MORPHOLOGY AND PATHOMORPHOLOGY

Apoptosis of Cardiomyocytes as Extreme Manifestation of Regeneration and Plastic Insufficiency of Myocardium

L. M. Nepomnyashchikh and D. E. Semenov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 9, pp. 336-341, September, 2000
Original article submitted July 21, 2000

Alkaline dissociation of the myocardium from rats with modeled anthracycline cardiomyopathy revealed decreased absolute number of cardiomyocytes, disturbances in their intracellular regeneration, although no sings of necrosis were observed. Regeneration and plastic insufficiency of the myocardium due to structural changes in the nuclei and disturbances in myofibril reproduction resulting from selective suppression of synthesis of contractile proteins in the cardiomyocytes leads to the death of up to 30% cardiomyocytes.

Key Words: *anthracycline cardiomyopathy; regeneration and plastic myocardium insufficiency; cardiomyocytes; absolute number of cardiomyocytes; apoptosis*

There are various ultrastructural manifestations of plastic insufficiency of cardiomyocytes (CMC) [2, 5,7], however, the contribution of these alterations into disturbances of the myocardial contractile function cannot be determined without quantitative stereological assessment of changes in the total weight of contractile myocardium and the total number of CMC [2].

Direct and irreversible disturbances in transcription and translation mechanisms result in rapid death of CMC [4,7,8]. However, this process cannot be revealed by light and electron microscopy, because cell death is not accompanied by coagulation or colliquative necrosis [8,9]. At the same time, true mechanisms underlying the dynamics of CMC population during pathological reactions remain unknown.

Our aim was to evaluate the absolute number of CMC in rat myocardium during regeneration and plastic insufficiency.

Laboratory of General Pathologic Anatomy, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (n=195) weighing 160-220 g. Anthracycline cardiomyopathy is the basic model allowing detailed chronometry of plastic deficiency of CMC, since it is accompanied by direct inhibition of DNA-dependent RNA synthesis [2,6,7]. In series I, the rats (n=60) were injected intraperitoneally with daunomycin hydrochloride in a single dose of 30 mg/kg. For light and electron microscopy, the rats were decapitated 1, 2, 3, 6, 9, 12, and 18 h and 1, 2, 3, 4, and 5 days postinjection. Control rats (n=42) were intraperitoneally injected with single or fractional dose of saline (total dose 15 ml/kg).

In series II, the rats were injected intraperitoneally with fractional daunomycin during 3, 5, and 6 weeks. When the total dose of 30 mg/kg (the maximum toxic dose) was attained, the rats were decapitated 1-5 or 3-6 days after the last injection.

In series I the rats were weighted daily. In series II the rats were weighted before each injection.

The qualitative parameters of CMC population in the left ventricle were studied by the method of alka-

0007-4888/00/0009-903$25.00

line dissociation of fixed myocardium [7] in 19 control and 19 experimental rats in series I and in 12 experimental rats in series II.

The tissue and ultrastructural stereological analysis was carried out as described elsewhere [3]. The results were analyzed statistically using Student's t test at $p<0.05$.

RESULTS

Initially, ultrastructural changes in rat myocardium first appear in the nuclei and nucleoli: heterochromatin lumps disappeared, while collapse and nucleolus annulation developed virtually in all CMC (Fig. 1, *a*, *b*). Fragmentation and nucleousi annulation (Fig. 1, *c*, *d*) appeared after inhibition of protein synthesis not only at the transcription, but also at the translation level and reflect the decrease in ATP concentration [2].

A direct correlation was revealed between synthetic activity in CMC nuclei and glycogen content in their cytoplasm. Initial increase in glycogen content (Fig. 2, *a*) followed by its sequestration and autophagy in CMC attest to reduced activity or synthesis of the enzymes responsible for its utilization. During moderation of synthetic and metabolic processes, glycogen becomes a ballast for the cells, and it is sequestrated and degraded in autophagosomes.

Two days after injection of the cardiotoxic dose of daunomycin, multiple concentric membrane structures around fragments of sarcoplasmic reticulum or sometimes mitochondria were regularly observed in rat CMC (Fig. 2, *b*). Activation of autophagy in CMC cytoplasm after inhibition of protein synthesis attests to regression or involution aimed at matching the cytoplasmic volume with the functional state of the nucleus.

The most prominent manifestation of disturbances in protein synthesis was observed in the contractile apparatus, *i.e.* myofibrils. Despite lysis and thinning of myofibrils (Fig. 2, *c*), the cytoplasm did not become empty, because the muscle fibers thinned.

Total swelling of mitochondria, clarification of their matrix, and fragmentation of their crystae were observed in some CMC starting from day 3 postinjection (Fig. 2, *d*).

Optic and polarized microscopy revealed no focal degenerative and necrobiotic alterations in CMC.

The comparative stereological analysis of myocardial structures showed that the volume density of muscle fibers significantly decreased in the test rats ($p<0.001$). On day 5 after administration of the cardiotoxic dose of daunomycin (either by single or fractional injections), the absolute total weight of ventricular myocardium in test rats decreased by 32.8-38.4% ($p<0.001$).

Alkaline dissociation of the myocardium showed that the concentration of CMC nuclei and total number of CMC in test rats decreased by 21% and 30%, respectively (Table 1).

Among known processes leading to rapid and synchronous decrease in the number of all CMC structures, drastic decrease of RNA synthesis is the most probable candidate for the role of proteolysis inductor. Based on this working hypothesis, we interpret the numerical deficiency of CMC population as an extreme manifestation of plastic insufficiency of cardiac muscle cells.

Generally, cell death during plastic insufficiency of the myocardium was similar to programmed cell death, *i.e.* apoptosis. We denoted this process as CMC "disappearance" [8], since the mechanism of elimination of numerous muscle cells in contractile myocardium is not clearly understood.

In contrast to necrosis, apoptosis and disappearance of CMC do not lead to the formation of leukocytic infiltrates and sclerotic foci. Evaluation of the total CMC count in the ventricular wall revealed that

TABLE 1. Population of CMC in Daunomycin-Treated Rats ($M\pm m$)

Index	Control	Test
Concentration of CMC nuclei, 10^3 mg	29.32±0.74	23.15±0.84**
Number of CMC nuclei per heart, 10^6	18.87±0.85	12.02±0.39**
Number of CMC per heart, 10^6	9.64±0.43	6.16±0.19*
Number per 1000 CMC:		
nuclei	1958.0±4.4	1949.0±4.8
cells mononuclear	88±3	90.8±2.3
binuclear	882.0±4.1	881.4±3.5
trinuclear	13.2±0.9	13±1
polynuclear	16.8±1.8	14.8±1.6

Note. **$p<0.001$, *$p<0.01$ compared to the control.

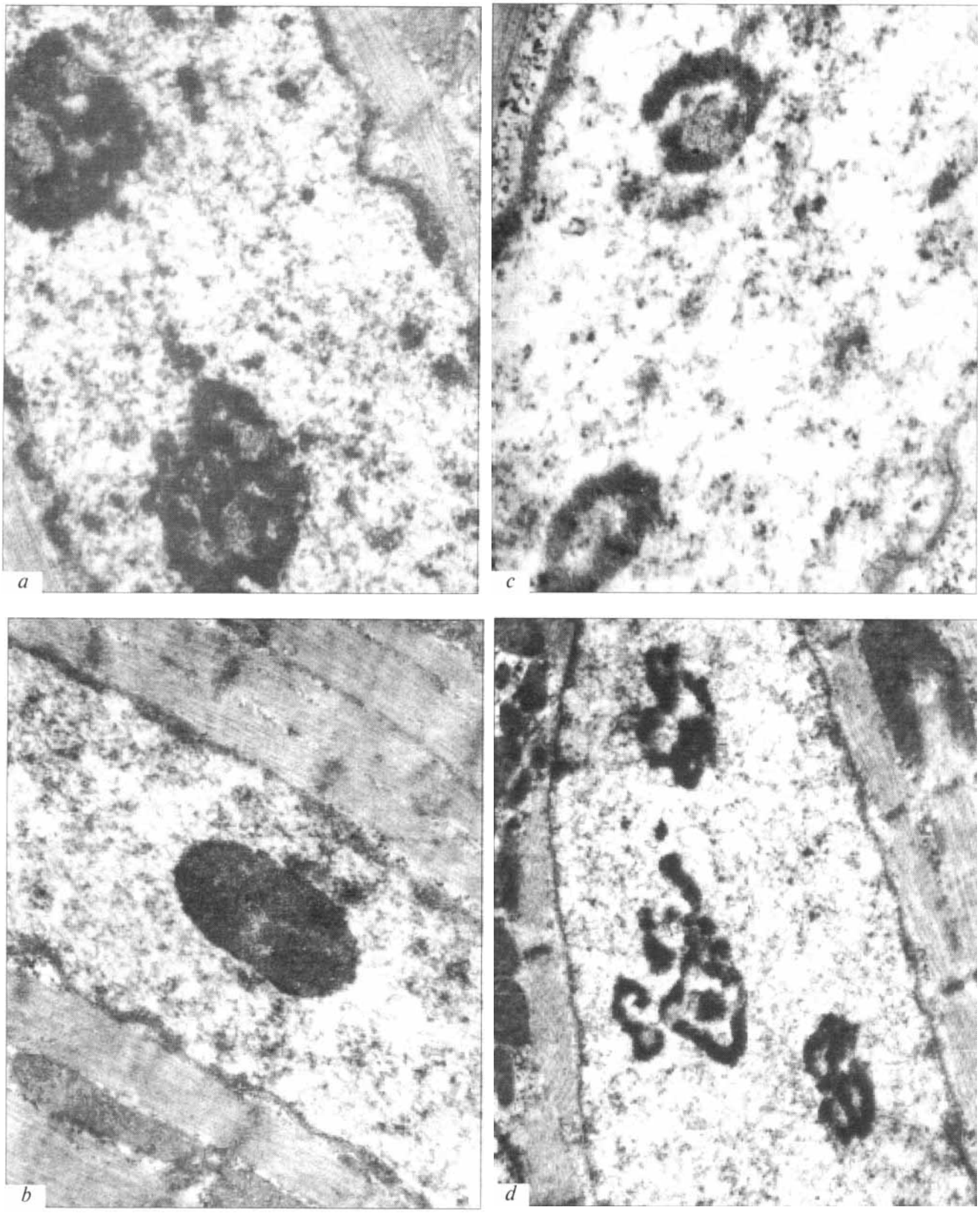

Fig. 1. Ultrastructural alterations in cardiomyocyte nucleoli in rat treated with daunomycin. *a*) fibrillar and granular components of nucleolus in control rat, ×20,000; *b*) nucleolus collapse 12 h postinjection, ×18,000; *c*) annular nucleoli 1 day postinjection, ×22,000; *d*) nucleolus fragmentation 2 days postinjection, ×16,000.

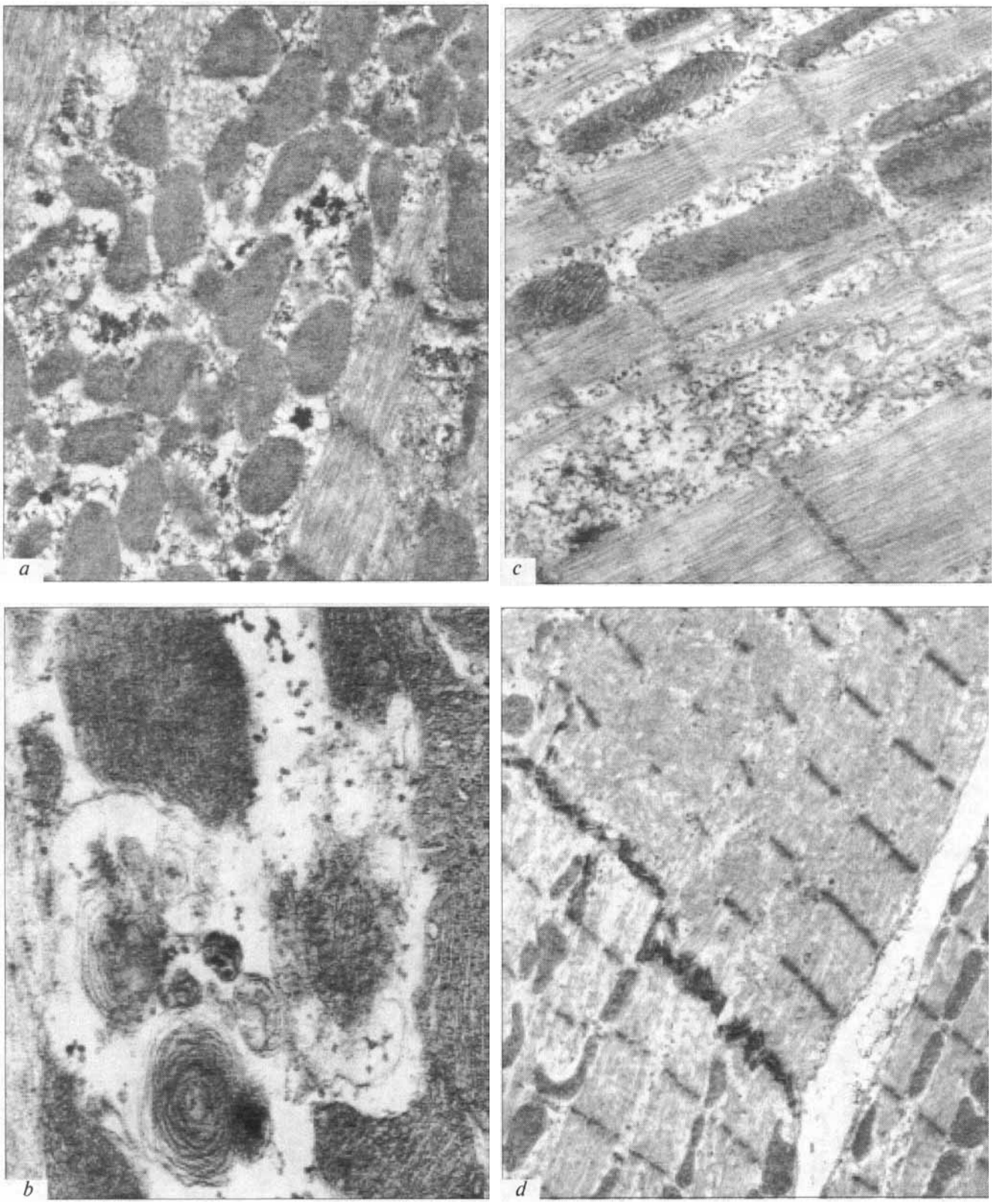

Fig. 2. Changes in cardiomyocyte cytoplasm in rats treated with daunomycin. *a*) lysis of myofilaments and α-glycogen rosettes 1 day postinjection, ×16,000; *b*) autophagosomes (myelin figures) among mitochondria 2 days postinjection, ×24,000; *c*) lysis of myofilaments along the perimeter of myofibrils and disintegration of mitochondria, ×18,000; *d*) total destruction of mitochondria in cardiomyocytes 2 days postinjection, ×10,000.

the heart rapidly lost about 30% cells without apparent disturbances in myocardial structure. Irreversible cardiac contractile insufficiency caused by disturbed plastic metabolism in the myocardium occurs after loosing of no less than $^1/_3$ absolute total weight of CMC.

Investigation of the effect of cytostatics on CMC number and DNA content in children and adults treated with various cytostatics (cyclophosphamide, vincristine, purinetol, and methotrexate) [10] revealed similar changes in CMC number in all children, while the number of CMC in adults did not significantly differ from normal.

Elimination of CMC with nuclei of different ploidy is little studied. The drastic decrease in the number of polyploid nuclei in human and rat myocardium under the effect of daunomycin can result from elimination of CMC with tetraploid nuclei, rather than from inhibition of DNA synthesis in the muscle nuclei. Our data showed that CMC with different number of nuclei are subjected to the same degree of elimination, which is confirmed by the constant ratio between mono-, bi-, tri-, and polynuclear CMC in the myocardium of test and control animals in all experimental models [2,6].

Elimination of CMC in the absence of necrosis is most likely based on rapid lysis and autophagy of intracellular structures with subsequent resorption of residual bodies and autophagosomes by macrophages, *i.e.* processes involved in genetically programmed renewal of cell population in normal tissues, cell elimination during early morphogenesis, and metamorphosis [11].

According to modern views [1], the most characteristic sign of programmed cell death is its dependence on *de novo* protein and mRNA synthesis. It is usually accompanied by DNA fragmentation between nucleosomes by Ca^{2+}- and Mg^{2+}-dependent endogenous endonucleases (electrophoretic separation yields "ladder" distribution of oligonucleosomal fragments corresponding to their molecular weight). This type of cell death is usually called apoptosis [1].

The cytological signs of apoptosis are condensation of nuclear chromatin and cytoplasm, formation of apoptotic bodies and vesicles on the membrane, and pronounced reduction of the cell volume [14]. It is important that programmed death can occur without DNA fragmentation (non-ladder type) and morphological signs characteristic of apoptosis [15]. The cases, when the morphological signs of apoptosis and internucleosomal DNA fragmentation are not accompanied by *de novo* protein and mRNA synthesis are not considered as programmed cell death [13].

The changes in expression pattern of the immediate early genes caused by endogenous and exogenous signals disturbing the process of re-programming of activity of delayed genes are considered as the molecular and genetic mechanisms of apoptosis during proliferation and differentiation. They lead to destabilization of chromatin, DNA, and genetic instability that may trigger the program of cell death [1].

All our experimental models of CMC plastic insufficiency [2] demonstrated ultrastructural signs of moderation of biosynthetic processes, while among the cytological sings characteristic of apoptosis only reduction of cell volume was observed. Nevertheless, the processes of self-organization of the supermolecular structures and macromolecules (*i.e.* specific program) underlie atrophic (involutional) changes in CMC caused by regeneration and plastic insufficiency. In this respect, the final stages of morphofunctional rearrangement in CMC during regeneration and plastic insufficiency resemble apoptosis.

REFERENCES

1. Yu. L. Volyanskii, T. Yu. Kolotova, and N. V. Vasil'ev, *Usp. Sovr. Biol.*, **114**, No. 6, 679-692.
2. L. M. Nepomnyashchikh, *Regeneration and Plastic Insufficiency of Cardiomyocytes during Disturbances in Protein Synthesis* [in Russian], Moscow (1998).
3. L. M. Nepomnyashchikh, E. L. Lushnikova, L. V. Kolesnikova, *et al.*, *Morphometric and Stereological Analysis of Myocardium: Tissue and Ultrastructural Organization* [in Russian], Novosibirsk (1984).
4. L. M. Nepomnyashchikh, G. I. Nepomnyashchikh, E. L. Lushnikova, *et al.*, *Morphogenesis of Vital General Pathologic Processes in Organs and Tissues of Humans and Animals: Five Discoveries in Biology and Medicine* [in Russian], Moscow (1998).
5. L. M. Nepomnyashchikh, D. E. Semenov, L. A. Semenova, and Yu. G. Tsellarius, *Arkh. Patol.*, No. 5, 19-26 (1983).
6. D. E. Semenov, *Pathomorphology of Plastic Insufficiency of Myocardium during Modeling of Cardiomyopathies*, Abstract of Doct. Med. Sci. Dissertation, Novosibirsk (1996).
7. L. A. Semenova, L. M. Nepomnyashchikh, and D. E. Semenov, *Morphology of Plastic Insufficiency of Cardiac Muscle Cells* [in Russian], Novosibirsk (1985).
8. D. E. Semenov, L. A. Semenova, L. M. Nepomnyashchikh, and Yu. G. Tsellarius, *Byull. Eksp. Biol. Med.*, **97**, No. 5, 629-633 (1984).
9. V. G. Tsyplenkova, *Arkh. Patol.*, No. 5, 71-74 (1996).
10. C.-P. Adler and U. Costabel, *Arch. Path. Anat. Abt. B.* **32**, 109-125 (1980).
11. *Cell Death in Biology*, Eds. I. D. Bowen and R. A. Lockshin, London (1981).
12. R. S. Duke, R. Chervenak, and J. J. Cohen, *Proc. Natl. Acad. Sci. USA*, **80**, 6361-6365 (1983).
13. D. S. Schmid, J. P. Tite, N. H. Ruddle, *Ibid*, **83**, 1881-1885 (1986).
14. K. Schulze-Osthoff, H. Walczak, W. Groge, and P. H. Krammer, *J. Cell. Biol.*, **127**, 15-20 (1994).
15. L. M. Schwartz, S. W. Smith, M. E. Jones, and B. A. Osborne, *Proc. Natl. Acad. Sci. USA*, **90**, 980-984 (1993).