MORPHOLOGY AND PATHOMORPHOLOGY

Parenchyma-Stroma Relationships in the Myocardium: Alterative Insufficiency of Cardiomyocytes and Morphogenesis of Focal Cardiosclerosis

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Remodeling of the myocardium during focal metabolic damages to cardiomyocytes is determined by contracture injuries to myofibrils, their lump degradation, and development of coagulation necrosis. Reactive changes in the stroma develop over the first hours and manifest in acute hemodynamic disturbances followed by proliferation of connective tissue cells. The immature granulation tissue is formed at the site of myocardial damages. Later this tissue is replaced by the mature connective tissue with the formation of small scars. It should be emphasized that sclerotic changes during remodeling of the myocardium after focal metabolic damages are reversible.

Key Words: *alterative cardiac insufficiency; focal metabolic damages; myocardium; parenchyma-stroma relationships; ultrastructure*

Studies of the parenchyma-stroma relationships in the myocardium during various pathological processes accompanied by the development of alterative and regenerative-and-plastic cardiac insufficiency would allow us to characterize remodeling of the heart and elaborate prognostic criteria [1-3]. Damages and death of cardiomyocytes (CM) produced by adverse factors are realized via various mechanisms, which determines different reactive changes in the myocardial stroma [2-4,12,13].

Extensive myocardial damages (*e.g.*, myocardial infarction) are accompanied by the development of substitutive cardiosclerosis, which results in deformation of heart regions with severe injuries [1,12]. These changes are accompanied by hemodynamic disturbances and decrease functional capacities of the heart and whole organism. Microfocal infarction-like damages to CM are similar to macrofocal injuries, but differ in their prognosis and changes in the stroma. Probably, coordinated reactions of parenchymal and stromal cells maintaining cytoarchitectonics of the heart play an important role in regenerative processes in the myocardium during microfocal metabolic damages.

Myocardial fibrosis accompanying remodeling of the heart in infarctions, infarction-like damages, cardiomyopathies, and other pathological states is a pathognomonic sign of congestive cardiac insufficiency [9]. Reparative regeneration of the myocardium during focal damages attracts much attention. Some aspects of this problem, including parenchyma-stroma relationships, remain unclear.

Here we studied changes in parenchyma-stroma relationships during remodeling of rat myocardium

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under conditions of focal metabolic damages to CM followed by the development of alterative cardiac insufficiency.

MATERIALS AND METHODS

Focal metabolic damages to the myocardium in 64 Wistar rats weighing 300-400 g were produced by subcutaneous injection of 0.1% epinephrine in a single dose of 0.5 ml per 100 g body weight. The control group included 5 intact animals. Twenty-nine of 64 rats died over the first 6 h after treatment. Survivors were decapitated 0.25-24 h and 2-45 days after epinephrine administration.

The hearts were examined by light and polarization microscopy [1]. Specimens were fixed in 10% neutral formalin. Deparaffinized sections were stained with hematoxylin and eosin, toluidine blue, and colloidal iron-PAS and by the method of van Gieson. The sections were examined under a Docuval universal light microscope.

For electron microscopy we used the hearts from 2 animals survived over the first 2 days. The specimens were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and processed routinely [1]. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under JEM 100B and JEM 1010 electron microscopes (acceleration voltage 80 and 40 kV).

RESULTS

Changes in rat myocardium over the first minutes and hours after epinephrine administration (up to 24 h) were characterized by acute hemodynamic disturbances and microfocal damages to CM. The severity of these changes progressively increased. CM damages were revealed by polarization microscopy and included the increase in anisotropy of myofibrils and lump degradation of the anisotropic substance. These signs corresponded to contracture injuries to myofibrils of different severity (Fig. 1, *a*, *b*) and their primary lump degradation (Fig. 1, *d*). Light microscopy showed that muscle segments with contractures and lump degradation were characterized by pronounced acidophily of the cytoplasm and diffuse PAS-positive reaction. Small zones of myocytolysis were found in the myocardium (Fig. 1, *c*). These zones were detected with acid and basic staining agents. In polarized light these zones were not anisotropic.

Hemodynamic disturbances in the myocardial stroma were most pronounced 4-6 h after epinephrine administration and included severe edema, minor hemorrhages, plasmorrhages, and insignificant polymorphonuclear infiltration. By the end of the 1st day the severity of edema increased, while the degree of perivascular infiltration increased. Polymorphonuclear leukocytes and mononuclear cells were concentrated around muscle segments with coagulation necroses. Individual leukocytes and mononuclear cells penetrated necrotized CM and destroyed or resorbed them. Accumulation of glycosaminoglycans was seen in colliquative CM necrosis foci; polymorphonuclear infiltration was absent (Fig. 2, *a*).

One day after epinephrine administration ultrastructural assay revealed dead CM with pronounced contracture of myofibrils (Fig. 3, *a*) and primary lump degradation in foci of coagulation necrosis. In CM with contractures individual myofibrils appeared as hypercontracted and electron dense structures. Mitochondria underwent most pronounced changes. Their electron density increased, small lysis foci were seen in their matrix. Numerous regions of partial sarcoplasm necrosis with the flake-like content were found in the intermyofibrillar zone. CM myofibrils with primary lump degradation were lysed and presented by clods of myofilaments. Leukocytes and macrophages penetrating these cells underwent degeneration and destruction (Fig. 3, *b*, *c*). Probably, necrotized cells were completely resorbed only after repeated attacks of macrophages.

CM with preserved ultrastructural characteristics were seen near the foci of coagulation necrosis. However, these cells formed numerous concentric osmiophilic membranes (Fig. 3, *d*). Individual mitochondria and degenerated sarcoplasmic regions were sequestered in these membranes.

After 2 days small foci of coagulation necrosis were found in the wall of the left ventricle, interventricular septum, and papillary muscles. Necrobiotic changes in CM were accompanied by proliferative processes in connective tissue cells and formation of granulomas (Fig. 4, *a*).

Polarization microscopy showed that most muscle fibers retained their normal structure on days 3-4. During this period granulomas of various sizes were formed at the site of dead muscle segments. Necrotized muscle fibers were completely destroyed and underwent intensive resorption. In the main substance glycosaminoglycans were accumulated between granulomas. The count of newly formed argyrophilic fibers increased, and bundles of collagen fibers appeared (Fig. 4, *b*).

After 5-6 days necrotic muscle fibers were completely resorbed. The immature granulation tissue with high content of glycosaminoglycans appeared (Fig. 4, *c*). In undamaged regions CM had normal structure. After 8-10 days proliferative activity of stromal cells increased, which was accompanied by maturation of the granulation tissue. Granulomas primarily contained fibroblasts. The main pathological changes included small scars and scarring granulomas (Fig. 4, *d*).

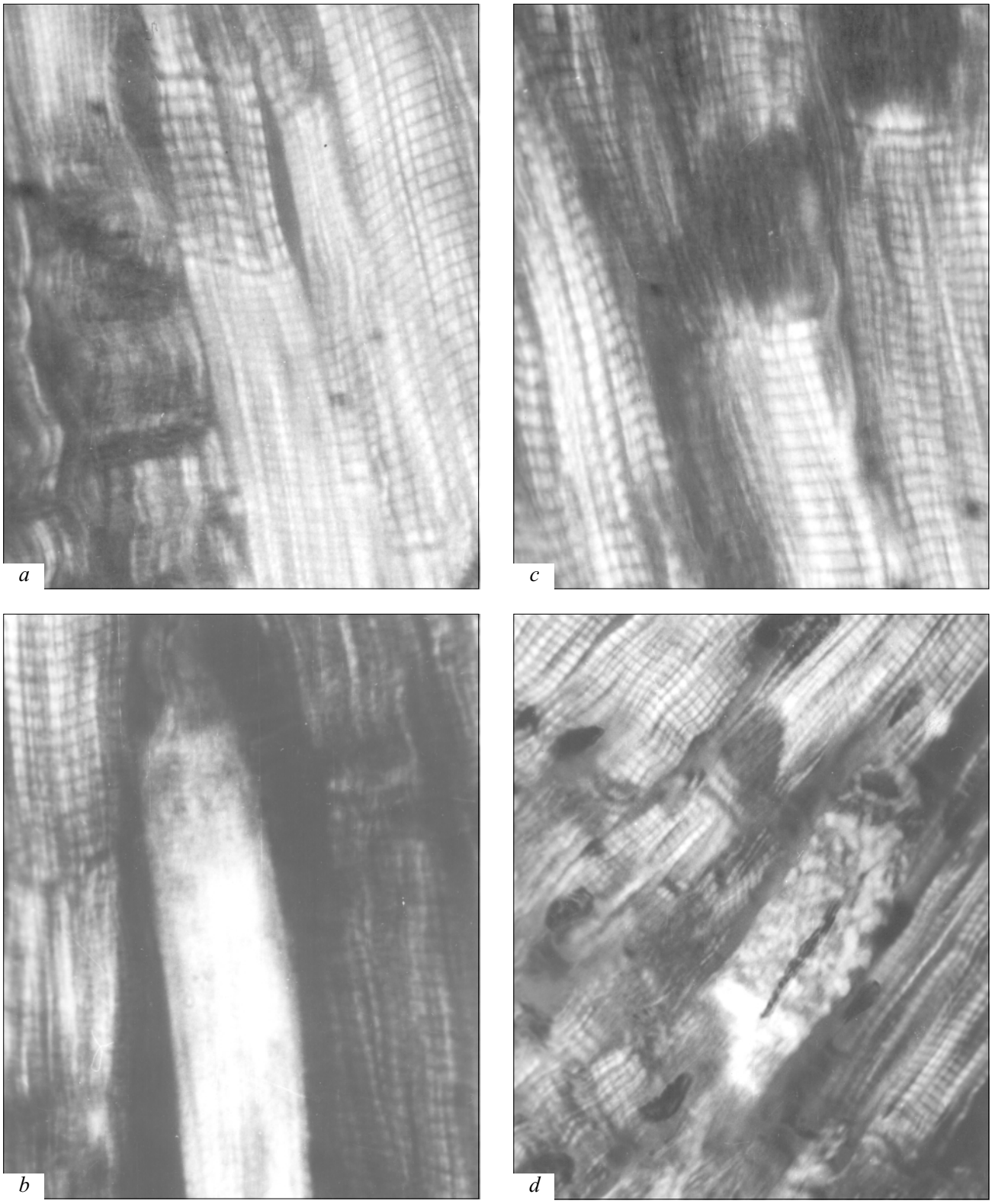

Fig. 1. Polarization microscopy of rat myocardium 1 h after epinephrine administration (×1600, *a*-*c*; ×1000, *d*). One elongated and one contracted muscle segments (center, *a*). Contracture of myofibrils in the muscle segment with increased anisotropy and fusion of anisotropic discs (*b*). Focus of myocytolysis without anisotropy (*c*). Muscle segment with primary lump degradation of myofibrils (center, *d*).

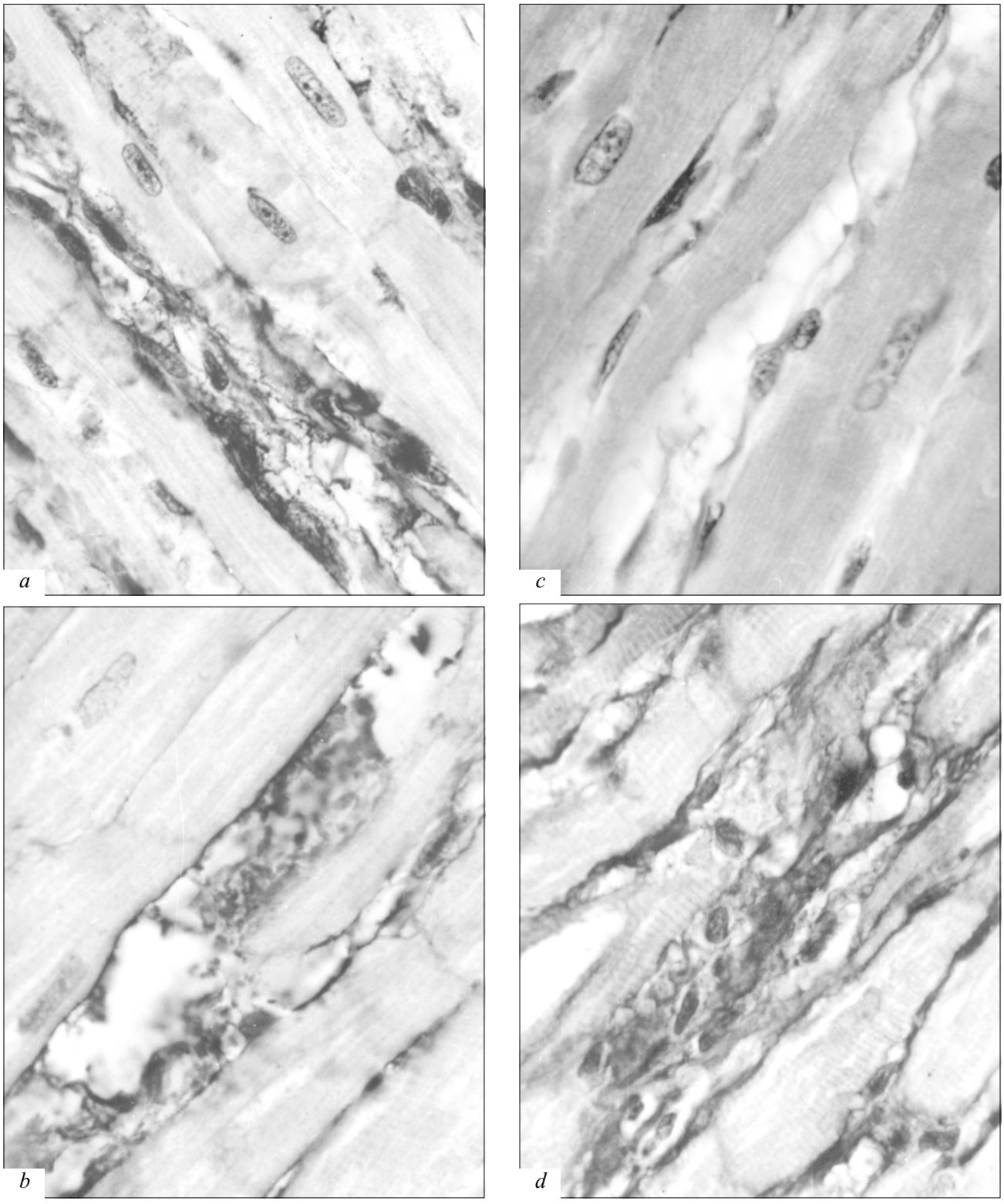

Fig. 2. Histochemical changes in rat myocardial stroma produced by epinephrine. Hale's reaction, PAS reaction, hematoxylin and orange staining (*a*, *b*, *d*), van Gieson staining (*c*); ×800 (*a*, *d*), ×1000 (*b*, *c*). Accumulation of glycosaminoglycans in the focus of colliquative necrosis in the muscle segment after 24 h (*a*). Glycosaminoglycans in muscle segments undergoing colliquative necrosis after 24 h (*b*). Migration of fibroblasts into the focus of cardiomyocyte damages after 2 days (*c*). Granuloma replacing dead muscle segments after 5 days (*d*).

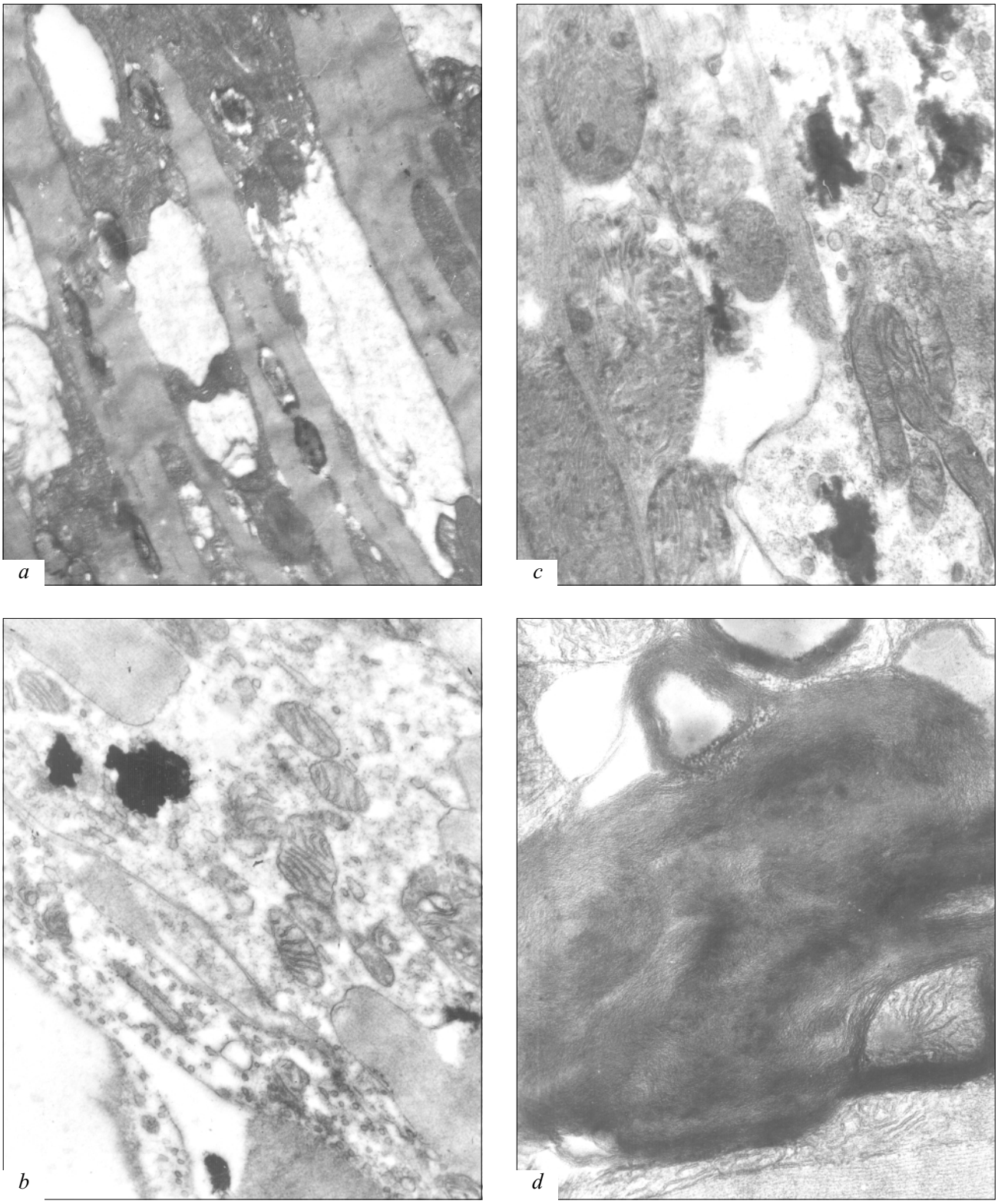

Fig. 3. Electron microscopy of rat myocardium 24 h after epinephrine administration. Necrotized cardiomyocyte with contracted myofibrils, focal degradation of mitochondria (*a*, ×10,000). Introduction of macrophage into destroyed cardiomyocyte (*b*, ×26,000). Thread-like lysosomes and mitochondria in the macrophage process (*c*, ×26,000). Degradation of mitochondria with the formation of osmiophilic myelin-like structures (*d*, ×45,000).

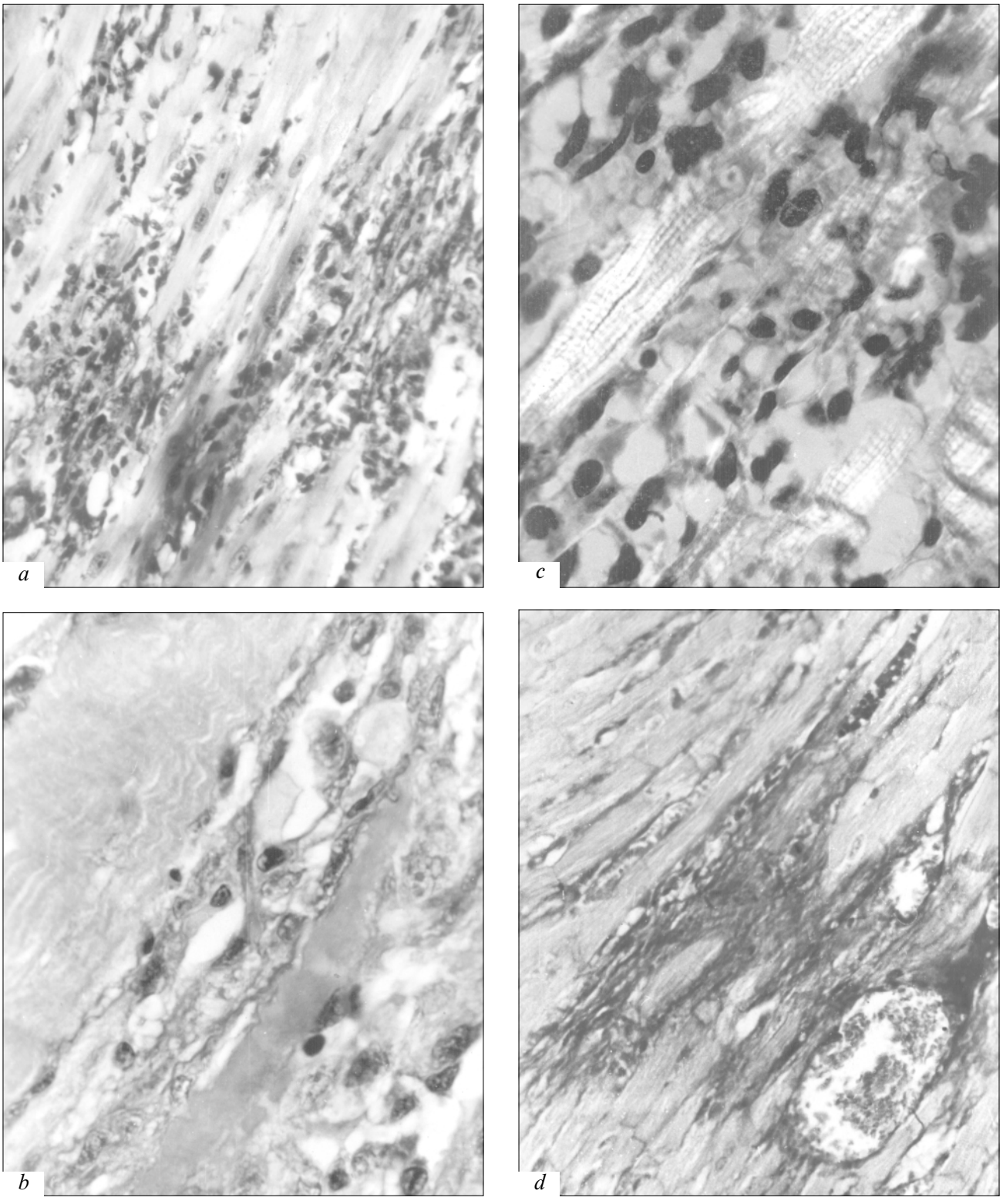

Fig. 4. Formation of connective tissue in rat myocardium after treatment with epinephrine. Granulomas at the site of dead cardiomyocytes after 2 days (*a*, ×300). Thin newly formed bundles of collagen fibers after 3 days (*b*, van Gieson staining, ×800). Granulomatous area with fragments of secondarily destroyed muscle segments after 5 days (*c*, hematoxylin and eosin, polarized light, ×800). Scar at the site of dead muscle segments in the wall of the left ventricle after 10 days (*d*, Hale's reaction, PAS reaction, and staining with hematoxylin and orange, ×250).

By the end of the 14th day we found not only focal scar changes, but also diffuse callousness of the stroma. In foci of coagulation necrosis, scarring granulomas were at various developmental stages. Large scars appeared as less mature structures. The reaction for glycosaminoglycans decreased with the progression of sclerosis in necrotic regions. By the 20th day the granulation tissue underwent pronounced scarring. After 25-30 days small scars consisting of collagen bundles, argyrophilic fibers, and individual elastic fibers were formed at the site of damaged muscle fibers. These changes were accompanied by diffuse callousness of the inter-bundle connective tissue in the myocardium and sclerosis of the epicardium. Moderate hyperplasia of muscle fibers was revealed near large foci of sclerosis. At the late stage (day 45) the number and size of sclerotic foci decreased; they underwent transformation into narrow fibrous layers.

Fibrosis is the major reparative process in tissues with low proliferative activity maintaining their integrity [15]. Fibrotic regions in the myocardium (scars) possess contractile properties due to the presence of myofibroblasts [14]. These cells are connected together and with extracellular matrix through gap junctions and fibronexus, respectively [15]. Myofibroblasts play an important role in paracrine regulation. They produce angiotensin II and stimulate synthesis of nitric oxide, bradykinin, prostaglandins, endothelins, steroids, and tissue metalloproteinase I inhibitor in endotheliocytes of newly formed capillaries [5,15]. These cytokines and hormones promote the inflammatory reaction and fibrogenesis.

Changes in the total content of collagen in the myocardium, ratio between collagen subtypes, degree of denaturation, and number of cross-links determine remodeling of the extracellular myocardial matrix, rigidity of the myocardium, and development of diastolic and systolic dysfunction [8,10]. According to modern notions, metalloproteinases cleave components of the matrix, promote denaturation and degradation of preexisting collagen fibers, regulate collagen synthesis by the feedback mechanism, and play an important role in remodeling of the myocardial matrix [6,7,9,11]. Metalloproteinases regulate the turnover of extracellular components in the connective tissue and are involved in morphogenesis.

Our results show that the major stages of myocardial remodeling during focal metabolic damages to CM are associated with contracture injuries, lump degradation of myofibrils, and development of coagulation necrosis. Reactive changes in the stroma develop over the first hours and are manifested in acute hemodynamic disturbances followed by proliferation of connective tissue cells. The immature granulation tissue is formed at the site of myocardial damages. This tissue is then replaced with mature connective tissue with the formation of small scars. It should be emphasized that remodeling of the myocardium during focal metabolic damages is accompanied by the development of reversible sclerotic changes.

REFERENCES

1. L. M. Nepomnyashchikh, *Morphogenesis of General Pathological Processes in the Heart* [in Russian], Novosibirsk (1991).
2. L. M. Nepomnyashchikh, *Alterative Insufficiency of Cardiomyocytes in Metabolic and Ischemic Damages* [in Russian], Moscow (1998).
3. L. M. Nepomnyashchikh, *Regenerative and Plastic Insufficiency of Cardiomyocytes in Impaired Protein Synthesis* [in Russian], Moscow (1998).
4. Yu. G. Tsellarius and L. A. Semenova, *Histopathology of Focal Metabolic Damages to the Myocardium* [in Russian], Novosibirsk (1972).
5. C. C. Chua, R. C. Hamdy, and B. H. L. Chua, *Biochim. Biophys. Acta*, **1311**, 175-180 (1996).
6. E. E. J. M. Creemers, J. P. M. Cleutjens, J. F. M. Smits, and M. J. A. P. Daemen, *Circ. Res*., **89**, 201-210 (2001).
7. C. M. Dollery, J. R. McEwan, and A. M. Henney, *Ibid*., **77**, 863-868 (1995).
8. Y. Y. Li, T. Q. Feng, T. Kadokami, *et al*., *Proc. Natl. Acad. Sci. USA*, **97**, 12,746-12,751 (2000).
9. Y. Y. Li, C. F. McTiernan, and A. M. Feldman, *Cardiovasc. Res*., **46**, 214-224 (2000).
10. L. Lu, Z. Gunja-Smith, J. F. Woessner, *et al*., *Am. J. Physiol. Heart Circ. Physiol*., **279**, H601-H609 (2000).
11. F. G. Spinale, *Circ. Res*., **90**, 520-530 (2002).
12. B. Swynghedauw, *Physiol. Rev*., **79**, 215-262 (1999).
13. J. R. Teerlink, J. M. Pfeffer, and M. A. Pfeffer, *Circ. Res*., **75**, 105-113 (1994).
14. R. Vracko and D. Thorning, *Lab. Invest*., **65**, 214-227 (1991).
15. K. T. Weber, *Circulation*, **96**, 4065-4082 (1997).