Comparative studies revealed a clear parallel between the concentration of intravitally formed zinc dithizonate in the cells, the duration of its stay there, and the degree of damage to these cells. In animals treated with powerful nucleophilic agents, the toxic action of this complex in the pancreatic B-cells was weakened.

The results of these experiments confirm the previous hypothesis that the mechanism of the pathological action of dithizone and other chelating agents on insulin-producing cells is based on the formation of an unsaturated (electrophilic) complex with zinc in them [5].

LITERATURE CITED

1. V. A. Eshchenko, Tsitologiya, No. 8, 927 (1978).
2. I. V. Toroptsev and V. A. Eshchenko, Tsitologiya, No. 11, 1481 (1970).
3. I. V. Toroptsev and V. A. Eshchenko, Arkh. Patol., No. 2, 31 (1972).
4. I. V. Toroptsev and V. A. Eshchenko, Experimental Dithizone Diabetes [in Russian], Tomsk (1975).
5. I. V. Toroptsev and V. A. Eshchenko, Byull. Éksp. Biol. Med., No. 7, 100 (1982).
6. A. Albert, Selective Toxicity and Related Topics, Methuen, London (1968).
7. J. Harwood, Industrial Applications of Organometallic Compounds, Chapman and Hall, London (1963).
8. K. Okamoto, Acta Schol. Med. Univ. Kyoto, 27, 43 (1949).
9. K. Okamoto, Tohoku J. Exp. Med., 61, Suppl. No. 3, 1 (1955).
10. K. Okamoto and H. Kawanishi, Endocrinol. Jpn., 13, 305 (1966).

STEREOLOGIC ANALYSIS OF MYOCARDIAL STRUCTURES IN PLASTIC CARDIAC INSUFFICIENCY (ABNORMAL ULTRASTRUCTURAL CARDIOMYOCYTE REGENERATION)

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KEY WORDS: Plastic cardiac insufficiency; myocardial parenchyma and stroma; cardiomyocyte ultrastructure; stereology.

Plastic insufficiency of the heart is associated with a disturbance of reproduction of the intracellular structures of cardiomyocytes, and it is to be distinguished from alterative myocardial insufficiency which develops as a result of injury to the muscle cells and the exclusion of some of them from contractile activity [2, 6]. The ultrastructural manifestations of plastic insufficiency were studied by the writers in experiments to determine the depression of protein synthesis in cardiomyocytes of albino rats treated with the anthracycline antibiotic rubomycin [5].

The object of the present investigation was a stereologic analysis of the myocardium in the same model in order to elucidate the role of cardiosclerosis in the development of plastic insufficiency of the cardiomyocytes.

EXPERIMENTAL METHODS

Eighteen male Wistar rats weighing 160-180 g were used. Rubomycin hydrochloride (0.2% solution) was injected intraperitoneally into nine rats in a single dose of 30 mg/kg body

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TABLE 1. Results of Stereologic Analysis of Rat Myocardium 5 Days after Injection of Rubomycin (M ± m)

Parameter	Control	Experiment	P
Body mass, g	172,0±4,9	132,6±3,99	<0,05
Absolute mass of heart, g	0,598±0,09	0,526±0,029	<0,05
Cardiac index	$31,8 \cdot 10^{-4} \pm 1,7 \cdot 10^{-4}$	$39,6 \cdot 10^{-4} \pm 2,2 \cdot 10^{-4}$	<0,05
Bulk density (V_V, fraction) of:			
cardiomyocytes	0,6672±0,0067	0,4710±0,0129	<0,05
blood vessels	0,1609±0,0044	0,1833±0,0122	<0,05
interstitial connective tissue	0,1107±0,0077	0,1704±0,0019	<0,05
intercellular spaces	0,0612±0,0021	0,1753±0,0067	<0,05
Surface density (S_V, cm^{-1}) of cardiomyocytes	142,480±2,978	155,200±7,238	<0,05
Ratio of surface density of cardiomyocytes to their bulk density (S_V/V_V, cm^{-1})	211,984±4,243	331,438±20,890	<0,01
Absolute total mass (M, 10^{-3} g) of:			
cardiac index	0,4026±0,0248	0,2480±0,0174	<0,01
blood vessels	0,0958±0,0044	0,0956±0,0064	
interstitial connective tissue	0,0660±0,0053	0,0922±0,0068	<0,05
tissue fluid and intercellular spaces	0,0352±0,0027	0,0922±0,0068	<0,01
Absolute total surface area (S, cm^2) of cardiomyocytes	85,456±5,788	81,238±4,496	
Ratio of number of nuclei of muscle and connective-tissue cells	1 : 2,4	1 : 3,5	

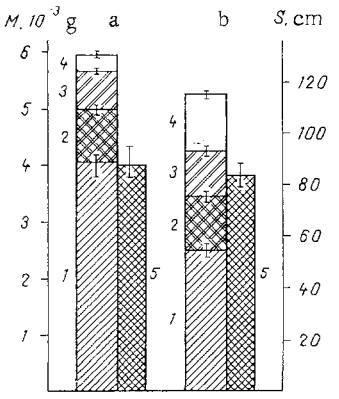

Fig. 1

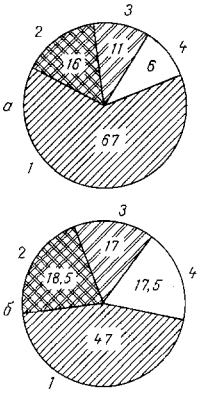

Fig. 2

Fig. 1. Absolute parameters of structural components of rat myocardium 5 days after injection of rubomycin. Abscissa: a) Control, b) experiment; ordinate; on left — absolute mass: 1) cardiomyocytes, 2) vessels, 3) interstitial connective tissue, 4) tissue fluid in intercellular spaces; on right — absolute total surface area of cardiomyocytes (5).

Fig. 2. Relative percentages of structural components of rat myocardium 5 days after injection of rubomycin. a) Control, b) experiment. 1) Cardiomyocytes, 2) vessels, 3) interstitial connective tissue, 4) intercellular spaces.

weight. The remaining animals, which acted as the control to the experimental series, were given an injection of the corresponding volume of physiological saline. At the end of the 5th day of the experiment all the rats were reweighed, anesthetized with chloroform, and decapitated. After the heart had stopped beating the atria were removed, the chambers of the heart were dried, and the heart was weighed and immersed in cold 4% paraformaldehyde, pH 8.0, in 0.1 M phosphate buffer. The fixed hearts were cut in the frontal plane across both ventricles and the ventricular septum 48 h later. One half was embedded in paraffin wax, and from the other half samples were taken for electron-microscopic study and were processed

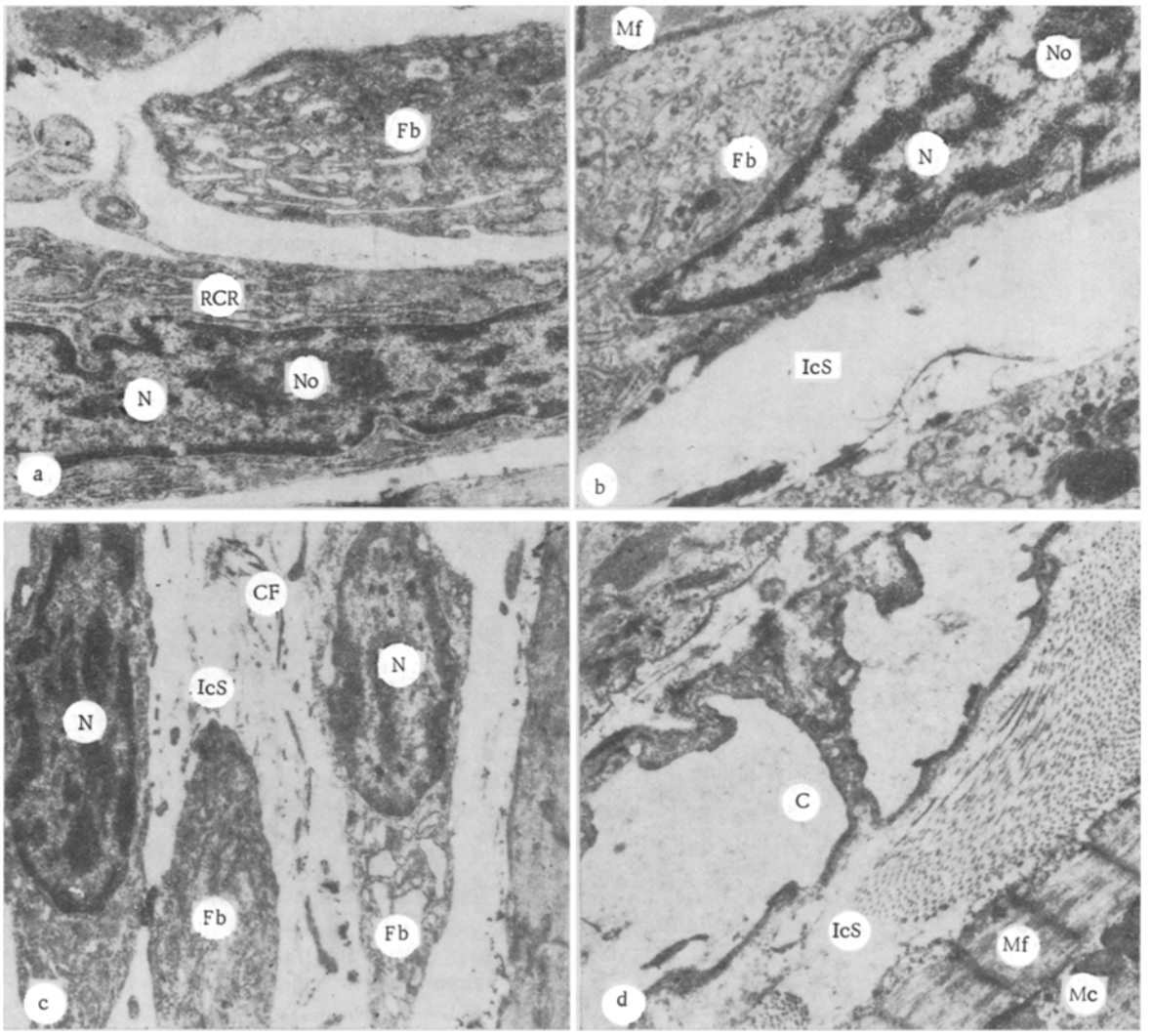

Fig. 3. Ultrastructural changes in stroma of rat myocardium 5 days after injection of rubomycin. a, b) Hyperplasia of cytoplasmic structures and enlargement of nucleoli (No) in nuclei (N) of fibroblasts (Fb); RCR — rough cytoplasmic reticulum. Magnification: a) 14,000, b) 12,000. c) Bundles of collagen fibers (CF) around synthetically active fibroblasts; IcS) intercellular spaces; magnification 9000. d) Massive bundles of collagen fibers in intercellular space; C) capillary, Mf) myofibrils; Mc) mitochondria; magnification 8000.

by the method described previously [5]. Paraffin sections 5 μ thick were stained with hematoxylin and eosin and by the colloidal iron—PAS—hematoxylin method. For the light-optical study, a universal NU-2 biological microscope was used.

The bulk density (V_v) of the cardiomyocytes, interstitial connective tissue (total of connective tissue cells, fibers, and ground substance), blood vessels (mainly capillaries), and intercellular spaces (the spaces widened with edema between the muscle and connective-tissue cells) was determined by means of a test system [1] at chosen points of the histological sections by the formula described previously [2]. The surface density of the cardiomyocytes (S_v) was determined on the basis of intersection of the muscle cells with lines, using the formula: $S_v = (4j/p \cdot Z)$, where j is the number of intersections, p the number of points (2000), and Z the length of the line (0.02 mm). The absolute mass (M) of the myocardial tissue components was calculated by the equation $M = M_t V_v$, where M_t is the total mass of the myocardium or absolute mass of the heart. The difference between mass and volume in this case lies within the limits of error of measurement. The difference between the specific gravity of the individual structural components of the myocardium does not exceed this value. When determining the absolute mass (volume) and surface area of the structures, contraction of the tissue during dehydration and embedding likewise was disregarded. Since we

were interested not in absolute values, but changes in them, such contraction is not important, but the units in which the results are presented must be regarded as conventional.

The absolute total surface area (S) of the cardiomyocytes was calculated by multiplying the surface density of the cardiomyocytes by the volume of myocardial tissue [7].

The ratio between the numbers of muscle and connective-tissue cells of the left ventricle of the heart was determined by counting 1000 nuclei of these cells in different fields of vision in the test system (endothelial and adventitial cells of blood vessels were disregarded). The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

At the time of their decapitation the rats of the experimental group exhibited severe acrocyanosis, dyspnea, lethargy, untidiness of the hair, and general lack of grooming. At autopsy severe edema of the subcutaneous areolar tissue, hydrothorax, hydropericardium, and ascites were observed. The body weight of the rats receiving rubomycin was significantly lower (Table 1) than in the control (132.6 ± 3.99 and 172.0 ± 4.9 g respectively). The absolute weight of the heart was reduced ($P < 0.05$) from 0.598 ± 0.09 (cardiac index $31.8 \times 10^{-4} \pm 1.7 \times 10^{-4}$) in the control to 0.526 ± 0.029 g (cardiac index $39.6 \times 10^{-4} \pm 2.2 \times 10^{-4}$) in the experiment.

Microscopic study of sections through the myocardium in ordinary and polarized light revealed no focal necrobiotic changes. The absence of glycogen and some widening of the perinuclear zone, which contained no myofibrils, of the cardiomyocytes, and the well-marked intermuscular edema and diffuse coarsening of the myocardial stroma were noted. Electron-microscopic investigation revealed changes in the nuclei and cytoplasmic structures characteristic of a disturbance of plastic processes in all the cardiomyocytes: segregation and fragmentation of the nucleoli, disappearance of glycogen granules, focal degradation and sequestration of elements of the sarcoplasmic reticulum, the formation of numerous secondary lysosomes, and thinning and focal lysis of the myofibrils. In the experimental rats the absolute total mass of the cardiomyocytes was significantly ($P < 0.01$) reduced (0.2480 ± 0.0174 g compared with 0.4026 ± 0.0248 g in the control), but the ratio of the surface density of the cardiomyocytes to their bulk density was increased from 211.984 ± 4.243 cm^{-1} in the control to 331.438 ± 20.890 cm^{-1} in the experiment, evidence of a significant ($P < 0.01$) decrease in diameter of the cardiomyocytes of the rats receiving rubomycin.

The decrease in total mass of the cardiomyocytes was accompanied by an increase in the mass of tissue fluid in the intercellular spaces from 0.0352 ± 0.0027 g in the control to 0.0922 ± 0.0068 g in the experiment ($P < 0.01$) and of the interstitial connective tissue from 0.0660 ± 0.0055 to 0.0922 ± 0.0068 g respectively ($P < 0.05$), whereas the total mass of the vessels (0.0958 ± 0.0044 and 0.0956 ± 0.0064 g respectively) showed no significant change (Figs. 1 and 2).

The decrease in mass of the contractile myocardium of the ventricles and thinning of the muscle fibers under the influence of rubomycin, which specifically inhibits DNA-dependent RNA synthesis in the cardiomyocytes [9], were due mainly to a reduction in the number of myofibrils as a result of their lysis [2, 5, 12-14] and focal degradation of cytoplasmic structures [5]. There are sufficient grounds on the whole for regarding this process as involution of the cytoplasm, connected with the low level of function of the genetic apparatus of the cell [4].

In the description of morphological changes in the myocardium under the influence of anthracycline antibiotics in a number of publications [10, 12, 14] fibrosis of the myocardial stroma with the development of cardiosclerosis was mentioned. In our own experiments, besides an increase in mass of the connective tissue, a significant increase also was found in the number of connective-tissue cells, as is clear from the change in the ratio of the number of nuclei of the muscle and connective-tissue cells in the myocardium of the left ventricle (1:2.4 in the control, 1:3.5 in the experiment).

At the light microscopic level, characteristic findings in the myocardium of the rats on the 5th day after injection of rubomycin were an increase in size of the fibroblast nuclei, collagenization of the stroma, and accumulation of glycosaminoglycans in the inter-

cellular spaces. In their ultrastructure the fibroblasts in the myocardium of the experimental animals differed from the controls by their larger nucleoli, loose arrangement of the concentrations of heterochromatin, and the abundant cytoplasm with well-developed rough cytoplasmic reticulum (Fig. 3a, b). Along the muscle cells and in the intercellular spaces around synthetically active fibroblasts, bundles of collagen fibers were constantly found (Fig. 3c, d). The absence of evidence of segregation and fragmentation in the nucleoli of the cardiac fibroblasts on the 5th day after injection of rubomycin agrees with data obtained by workers [11] who studied the dynamics of anthracycline-specific fluorescence of cardiomyocyte and connective-tissue cell nuclei in tissue culture, and who showed that the antibiotic adriamycin (an analog of rubomycin) is released from fibroblast nuclei toward the end of the 1st day, whereas it is retained in cardiomyocyte nuclei. These same workers studied restoration of the structure of the nucleolus of the nonmuscle cells of the myocardium, which takes place immediately after release of the antibiotic from the cells.

The increase in the fibroblast population in the myocardium is evidently connected with multiplication of local fibroblasts, for anthracycline antibiotics inhibit mitotic activity of bone marrow cells most effectively and for the longest time [9].

Diffuse activation of protein synthesis in fibroblasts and new collagen formation (in the absence of focal necrotic changes in the myocardium inducing this process) is at first sight hard to explain unless involutional and atrophic changes taking place in the cardiomyocytes are taken into account.

In the normal myocardium, just as in other organs of mature organisms, the absolute quantity of intercellular structures in the stroma does not increase because of dynamic equilibrium between desmolytic and desmoplastic processes. Since desmolytic processes are connected with the function of parenchymatous cells [8] it can be tentatively suggested that inhibition of cardiomyocyte functions as a result of exposure to hormonal, toxic, and radiation (acting on genes in particular) factors and chronic circulatory disorders leads to the development of diffuse sclerosis of the stroma of the organ [2, 3].

The results of stereologic analysis of structural components of the myocardium injured by rubomycin indicate a genetic association between plastic insufficiency of the cardiomyocytes and the process of diffuse cardiosclerosis.

LITERATURE CITED

1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973).
2. L. M. Nepomnyashchikh, Pathological Anatomy and Ultrastructure of the Heart [in Russian], Novosibirsk (1981).
3. V. S. Paukov and V. A. Frolov, Elements of a Theory of Pathology of the Heart [in Russian], Moscow (1982).
4. D. S. Sarkisov, Outlines of Structural Principles of Homeostasis [in Russian], Moscow (1977).
5. D. E. Semenov, L. M. Nepomnyashchikh, and V. P. Tumanov, Byull. Éksp. Biol. Med., No. 9, 102 (1982).
6. L. A. Semenova and Yu. G. Tsellarius, Ultrastructure of Heart Muscle Cells in Focal Metabolic Lesions [in Russian], Novosibirsk (1978).
7. Yu. G. Tsellarius and N. K. Eriskovskaya, Byull. Éksp. Biol. Med., No. 6, 627 (1979).
8. Yu. G. Tsellarius and L. A. Semenova, Histopathology of Focal Metabolic Lesions of the Myocardium [in Russian], Novosibirsk (1972).
9. A. Di Marco, Pathol. Biol., 5, 897 (1967).
10. R. S. Jaenke, Cancer Res., 36, 2958 (1976).
11. T. J. Lampidis, L. V. Johnson, and M. Israel, J. Mol. Cell. Cardiol., 13, 913 (1981).
12. F. P. Mettler, D. M. Young, and J. M. Ward, Cancer Res., 37, 2705 (1977).
13. H. M. Olson, D. M. Young, D. J. Prieur, et al., Am. J. Pathol., 77, 439 (1974).
14. J. F. Van Vleet, V. J. Ferrans, and W. E. Weirich, Am. J. Pathol., 99, 13 (1980).