THE "DISAPPEARING CARDIOMYOCYTES" PHENOMENON IN PLASTIC MYOCARDIAL INSUFFICIENCY

D E. Semenov, L. A. Semenova,
L. M. Nepomnyashchikh, and Yu. G. Tsellarius

UDC 616.127.018.1-02:
615.277.3]-092.9

KEY WORDS: plastic myocardial insufficiency; "dsappearing cardiomyocytes" phenomenon; isolated heart muscle cells; quantitative morphology.

Structural changes in the cardiomyocytes of experimental animals caused by the action of anthracycline cytostatics are evidence of a profound disturbance of the structural metabolism in these cells — the condition of plastic cardiac insufficiency [4, 8]. In some cases of treatment with these preparations, the number of muscle cells in the ventricular myocardium of children has been shown to be reduced [14].

The aim of this investigation was to study the "disappearing cardiomyocytes" phenomenon during disturbance of the synthesis of contractile proteins, leading to plastic insufficiency of the myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on 47 male Wistar rats weighing 180 ± 20 g, divided into two groups. Rubomycin hydrochloride was injected into the animals of group 1 (n = 20) in the form of a 2% aqueous solution, intraperitoneally, in a single dose of 30 mg/kg. The 17 rats still alive 5 days after injection of the substance were decapitated under chloroform anesthesia. The rats of group 2 (n = 10) were given three injections, each of 10 mg/kg, of the substance at intervals of 7 days; eight rats surviving 5 days after the third injection of rubomycin were decapitated. Control rats (n = 17) received an intraperitoneal injection of physiological saline in a volume corresponding to their body weight. The hearts were removed from the cadavers, contractions were stopped by cold, the atria were carefully removed, and the ventricles were weighed on torsion scales and fixed in cold 4% paraformaldehyde solution in 0.1 M phosphate buffer, pH 8.8 [9] for 10 days. At the end of fixation the ventricles were reweighed and the values obtained for their weight were used in subsequent calculations. A piece weighing 20-25 mg was excised from the wall of the left ventricle and subjected to alkaline dissociation into cells [1]. The cell suspension was stained with orcein and the number of cardiomyocyte nuclei was counted in a Fuchs—Rosenthal chamber. In films of the suspension stained with orcein and Light green the number of nuclei in 1000 cardiomyocytes was counted and the ratio between cells with 1, 2, 3, or more nuclei was calculated. The number of cardiomyocyte nuclei in 1 mg tissue was determined by the equation:

$$n = \frac{a \cdot 100}{3.2 \cdot C},$$

where a is the mean number of nuclei per chamber (six chambers were counted) and C the concentration of the suspension.

The total number of cardiomyocytes in the ventricles was calculated by the equation:

$$N = n \cdot W,$$

where W is the weight of the ventricles after fixation.

The total number of cardiomyocytes in the ventricles was calculated by the equation:

Department of Pathomorphology and Morphometry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the **USSR. Laboratory of Pathomorphology**, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 5, pp. 629-633, May, 1984. Original article submitted October 27, 1983.

0007-4888/84/9705-0689$08.50

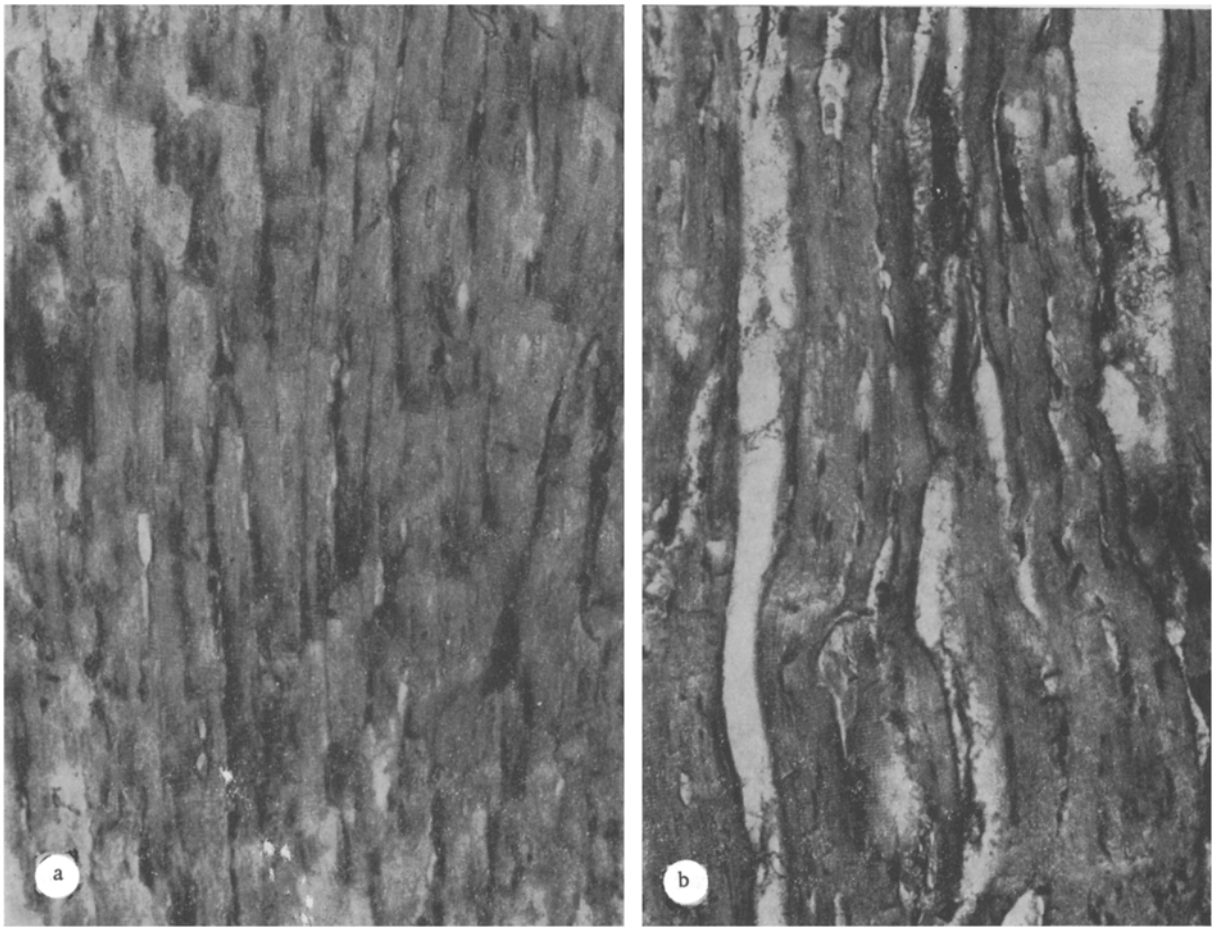

Fig. 1. Changes in myocardium during plastic insufficiency of heart muscle: a) subendocardial layer of left ventricular myocardium of control rat: muscle cells packed closely together, concentrations of glycogen granules in cardiomyocytes; b) the same layer of myocardium from a rat killed 5 days after a single injection of a cardiotoxic dose of rubomycin: marked edema of stroma, separating fibers, absence of glycogen granules in cardiomyocytes. Stained with colloidal iron–PAS–hematoxylin, 320 ×.

$$M = \frac{N \cdot 1000}{m},$$

where m is the number of nuclei in 1000 cardiomyocytes. The results were subjected to statistical analysis by Student's test. Myocardium from decapitated rats only was used for electron microscopy [8]. Histological investigations of myocardium was carried out also on animals which died in the course of the experiment. Histo-topographic sections of the right and left ventricles and ventricular septum, stained with hematoxylin and eosin and with colloidal iron–PAS–hematoxylin, were studied in ordinary and polarized light in the NU-2 biological microscope (Carl Zeiss, East Germany).

EXPERIMENTAL RESULTS

In rats of experimental groups 1 and 2 marked signs of stasis were present in the systemic circulation at the time of sacrifice, and the body weight was reduced by 15 and 21% respectively. The gain in weight of the control animals, kept in the animal house on the same standard diet, averaged 20%.

Under the light microscope sections of the myocardium from animals of both experimental groups revealed intermuscular edema, most marked in the rats of group 1, and absence of glycogen in the cytoplasm of the cardiomyocytes (Fig. 1a, b).

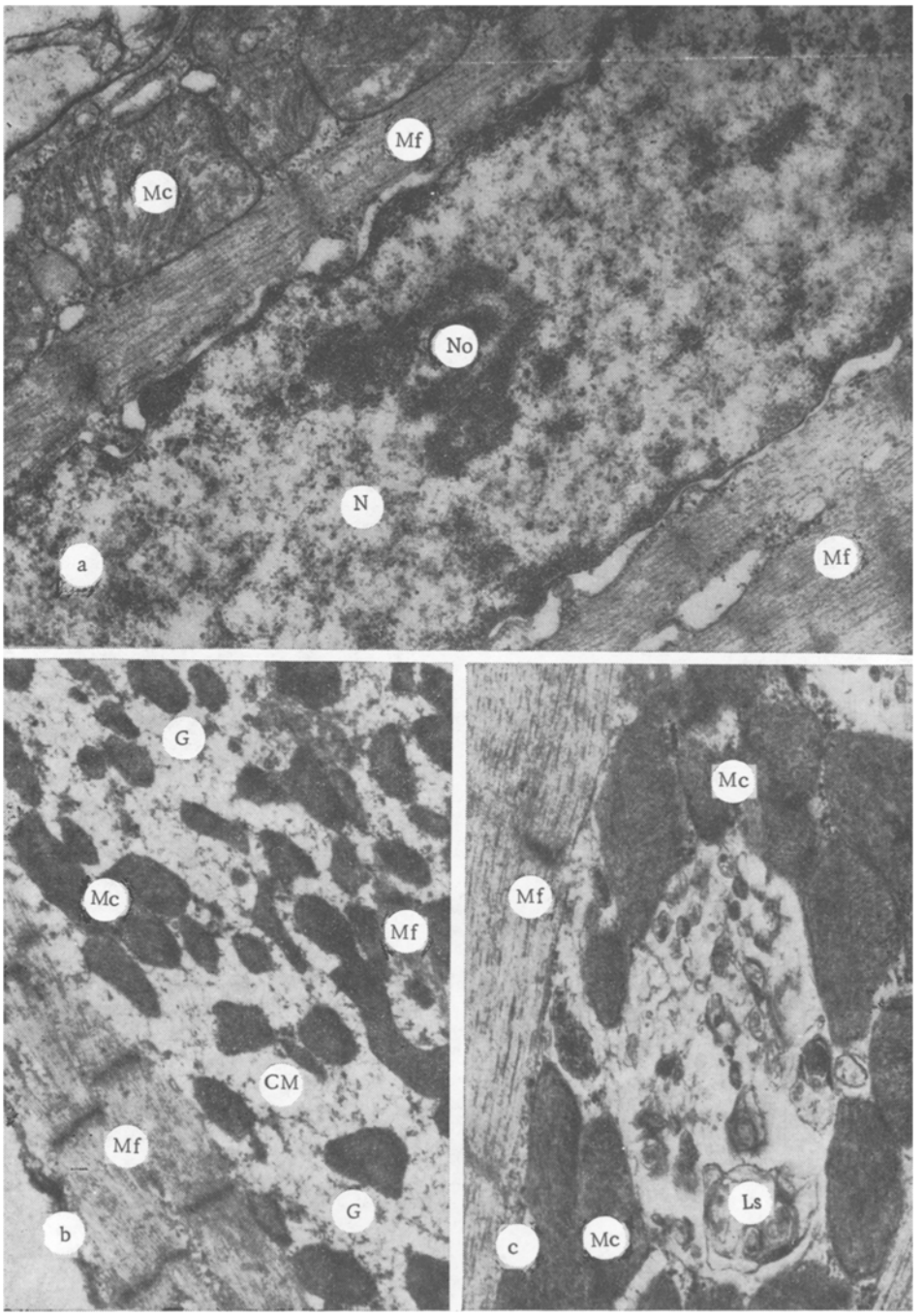

Fig. 2. Ultrastructural changes in cardiomyocytes in plastic myocardial insufficiency: a) fragmentation of nucleolus in cardiomyocyte nucleus and swelling of mitochondria during processing of material, reflecting instability of their membranes; b) thinning and lysis of **myofibrils** with denudation of cytoplasmic matrix (glycogen in cytoplasm represented by reticular β-form; no free ribosomes can be seen; c) focal degradation of cardiomyocyte cytoplasm (formation of autophagosomes and secondary lysosomes); d) glycogen, Ls) lysosomes; Mf) myofibrils, Mc) mitochondria, CM) cytoplasmic matrix, N) nucleus, No) nucleolus, G) **glycogen.** Magnification: a) 35,000, b) 25,000, c) 40,000 ×.

TABLE 1. Characteristics of Ventricular Myocardium of Control Rats and Animals Receiving Rubomycin ($\bar{x} \pm m$)

Parameter	Group of animals		
	control n = 17	1 n = 17	2 n = 8
Weight of ventricles, mg	646,7±24,8	511,1±19,0***	534,0±26,1**
Ventricular index	3,05±0,10	3,14±0,10	3,69±0,22***
Number of cardiomyocyte nuclei in 1 mg x 10^3	29,32±0,71	23,15±0,84***	28,68±1,50
Number of cardiomyocyte nuclei in ventricles x 10^6	18,86±0,85	12,02±0,39***	15,46±1,03*
Number of cardiomyocytes in ventricles x 10^6	9,64±0,43	6,16±0,19***	7,89±0,52**
Number of nuclei in 1000 cardiomyocytes	1958±4	1949±5	1947±5

Legend. *P < 0.05; **P < 0.02; ***P < 0.01.

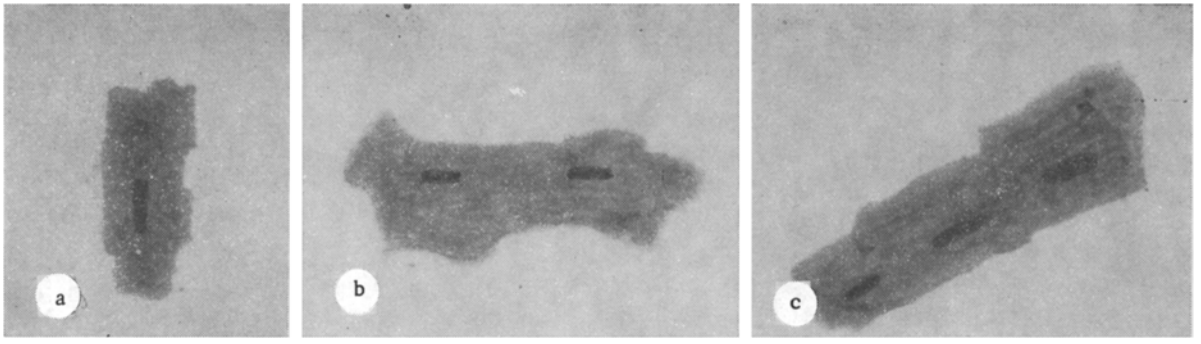

Fig. 3. Isolated cardiomyocytes with different numbers of nuclei: a, b, c) heart muscle cells from film of cardiomyocyte suspension obtained by alkaline dissociation of myocardium and containing 1, 2, and 3 nuclei **respectively.** Stained with orcein and Light green, 1250 ×.

Ultrastructural changes in the cardiomyocytes developing under the influence of rubomycin [4, 8] could be reduced to a combination of features demonstrating depression of synthetic activity of the cells: segregation and fragmentation of nucleoli, thinning and lysis of myofibrils, focal degradation of the cytoplasm, instability of the mitochondrial membranes, disappearance of granular glycogen, and a decrease in the number of free ribosomes in the cytoplasm (Fig. 2).

As already stated [3, 4, 8], a characteristic feature of plastic myocardial insufficiency due to the action of rubomycin is absence of focal necrobiotic lesions of the contractile myocardium. In individual rats killed at the end of the experiment or dying before it ended, single cardiomyocytes could be seen in the section with fragmentation of the myofibrils into clumps. These changes were evidently agonal in origin for in all cases the cytoplasm of cells with fragmentation of the myofibrils was PAS-negative; there were no reaction of the stroma to injury and no microfoci of macrophagal-leukocytic infiltration.

Analysis of the quantitative data (Table 1) shows that the weight of the ventricles of the experimental animals of both groups was significantly lower than in the control; the decrease was more marked in the rats of group 1, receiving a single cardiotoxic dose of rubomycin.

The cardiac index (the ratio of the weight of the ventricles to body weight) did not differ significantly from the control in rats of group 1, but in animals of group 2 it was increased a little, due to the longer duration of the experiment and, correspondingly, the greater emaciation of the animals.

The concentration of muscle nuclei per unit volume of heart tissue in rats of group 1 was 22% less than in the control, but after injection of rubomycin in repeated small doses it did not differ significantly from the control. The greater decrease in the concentration of nuclei in the rats of group 1 can evidently be explained by considerable edema of the myocardial connective tissue. According to the results of a morphometric investigation [3] the increase in volume of the intermuscular spaces in the myocardium of rats killed on the 5th day after a single injection of the complete dose of rubomycin averaged 11.5% ($P < 0.01$).

The absolute number of muscle nuclei and the absolute number of cardiomyocytes in the ventricles of rats of groups 1 and 2 were reduced compared with the control by 37 and 19% respectively. Similar results were obtained when the total number of muscle cells was determined by a combination of biochemical and cytophotometric estimation of DNA in the myocardium of persons receiving anthracycline cytostatics [14, 15].

The multinuclearity index (the number of nuclei in 1000 cardiomyocytes) in rats of all three groups did not differ significantly, i.e., the number of predominantly mononuclear, binuclear, and multinuclear cardiomyocytes was not reduced.

The results of this investigation, using the method of alkaline dissociation of the myocardium (Fig. 3), add details to the picture of qualitative and quantitative (stereologic) changes in the cardiomyocyte population of the ventricles of rats with depressed DNA-dependent RNA synthesis and specific protein synthesis.

Besides processes of involution in the cytoplasm of the cardiomyocytes [8], leading to a significant reduction in total weight and a reduction in diameter of the heart muscle fibers [3], a numerical deficit of cardiomyocytes also arises in the contractile myocardium, which we describe as the "disappearing cardiomyocytes" phenomenon, since the mechanism of elimination of a considerable proportion of cardiomyocytes is not yet clear.

The cardiomyocyte deficit in the degenerative form of contractile myocardial insufficiency is caused by injury and necrosis of the cells (focal metabolic injuries, myocardial infarction). Types of acute cardiomyocyte damage leading to the appearance of coagulative or colliquative necrosis, and the reaction of the myocardial stroma to these types of necrosis have been studied in detail [2, 10, 11] and they can be revealed by the methods of light microscopy at any stage of the process [12, 13]. Disappearance of cardiomyocytes in plastic myocardial insufficiency is not connected with their necrosis in the usual meaning of this term, for no necrobiotic or sclerotic changes could be found in the myocardium of animals dying or killed at different stages of the experiment.

It can be tentatively suggested that the "disappearing cardiomyocytes" phenomenon in plastic myocardial insufficiency is based on lysis of the cells, unaccompanied by any of the known pictures of necrobiotic changes. This cell lysis evidently takes place sufficiently

rapidly, and it has not yet proved possible to detect it in sections, for the further reason that the initial stages of the process are indistinguishable from the usual picture of plastic insufficiency, while the final stages are taken for tangential sections through areas of cells with insufficiency.

The phenomenon described above probably also takes place in other organs and tissues subjected to reduction in the absence of any visible necrobiotic changes in the cells. This may apply, in particular, to reduction of organs under the influence of endocrine factors [5, 7].

LITERATURE CITED

1. L. M. Belov, M. E. Kogan, T. A. Leont'eva, et al., Tsitologiya, No. 11, 1332 (1975).
2. L. M. Nepomnyashchikh, Pathological Anatomy and Ultrastructure of the Heart [in Russian], Novosibirsk (1981).
3. L. M. Nepomnyashchikh, D. E. Semenov, V. P. Tumanov, et al., Byull. Éksp. Biol. Med., No. 9, 25 (1983).
4. L. M. Nepomnyashchikh, D. E. Semenov, L. A. Semenova, et al., Arkh. Patol., No. 5, 19 (1983).
5. V. S. Paukov and V. A. Frolov, Elements of a Theory of Pathology of the Heart [in Russian], Moscow (1982).
6. P. P. Rumyantsev, Cardiomyocytes in Processes of Reproduction, Differentiation, and Regeneration [in Russian], Leningrad (1982).
7. D. S. Sarkisov, Essays on the Structural Bases of Homeostasis [in Russian], Moscow (1977).
8. D. E. Semenov, L. M. Nepomnyashchikh, and V. P. Tumanov, Byull. Éksp. Biol. Med., No. 9, 102 (1982).
9. L. A. Semenova, Arkh. Patol., No. 9, 61 (1976).
10. L. A. Semenova and Yu. G. Tsellarius, Ultrastructure of Heart Muscle Cells in Focal Metabolic Lesions [in Russian], Novosibirsk (1978).
11. Yu. G. Tsellarius and L. A. Semenova, Histopathology of Focal Metabolic Lesions of the Myocardium [in Russian], Novosibirsk (1972).
12. Yu. G. Tsellarius, L. A. Semenova, and L. M. Nepomnyashchikh, Morbid Anatomical Diagnosis of Prenecrotic Changes and Infarcts of the Myocardium by Polarization Microscopy. Technical Recommendation [in Russian], Moscow (1979).
13. Yu. G. Tsellarius, L. A. Semenova, and L. M. Nepomnyashchikh, Focal Lesions and Myocardial Infarction. Light, Polarization, and Electron Microscopy [in Russian], Novosibirsk (1980).
14. C.-P. Adler and U. Costabel, Recent Adv. Stud. Card. Struct. Metab., 6, 343 (1975).
15. C.-P. Adler and U. Costabel, Arch. Path. Anat. Abt. B, 32, 109 (1980).