtheritic intoxication and experimental hypercholesterolemia [3]. Our results indicate that ultrastructural changes in myofibrils during anthracycline-induced cardiomyopathy reflect the major mechanisms underlying their degradation and regeneration during impairment of biosynthetic processes at the posttranscriptional level.

Fig. 1. Ultrastructural changes in cardiomyocyte myofibrils after single administration of daunomycin hydrochloride in a cardiotoxic dose: *a*) lysis of sarcomeres, exposed T system tubules, small glycogen granules in the cytoplasm, and the absence of ribosomes (×25,800); *b*) agglomerates of ribosomes and polyribosomes in the peripheral region of thinned myofibrils and cytoplasm along newly formed myofilaments (×29,000).

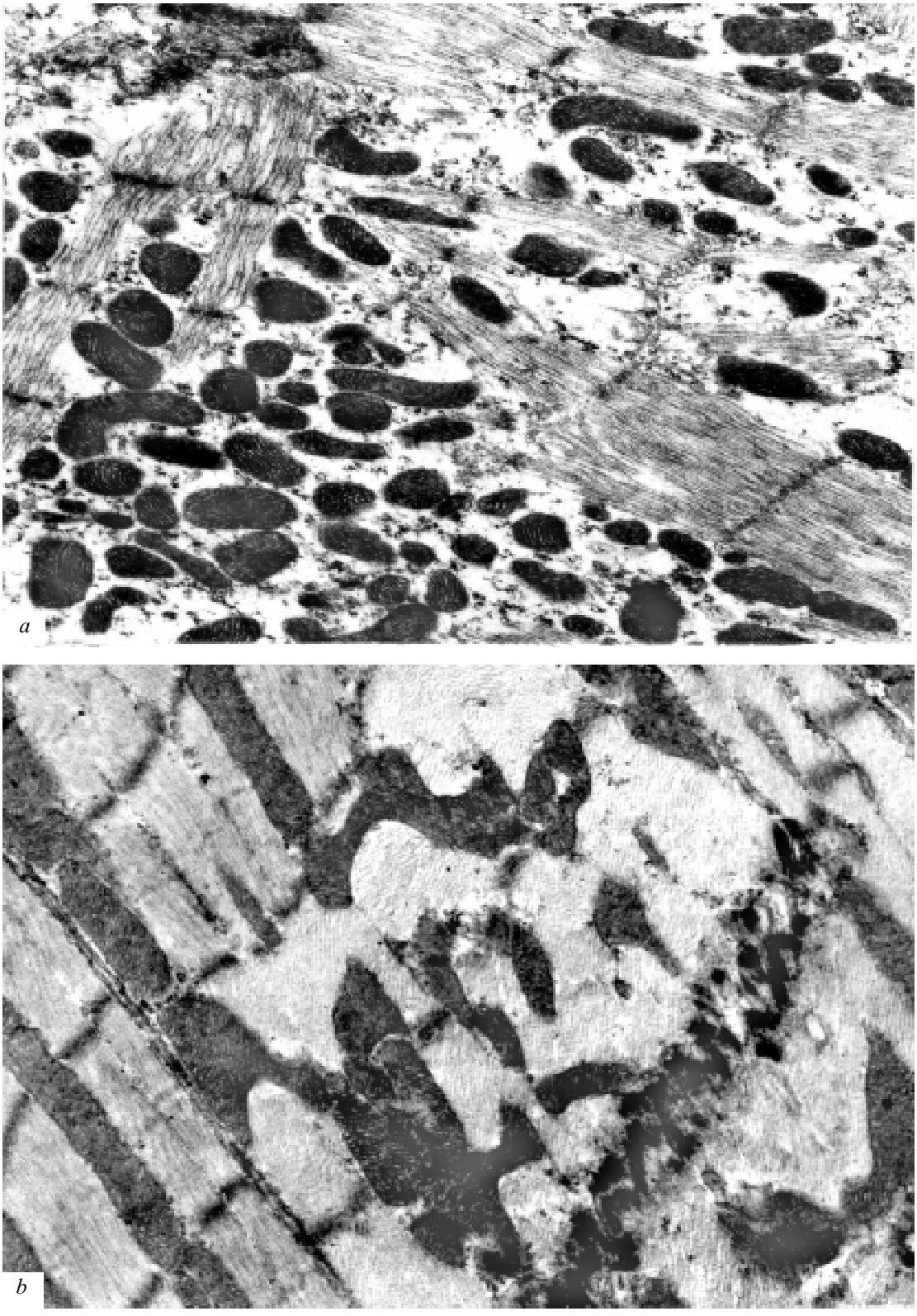

Fig. 2. Disorientation of newly formed myofibrils during anthracycline-induced cardiomyopathy: myofibrils extended in a fan-like manner (×20,000, *a*); myofilaments positioned at angle to the long axis of muscle fibers in 2 sarcomeres (longitudinal section of cardiomyocyte), the same distance between Z lines in myofibrils (×16,100, *b*).

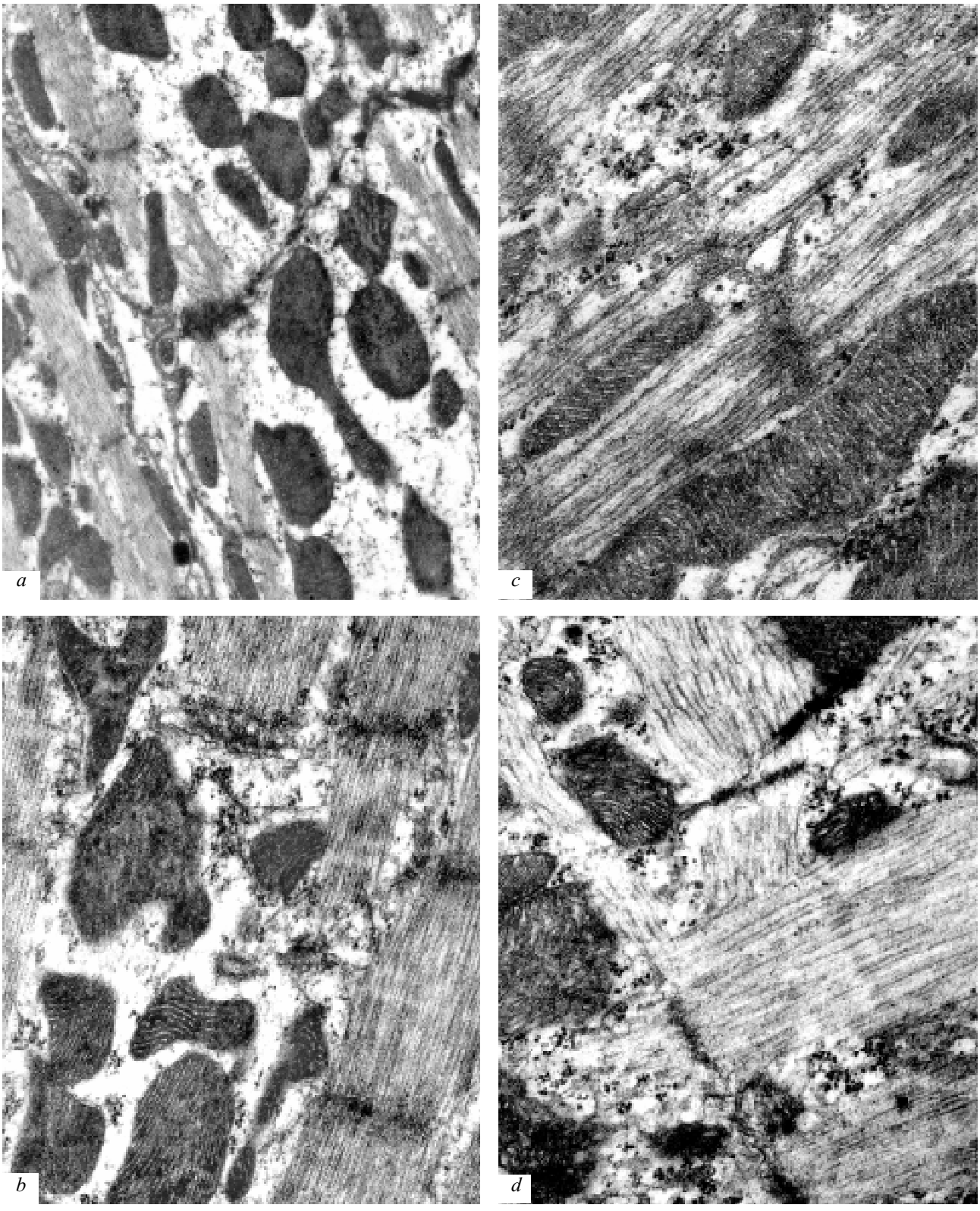

Fig. 3. Ultrastructural changes in cardiomyocyte myofibrils after fractional administration of daunomycin hydrochloride: decreased number of myofibrils and unchanged cytoplasmic volume (×17,100, *a*); initial stage of myofibril regeneration, ribosomes and polyribosomes in the cytoplasm of lysed sarcomeres (×27,300, *b*); chains of polyribosomes between preserved myofibril myofilaments (×41,000, *c*); newly formed myofilaments on ribosomes and disorientation of myofibrils (×41,000, *d*).

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