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Focal Degradation of Cytoplasmic Organelles in Cardiomyocytes during Regenerative and Plastic Myocardial Insufficiency

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Focal degradation of cardiomyocyte ultrastructures and cytoplasm, their sequestration, and autophagy were found in Wistar rats with daunomycin-induced regenerative and plastic myocardial insufficiency starting from day 1 after cytostatic treatment. These changes were morphologically manifested in the formation of myelin-like structures, autophagosomes, and secondary lysosomes. Sequestration and partial autophagy of the cytoplasm in cardiomyocytes with diminished or blocked protein synthesis reflect cell regression or involution directed to the adjustment of the cytoplasm volume to functional state of the nucleus.

Key Words: *anthracycline antibiotics; regenerative and plastic myocardial insufficiency; cardiomyocyte; cytoplasm; ultrastructure*

Synthesis and degradation of cell proteins, organellogenesis, and destruction under physiological and pathological conditions is an urgent problems of cell biology. Degradation of structures is less studied than their biosynthesis. This concerns molecular and cellular processes and the dynamics of morphological changes in various cells. It was reported that not only biosynthesis, but also degradation of intracellular structures is an ATP-dependent multistage process manifested in focal or total destruction and lysis of organelles and cytoplasmic matrix [11,14]. Total lysis and destruction of intracellular structures result from cell necrosis and autolysis. Focal destructive and lytic changes in the cytoplasm and organelles are related to compartmentalization in damaged, but viable cells.

Focal degradation of the cytoplasm is a reaction of cells to degeneration and necrosis of subcellular structures [1]. The formation of concentric membranes around damaged cytoplasmic regions (sequestration) leads to the appearance of autophagosomes and secondary lysosomes. Autophagy plays an important role in the maintenance of homeostasis in organs and tissues, underlies removal of damaged structures, macromolecules, excessive glycogen, and lipids from cells, and contributes to the metabolism and utilization of nucleic acids, proteins, and other biological substrates. The biological importance and peculiarities of this process under conditions of impaired biosynthesis are poorly understood. This concerns the dynamics and intensity of destruction and autophagy in cardiomyocytes (CM) during their regenerative and plastic insufficiency (RPI).

Here we studied the dynamics of destructive changes in the cytoplasm of CM during anthracycline-induced myocardial RPI.

MATERIALS AND METHODS

Experiments were performed on 96 male Wistar rats weighing 180±20 g with anthracycline cardiomyo-

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pathy. The samples were taken 1-24 h and 2-7 days after single or fractional administration of daunomycin hydrochloride in a cardiotoxic dose (30 mg/kg) [6]. For electron microscopy, myocardial samples were fixed in 4% paraformaldehyde, postfixed in 2% OsO_4, and processed routinely [7]. Ultrathin slices were contrasted with uranyl acetate and lead citrate and examined under Tesla BS500, JEM 100B, and JEM 1010 electron microscopes.

RESULTS

Intracellular organization of CM remained unchanged over the first hours after daunomycin administration. The very early ultrastructural changes included heterochromatin decondensation and segregation of the fibrillar and granular nucleolar components. Glycogen appeared as small agglomerates of β-granules in the cytoplasm of CM. Ribosomes were found only between myofilaments of myofibrils.

The amount of granular glycogen in the cytoplasm markedly increased 6 h after daunomycin administration. Large agglomerates of glycogen granules were surrounded by monolayer membranes (Fig. 1, *a*). Multilayer membranes segregating (sequestering) glycogen from surrounding structures were found around small glycogen agglomerates (Fig. 1, *b*).

Numerous osmiophilic myelin-like structures (MLS) containing glycogen granules or unidentified cytoplasmic structures were found in CM cytoplasm 9-12 h after daunomycin administration. MLS were localized in the subsarcolemmal (Fig. 1, *c*) and intermuscular spaces or adjacent to intercalated discs (Fig. 1, *d*). It is believed that MLS are products of mitochondrial degradation (taking into account their localization) [1]. Many CM contained considerable amounts of granular glycogen with signs of initiating sequestration (Fig. 2, *a*). Lysis of myofilaments and sequestration of glycogen were accompanied by the appearance of numerous secondary lysosomes (Fig. 2, *b*) surrounded by monolayer membranes and containing osmiophilic structures (Fig. 2, *c*).

Two days after daunomycin administration, pronounced lysis of myofibrils was accompanied by a decrease in the number of MLS in CM cytoplasm. These structures were not found in some CM, while in others they were localized in the intermyofibrillar and subsarcolemmal spaces or adjacent to intercalated discs (Fig. 2, *d*). Secondary lysosomes were localized in the peripheral region of CM. Destruction and autophagy of organelles with the formation of MLS and secondary lysosomes were more pronounced 4-5 days after daunomycin administration.

Fractional administration of daunomycin produced similar, but less pronounced changes in CM cytoplasm. Focal degradation of organelles with the formation of MLS and autophagosomes was found in some CM (Fig. 3, *a*). Ultrastructural changes in CM correlated with the severity of damages to the nuclei and nucleoli. CM with damaged nucleoli contained dense and lamellar osmiophilic residual bodies, autophagosomes, and secondary lysosomes (Fig. 3, *b*). Myelin-like residual bodies were localized in the intermuscular space and around capillaries (Fig. 3, *c*). Large autophagosomes were occasionally seen (Fig. 3, *d*).

Focal degradation and autophagy accompany CM damages during myocardial insufficiency (*e.g.*, anthracycline-induced RPI) [3,8,15] and acute alterative injuries [2,8]. Autophagosomes are less typical of acute CM alteration. During RPI, autophagy of degraded organelles is a systemic process manifested at all periods of observations.

Intracellular degradation begins from sequestration (segregation) of cytoplasmic regions and CM organelles followed by their fusion with lysosomes and formation of autophagolysosomes. Autophagolysosomes are transformed into residual bodies (osmiophilic membrane glomeruli) with degradation of segregated cytoplasmic regions. These multimembrane residual bodies are removed from cells through the sarcolemma and intercalated discs by exocytosis, which is confirmed by their localization. It should be emphasized that myofibrils do not undergo sequestration and autophagy. Their primary destruction (disassembly) is catalyzed by neutral or alkaline proteases [12] and follows by lysosomal degradation [10,13].

Focal degradation of the cytoplasm in CM was accompanied by an increase in the number of secondary lysosomes having other composition. These lysosomes were oval or round in shape, surrounded by monolayer membranes, and contained osmiophilic granular component. Secondary lysosomes were localized in the peripheral and central regions of CM. By the end of observations, these lysosomes were found only in the perinuclear space. Some lysosomes lost their membranes and osmiophilic components; residual bodies appeared as osmiophilic granules (lipofuscin). Lipofuscin was not (or very slowly) removed from the cytoplasm of CM. Lipofuscin agglomerates are formed in decompensated hypertrophic heart, senile myocardium [5], and during anthracycline cardiomyopathy [7,9].

Sequestration and partial autophagy of the cytoplasm in CM with suppressed or blocked protein synthesis probably reflect cell regression and/or involution directed to the adjustment of the cytoplasm volume to functional state of the nucleus. This assumption is confirmed by the dynamics of ultrastructural changes in CM during starvation and aging accompanied by impaired protein synthesis and autophagy of excessive subcellular components [4,5].

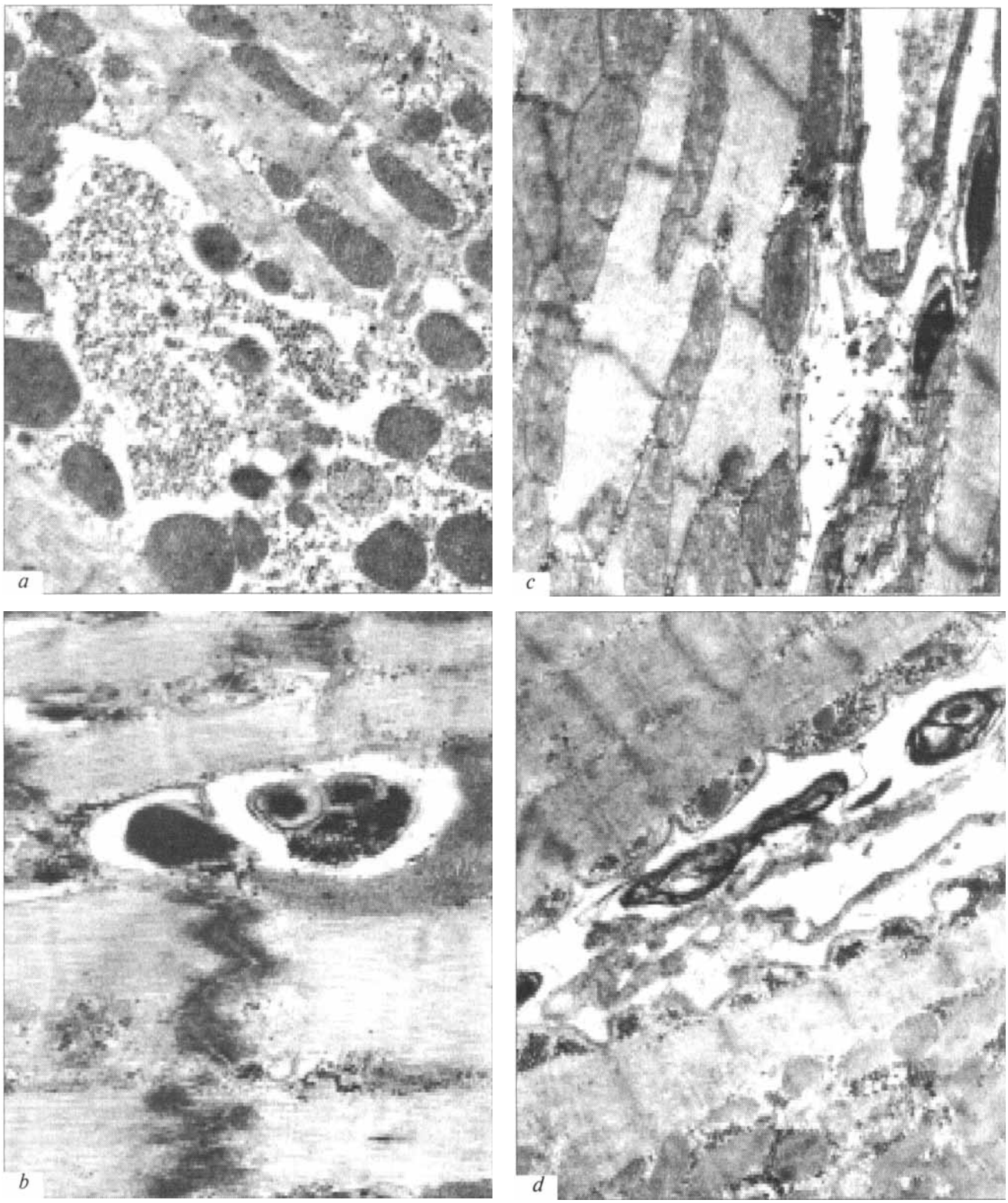

Fig. 1. Ultrastructure of cytoplasmic organelles in cardiomyocytes 6-24 h after single injection of daunomycin in a cardiotoxic dose: optically empty rings and monolayer membranes around granular glycogen agglomerates (glycogen sequestration, *a*, ×16,100); granular material (the intensity of staining is similar to that of glycogen) in a autophagolysosome incorporated into vacuole of intercalated disc (*b*, ×29,000); subsarcolemmal residual osmiophilic lamellar bodies (*c*, ×16,100); and residual bodies in the intermuscular space near capillaries (*d*, ×12,900).

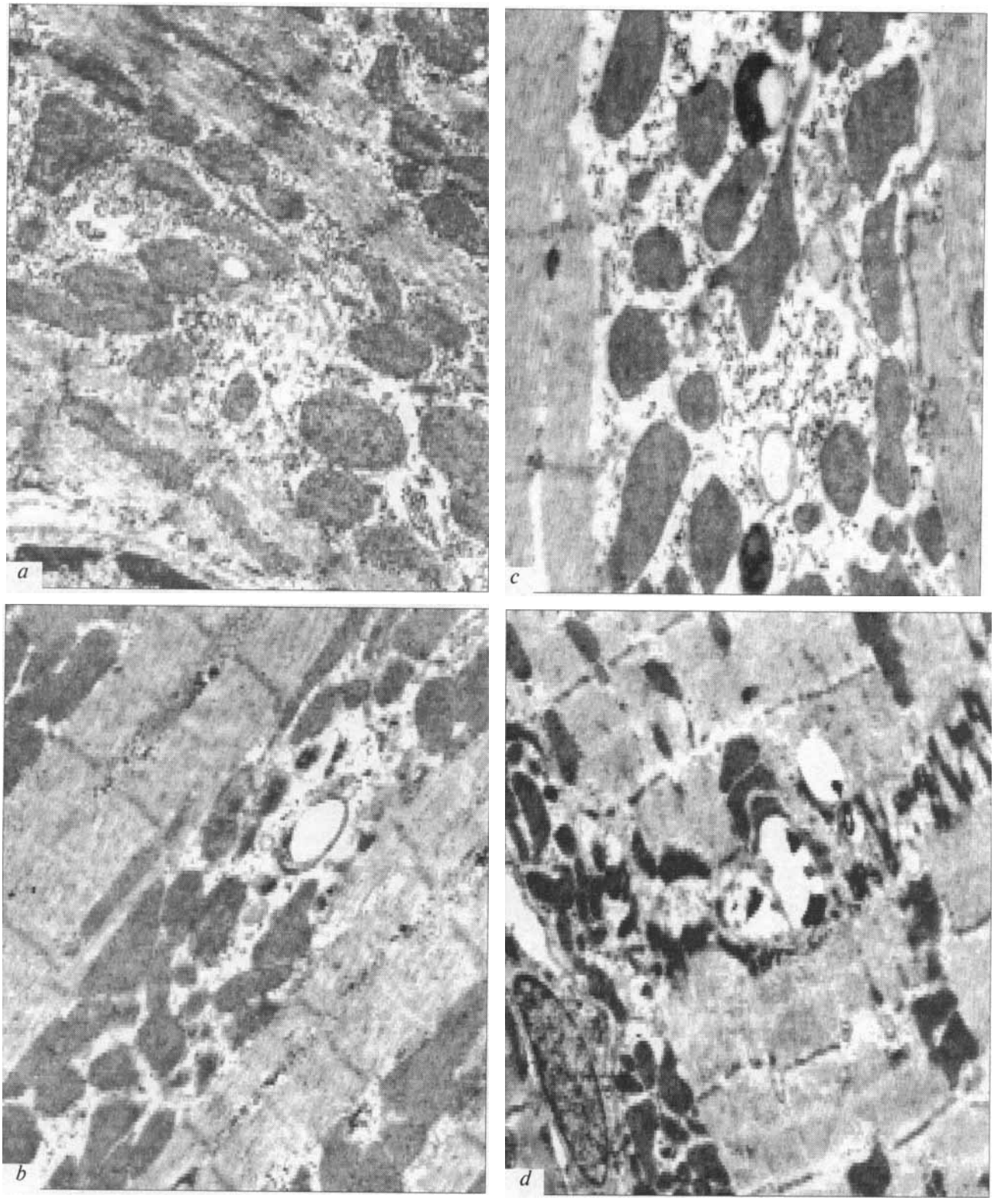

Fig. 2. Ultrastructure of cytoplasmic organelles in cardiomyocytes 1-2 days after single injection of daunomycin in a cardiotoxic dose accumulation of glycogen in the cytoplasm; light rings around glycogen agglomerates reflect sequestration (*a*, ×16,100); secondary lysosomes in the cytoplasm (*b*, ×16,100); secondary lysosomes and small autophagosomes (*c*, ×19,300); and dense osmiophilic residual bodies in a vacuole of intercalated disc (*d*, ×9700).

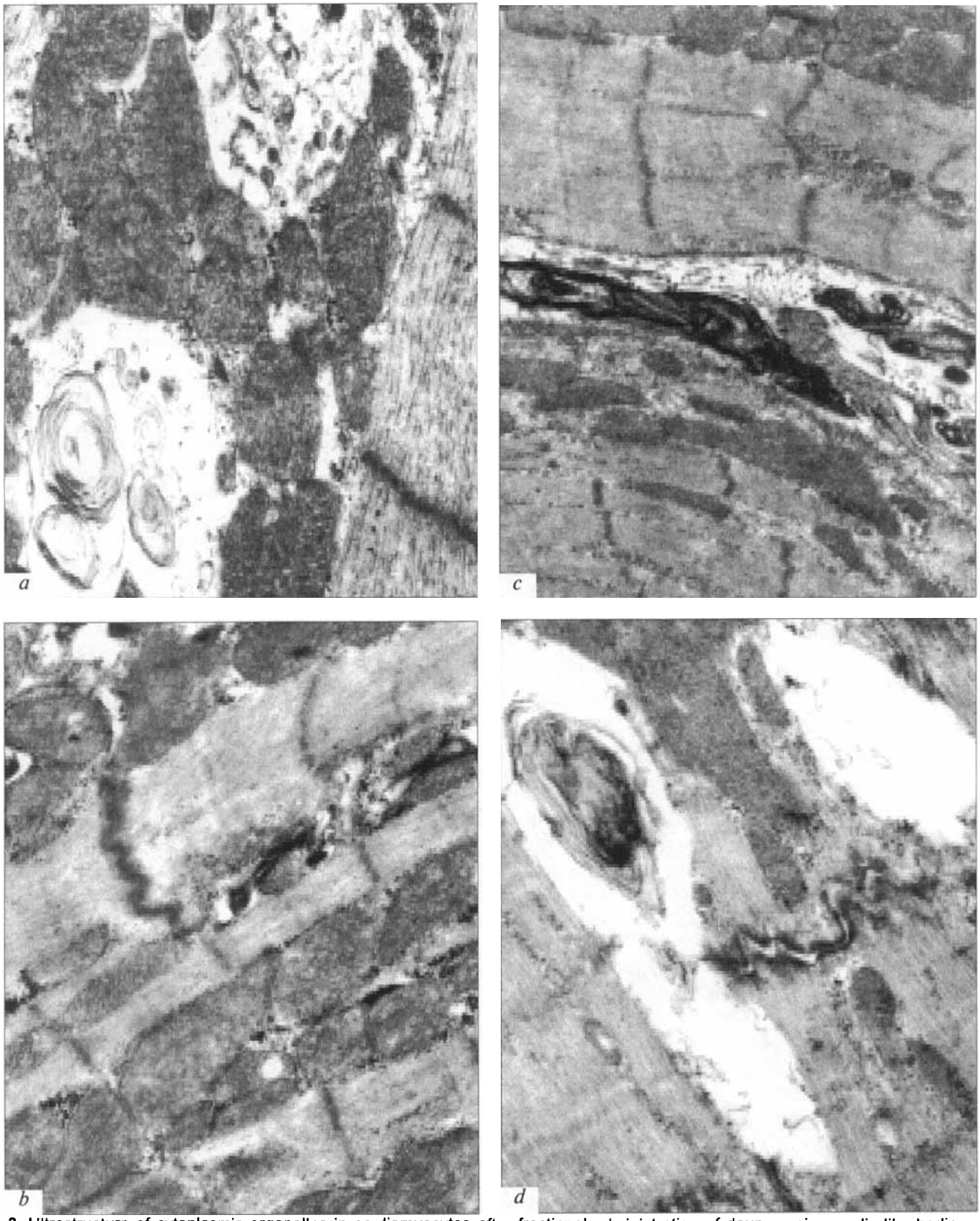

Fig. 3. Ultrastructure of cytoplasmic organelles in cardiomyocytes after fractional administration of daunomycin: myelin-like bodies and autophagosomes, absence of glycogen and ribosomes (*a*, ×25,800); residual myelin-like bodies (*b*, ×20,500); residual myelin-like bodies in the intercellular space (*c*, ×15,400); and residual myelin-like bodies in enlarged intercalated discs (*d*, ×30,000).

It should be emphasized that CM necrosis is not typical of anthracycline-induced myocardial RPI. Under these conditions, apoptosis underlies CM death; (up to 30% parenchymal cells). Pronounced degradation and autophagy of intracellular structures lead to CM atrophy. The number of CM decreases, and these cells undergo involution during anthracycline-induced myocardial RPI. Intensification of biosynthetic processes in CM (*e.g.*, during hypertrophy) is accompanied by inhibition of degradation [11]. Disturbances in nucleic acid and protein biosynthesis in CM are accompanied by activation of autophagy (opposite regulation of anabolic and catabolic processes).

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