It should be pointed out that the type of PAG neurons as defined by Ramon-Moliner [13] corresponds to the isodendritic type of neurons forming the "brain core." This is the simplest type of neurons in the CNS and it is characterized by straight, long dendrites with few branches, which gave rise to nerve cells with a complex dendritic pattern. Our preliminary electron-microscopic studies have shown that PAG contains pectiniform synapses such as are described for phylogenetically old brain formations.

It can thus be concluded from these results that there are direct descending connections between the CM–Pf complex and the lateral nucleus of PAG, and data in the literature indicate the presence of ascending projections of PAG to that thalamic structure, thus confirming the general principle of two-way connections for brain structures.

Considering data in the literature showing that neurons of CM–Pf and PAG respond to nociceptive stimuli and that these formations received projections or collaterals from the paleo-spino-thalamic bundle, it can be suggested that the brain structures which we have investigated, located in the "brain core," are directly concerned with the transmission and reception of slow, diffuse pain, on account of which the patient most frequently seeks medical advice.

## LITERATURE CITED


1. T. A. Leontovich, in: Structure and Function of the Reticular Formation and Its Place in the System of Analyzers, S. A. Sarkisov, ed. [in Russian], Moscow (1959), pp. 91-126.
2. T. A. Leontovich, Neuronal Organization of Subcortical Forebrain Formations [in Russian], Moscow (1978).
3. H. Chang, Sci. Sin., 16, 26 (1973).
4. B. L. Hamilton, J. Comp. Neurol., 149, 1 (1973).
5. B. L. Hamilton, J. Comp. Neurol., 152, 45 (1973).
6. D. L. Marburg, Int. J. Neurosci., 5, 153 (1973).
7. R. Simantov, M. T. Kupar, G. W. Pasternak, et al., Brain Res., 106, 189 (1976).


# ULTRASTRUCTURAL STEREOLOGIC STUDY OF CARDIAC MYOCYTES IN MYOCARDIAL ATROPHY


L. M. Nepomnyashchikh and L. V. Kolesnikova



UDC 616.127-007.23-092.9-02:616.393]

KEY WORDS: stereology; myocardial atrophy; total starvation; ultrastructure of cardiac myocytes.


To stimulate general pathological processes or the action of extremal factors, starvation is frequently used [4-6, 8]. In the investigation described below a quantitative morphological study was made of the principal ultrastructures of the cardiomyocytes of the left ventricular myocardium of rats in the course of total starvation.

## EXPERIMENTAL METHOD

Experiments were carried out on 19 sexually mature Wistar rats (females) weighing initially 237.1 ± 3.3 g. The experimental rats were divided into three groups and totally deprived of food (but allowed water ad libitum): the animals of group 1 were starved for 2 days, those of group 2 for 5 days, those of group 3 for 10 days. Control animals were given a balanced diet of adequate amount. The experimental and control animals were weighed and decapitated at the end of the experiment. After rapid removal of the heart from the chest and determination of the absolute weight of the heart, the relative weight of the organ was calculated.


Laboratory of Pathological Anatomy and Histocytometry, Department of General and Clinical Pathology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.)


Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 7, pp. 107-111, July, 1980. Original article submitted November 28, 1979.




0007-4888/80/8907-0994$07.50

TABLE 1. Weight of Heart and Diameter of Cardiac Myocytes of Rats Exposed to Total Starvation (M ± m)

| Parameter | Control (4) | Duration of starvation, days | | |
|---|---|---|---|---|
| | | 2 (5) | 5 (5) | 10 (4) |
| Body weight at beginning of experiment, g | 236,00±9,89 | 243,20±5,64 | 232,80±5,82 | 228,50±7,37 |
| Body weight at end of experiment, g | 237,15±8,23 | 207,80±5,73† | 188,80±7,55† | 138,50±3,18† |
| Absolute weight of heart, mg | 617,75±22,96 | 739,60±39,5* | 599,00±24,1 | 453,50±9,35† |
| Relative weight of heart per gram body weight, mg | 2,630±0,203 | 3,549±0,09† | 3,176±0,145 | 3,275±0,047* |
| Diameter of cardiac myocytes, μ | 14,14±0,31 | 14,53±0,47 | 10,74±0,20* | 10,45±0,43 |

Legend. Number of animals in parentheses; *) differences significant at P < 0.05 level; †) differences significant at P < 0.01 level compared with control.

TABLE 2. Results of Stereologic Investigations of Ultrastructures of Cardiomyocytes of Papillary Muscle in Rats Exposed to Total Starvation (M ± m)

| Component | Parameter | Control | Duration of starvation, days | | |
|---|---|---|---|---|---|
| | | | 2 (5) | 5 (5) | 10 (4) |
| Myofibrils | $V_{v_{mf}}$, $mm^3/cm^3$ | 497,9±8,3 | 550,5±14,4* | 572,2±25,6 | 623,2±8,5† |
| | $S_{v_{mf}}$, $m^2/cm^3$ | 1,304±0,099 | 1,269±0,082 | 1,670±0,259 | 0.772±0,040* |
| Mitochondria | $V_{v_{mc}}$, $mm^3/cm^3$ | 317,9±4,7 | 272,8±11,5* | 236,2±14,1* | 241,7±2,9† |
| | $S_{v_{mc}}$, $m^2/cm^3$ | 1,557±0,006 | 1,459±0,055 | 1,408±0,116 | 0,862±0.065† |
| T system | $V_{v_{ts}}$, $mm^3/cm^3$ | 21,8±1,7 | 21,2±1,0 | 21,1±0,7 | 20,4±0,2 |
| | $S_{v_{ts}}$, $m^2/cm^3$ | 0,398±0,013 | 0,260±0,072* | 0,443±0,032 | 0,387±0,021 |
| Sarcoplasmic reticulum | $V_{v_{sr}}$, $mm^3/cm^3$ | 11,5±1,3 | 9,8±0,5* | 9,8±0,9 | 11,0±0,9 |
| | $S_{v_{sr}}$, $m^2/cm^3$ | 0,250±0,013 | 0,197±0,014 | 0,292±0,018 | 0,273±0,043 |
| Remaining structures of cell | $V_{v_{rem}}$, $mm^3/cm^3$ | 150,9±5,3 | 145,7±2,7 | 160,7±16,0 | 103,7±10,0* |
| Ratio between bulk densities of mitochondria and myofibrils | $V_{v_{mc}}/V_{v_{mf}}$ | 0,64±0,02 | 0,50±0,03* | 0,42±0,04* | 0,39±0,01† |
| Ratio between bulk densities of sarcoplasmic reticulum and myofibrils | $V_{v_{sr}}/V_{v_{mf}}$ | 0,023±0,007 | 0,018±0,001* | 0,017±0,002 | 0,018±0,002 |
| Ratio between bulk densities of T system and myofibrils | $V_{v_{ts}}/V_{v_{mf}}$ | 0,044±0,004 | 0,039±0,003 | 0,037±0,004 | 0,033±0,0006* |
| Surface–volume ratio of myofibrils | $S_{v_{mf}}/V_{v_{mf}}$, $m^2/cm^3$ | 2,611±0,158 | 2,291±0,092 | 2,892±0,318 | 1,244±0,072* |
| Surface–volume ratio of mitochondria | $S_{v_{mc}}/V_{v_{mc}}$, $m^2/cm^3$ | 4,910±0,064 | 5,407±0,164* | 6,038±0,764 | 3,612±0,269* |
| Surface–volume ratio of T system | $S_{v_{ts}}/V_{v_{ts}}$, $m^2/cm^3$ | 18,401±1,00 | 20,550±2,172 | 21,597±1,935 | 19,377±1,215 |
| Surface–volume ratio of sarcoplasmic reticulum | $S_{v_{sr}}/V_{v_{sr}}$, $m^2/cm^3$ | 22,596±3,170 | 20,434±1,107 | 30,949±3,722 | 25,518±2,094 |

Legend. Number of animals given in parentheses. *) Differences significant at P < 0.05 level, †) differences significant at P < 0.001 level compared with data for intact rats.

Pieces of the left papillary muscle for electron microscopy and for preparation of semithin (1 μ) sections were treated with paraform, postfixed in 1% osmium tetroxide solution, and subjected to standard dehydration; the material was embedded in a mixture of Epon and Araldite. The semithin sections were stained with toluidine blue and used for morphometric determination of the diameter of the muscle fibers. Ultrathin sections were obtained on the LKB Ultrotome, stained with uranyl acetate and lead citrate, and examined in the IEM 100B electron microscope.

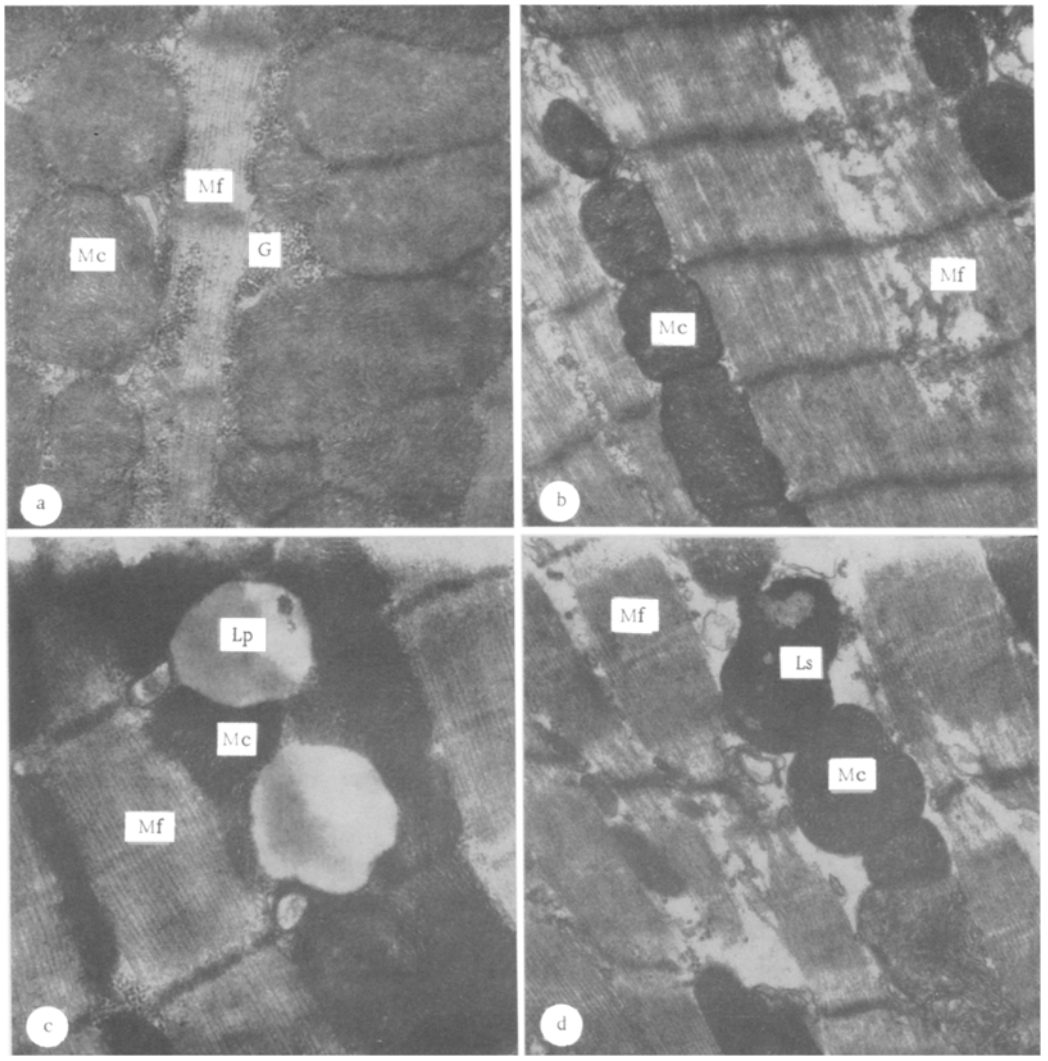


Fig. 1. Ultrastructure of cardiac myocytes from left ventricle of rats after total starvation: a) increase in size of glycogen granules at sites of concentration of mitochondria after 2 days of experiment; b) injuries to myofibrils (5 days); c) formation of secondary lysosomes (5 days); d) appearance of lipid droplets (10 days). Magnification: a, b, c) 8300; d) 13,300. Mf) Myofibrils, Mc) mitochondria, G) glycogen, Ls) lysosomes, Lp) lipids.

Ultrastructural stereologic analysis was carried out on negative electron micrographs of longitudinal sections through the cardiomyocytes; the final magnification was 16,600. The following parameters were determined: the bulk and surface density ($V_V$ and $S_V$) of the myofibrils, mitochondria, T system, and sarcoplasmic reticulum, and also the bulk density of the remaining cell structures. Secondary indices were obtained by calculation: the ratio of the bulk density of the mitochondria, sarcoplasmic reticulum, and T system to the bulk density of the myofibrils, and surface–volume ratios of the myofibrils, mitochondria, T system, and sarcoplasmic reticulum. The method of measuring and counting the ultrastructural components was described in [1] and the test systems used also were described previously [2]. The significance of differences was determined by Student's t test. The data were calculated relative to 1 $cm^3$ of heart muscle cells, according to the recommendations of the International Society of Stereology.

## EXPERIMENTAL RESULTS

During total starvation of the rats for 5 and 10 days general atrophy took place with a decrease in the body weight and in the absolute weight of the heart. The decrease in the diameter of the cardiac myocytes under these circumstances was evidence of developing myocardial atrophy (Table 1). The relative weight of the heart increased somewhat during starvation, because of the more marked decrease in the body weight.

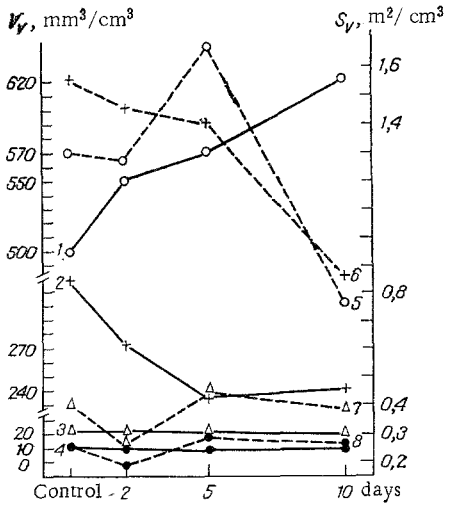


Fig. 2

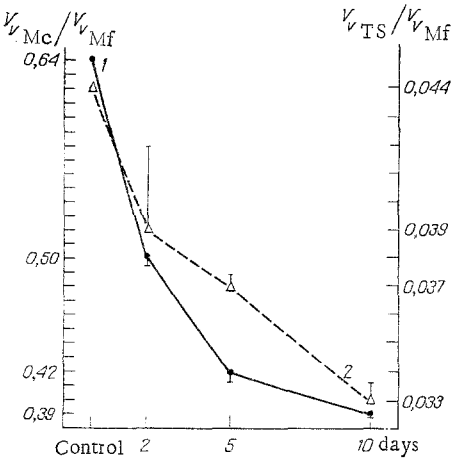


Fig. 3

Fig. 2. Results of measurements of primary stereologic parameters of ultrastructures of cardiac myocytes of starved rats. Abscissa, duration of starvation (in days); ordinate, bulk density (in $mm^3/cm^3$) and surface density (in $m^2/cm^3$). 1, 5) Myofibrils; 2, 6) mitochondria; 3, 7) T system; 4, 8) sarcoplasmic reticulum.

Fig. 3. Results of calculations of secondary stereologic parameters of ultrastructures of cardiac myocytes of starved rats. Abscissa, duration of starvation (in days); ordinate: ratio between bulk densities of (1) mitochondria and myofibrils, and 2) of T system and myofibrils.

After starvation for 2 days no structural changes could be detected in the myocardium of the rats. Only an increase in the number of glycogen granules was found, especially where there were concentrations of mitochondria (Fig. 1a).

The most marked ultrastructural changes were found in heart muscle cells of rats starved for 5 days. Areas of liquefaction of myofibrils (Fig. 1b) were observed, with myofibrillolysis localized between the Z disks. Cisterns of the T system and sarcoplasmic reticulum were dilated.

The number of lysosomes was increased (Fig. 1c) and they accumulated not only in the perinuclear zone, but also in the myofibrillary zone around the mitochondria. Smith [15] suggests that these ultrastructural changes are evidence of the development of autolysis taking place at the subcellular level.

It has been shown biochemically [7] and morphologically [5] that with a change to endogenous feeding cell metabolism switches from carbohydrate to fat. Clear evidence of this switch was observed after 5 and 10 days of total starvation, which was accompanied by a considerable increase in the number of lipid droplets (Fig. 1d). Lipid inclusions were distributed mainly in the myofibrillary zone, often in direct contact with the mitochondria.

The stereologic investigation showed that the structural organization of the cardiomyocytes underwent considerable changes (Table 2, Fig. 2). As early as after 2 days of starvation the bulk density of the mitochondria was reduced from $317.9 \pm 4.7$ $mm^3/cm^3$ in the control to $272.8 \pm 11.5$ $mm^3/cm^3$ and the relative volume of the myofibrils was increased from $497.9 \pm 8.3$ $mm^3/cm^3$ in the control to $550.5 \pm 14.4$ $mm^3/cm^3$. By the end of the experiment (10th day) the relative volume of the mitochondria was reduced by 24% compared with normal, and the bulk density of the myofibrils was increased by 25.2%. A similar imbalance in ultrastructural organization is observed during progressive hypertrophy [3, 10, 12, 14]. Consequently, both during progressive hypertrophy and during atrophy of heart muscle cells quantitative changes in the mitochondria are disproportionate to changes in the quantity of contractile substance and indicate a reduction in the energy resources to cope with the increased load.

A decrease in the relative volume of the mitochondria may be accompanied by an increase in their surface area, a matter of great biological importance to adaptation of the myocardium [9]. The surface density of the mitochondria after starvation for 2 and 5 days was maintained at about the same level as the control,

but on the 10th day of starvation it fell to $0.862 \pm 0.065$ $m^2/cm^3$ ($1.557 \pm 0.006$ $m^2/cm^3$ in the control). During atrophy of the myocardium, the change in the surface density of the other organelles likewise was evidently of no adaptive importance. For instance, the surface density of the myofibrils showed a tendency to increase after total starvation for 5 days, but these differences were not statistically significant; after 10 days this parameter was reduced to $0.772 \pm 0.040$ $m^2/cm^3$ (in the control $1.304 \pm 0.099$ $m^2/cm^3$). The surface density of the T system and sarcoplasmic reticulum was reduced after 2 days of the experiment, after which it returned to its initial values.

The calculated data obtained for volume and surface–volume ratios (Table 2, Fig. 3) showed that the ratio between the volume of the mitochondria and volume of the myofibrils decreased in the course of the experiment from $0.64 \pm 0.02$ to $0.39 \pm 0.01$. Some workers have suggested [13] that the higher the degree of atrophy, the smaller this ratio becomes. The ratio between the volumes of the sarcoplasmic reticulum and myofibrils did not change significantly. The ratio between the volume of the T system and that of the myofibrils decreased, to reach $0.033 \pm 0.0006$ after 10 days of the experiment ($0.044 \pm 0.004$ in the control). The surface–volume ratio of the myofbrils remained at approximately the same level until the 5th day and was reduced after 10 days. The surface–volume ratio of the mitochondria increased gradually to $6.038 \pm 0.764$ $m^2/cm^3$ after 5 days of starvation ($4.910 \pm 0.064$ $m^2/cm^3$ in the control), but this increase was not statistically significant. After starvation for 10 days the surface–volume ratio of the mitochondria decreased to $3.612 \pm 0.269$ $m^2/cm^3$. The surface–volume ratio of the T system and sarcoplasmic reticulum showed a tendency to rise.

The quantitative stereologic investigation of the ultrastructures of the cardiac myocytes of the left ventricle of rats during total starvation showed that the compensatory mechanisms in myocardial atrophy are aimed at preserving the fundamental spatial relationship and the density characteristics of the sarcoplasmic reticulum, T system, and myofibrils, which are probably unique constants of the architectural structure of the cardiomyocytes and which are essential for the maintenance of coordination between excitation and contraction. The higher the degree of atrophy, the greater the fall in the ratio between the volumes of mitochondria and myofbrils. On exhaustion of the reserves of the body, the most drastic changes are observed in the architecture of the membranous components of the cardiomyocytes, manifested in particular as a decrease in the surface density of the mitochondria, evidence of decompensation of the atrophied myocardium.

## LITERATURE CITED


1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973).
2. L. V. Kolesnikova and L. M. Nepomnyashchikh, Arkh. Anat., No. 4, 28 (1978).
3. F. Z. Meerson, Adaptation, Disadaptation, and Failure of the Heart [in Russian], Moscow (1978).
4. L. M. Nepomnyashchikh and L. V. Kolesnikova, Byull. Éksp. Biol. Med., No. 8, 247 (1977).
5. L. M. Nepomnyashchikh, S. V. Mishina, and E. E. Filyushina, Arkh. Anat., No. 7, 40 (1979).
6. L. M. Nepomnyashchikh, E. E. Filyushina, S. V. Mishina, et al., Arkh. Patol., No. 5, 42 (1976).
7. L. E. Panin, Energetic Aspects of Adaptation [in Russian], Leningrad (1978).
8. D. S. Sarkisov and P. I. Remezov, Experimental Reproduction of Human Diseases [in Russian], Moscow (1960).
9. Yu. G. Tsellarius and N. K. Eriskovskaya, Byull. Éksp. Biol. Med., No. 6, 627 (1979).
10. P. Anversa, L. Bitali-Mazza, O. Visioli, et al., J. Mol. Cell. Cardiol., 3, 213 (1971).
11. M. A. Goldstein, L. A. Sordahl, and A. Schwartz, J. Mol. Cell. Cardiol., 6, 265 (1974).
12. L. P. McCallister and E. Page, J. Ultrastr. Res., 42, 136 (1973).
13. R. Poche and D. Monkemeier, Arch. Pathol. Anat., 335, 271 (1962).
14. R. Poche, C. Moraes de Mello Mattos, H. W. Rembarz et al., Arch. Pathol. Anat., 344, 100 (1968).
15. A-L. N. Smith, Cytobios, 18, 111 (1978).