Ultrastructural Changes in Cardiomyocyte Mitochondria during Regenerative and Plastic Insufficiency of the Myocardium

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Daunomycin-induced regenerative and plastic insufficiency of the myocardium was accompanied by accumulation of cardiomyocytes with unstable mitochondrial membranes containing enlarged mitochondria with lightened matrix and fragmented cristae. Total destabilization of mitochondrial membranes was found in cardiomyocytes with most pronounced ultrastructural signs of impaired protein synthesis. These changes in mitochondria were permanent, which suggested that swelling and destruction of cristae were related to intravital decrease in mitochondrial membrane stability.

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

Mitochondria (MC) provide the energy for cells and play a central role in the initiation of apoptosis in response to various extracellular and intracellular stimuli [10]. Functional state of MC and ultrastructural criteria characterizing various types of MC damages attract much attention. Some authors believe that mitochondria are sensitive to cytopathic and stress factors [1], while others report that MC are the most stable cell organelles that undergo changes only after irreversible damages [9].

Ultrastructural changes in cardiomyocytes (CM) include lightening of mitochondrial matrix and destruction and reduction of their cristae [16]. Total swelling of MC accompanied by pronounced lightening of the matrix and destruction of cristae is the ultrastructural criterion of cell necrosis [8]. However, the presence of individual MC with lysed matrix and destructed cristae does not necessarily indicate cell death or irreversible changes.

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The increasing interest in morphological criteria of apoptosis in MC is determined by intensive studies of apoptosis and the role of MC in this process. It was hypothesized that MC do not undergo morphological changes during apoptosis. Recent studies showed that apoptosis is preceded by the appearance of small MC with dense matrix. This process was named "mitochondrial pyknosis" [10]. It remains unclear whether this ultrastructural reorganization of MC during apoptosis is universal for all cells and factors inducing programmed cell death. At the same time, ultrastructural changes in various cells during apoptosis may differ from those taken as morphological criteria for apoptosis [15].

Here we studied the type and dynamics of ultrastructural changes in MC of CM during anthracycline-induced 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

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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) intraperitoneally administered with 10 mg/kg daunomycin hydrochloride at 7-day intervals and decapitated 5 days after the last injection. Control animals received intraperitoneal injections of physiological saline in an equivalent volume.

For electron microscopy, myocardial samples were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and treated by routine methods [6]. 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

The very early ultrastructural changes in CM caused by daunomycin included reorganization of nuclei and nucleoli. Heterochromatin was absent in some CM nuclei 1 h after daunomycin administration; segregation of the granular and fibrillar nucleolonema components was seen in nucleoli. More severe damages to nucleoli (fragmentation and annulation) developed 12 h after daunomycin administration, which correlated with pronounced ultrastructural changes in CM: progressive lysis of myofilaments, reduction of organelles, and intensive autophagy.

The ultrastructure of MC practically did not differ from normal over the first 2 days after daunomycin treatment. The most pronounced lytic changes were found in myofibrils (MF). Progressive lysis of MF myofilaments was accompanied by sequestration and focal degradation of ultrastructures. Numerous myelin figures and secondary lysosomes containing lipofuscin appeared in the sarcoplasm.

On day 3 after daunomycin administration, individual CM contained enlarged MC with lightened matrix and fragmented cristae (Fig. 1, *a*, Fig. 2, *b*). Other ultrastructural parameters did not differ from normal. Ultrastructural changes in MC reflected their intensive autolysis (Fig. 2, *a*). However, the dynamics of ultrastructural changes in CM indicated that progressive decrease in the content of sarcoplasmic elements and MF was accompanied by a slight decrease in the number of cell MC.

It should be emphasized that these changes in MC of viable cells were permanent, which suggested that swelling and destruction of cristae proceeded during preparation of samples for electron microscopy and were related to intravital decrease in mitochondrial membrane stability. Total swelling of MC and fragmentation of cristae can be revealed in normal myocardial samples after their inadequate treatment; in this case, MC in all CM undergo similar changes. Since in our experiments ultrastructural changes were found only in individual CM, they were related to decreased mitochondrial membrane stability. Similar changes in MC were found in CM of rats treated with doxorubicin [11].

Destabilization of all mitochondrial membranes in individual CM is probably associated with suppressed synthesis of proteins (including mitochondrial) in the cytoplasm [2,3]. Since cytostatics produce different damages to the genetic apparatus of CM, total destabilization of mitochondrial membranes is observed in CM with most pronounced inhibition of protein synthesis.

Reciprocal localization of MC and MF reflects contractile properties of CM at the ultrastructural level. In normal myocardium, MC form chains, in which they are in contact with each other and contractile regions of MF (sarcomeres). Large contact surface between MC and MF provides maximum energy transfer from MC to MF and high rate of muscle contractions. In our experiments, the most pronounced ultrastructural changes in nuclei, nucleoli, and MF were accompanied by disorganization of MC in the perinuclear zone. There were single MC in the sarcoplasm, which make practically no contacts with each other and outer nuclear membrane. In the space between MF, the area of contacts between MC and sarcomeres was small (Fig. 1, *b*). Since anthracycline antibiotics impair ATP synthesis in CM, this process can serve as an indirect morphological criterion for abnormal energy consumption by the nucleus and MF and inhibition of ATP synthesis in MC.

Anthracycline antibiotics lead to dose-dependent cardiomyopathy, cardiac insufficiency [13], and apoptotic death of CM [4,5]. Molecular mechanisms underlying cardiotoxic effects of anthracycline antibiotics are poorly understood. Since anthracycline antibiotics display high affinity for lipids (in particular, cardiolipin, a component of the inner mitochondrial membrane [7]), they probably impair cardiospecific gene expression [14] and intensify lipid peroxidation in membrane structures [12]. Although CM death during anthracycline-induced cardiomyopathy is realized via apoptosis, the ultrastructural changes in MC caused by daunomycin hydrochloride do not correspond to mitochondrial pyknosis and massive total swelling typical of apoptosis and CM necrosis, respectively. Anthracycline-induced cardiomyopathy is probably accompanied by specific damages to MC related to a direct effect of antibiotics on mitochondrial structures and total impairment of biosynthetic processes. These data suggest that the appearance of CM with altered MC is a stage of programmed cell death or a sign reflecting structural and functional heterogeneity of cells.

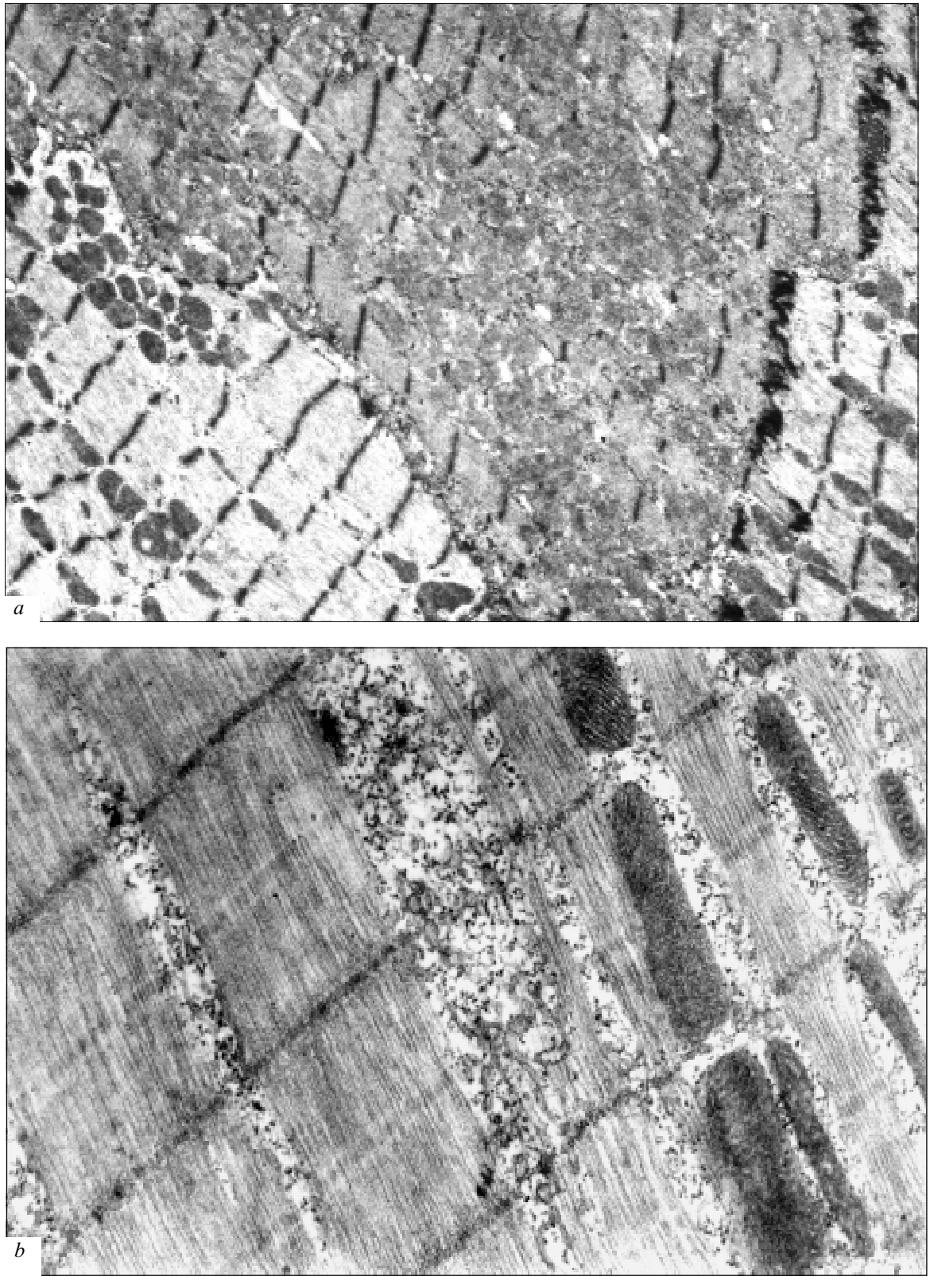

Fig. 1. Ultrastructural changes in cardiomyocyte mitochondria 1-3 days after administration of daunomycin hydrochloride in a cardiotoxic dose: total swelling and destruction of mitochondria, preserved structure of mitochondria in neighboring myocytes (×9700, *a*); and small areas of myofibril-mitochondrion and mitochondrion-mitochondrion contacts in the intermyofibrillar space, lysis of myofilaments, and thinning of myofibrils (×25,800, *b*).

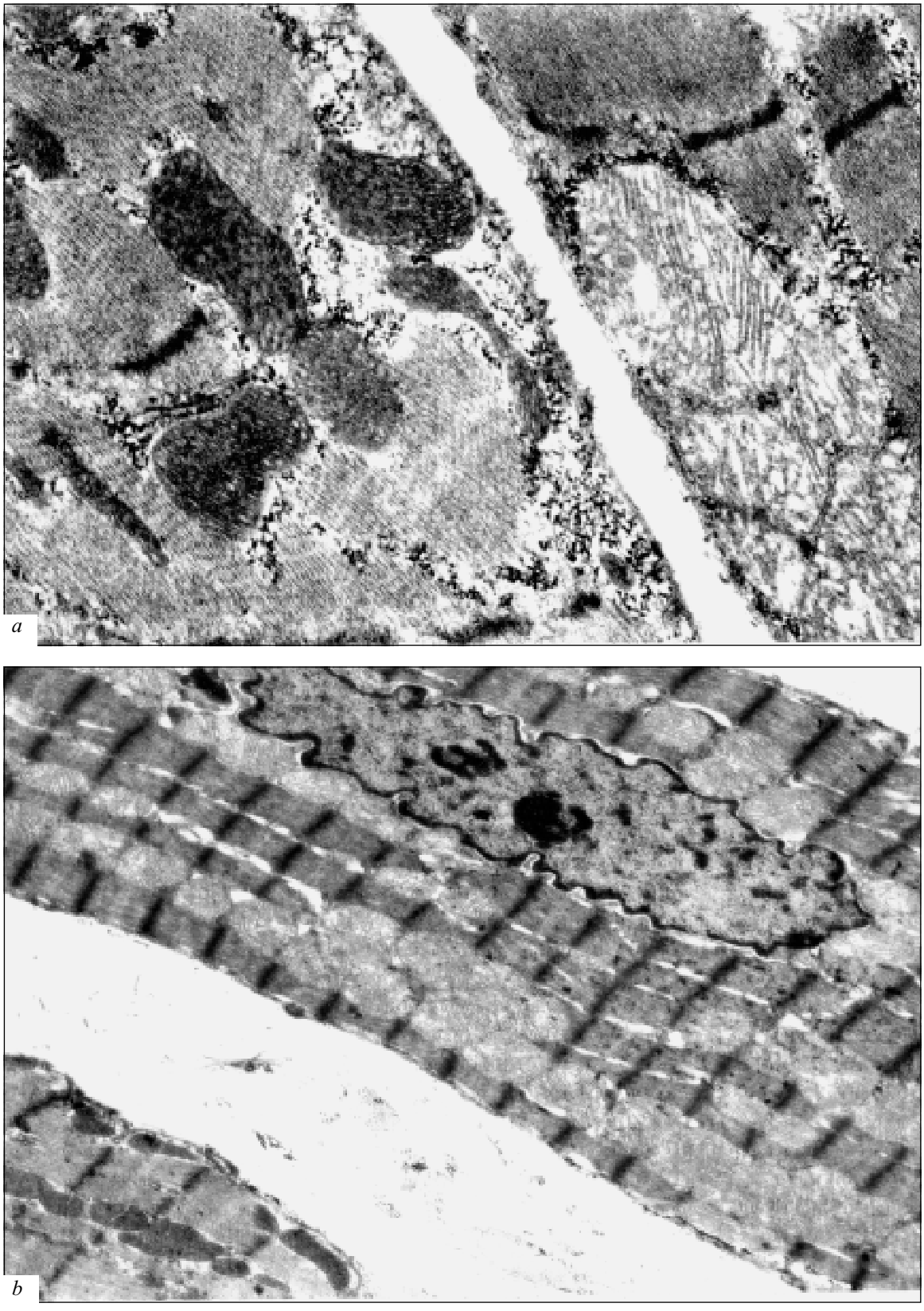

Fig. 2. Ultrastructural changes in cardiomyocyte mitochondria 4-5 days after administration of daunomycin hydrochloride in a cardiotoxic dose: various structures of mitochondria in 2 cardiomyocytes lying in parallel muscle fibers (×22,500, *a*); and total swelling of mitochondria (×9700, *b*).

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