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Effect of Repeated Complete Starvation on Quantitative Parameters of Rat Cardiomyocytes

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Sixty-four male Wistar rats were subjected to repeated complete starvation with free access to water. Each starvation period lasted 6 days and was followed by a 7-day recovery period. The concentrationof cardiomyocytes (CMC) and their total number in the left ventricle (LV) were measured after alkaline dissociation of the myocardium. The volume density of muscle fibers and their absolute total mass in the LV were determined by stereological analysis. It is shown that after 6 successive starvation periods the proliferative potential of the CMC population is reduced.

Key Words: *starvation period; rat myocardium; cardiomyocytes, cell population*

Starvation is one of the factors reducing the synthesis of structural proteins in animal tissues and organs with a parallel decrease in the mass of parenchymatous organs. The latter phenomenon has been less studied than the body mass loss. In numerous experiments on albino mice and rats it was demonstrated that after starvation is discontinued the animals grow in accordance with their body weight [12]. However, in the case of repeated starvation the compensatory growth slows down, and after several starvation periods (5-7 for rats) free access to food does not provide for the full recovery of body weight, and the animals die of unknown causes [2].

It is generally accepted that the decrease in the heart mass is determined by the total decrease in the mass of the parenchymatous cells; however, the cellular mechanisms underlying this decrease remain unclear, namely, which event: the decrease in the number of CMC or the decrease in their volume, predominates.

Modeled fast has often been used for morphological studies of the myocardium [3-7,10]. The objective of this study was to investigate the dynamics of the number of the parenchymatous cells of the heart during repeated starvation periods and to find out which processes are responsible for the compensatory growth of the mass of the myocardial muscular component during the recovery periods after starvation.

MATERIALS AND METHODS

The experiments were performed on 64 male Wistar rats. The animals were assigned to 8 equal groups (n=8): 2 control and 6 experimental. The rats of the experimental groups were subjected to a 6-day total starvation with free access to water [7]. The animals were kept in individual wire bottom cages with to prevent cannibalism and co-

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TABLE 1. Dynamics of Body Mass of Albino Rats after 6 Periods of Total Starvation ($M \pm m$)

Group No.	Group characteristics	Body mass, g		Changes in body mass
		initial	finall	
1	Control I	204±3.1	204±3.1	0
2	1 starvation period	203±3.1	142±4.0	−30%
3	1 starvation period + recovery	222±1.3	214±6.5	−3.6%
4	3 starvation periods + 2 recoveries	218±2.9	193±3.5	−12%
5	3 starvation periods + 3 recoveries	211±5.7	239±8.8	+13%
6	6 starvation periods + 5 recoveries	221±2.1	189±8.9	−14%
7	6 starvation period + 6 recoveries	198±2.5	268±7.6	+35%
8	Control II	218±9.1	355±11.1	+63%

prophagy. Each recovery period lasted 7 days, the amount of time required for the body weight normalization after a single starvation. The experiments were performed according to the following scheme: group I intact animals served as controls; group 2 animals were starved for 6 days and were sacrificed together with the controls; group 3 animals were starved for 6 days and were sacrificed after a 7-day recovery period; group 4 animals underwent 3 starvation periods with 2 recovery periods; group 5 animals had 3 repeated starvation periods with 3 recovery periods, group 6 went through 6 starvation periods with 5 recovery periods; group 7 had 6 starvation periods with 6 recovery periods, and group 8 rats served as a second control group (these rats were sacrificed together with group 7 rats, i.e., after 12 weeks of the experiment, Table 1).

After the animals had been sacrificed, the mass of the body, heart, and the LV myocardium was determined. Alkaline dissociation of the myocardium was performed after a 14-day fixation with 10% paraformaldehyde in 0.1 M phosphate buffer (pH 8.0) [1], and the concentration of nuclei, and share of mono-, bi-, tri-, and polynuclear CMC per 1000 cells were determined, as well as the CMC concentration and the total number of CMC in the LV.

The methods of light and electron microscopy were described previously [7].

Stereological analysis was performed on histological sections stained with PAS-hematoxylin; the volume density of muscle fibers (V_v) and absolute total mass of myofibrils were determined [9]. The mass of 1 mln. CMC was determined by dividing the absolute total mass of myofibrils by the total number of CMC. The data were analyzed using Student's t test.

RESULTS

A considerable (30%) body weight loss occurred in rats after a 6-day single starvation (Table 1). Myocardial microscopy revealed the absence of glycogen granules from the CMC cytoplasm and a considerable interstitial edema. Light microscopy revealed no dystrophic, necrotic, or sclerotic changes in CMC. Electron microscopy revealed signs of reduced synthesis of structural proteins and the changes in capillaries and stromal cells [6].

Repeated total starvation with free access to water did not cause the development of dystrophic,

TABLE 2. Dynamics of Cardiac Structures of Albino Rats after 6 Periods of Total Starvation ($M \pm m$)

Group No.	Mass of heart	Mass of LV	Volume density of CMC	Mass of LV CMC
1	675±28.6	493±32.1	0.655±0.013	322±20.5
2	469±16.9	375±17.1	0.618±0.008	232±10.4
3	569±22.2	486±20.6	0.647±0.014	315±14.5
4	562±11.0	470±13.0	0.709±0.012	333±8.9
5	642±14.3	530±11.1	0.700±0.009	371±9.4
6	550±19.2	470±20.9	0.709±0.015	332±12.4
7	671±12.5	551±15.5	0.710±0.017	391±10.8
8	818±15.8	680±28.6	0.693±0.009	445±23.0

TABLE 3. Dynamics of the LV CMC Population in Albino Rats after 6 Periods of Total Starvation ($M \pm m$)

Group No.	Concentration of CMC nuclei, thous./mg	Concentration of CMC, thous./mg	Number of CMC in LV, mln.	Mass of 1 mln. CMC, mg
1	30.71±1.09	15.58±0.55	7.75±0.66	42.31±1.78
2	31.30±2.45	16.14±1.87	5.94±0.31	39.91±2.99
3	30.28±1.21	15.74±0.68	7.68±0.53	40.98±2.08
4	30.00±2.15	15.40±1.23	7.24±0.64	47.80±2.59
5	30.00±2.24	15.69±1.20	8.26±0.55	46.34±2.49
6	31.81±1.29	14.87±0.72	7.81±0.57	43.46±1.55
7	25.51±0.92	14.03±0.49	7.36±0.30	53.66±2.38
8	31.22±0.89	16.27±0.50	11.05±0.50	46.01±1.40

necrotic, or atrophic alterations at the light microscopy level. When viewed under the light microscope, the myocardium of control and experimental animals had almost the same appearance.

After a single total fasting (Table 2) the mass of the LV myocardium mass decreased 24% with a simultaneous drop of the total mass (28%) and number of CMC (23%) in the LV (Table 3). Comparison of these changes with the CMC concentration, which was equal in the control and experimental animals, showed that the decrease in the mass of muscle tissue is predominantly due to the elimination of CMC accompanied by a slight decrease (6%) in the average mass of one CMC.

The morphometric parameters returned to the initial level after a 7-day recovery period during which the animals received a balanced diet. After 3 complete starvation periods with 2 recovery periods the body weight loss was 12% of the initial weight. There was an insignificant decrease in the mass (5%) of the LV and in the number of CMC (6%) against the background of an increased (by 8%) volume density of CMC compared with the first control group. A 13% increase in the mean CMC mass was observed in group 4 animals.

After 3 starvation periods with 3 recovery periods the body weight of rats increased 13% compared with the initial value. The mass of the LV and of the LV muscle cells increased 7.5 and 15%, respectively, compared with the first control group; the number of CMC increased 6.5% and the mass of one CMC increased 9.5%.

After 6 starvation periods and 5 recovery periods (group 6) the body weigh loss was 14% compared with the initial value, which is consistent with the body weight loss after 3 starvation periods with 2 recovery periods (group 4). The mass of the heart and LV and the total mass of the LV CMC in group 6 rats were the same as in group 4 rats. The concentration and the number of CMC in the LV did not differ significantly in comparison with the first control group, but were lower (by 9 and 29%, respectively) than in the 8th control group.

After 6 starvation periods and 6 recovery periods (group 7) body weight increased 35%, while in the 8th control group body mass increased 63%. The compensatory growth of the mass of muscle tissue differed from that observed in the recovery period after the 3rd starvation period. First, the number of CMC in the LV during recovery after the 6th starvation period did not change compared with the 6th starvation period (Table 3), i.e., the increase in the muscle tissue mass for the first time appeared not to be related to CMC proliferation. Second, a statistically significant inversely proportional decrease in the concentration of CMC nuclei was observed during the growth of the mass of CMC in the LV.

Thus, after 6 starvation periods the proliferative potential of the LV CMC population proved to be exhausted, and the compensatory growth of the mass of muscle tissue dictated by the regularities of allometric growth of the heart was realized via the universal process, that is, CMC hypertrophy: the mean mass of 1 mln. CMC after the 6th recovery increased 23-27% compared with the 6th starvation period and the 1st control and was 17% higher than the age norm (the 2nd control).

After the 78-day experimental period the body weight of experimental and animals had increased 35%, while in intact animals the increase was 63%, i.e., the growth rate of starving animals was lowered almost 2-fold. In the control animals, the growth of the myocardium mass was predominantly due to the increase in CMC population (42%), whereas in the experimental animals the mean mass of individual CMC increased after the proliferative ability of the CMC population had been exhausted.

The mechanism of CMC elimination is not open to question: it is the phenomenon of "disappearance" [8] or programmed cell death [11]. This conclusion is supported by the evidence that there were no morphological indications of random CMC death via necrosis or the consequences of such death manifested as focal atherosclerosis.

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