Ultrastructure of Nuclear Compartment in Cardiomyocytes during Regenerative and Plastic Insufficiency of the Myocardium

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We studied ultrastructure of nuclear compartment in cardiomyocytes during regenerative and plastic insufficiency of the myocardium induced by anthracycline antibiotic daunomycin. A peculiarity of ultrastructural organization of cardiomyocyte nuclei under these conditions is almost complete disappearance of the heterochromatin lumps. The earliest changes in nucleoli under conditions of disturbed DNA-dependent RNA synthesis are segregation of the granular and fibrillar nucleolonema components. Deep alterations in the nucleoli manifested by fragmentation and annulation correlate with pronounced changes in cardiomyocytes ultrastructure, intensive lysis of the myofilaments, reduction of the organelles, and enhanced autophagocytosis.

Key Words: *anthracycline antibiotics; plastic myocardium insufficiency; cardiomyocyte nuclei and nucleoli; ultrastructure*

Nuclear compartment is one of the most important cell subdivisions, which regulates and integrates the major metabolic processes in cells. Alterations of the nuclear compartment play an important role during general biological and pathological processes [1,4]. Numerous cytological studies revealed the basic features in the reorganization of the nuclear apparatus in various cells during functional modification or under the effect of damaging factors [1,3,10,12-14]. However, many of these data are controversial and cannot be unambiguously explained entirely by the character of morphological alterations without considering the molecular, genetic, and biochemical processes [6,7,9]. This is also true for states accompanied by progressive decrease or disturbances in protein synthesis leading to regenerative and plastic insufficiency (RPI) in organs and tissues [2]. One of the most efficient RPI models is anthracycline cardiomyopathy [4], which results from inhibition of DNA-dependent RNA synthesis in cardiomyocytes (CMC). The regularities and peculiarities of ultrastructural reorganization of the nuclear compartment during RPI of the myocardium are of great interest for elucidation of the basic principles of reorganization of the nuclear apparatus and elaboration of diagnostic cytological criteria of RPI of CMC.

Our aim was to study ultrastructure of the nuclear compartment of CMC during RPI of the myocardium induced by anthracycline antibiotic daunomycin.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (n=96) weighing 180±20 g. Group 1 rats (n=60) were injected intraperitoneally with daunomycin hydrochloride in a single dose of 30 mg/kg (2% water solution). On postinjection day 5, survivors (n=49) were decapitated under chloroform narcosis. Group 2 rats (n=36) were fractionally injected with daunomycin (10 mg/kg

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3 times with 7-day intervals). Five days after the third injection 28 survivors were decapitated. The control rats were simultaneously injected with physiological saline in a volume corresponding to their weight. The hearts were isolated and arrested by cold. The atria were removed, and the ventricles were weighed and fixed in cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 8.0) [5].

Specimens for electron microscopy were processed routinely [5]. The myocardium was taken only from decapitated rats. The animals died during the experiments were histologically examined. The histotopographic sections of the right and left ventricles and interventricular septum stained with hematoxylin and eosin or colloid iron—PAS—hematoxylin were examined in non-polarized and polarized light under NU-2 and Docuval (Carl Zeiss) microscopes. Ultrathin sections were contrasted with uranyl acetate and lead citrate and analyzed under Tesla BS500, JEM 100B, and JEM 1010 electron microscopes.

RESULTS

One and 2 days after a single cardiotoxic dose of daunomycin, myocardium architectonics virtually did not differ from that in the control rats, and only slight widening of the intermuscular spaces was noted. In contrast to tissue architectonics, the ultrastructural organization of CMC changed in the first hours postinjection. In some CMC heterochromatin disappeared and the structure of nucleoli changed: nucleolonema loops were disorganized, the granular and fibrillar components were separated and segregated (Fig. 1, *a*, *b*). Many CMC contained glycogen in clusters of β-granules, while ribosomes were revealed only between the myofilaments.

Six hours postinjection, the number of CMC with segregated nucleoli increased. In some CMC the nucleolar nucleolonema was fragmented (Fig. 1, *c*); the fragments had a fibrillar structure without ribosome-like granules. Other CMC contained very dense round nucleoli, and their fine structure could not be resolved. In addition, they shrank and collapsed (Fig. 1, *d*).

On 9-12 h postinjection, heterochromatin was absent in almost all CMC nuclei. The nuclei were enlarged and the nuclear membrane was smooth. At the same time, all nuclei demonstrated alterations in the nucleoli: in some nuclei they were segregated and formed by a fibrillar component with small clusters of the granular components (ribonucleoprotein granules), while in other nuclei the nucleoli were fragmented (Fig. 2, *a*) or annular with a light center (Fig. 2, *b*).

These changes persisted during postinjection day 1, the annular nucleoli became a predominant form of damage to the nucleolar apparatus.

On postinjection day 2, the nuclei of CMC retained the described alterations: they had no heterochromatin lumps, and their nucleoli were fragmented. At this period, the most pronounced pathological changes were observed in myofibrils. Some CMC retained initial volume, but the perinuclear spaces in these cells were widened, and the number of organelles (mitochondria) was decreased (Fig. 2, *c*).

On postinjection day 3, the normal structure of nucleoli in some CMC began to restore, narrow heterochromatin bands appeared along the internal surface of the nuclear membrane (Fig. 2, *d*). However, in most CMC the nucleoli were still fragmented or annular. Some nucleoli were collapsed. Free ribosomes appeared in the cytoplasm of CMC with normal structure. Regeneration of myofilaments was observed in sarcomeres: multiple ribosomes in the wide space between preserved myofilaments sometimes formed polyribosome chains. However, myofibril bundles in CMC remained thin.

On days 4-5 after daunomycin injection, the number of CMC with normal nucleoli, and that with collapsed, fragmented, or segregated nucleoli varied. In dead rats these alterations were expressed to the same degree as in decapitated rats. The common feature was the predominance of euchromatin. Fragmentation and annulation of the nucleoli were accompanied by myofibril lysis, low content of ribosomes in sarcoplasm, and formation of myelin-like structures, the secondary lysosomes, and autophagosomes.

Ultrastructural alterations in CMC were less pronounced after fractional injections of daunomycin in a cardiotoxic dose. In this case, CMC without visible alterations or with minimal structural modifications were observed more frequently than after single daunomycin injection. Most CMC nuclei contained narrow heterochromatin strips adjacent to the inner nuclear membrane (Fig. 3, *a*). This feature was characteristic of the nuclei with damaged and with restoring nucleoli. The annular nucleoli were observed most frequently (Fig. 3, *b*), while nucleolar fragmentation was a more rare variant of nucleolar damage. There was no segregation of the fibrillar and granular components in the nucleolonema. In all rats, mosaic disposition of CMC were frequently observed, which had almost normal nucleolar structure with appearing granules (Fig. 3, *d*).

The data show that the most early stages of nucleolar alterations are characterized by segregation of the granular and fibrillar components. This phenomenon was first observed in experiments with actinomycin D, a potent specific inhibitor of DNA-dependent RNA synthesis and RNA polymerase activity in virtually all types of the cells [13]. It was found that the inhibitors of RNA or protein syntheses with other mode

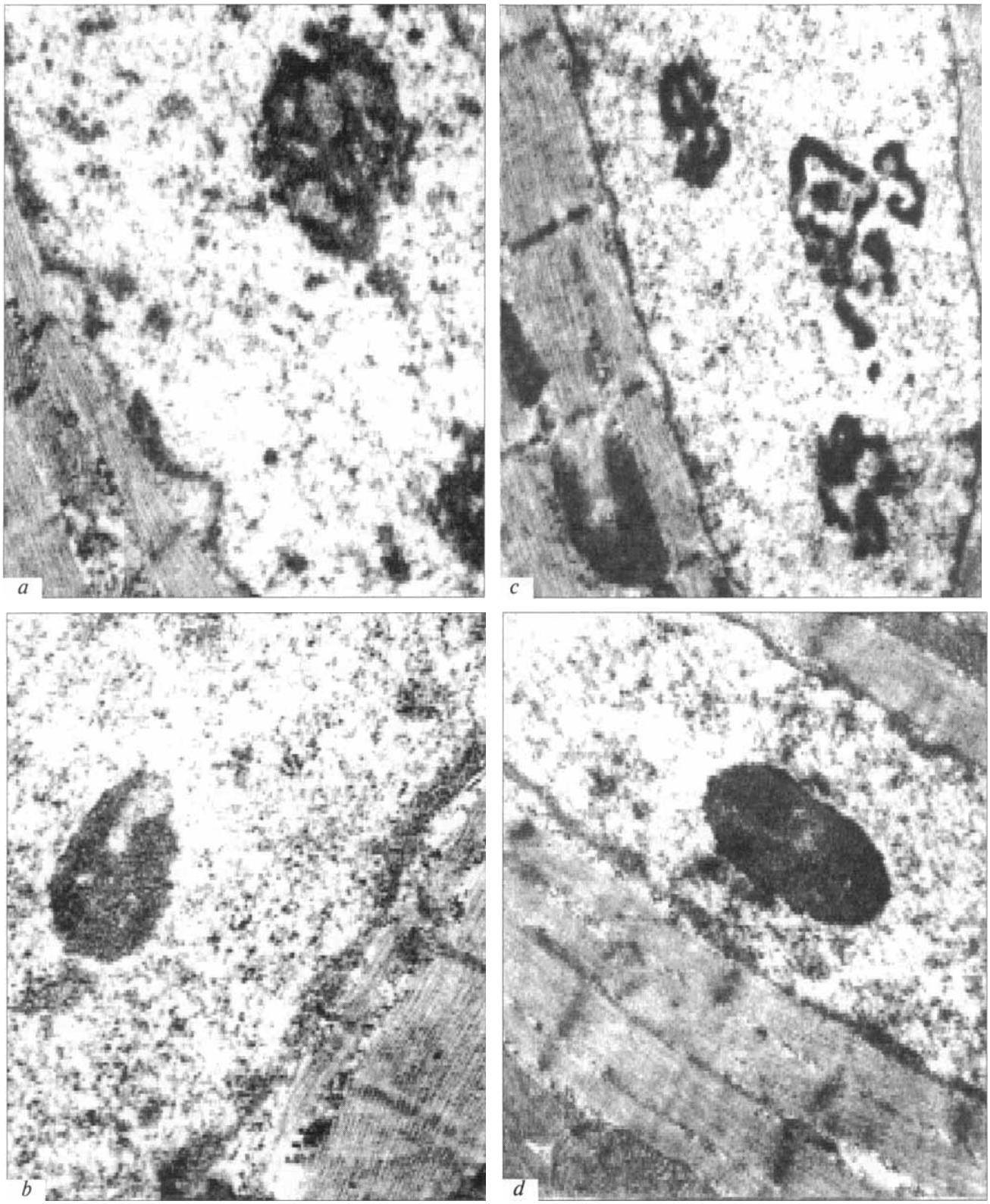

Fig. 1. Ultrastructure of cardiomyocyte nuclei on 1-6 h after injection of daunomycin in a cardiotoxic dose. *a*) ultrastructure of normal nucleolus, ×28,000; *b*) segregation of granular and fibrillar components and decreased density of chromatin lumps, ×25,800; *c*) fragmentation of nucleolar nucleolonema, ×16,000; *d*) shrinkage, collapse, and degranulation of nucleolus, ×26,000.

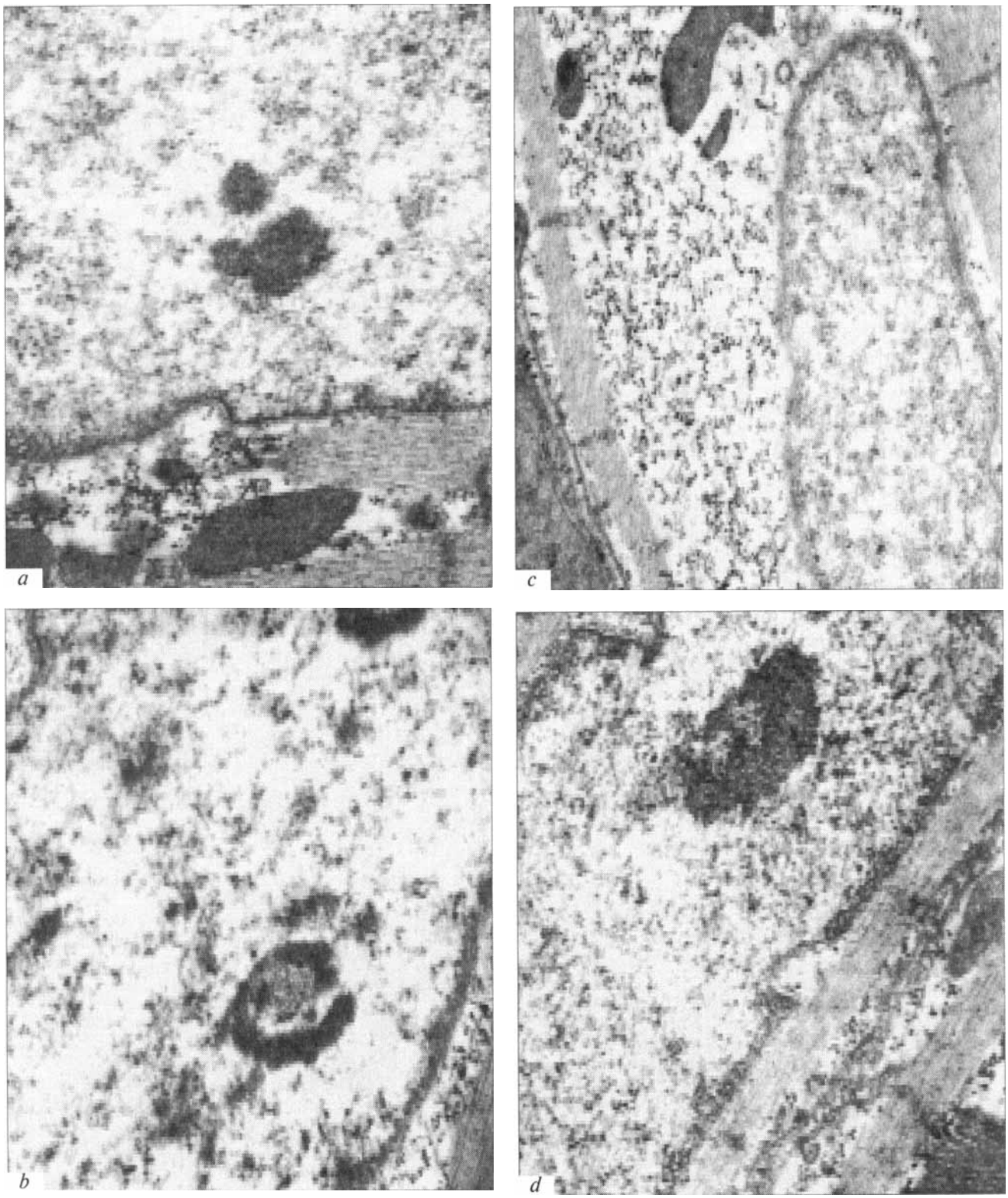

Fig. 2. Ultrastructure of cardiomyocyte nuclei 9 h–3 days after injection of daunomycin in a cardiotoxic dose. *a*) fibrillar component of fragmented nucleolus. Nuclear chromatin is dispersed, ×25,800; *b*) annular nucleolus. Nuclear chromatin is presented by euchromatin, ×25,000; *c*) widening of the perinuclear space due to myofibril lysis, ×19,300; *d*) initial stage of structural recovery in nucleus and nucleolus: accumulation of heterochromatin near the nuclear membrane and appearance of granules in the nucleolus, ×25,800.

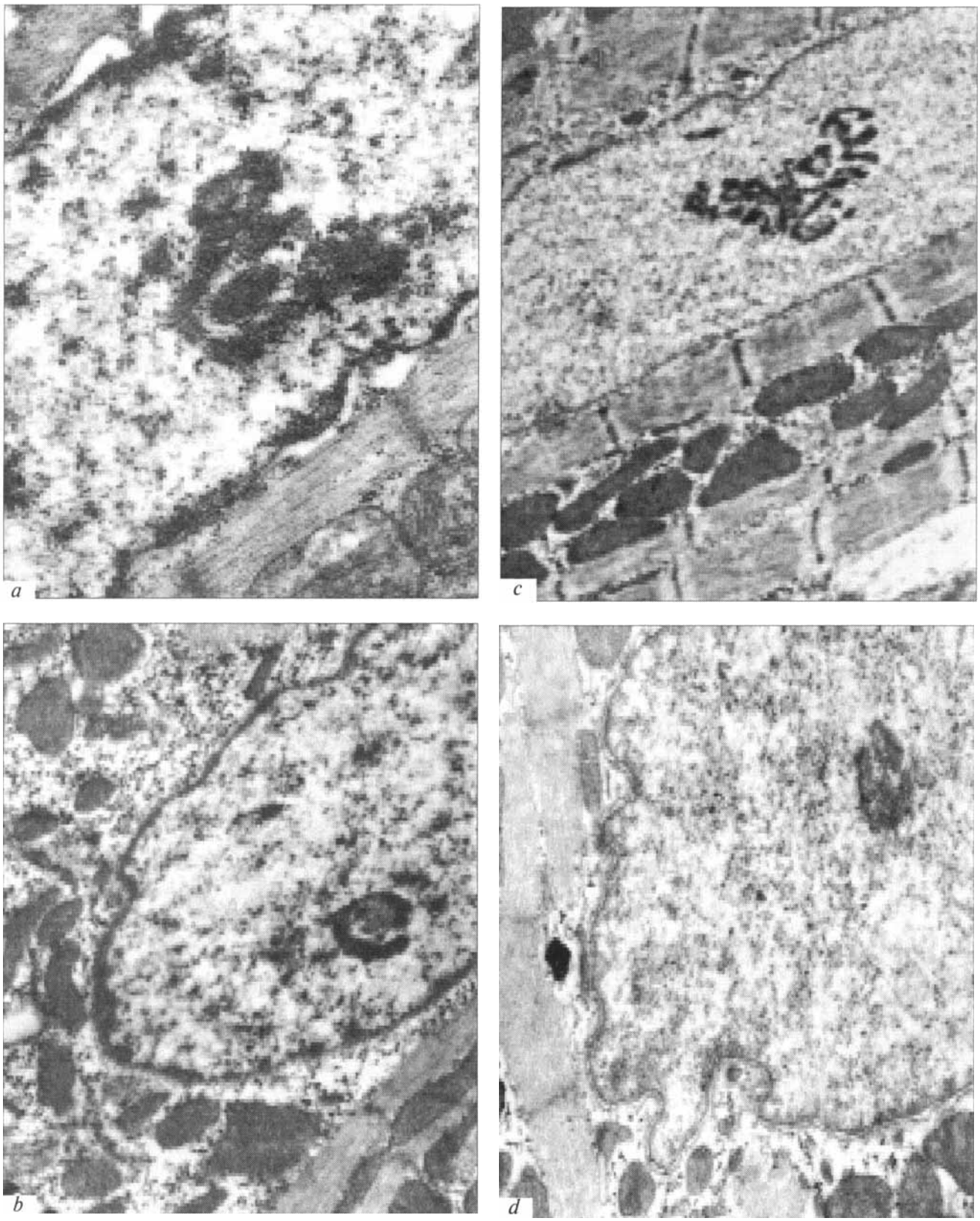

Fig. 3. Ultrastructure of cardiomyocyte nuclei after fractional administration of daunomycin. *a*) large heterochromatin lumps in the nucleus, and fibrillar and granular structures in the nucleolus, ×28,000; *b*) annular nucleolus. Heterochromatin forms a narrow band adjacent to nucleolonema, ×17,100; *c*) fragmentation of nucleolar nucleolonema, ×13,700; *d*) granular structure of a recovering nucleolus, ×20,500.

of action than inhibition of DNA-dependent RNA synthesis induce no nucleolar segregation [14].

Fragmentation and annulation of the nucleoli are less specific for disturbances of DNA matrix properties. These changes accompany inhibition of protein synthesis at the stage of transcription, disturbances of the translation [4], and acute ATP deficiency [12]. Nucleolar fragmentation in ATP deficiency is usually considered as a sign of disturbed energy metabolism [10]. In our experiments, fragmentation and annulation of nucleoli in some CMC persisted 5 days after single or fractional injection of daunomycin in the cardiotoxic dose. ATP deficiency in CMC during anthracycline cardiomyopathy results from cardiospecific suppression of genes encoding the key enzymes of ATP synthesis (ADP/ATP-translocase and phosphofructokinase) [6].

Disappearance of heterochromatin starting from 1 h postinjection is a peculiar ultrastructural alteration of the nuclear compartment in CMC provoked by anthracycline antibiotics, which can be considered as a result of intercalation of these antibiotics between the DNA base pairs. The paradoxical predominance of euchromatin over heterochromatin in the nuclei with inhibited RNA synthesis takes place.

Therefore, the ultrastructure of the nuclear compartment in CMC damaged by anthracycline is characterized by combination of "active" nucleus and "passive" nucleolus. Partial or complete inhibition of RNA synthesis results in ATP deficiency, drop in ribosome and polysome content in CMC sarcoplasm, and disturbances of intracellular regeneration. The terminal stages of such disturbances are atrophy of some CMC followed by their apoptosis [8,11,15] and resorption by mononuclear cells, which are the processes underlying the successive stages of RPI in the myocardium.

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