Peculiarities of Intracellular Regeneration of Cardiomyocytes during Plastic Myocardial Insufficiency

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We performed morphological assay of the myocardium in Wistar rats with anthracycline cardiomyopathy and SHR rats with genetically determined arterial hypertension causing hypertrophic cardiomyopathy. Both disorders were accompanied by a decrease in protein synthesis and development of plastic cardiomyocyte insufficiency. Electron microscopy revealed peculiarities of intracellular regeneration of cardiomyocytes. We observed disorientation of newly formed myofibrils lying between preserved myofibrils. These myofibrils were positioned perpendicular or at angle to the long axis of muscle fibers, or extended from the Z line (in a fan-like manner) crossing each other. Regeneration disturbances also included excessive elongation of myofibrils. These abnormalities of myofibril regeneration were related to changes in transcription and translation in cardiomyocytes, due to DNA damages caused by cardiotoxic doses of rubomycin.

Key Words: *anthracycline cardiomyopathy; SHR rats; plastic myocardial insufficiency; cardiomyocytes; intracellular regeneration*

Plastic myocardial insufficiency (PMI) is accompanied by abnormal regeneration of intracellular structures [3,8] providing normal functioning of cardiomyocytes (CM) rather than by their direct damage [7]. Diffuse changes lead to contractile dysfunction of CM. The increasing interest in PMI is due to the development of cytostatics displaying considerable cardiotoxic effects [8] and damaging various systems in the body [1]. There is a variety of compounds directly inhibiting DNA properties.

PMI is often associated with direct effects of anthracycline antibiotics on the genetic apparatus of CM. These drugs specifically bind to DNA by intercalating between base pairs in the double helix [15], inhibit functions of DNA-dependent RNA polymerase, and impair intracellular regeneration.

PMI is reversible in the case of early recovery of protein synthesis. However, some CM loose the ability to regenerate after long-lasting and profound changes in mRNA synthesis or translation. The dynamics of myofibril regeneration after their partial lysis was studied in experiments with acute damages to the myocardium [6,9]. Regenerative processes start at the ends or on the surface of preserved myofibrils and lead to restoration of their continuity and diameters. The peculiarities of intracellular regeneration of CM during PMI are poorly understood.

Here we studied the peculiarities of intracellular regeneration of CM during various forms of PMI (anthracycline cardiomyopathy in Wistar rats and genetically determined hypertrophic cardiomyopathy in SHR rats).

MATERIALS AND METHODS

Anthracycline cardiomyopathy was induced in 65 male Wistar rats weighing 160-220 g. The animals were decapitated 1-24 h and 1-5 days after single intraperitoneal injection of 30 mg/kg rubomycin hydrochloride. Genetically determined hypertrophic cardiomyo-

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pathy was studied on 11-month-old SHR rats (n=25) with spontaneous arterial hypertension [4].

Paraffin sections were stained with hematoxylin and eosin, colloidal iron-PAS-hematoxylin, and by the method of van Gieson.

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

RESULTS

Regenerative and plastic insufficiency of CM is related to impaired protein synthesis caused by cytopathic effects of cardiotropic factors. Disturbances in ultrastructural regeneration lead to a decrease in the total number of CM due to their atrophy and elimination (without coagulation and colliquative necroses).

Qualitative changes in ultrastructural components and their dynamics suggest that the number of myofibrils and cytoplasmic elements progressively decreases, while the content of cell mitochondria changes insignificantly. This assumption is confirmed by stereological studies of the myocardium showing that the volume density and absolute total weight of CM in these animals are much lower than in the control [8]. These data indicate that cytostatics cause regressive changes in the cytoplasm of CM.

The very early changes in the nucleus and nucleolus followed by removal of glycogen stores, partial sequestration of the cytoplasm, lysis of myofibrils, and impairment of mitochondrial functions over the first 2 days of observations indicate damages to the genetic apparatus of CM.

The number of stromal cells increased during PMI, which was accompanied by the appearance of large glycosaminoglycan agglomerates in the intermuscular space and diffuse collagenation of the stroma. Ultrastructural assay revealed a considerable number of activated macrophages adjacent to CM. Myelin-like residual bodies and secondary lysosomes found in the cytoplasm of macrophages were similar to those formed in CM.

Morphological signs of intracellular regeneration of CM (formation of new myofilaments on polyribosomes and normalization of the structure and number of myofibrils) were observed after the appearance of ribosomes in their cytoplasm. Two-four days after administration of rubomycin in cardiotoxic doses, the structure of nucleoli in some CM was restored (Fig. 1, *a*), and free lysosomes appeared in the cytoplasm (especially in the site of lysis of myofilaments). The number of ribosomes and polyribosomes in the subsarcolemmal space peaked on day 4-5 (Fig. 1, *b*). The diameter of myofibrils oriented parallel to the long axis of CM was nearly normal or even increased. In the majority of cells organelles lay close to each other.

The peculiarity of intracellular regeneration of CM during anthracycline cardiomyopathy in Wistar rats and hypertrophic cardiomyopathy in SHR rat was disorientation of newly formed myofibrils (Figs. 2, 3). In the subsarcolemmal and perinuclear spaces and between preserved myofibrils, newly formed myofibrils were oriented perpendicular or at an angle to the long axis of muscle fibers, or extended from the Z line (in a fan-like manner) crossing each other. Disoriented myofibrils were also found within individual CM.

Regenerative disturbances also manifested in excessive elongation of myofibrils in CM of rats with anthracycline cardiomyopathy, which was detected on days 3-5 of the experiment and led to winding of myofibrils. Electron microscopy of longitudinal myofibrils with the same distances between Z lines showed that in some sarcomeres myofilaments run parallel to each other, while in others they were positioned perpendicular or at an angle to the section. This orientation of myofibrils did not correspond to their extension or contraction, because the length of sarcomeres was the same and did not differ from that in adjacent CM with normal structure of contractile elements. This fact can be explained by helical orientation of myofibrils [2]. We believe that these changes reflect excessive elongation of myofibrils. Disorientation of myofilaments in sarcomeres was most pronounced near intercalated discs.

These abnormalities of myofibril regeneration are probably related to dysregulation of transcription and translation in CM, which follows anthracycline-induced DNA damages or disturbances in constitutive metabolic processes during hypertrophic cardiomyopathy [5].

The similarity of morphological signs in these disorders can be explained by common molecular and cellular mechanisms determining survival of CM during anthracycline cardiomyopathy and genetically determined hypertrophic cardiomyopathy. It was shown that p38 mitogen-activated protein kinases (*e.g.*, MKK6) attenuate apoptosis after anisomycin-induced inhibition of protein synthesis in CM [13]. In addition, intensive expression of MKK6 *in vitro* leads to hypertrophy of CM [14]. The involvement of mitogen-activated protein kinases in various functions of CM underlies the mechanism of regulation of constitutive processes in cells, including proliferation, differentiation, functioning, and apoptosis [10-12].

The interaction between regulatory systems in cells, which provides normal functioning under the effect of exo- and endogenous factors, determines their regeneration (at the cellular and intracellular levels) or

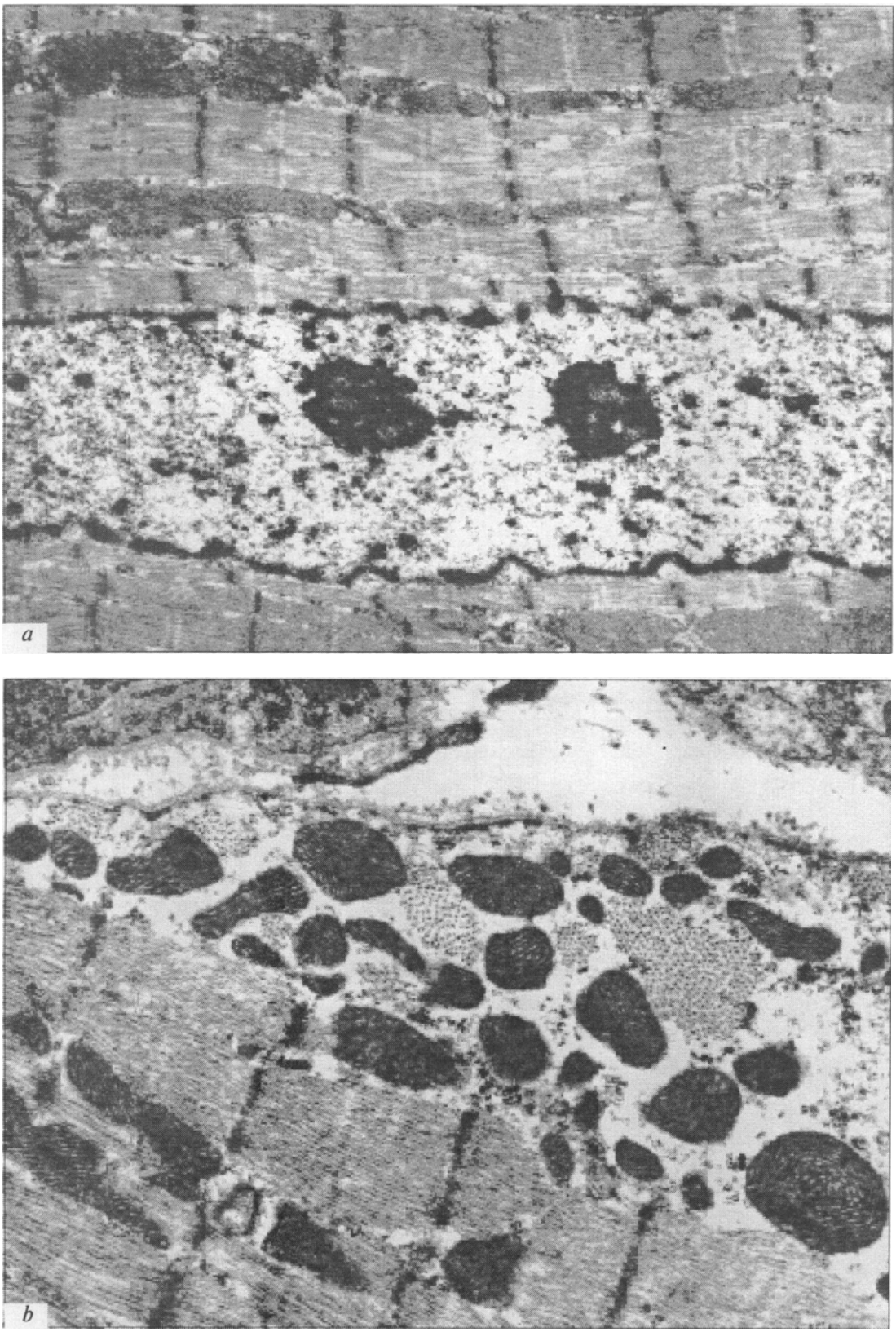

Fig. 1. Intracellular regeneration of cardiomyocytes (CM) in rats injected with rubomycin. Recovery of structure of the nucleolus and heterochromatin on day 4, diameter of myofibrils is practically normal, Z lines are fragmented (*a*, ×11,300); subsarcolemmal zone of CM in the same rat, agglomerates of ribosomes and polyribosomes in the cytoplasm, newly formed myofibrils are oriented perpendicular to the long axis of CM (*b*, ×26,700).

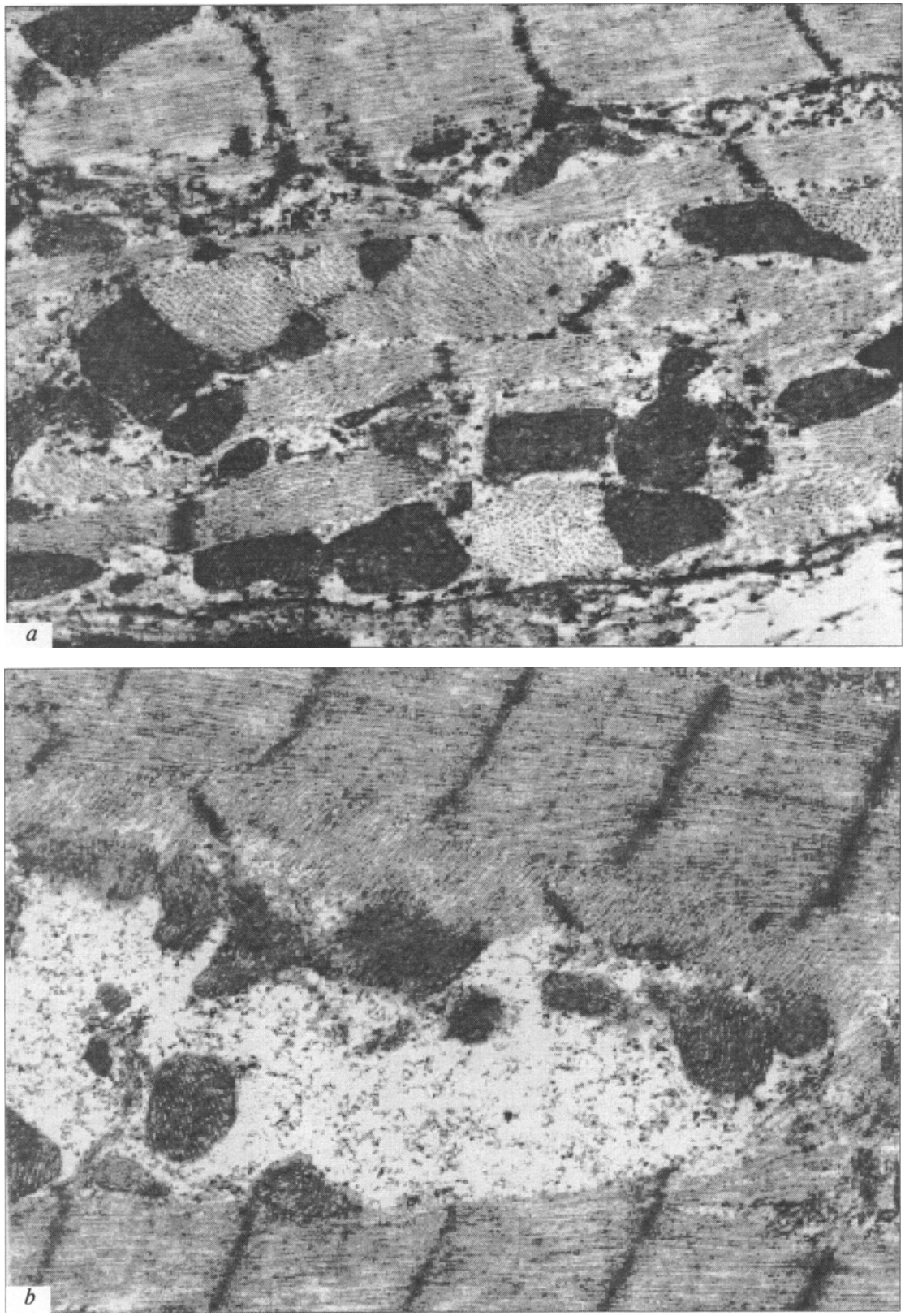

Fig. 2. Abnormal localization of myofibrils in CM 5 days after rubomycin administration. Myofibrils lie perpendicular or at an angle to the section (*a*, ×19,300); newly formed myofibrils around the perinuclear space (*b*, ×22,500).

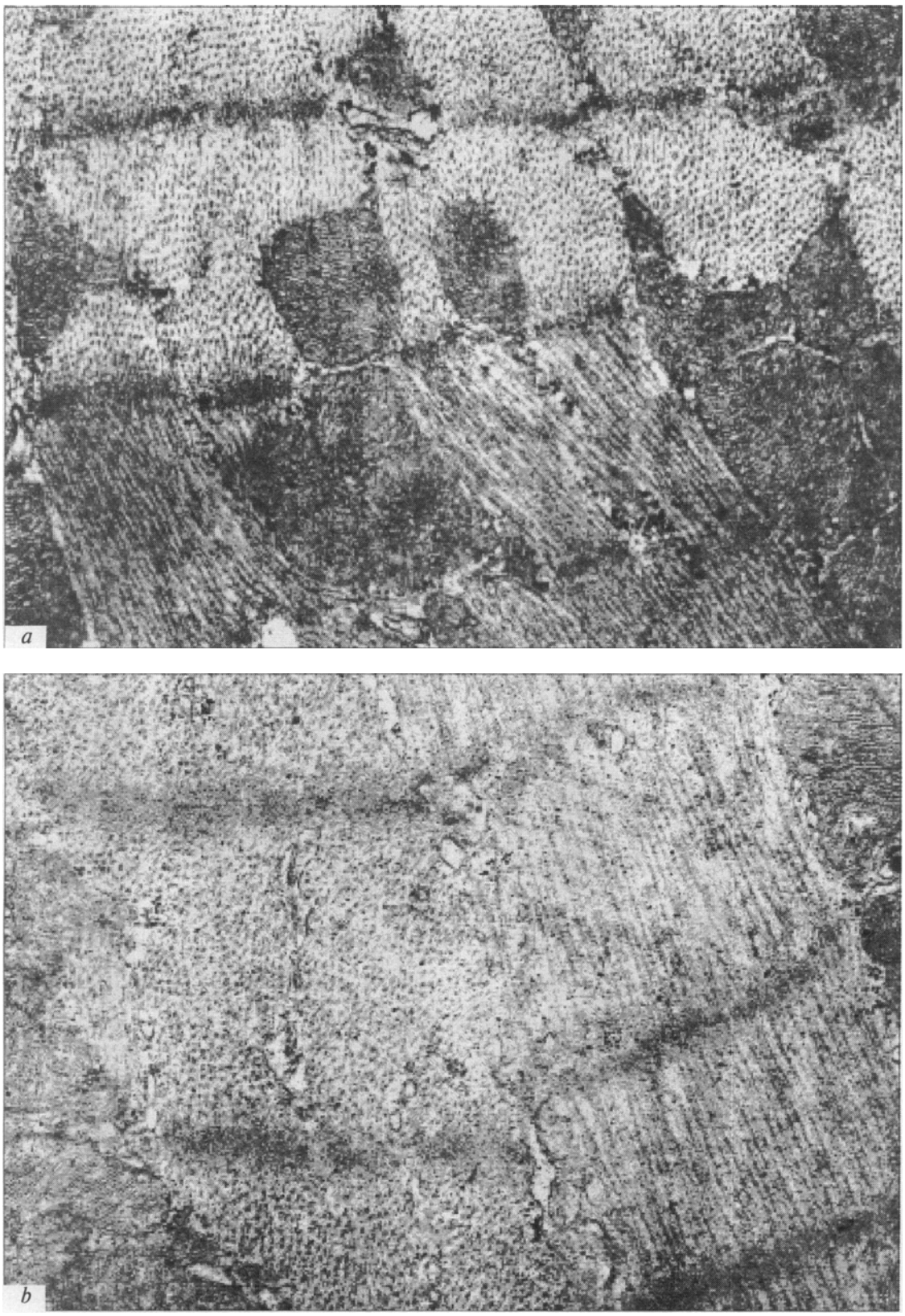

Fig. 3. Pathological intracellular regeneration of CM. Agglomerates of ribosomes around myofibrils with disoriented myofilaments in sarcomeres of the myocardium in rats injected with rubomycin (*a*, ×35,200); ultrastructure of CM in 11-month-old SHR rat, disorientation of myofibrils positioned perpendicular to the long axis of CM (*b*, ×16,000).

death. Despite various mechanisms underlying the pathogenesis of cardiomyopathy caused by anthracycline antibiotics or accompanying genetically determined arterial hypertension, intracellular regeneration of CM can be realized via the same molecular and cellular mechanisms, which explains the similarity of ultrastructural signs of regenerative processes. Our results contribute to the understanding of the mechanisms involved in specific regeneration of myofibrils and can be used for the diagnostics of various cardiomyopathies.

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