Ultrastructural Cytochemical Analysis of Cardiomyocytes in Young and Old Rats Subjected to General Overheating

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It is shown that regenerative plastic insufficiency develops in cardiomyocytes of young and old rats after a one-time general overheating. Atrophic and necrobiotic alterations of the cardiomyocytes together with a decrease of the heart weight are noted as well. Intracellular alterations of cardiomyocytes of young and old rats are accompanied by a marked decrease of succinate dehydrogenase activity under these conditions. Thereafter its activity rises appreciably in young animals during the course of postheating restitution, while in old rats the activity of this enzyme remains low.

Key Words: *general overheating; cardiomyocyte; ultrastructure and cytochemistry; succinate dehydrogenase activity*

General overheating is a major ecological impact and causes substantial reorganizations in the key morphofunctional systems of the organism [2,3, 10], particularly in the cardiovascular system [9,11, 12]. Under these conditions the nature of the adaptive-compensatory reactions occurring in the myocardium is mainly dictated by the state of cardiomyocytes (CM) and the specific features of intracellular regeneration [4,7]. The ultrastructural alterations of CM after general overheating of the organism, especially during aging, have not been adequately studied. There is still no clear understanding of the correlations between ultrastructural reorganizations and changes in the activities of enzymes which are crucial to the intracellular metabolism of the myocardium.

The aim of the present study was to analyze the special features of intracellular reorganization of CM in young and old Wistar rats during the course of postheating restitution and to perform an electron-cytochemical assessment of succinate dehydrogenase (SDH) activity in the mitochondria.

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MATERIALS AND METHODS

Thirty-six male Wistar rats aged 2 months (9 of which served as a control) and 20 male rats aged 20 months (9 controls) were used in the experiment modeling postheating restitution of the myocardium after a one-time general overheating. The time of exposure to high temperatures was dictated by the survival of the animals and the cutoff point after which large-scale mortality occurred. Young rats spent 45 min in the thermal chamber, whereas the older animals were held for 40 min. The material was sampled for electron-microscopic and electron-cytochemical analysis on the 3rd and 7th day after overheating.

The weight of the heart and body was determined in each experimental group. For the survey electron microscopy, myocardial samples from the left ventricle were fixed in 4% paraformaldehyde,

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postfixed in 1% osmium tetroxide, and, after dehydration, embedded in an Epon-Araldite mixture.

SDH activity was determined in the CM of experimental animals by the electron-cytochemical method with copper ferrocyanide [13]. Postreaction, the samples of the left ventricle myocardium were fixed in 1% osmium tetroxide solution in Millonig buffer at pH 7.4, dehydrated, and embedded in Epon-Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM 100S under an accelerating voltage of 80 kV.

Quantitative analysis of the product of the cytochemical reaction was performed by counting the number of grains of copper ferrocyanide in the mitochondria per 100 μ^2 of mitochondrial compartment. For this purpose the area of mitochondrial profiles was estimated on negatives according to the planimetric method [1] using a square grid with a known distance between lines. Representative regions of CM were photographed at an initial magnification of 15,000 and the final enlargement of negatives upon projection was 45,000. For analysis the total area of the mitochondrial compartment was no less than 200 μ^2 for each group. The results of the calculations were expressed as the mean value and compared according to the Student test.

RESULTS

One-trial general overheating caused a modest drop of the body weight of young (by 12%) and old (by 5%) animals on the 7th day after treatment. At the same time the weight of the heart of young rats reliably decreased (by 24%, $p<0.05$) and of old animals by 13% beginning from the 3rd day after overheating (Fig. 1).

An ultrastructural analysis of the CM revealed insignificant changes in young rats on the 3rd day after overheating. Small foci of lysis of sarcoplasmic matrix and myofibrils and in some cases of mitochondrial destruction (Fig. 2, *a*) were found in the CM. Cardiomyocytes with contracture damage of myofibrils were noted as well. Lytic changes manifested in local lysis of myofibrillar bundles and sarcoplasm and focal destruction of mitochondria mainly in the perinuclear (Fig. 2, *b*) and subsarcolemmal regions were found in the majority of cardiomyocytes in old animals on the 3rd day of the experiment. The number of cells with contracture lesions of myofibrils was greater than in young animals.

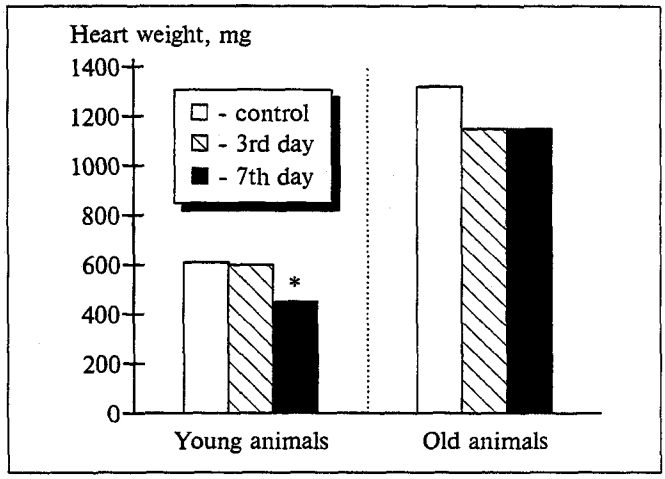

Fig. 1. Changes of the heart weight of young and old rats after a one-time general overheating. *$p<0.05$

The most significant ultrastuctural damage to CM was found in young and old animals on the 7th day of postheating restitution. The heterogeneity of the damage was best demonstrated in the CM of young animals. Destruction and lysis of organelles were boosted in the majority of cells. Focal lysis of myofibrils (Fig. 2, *c*), partial necrosis of thec sarcoplasm (predominantly in the perinuclear region), and a large number of myelinlike structures (Fig. 2, *d*) were noted in virtually all CM. Fragmentation of the nucleoli and a predominance of the fibrillar component in them were frequently shown in such cells. A marked widening of the sarcoplasmic vesicles and tubules of the T-system should be particularly noted. Sequestration of glycogen was noted in old animals. Numerous polysomes appeared in some CM of young rats at this time of the experiment, attesting to a resumption of

TABLE 1. Activity of SDH in Rat CM Mitochondria after a One-Time General Overheating ($M \pm m$)

Age of animals	Time after treatment, days	Number of granules of reaction product per 100 μ^2 of mitochondrion area
2 months	Control	134.0±3.6
	3rd	42.8±4.8***
	7th	195.5±10.5**
20 months	Control	64.7±3.8
	3rd	34.0±11.0*
	7th	30.5±9.5*

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ in comparison with the control.

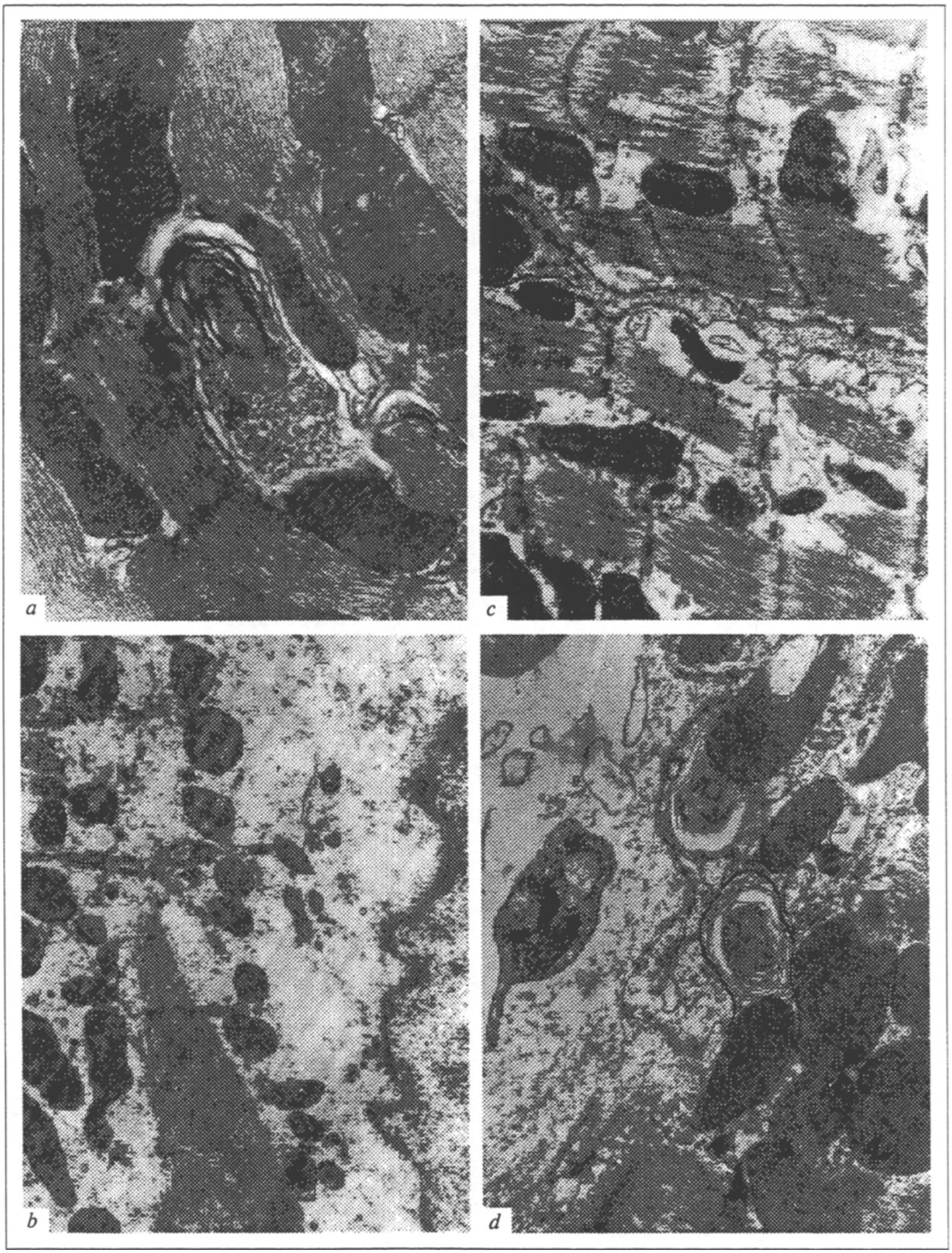

Fig. 2. Ultrastructural changes of CM in young and old rats after a one-time general overheating. *a*) focal destruction and autophagocytosis of mitochondria in the CM of a young rat on the 3rd day after overheating. ×15,000; *b*) focal lysis of myofibrillar bundles and "devastation" of perinuclear region in CM of an old rat on the 3rd day after overheating. ×8000; *c*) focal lysis of myofibrils and marked lysis of sarcoplasmic matrix in CM of an old rat on the 7th day after overheating. ×8000; *d*) myelinlike structures in subsarcolemmal zone of CM of an old rat on the 7th day after overheating. ×10,000.

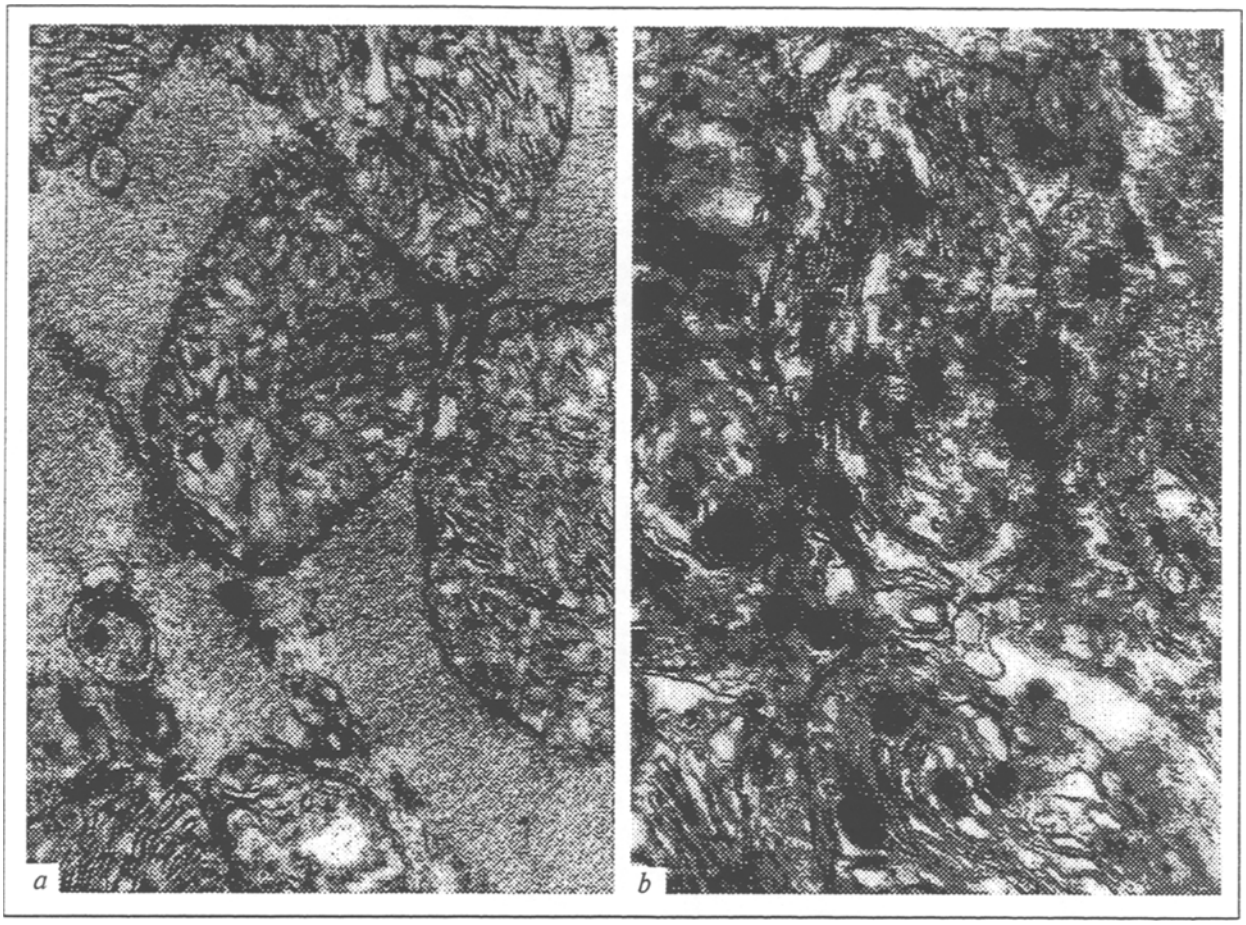

Fig. 3. Electron-cytochemical demonstration of SDH activity in CM mitochondria of young rats after a one-time general overheating. ×20,000. 3rd (*a*) and 7th (*b*) day after overheating.

intracellular regeneration. Polysomes and cisternae of the granular sarcoplasmic reticulum were practically absent in the CM of the old rats.

The revealed ultrastructural changes of CM, among which fragmentation of nucleoli and the disappearance of their granular component dominated, along with focal destruction and lysis of organelles as well as a boost of autophagocytosis, testify to the development of regenerative plastic insufficiency of cardiomyocytes after general overheating of the organism [5]. The nature of these alterations, particularly the fragmentation of the nucleoli, reflects a decrease not only of plastic, but also of energy metabolism in the CM [14].

Electron-cytochemical determination of SDH activity in the CM of young rats revealed a decline on the 3rd day of postheating restitution: the number of granules of reaction product (per 100 μ^2 of the mitochondrial compartment) was 3 times smaller than in the control (Table 1). After 7 days of overheating SDH activity rose markedly in the CM mitochondria and exceeded (by 46%) the control value. It should be noted that on the 3rd day after overheating granules of reaction product were not found in all mitochondria (Fig. 3, *a*), whereas on the 7th day numerous granules were present in practically all mitochondria (Fig. 3, *b*).

In old rats SDH activity was 50% lower on the 3rd day after exposure to high temperatures and remained at this level until the end of the experiment (Table 1). It should be noted that SDH activity in intact old rats was only half that in the intact young animals, as was previously demonstrated by us [8].

The decrease of SDH activity in the mitochondria of CM after the thermal impact probably stems from the change of the phase state of lipids in the cell membranes, followed by a boost of lipid peroxidation and disturbance in the lipid-protein interactions [3]. These disorders are preserved after the cessation of high temperature action, leading to destabilization of the membrane-bound enzymes and, indirectly, to the inhibition of SDH activity in the CM mitochondria [6]. At later times (7 days) after cessation of thermal action a rise of SDH activity is evidence of stabilization of the energy processes in the myocardium of young animals, despite the lytic damage to the CM. It should be

noted that in young animals such stabilization occurred simultaneously with the boosting of intracellular regeneration in the CM. In old animals the ultrastructural features of intracellular regeneration were not found at the studied times of postheating restitution, attesting to a more prolonged action of general overheating on metabolic processes in the myocardium in aging.

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