# Relationships between Myocardial Parenchyma and Stroma: Regenerative and Plastic Insufficiency of Cardiomyocytes and Development of Diffuse Cardiosclerosis

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Regenerative and plastic myocardial insufficiency characterized by impaired intracellular regeneration, progressive involution and apoptosis of cardiomyocytes associated with selective cardiotoxic effect of anthracycline antibiotic rubomycin is accompanied by enhanced proliferative and functional activities of fibroblasts and other stromal cells. Elimination of 30% cardiomyocytes and their atrophy are accompanied by the development of diffuse myocardial sclerosis, which is considered as a compensatory reaction of the connective tissue to the decrease in the weight of muscle fibers. Remodeling of the myocardium during anthracycline-induced cardiomyopathy due to changes in the parenchyma-stroma relationships does not lead to severe deformation of the heart, which is a favorable factor for normalization of myocardial architectonics after initiation of regenerative processes.

**Key Words:** *regenerative and plastic insufficiency; anthracycline-induced cardiomyopathy; myocardium; parenchyma-stroma relationships*

The regulatory interaction between the parenchyma and stroma is a key problem of general pathology and a key for understanding of the mechanisms underlying physiological and reparative regeneration, remodeling of organs and tissues during ontogeny and under pathological conditions, reversibility of pathological changes, and recovery of normal tissue architectonics and specialization. The mechanisms of changes in parenchyma-stroma relationships during inflammatory, compensatory, adaptive, involutional, and other general pathological processes were extensively studied [1,7,11,12].

Relationships between the myocardial parenchyma and stroma during ischemic and metabolic damages attract much attention due to low proliferative activity of cardiomyocytes (CM) in the postnatal ontogeny and development substitutive focal or diffuse cardiosclerosis after CM death. The development of extensive focal cardiosclerosis during myocardial infarction and infarct-like damages leads to severe deformation of the heart, exhaustion of its functional reserves, and hemodynamic disturbances [6]. Postinfarction scars in the myocardium persist for a long time and do not regress, while myocardial scars in regions of infarct-like damages can disappear after several months [12,13]. Changes in the parenchyma-stroma relationships during cardiomyopathies of various geneses are poorly understood, which makes it difficult to study regenerative properties of the myocardium and evaluate reversibility of sclerotic damages.

Here we studied changes in the parenchyma-stroma relationships in rat myocardium during anthracycline-induced regenerative and plastic insufficiency (RPI).

## MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 160-220 g. RPI of the myocardium was induced by single or fractional administration of

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daunomycin hydrochloride (rubomycin). Group 1 rats ($n$=36) were decapitated 1-24 h and 1-5 days after single intraperitoneal injection of rubomycin in a cardiotoxic dose of 30 mg/kg. Group 2 rats ($n$=24) received 3 intraperitoneal injections of 10 mg/kg rubomycin at 7-day intervals and were decapitated 5 days after the last injection.

For light and polarization microscopy, the specimens were fixed in 10% neutral formalin. Deparaffinized sections were stained with hematoxylin and eosin by the van Gieson's method, and by a combined method including PAS reaction and colloidal iron staining. The sections were examined under a Docuval light microscope. Myocardial samples from survivors were used for electron microscopy. The samples were fixed in 4% paraformaldehyde, postfixed in 1% $OsO_4$, and treated by routine methods [10]. Ultrathin sections 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

Single and fractional administration of rubomycin in a cardiotoxic dose produced similar qualitative changes in the myocardial parenchyma-stroma relationships. Tinctorial properties of the myocardium 24 and 48 h after single injection of rubomycin did not differ from normal. CM in the internal and external layers of ventricular myocardium contained numerous glycogen granules. Electron microscopy of CM nuclei revealed disappearance of heterochromatin, segregation and fragmentation of nucleoli, and appearance of ring-like nucleoli (main type of nucleolar damages). In many CM microfocal lysis of myofilaments within one or several sarcomeres and in the marginal zone of myofibrils was observed. Numerous osmiophilic myelin-like bodies containing glycogen granules and unidentified cytoplasmic structures were found in the cytoplasm of CM. Thus, ultrastructural signs of decreased protein synthesis, intensive autophagy, and impaired intracellular regeneration were observed in most CM.

The severity of intermuscular edema in the myocardium progressively increased from day 3 after rubomycin administration to the end of observations. Enlarged intermuscular spaces contained glycosaminoglycans, which looked like narrow strips contouring the muscle fibers (Fig. 1, *a*); on days 4-5 glycosaminoglycan threads and granules occupies all intermuscular spaces (Fig. 1, *b*). Thinned CM surrounded and partially masked by glycosaminoglycan agglomerates were found in the intermuscular space (Fig. 1, *c*). Polarization microscopy of these CM revealed anisotropy typical of myofibrils, but their nuclei were weakly stained with hematoxylin and contained no chromatin lumps; nucleoli were not seen.

Ultrastructural changes in CM progressed 3-5 days after rubomycin administration. Most pronounced pathological changes were found in myofibrils, which became thinned and disintegrated into thin bundles. Despite lysis and thinning of myofibrils, most CM were not characterized by loosening of the cytoplasm. Muscle fibers looked like narrow structures.

The content of connective tissue cells (particularly fibroblasts and macrophages) in the stroma increased 3-5 days after rubomycin administration. It should be emphasized that fibroblast nuclei were enlarged, and cytoplasmic processes of fibroblasts were clearly seen. Fibroblasts with large nuclei and glycosaminoglycan agglomerates were found in the adventitia of muscular vessels.

The ultrastructure of fibroblasts did not differ from normal over the first 2 days after rubomycin administration (Fig. 2, *a*). Fibroblast nuclei contained considerable amounts of heterochromatin. Individual short tubules of the granular endoplasmic reticulum (GER) were seen in the cytoplasm. Three days after rubomycin administration the ultrastructure of fibroblasts underwent pronounced changes (Fig. 2, *b*). Enlarged and round nuclei contained loosened nucleoli. The cytoplasm formed long and wide processes, and its volume increased. The number of GER elements in the cytoplasm increased, cisternae were widened. Flake-like substances with low electron density were found in the intermuscular space around these fibroblasts. It corresponded to glycosaminoglycans suspended in the intercellular fluid. These changes were typical of most stromal fibroblasts. Therefore, pronounced structural and functional reconstruction of these cells occurred 3 days after rubomycin administration. Ultrastructural changes in fibroblasts were accompanied by the appearance of numerous macrophages near the CM sarcolemma.

The number of CM with normal nuclei and collapsed, fragmented, or segregated nucleoli 4-5 days after single administration of rubomycin in a cardiotoxic dose greatly varied. Fragmentation and annulation of nucleoli were accompanied by lysis of myofibrils, focal degradation of the cytoplasm, and intensive autophagy. Reparative processes were initiated in individual CM. Ribosomes and polyribosomes were accumulated in the cytoplasm, and the diameter of myofibrils approached the normal [4]. Functionally active fibroblasts were still seen in the stroma. These cells had a well-developed GER with widened cisternae containing homogenous substance with moderate electron density (Fig. 2, *c*). Fibroblasts were surrounded by collagen bundles. The count of collagen fibers twined into CM sarcolemma increased (Fig. 2, *d*). Fractional administration of rubomycin produced simi-

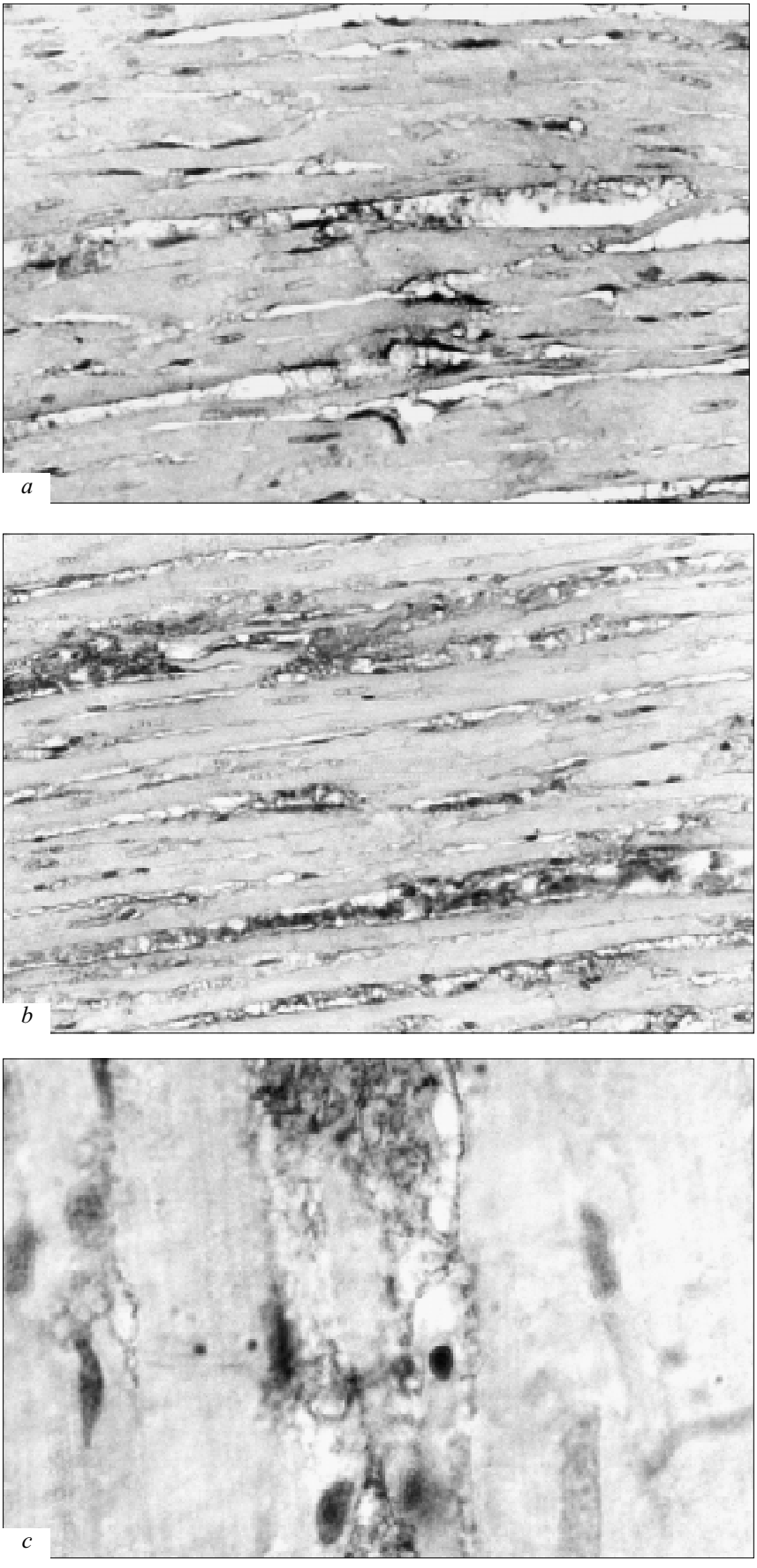


**Fig. 1.** Histochemical changes in the myocardial stroma 3 (*a*) and 5 (*b*, *c*) days after single administration of rubomycin in a cardiotoxic dose (colloidal iron-PAS-hematoxylin): reaction of glycosaminoglycans with colloidal iron (*a*, ×400); increased amount of glycosaminoglycans compared to previous term (*b*, ×320); and agglomerates of glycosaminoglycans around atrophic muscle fiber (*c*, ×800).

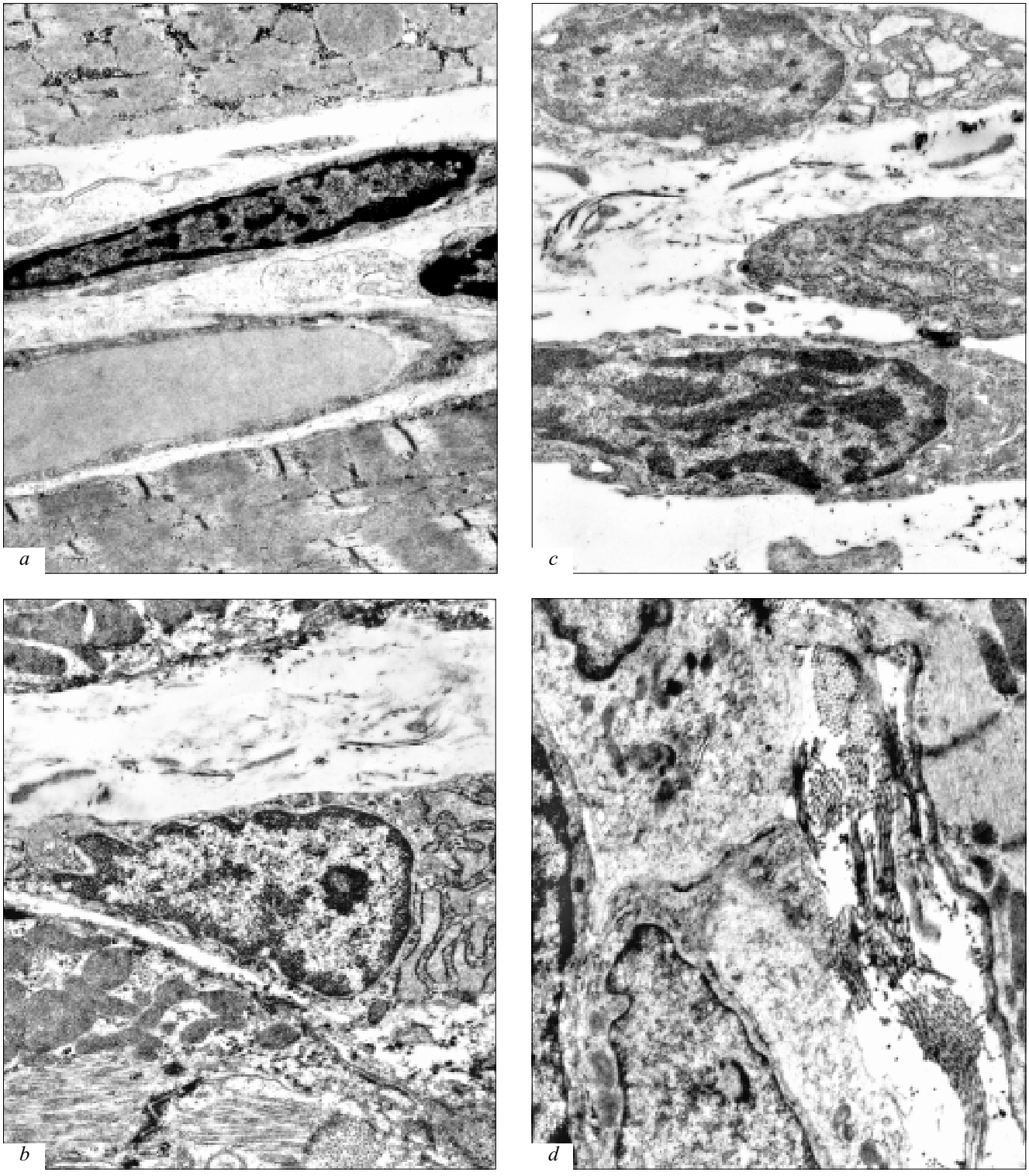
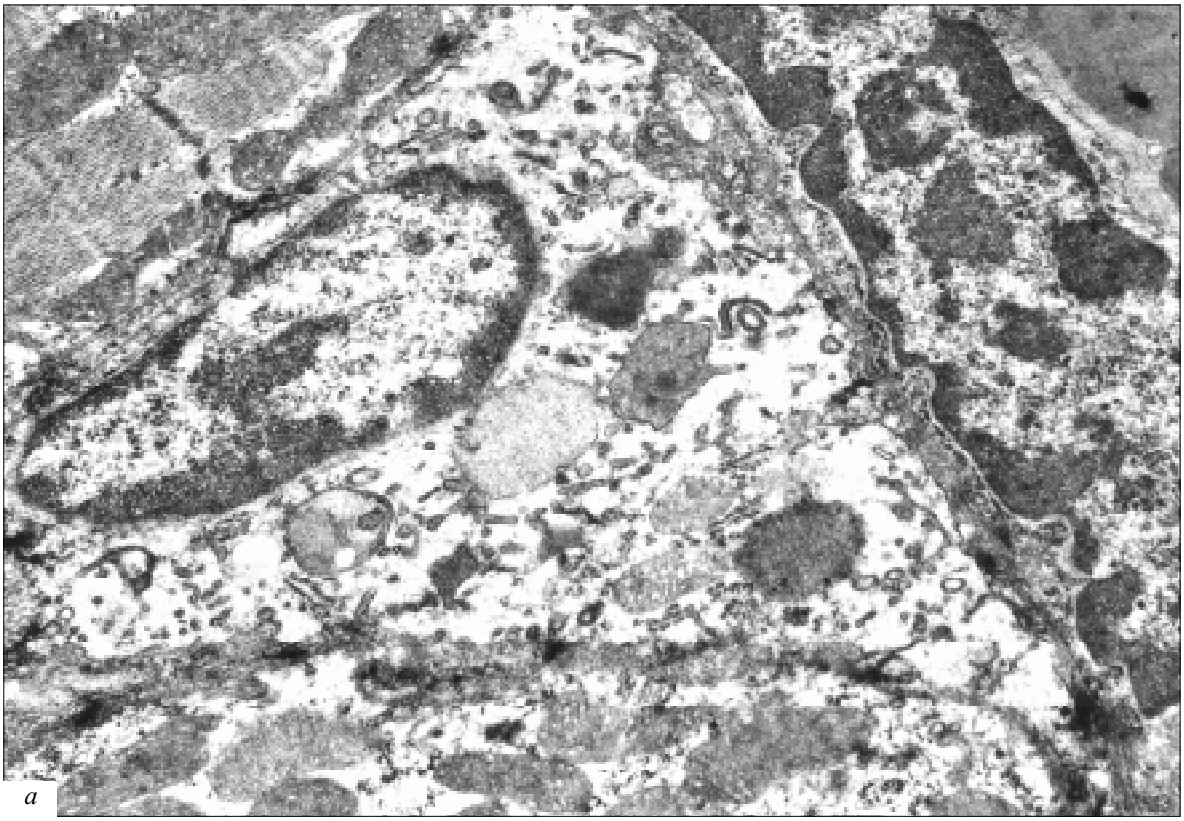



**Fig. 2.** Ultrastructural changes in the myocardial connective tissue 3 (*a*, *b*), 5 (*c*), and 6 (*d*) days after single administration of rubomycin in a cardiotoxic dose: resting fibroblast (*a*, ×10,000); structure of fibroblast on day 3: increased amount of nuclear euchromatin, loosened nucleolus, and granular endoplasmic reticulum with widened cisternae in the cytoplasm (*b*, ×16,100); synthetically active fibroblasts surrounded by thin collagen bundles and intermuscular edema of the stroma (*c*, ×9000); thick collagen bundles in the intermuscular space and numerous collagen fibers twined into cardiomyocyte sarcolemma (*d*, ×12,000).



*a*

*b*

**Fig. 3.** Ultrastructural changes in the myocardial connective tissue 5 days after fractional administration of rubomycin: activated macrophages (*a*, ×17,100), widened collagen fibers and macrophage (*b*, ×22,700).

lar ultrastructural changes in fibroblasts and extracellular spaces. The ultrastructure of macrophages (numerous cytoplasmic organelles and phagolysosomes and prevalence of nuclear euchromatin) attested to intensive phagocytosis (Fig. 3).

Quantitative morphological analysis showed that in rats receiving single or fractional injections of rubomycin the ratio of CM to connective tissue nuclei decreased by 50% by the end of observations. Single and fractional injections of rubomycin increased the absolute weight of the stroma by 22 and 35%, respectively, compared to the control. Signs of decreased synthetic activity in CM nuclei were found after fractional administration of rubomycin in a dose of 20 mg/kg. Long-lasting disturbances of CM functions and more pronounced fibrosis of the stroma were seen in these rats.

Rubomycin-induced intracellular reconstruction of CM can be considered as involution, while ultrastructural changes in CM are manifestations of RPI [1-3,10]. Selective inhibition of cardiospecific protein synthesis and intensification of free radical oxidation in CM caused by anthracycline antibiotics [14, 16] lead to apoptosis of individual CM and more than 30% decrease in their count [5,9,17]. Parenchymal cells play a key role in regulatory relationships between the parenchyma and stroma. It can be suggested that impaired functional activity of most CM contributes to activation of protein synthesis and formation of collagen in fibroblasts and, therefore, determines the development of diffuse cardiosclerosis.

The peculiarity of cardiosclerosis associated with diffuse desmoplastic stromal reactions is its reversibility [12]. The connective tissue stroma is partially or completely restored after normalization of metabolism and parenchymal cell count in sclerotic organs and tissues [8,15]. As differentiated from postinfarction remodeling of the myocardium characterized by pronounced changes in the size and geometry of various heart chambers due to scarring, tissue architectonics practically does not differ from normal after myocardial remodeling during RPI.

Thus, involutional (atrophic) changes in CM during anthracycline-induced myocardial RPI induce synthesis of glycosaminoglycans and collagen proteins in stromal fibroblasts. The severity of diffuse collagenation of the stroma and increase in the absolute weight of the connective tissue depend on the duration of disturbances in plastic metabolism in CM. It should be emphasized that remodeling of the myocardium during anthracycline-induced cardiomyopathy associated with changes in parenchyma-stroma relationships is not accompanied by severe deformation of the heart, which is a favorable factor for normalization of myocardial architectonics after initiation of regenerative processes.

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