ULTRASTRUCTURAL MECHANISMS OF MYOCARDIAL ATROPHY IN ALBINO RATS DURING STARVATION

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The basis of the atrophic changes arising in the myocardium during realization of genetic programs (differentiation, metamorphosis, aging) and in pathological situations (starvation, malnutrition, chronic poisoning, and circulatory disturbances), is reduction or suppression of synthesis of structural and tissue-specific proteins in muscle cells [4, 9]. In the study of the morphological principles governing development of myocardial atrophy of varied genesis, a model of atrophy of cardiomyocytes due to intracellular disturbances, preventing adequate plastic metabolism, by selective depression of DNA-dependent RNA synthesis [9], has been investigated in detail. The question of cellular and tissue reactions of the myocardium to partial or complete starvation, leading to myocardial atrophy as a result of a general deficiency of plastic materials, has received less study [3, 5, 7].

The aim of this investigation was to study the myocardium of rats during the most complete possible starvation, using a combined morphological, morphometric, and stereologic analysis.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male Wistar rats weighing initially 196.1 ± 2.4 g. Since the average 100% survival period of rats during complete starvation, with free access to water, is 6-7 days [3], 10 rats kept in individual cages with wire mesh floor were subjected to total starvation, but with unrestricted access to water, for 6 days. Intact rats of the control group were kept on the normal animal house diet. All the animals were weighed daily in the morning. The animals were removed from the experiment by decapitation under superficial chloroform anesthesia, the cadavers were autopsied, and the hearts removed and immersed in cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 8.0). The atria and great vessels were carefully removed after 1 h, the ventricular myocardium was weighed, and the heart was divided in the frontal plane from the base to the apex into two halves. One half was weighed and kept in fixative for 14 days for subsequent alkaline dissociation and quantitative analysis of the cardiomyocyte population [1]; the papillary muscle of the left ventricle was excised from the other half of the heart for electron microscopy [12]; the remaining material was embedded in paraffin wax. Paraffin sections were stained with hematoxylin and eosin and also by a combined histochemical method: colloidal iron–PAS–hematoxylin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the Tesla BS 500 electron microscope. Quantitative parameters of stereologic analysis of the tissue components of the myocardium and of cardiomyocyte ultrastructure [2] and data on the population of muscle cells in the ventricular myocardium were analyzed by parametric statistical methods, using Student's test [10].

EXPERIMENTAL RESULTS

Toward the end of the 6th day of starvation the animals were in a precomatose state: apathetic, motionless, and responding weakly to external stimulation. The body weight of

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TABLE 1. Results of Morphometric Investigation of Heart of Albino Rats after Complete Alimentary Starvation (M ± m)

Parameter	Intact animals	Animals starved for 6 days
Number of animals	10	10
Body weight, g	189,4±2,6	142,0±3,2***
Weight of ventricles of heart, mg	598,5±16,1	473,6±21,6***
Cardiac index, mg/g	3,16±0,089	3,34±0,14
Concn. of cardiomyocyte nuclei in 1 mg tissue, $\cdot 10^3$	27,82±0,47	30,95±1,97
Number of cardiomyocyte nuclei in heart, $\cdot 10^6$	16,65±0,52	14,40±0,62**
Number of cardiomyocytes in heart, $\cdot 10^6$	8,59±0,26	7,42±0,35*
Number of cardiomyocytes in 1000 cells		
Mononuclear	97,8±2,8	93,0±6,4
Binuclear	877,1±3,7	883,7±6,7
Trinuclear	11,6±1,3	17,1±2,5
Multinuclear	16,3±2,3	11,3±1,8

Legend. *p < 0.05; **p < 0.02; ***p < 0.01 compared with control.

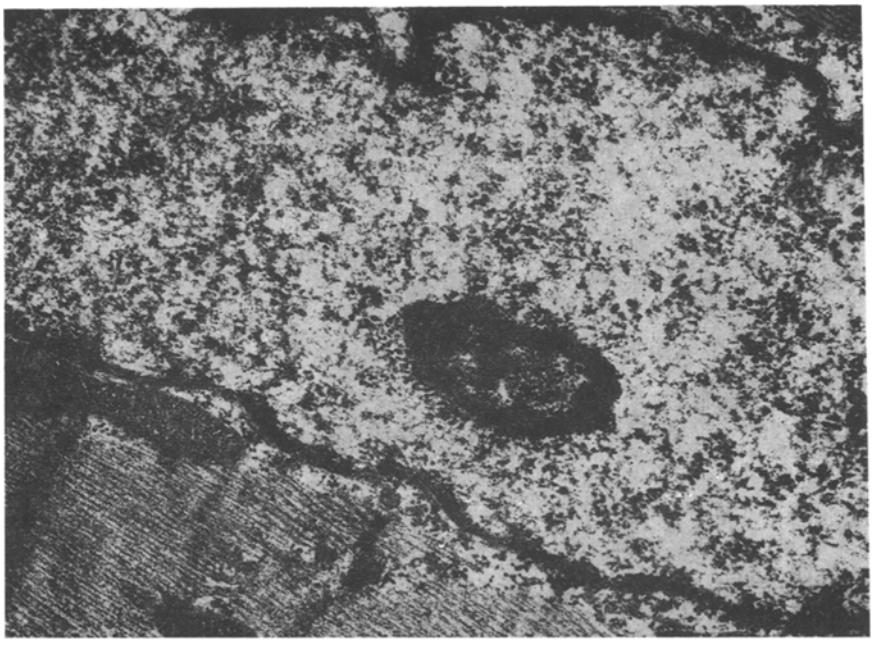

Fig. 1. Simplified structure of nucleolus in cardiomyocyte of rat after 6 days of total starvation: fibrillar structure of nucleolonema and condensed perinucleolar chromatin; absence of any significant granular component (64,000×).

the experimental rats fell progressively and by the end of the 6th day it was 70% of its initial value (142.0 ± 3.2 g). The body weight of the control rats in this period remained substantially unchanged.

At autopsy on the cadavers of the starving animals no signs of circulatory failure could be found. The weight of the ventricular myocardium was reduced on average by 20.7% ($p < 0.01$). The cardiac index was 3.16 ± 0.089 in the control and 3.37 ± 0.14 in the experiment, evidence of a greater reduction of body weight than of the weight of the heart.

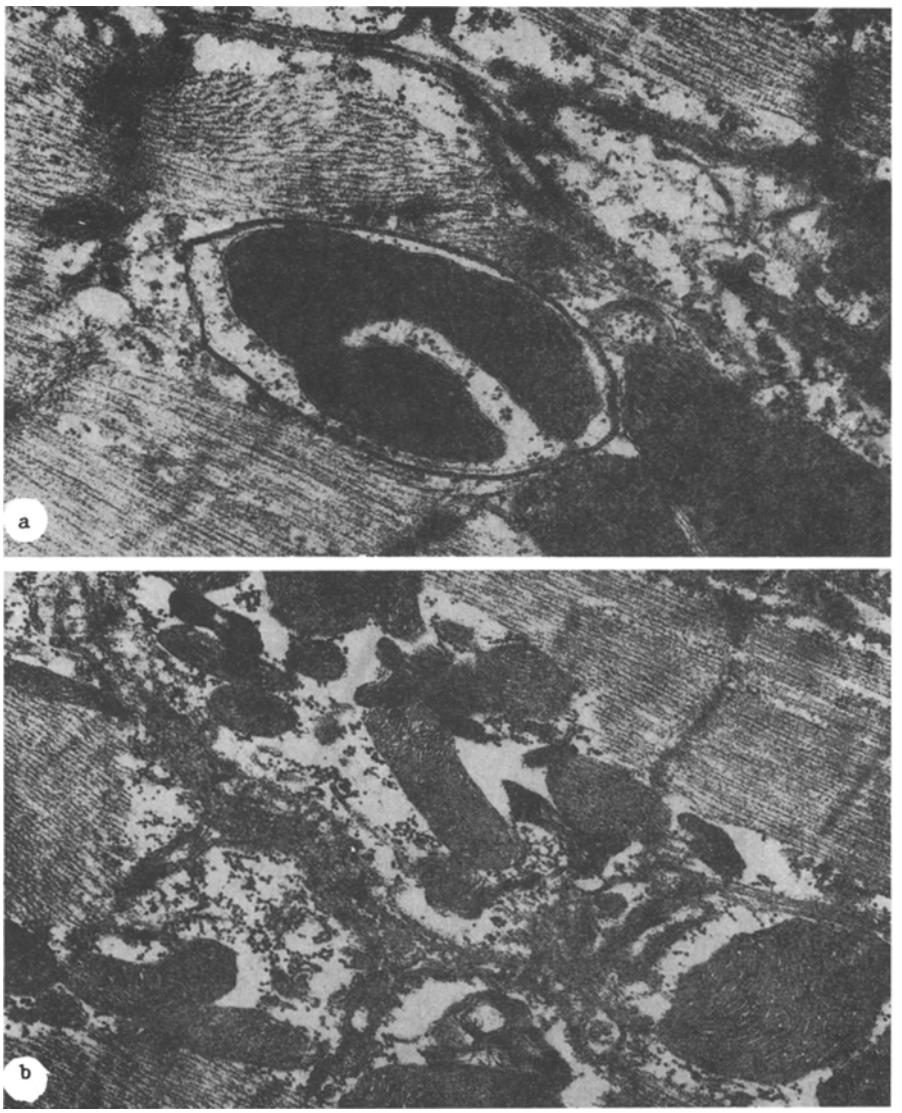

Fig. 2. Ultrastructural manifestations of focal degradation of sarcoplasm of cardiomyocytes after total starvation. a) Residual bodies and autophagosomes in subsarcolemmal zones of two neighboring cardiomyocytes (56,000×); b) initial stage of autophagosome formation. Region of cytoplasm with mitochondria and glycogen surrounded by a stratified membrane (72,000×).

Microscopic investigation of the myocardium of the starving rats showed that it differed from the control in the absence of glycogen granules in the cytoplasm of the cardiomyocytes and the considerable intermuscular edema. No appreciable reduction of the thickness of the muscle fibers of dystrophic, necrotic, and sclerotic changes could be found.

Ultrastructural investigation of the cardiomyocytes revealed definite signs of reduction of structural protein synthesis: a decrease in size of the nucleoli and absence of their granular component (Fig. 1), a small number of free ribosomes and polysomes, and absence of large β-glycogen particles (simple atrophy). Patterns of reduction of intracellular organelles, in the form of autophagosomes containing undifferentiated components of the sarcoplasm and mitochondria (Fig. 2) were observed in the cytoplasm of most cardiomyocytes. The structural organization of the myofibrils was preserved, but the myofilaments in them showed focal lysis or reduced density. The mitochondria were enlarged but of the usual structure.

Fibroblasts of the myocardial stroma were in a resting state: the chromatin in the nuclei formed large masses, nucleoli were discovered with difficulty, the cytoplasm formed fine processes, the rough endoplasmic reticulum was poorly developed, and there were few mitochondria. Destruction and death of single endotheliocytes could be observed occasionally in the capillaries (Fig. 3).

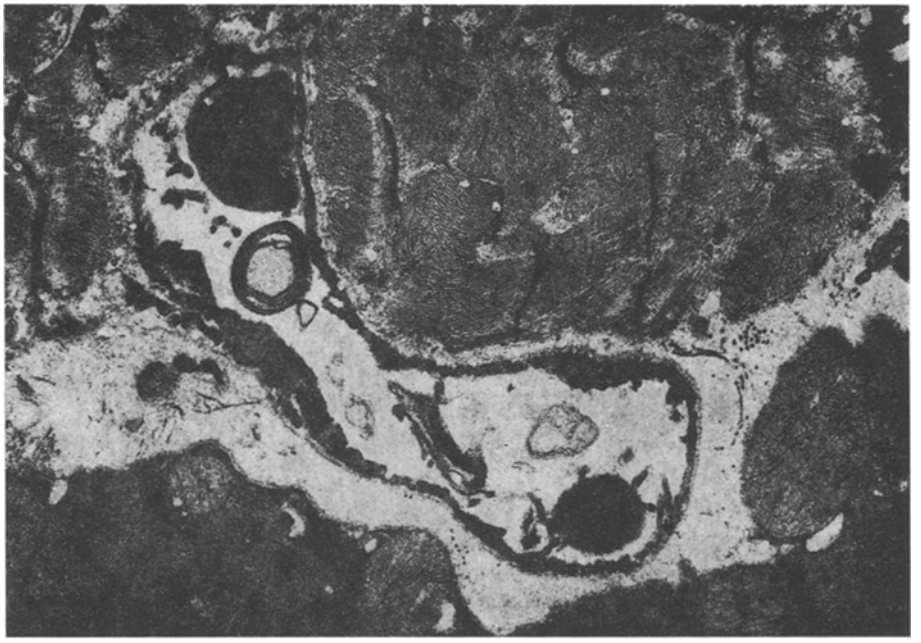

Fig. 3. Ultrastructure of capillary in rat myocardium after 5 days of total starvation: destruction of endotheliocytes, condensation and homogenization of cytoplasm, pycnosis of nucleus, artificial myelin-like structures in capillary lumen (32,000×).

Comparative quantitative analysis of the cardiomyocyte population in the ventricles of the heart of the control and experimental animals revealed a significant decrease by 13.6% in the starving rats (Table 1), with no change in the ratio between the numbers of cells with different numbers of nuclei, an indication of the planned elimination of a certain number of muscle cells from the myocardium (numerical atrophy). The reduction in the number of cardiomyocytes in the absence of necrotic lesion can be explained by activation of a mechanism of apoptosis, which plays the principal role in the maintenance of homeostasis of several organs and tissues [14]. Until recently, the participation of apoptosis in myocardial pathology of adult mammals has not been contemplated. This phenomenon was described for the first time by the present writers in anthracycline-induced cardiomyopathy under the name of the cardiomyocyte "disappearance" phenomenon [8].

According to the results of stereologic analysis (Fig. 4) reduction of the absolute total weight of the muscle fibers (from 418.2 ± 13.3 to 296.5 ± 13.7 mg is the result of both numerical and simple atrophy: the contribution of the latter was an additional decrease by 15.5% of the initial weight of the muscle tissue ($p < 0.01$). In simple atrophy the absolute total weight of the myofibrils was reduced by 15.1% ($p < 0.01$), whereas the total weight of the mitochondria, by a similar calculation, was reduced by 23.4% ($p < 0.01$). Together with autophagy of the excess number of mitochondria, there was an additional reduction of 29.1% ($p < 0.01$) in their absolute total surface area on account of their enlargement.

The changes described above led to comparatively small changes in the bulk density of the tissue components of the myocardium and of the intracellular structures of the cardiomyocytes. For instance, the relative volume of the muscle fibers was reduced by only 10.4% ($p < 0.01$), the volume of the tissue fluid was almost doubled ($p < 0.01$), and the volume of the vessels and connective tissue was unchanged compared with the control. In the cardiomyocytes of the starving rats, myofibrils accounted for 50% of the total volume, as in the normal animal, the contribution of mitochondria was reduced by 10.7% ($p < 0.01$), and that of the sarcoplasm was increased 26% ($p < 0.05$). This change in the relative volumes of the myocardial structures evidently did not prevent maintenance of an adequate blood supply under conditions of sharply depressed metabolism.

The results show that during the most complete possible starvation the absolute combined weight of the myocardial muscle fibers fell parallel with the decrease in the animals' body weight, on account of a proportional reduction of the absolute total weight of the myofibrils in the cardiomyocytes (Table 1).

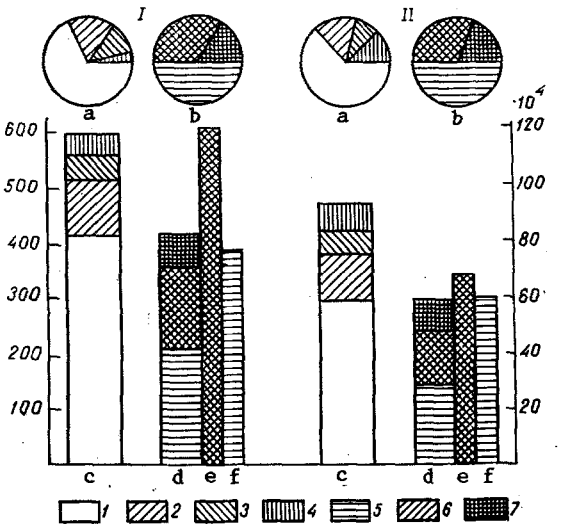

Fig. 4. Ratio between stereologic parameters of structural components of the myocardium and cardiomyocyte ultrastructure after 6 days of total starvation. I) Control; II) experiment. 1) Cardiomyocytes; 2) vessels; 3) connective tissue; 4) tissue fluid; 5) myofibrils; 6) mitochondria; 7) cytoplasmic matrix. a) Relative total weight of tissue components of myocardium; b) relative total weight of ultrastructural components of cardiomyocytes. Ordinate: on left (in mg), absolute total weight of tissue components of myocardium (c) and absolute total weight of ultrastructural components of cardiomyocytes (d); on right (in mm^2), total surface area of mitochondria (e) and total surface area of myofibrils (f).

Thus during the development of myocardial atrophy under the conditions of this investigation, as also in hypertrophy of the heart [6, 11], the basic controlled parameters are the weight of the myofibrils and the weight and surface area of the mitochondria.

The increase of 26% ($p < 0.05$) in the relative volume of the cytoplasm in the cardiomyocytes of the starving rats evidently also plays a role in homeostasis, by preventing an excessive decrease in cell volume. The adaptive importance of the combined participation of the two types of atrophy in the reduction in the weight of the myocardial muscle fibers proportionally to the reduction in body weight probably lies in the unprofitable nature of its realization by only one of these ways. For instance, in the case of numerical atrophy alone, the cardiomyocyte population would have to fall to a critical level [8]. Since cardiomyocytes are highly differentiated postmitotic cells their loss cannot be made good, and to restore the body weight after the end of starvation, this would have led to the development of fatal heart failure. Meanwhile simple atrophy alone by the end of starvation would have inevitably disturbed the contractile function of the cardiomyocytes due to the excessively great deviations of the surface to volume ratios of the intracellular organelles.

The absolute total weight and the fraction of intramural vessels depend directly on the weight and volume of the myocardium as a whole, i.e., they are under remote control, effected through the constancy of the weight/volume ratios of the organs and tissues during starvation. The increase in the relative volume of intracellular fluid in the myocardium, observed in total starvation [13] and other pathological situations, may perhaps help to maintain its blood supply within optimal limits.

Reduction of the absolute total weight of the vessels by 16% during starvation ($p < 0.02$), judging from the results of the ultrastructural investigation, takes place on account of death of the endotheliocytes (Fig. 3).

Unlike blood vessels, the state of the connective tissue is under dynamic control by the level of metabolism and function of the cardiomyocytes [4, 9]. The absence of proliferation and of an increase in synthetic activity of the fibroblasts in the myocardium of the starving rats is a morphological reflection of the state of compensation of cardiac activity.

In conclusion it must be emphasized that the possibility of detecting two mechanisms of myocardial atrophy during adaptation to total starvation, demonstrated in this investigation, can be used to study the myocardium and other organs under conditions of malnutrition of varied genesis.

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