deposits of different sizes. The largest concentration of fibronectin was found at points of intersection of several fibers where, as a rule, actively protein-synthesizing fibroblasts are located.

LITERATURE CITED

1. L. V. Beletskaya and T. A. Danilova, in: Immunoluminescence in Medicine [in Russian], Moscow (1977), pp. 145-183.
2. L. V. Beletskaya, A. A. Ivanov-Smolenskii, O. V. Repko, et al., in: Physiology and Pathology of Connective Tissue [in Russian], Vol. 1, Novosibirsk (1980), pp. 26-27.
3. L. V. Beletskaya, Byull. Éksp. Biol. Med., No. 7, 102 (1981).
4. O. D. Zinkevich, R. I. Litvinov, and M. S. Kuravskaya, Byull. Éksp. Biol. Med., No. 7, 86 (1982).
5. A. A. Ivanov-Smolenskii, Byull. Éksp. Biol. Med., No. 10, 454 (1978).
6. A. A. Ivanov-Smolenskii and A. G. Grosheva, Byull. Éksp. Biol. Med., No. 4, 451 (1978).
7. C. L. Anderson and H. M. Grey, J. Immunol., 118, 819 (1977).
8. Z. Avnur and B. Geiger, Cell, 25, 121 (1981).
9. A. B. Chen and M. W. Mosesson, Anal. Biochem., 79, 144 (1977).
10. C. H. Fox, U. H. Coffler-Fox, and K. M. Yamada, Exp. Cell Res., 130, 477 (1980).
11. R. O. Hynes, Biochim. Biophys. Acta, 458, 73 (1976).
12. K. M. Yamada and K. Olden, Nature, 275, 179 (1978).

ULTRASTRUCTURAL STEREOLOGIC ANALYSIS OF ACUTE HYPERTENSIVE MYOCARDIAL HYPERTROPHY

E. L. Lushnikova, L. M. Nepomnyashchikh, V. P. Tumanov, G. I. Nepomnyashchikh, and A. M. Gonchar

UDC 616.12-008.331.1-06: 616.127-007.61.091.8

KEY WORDS: arterial hypertension; myocardial hypertrophy; cardiomyocyte ultrastructure; morphometry and stereology.

A detailed explanation of the mechanisms of development of compensatory and adaptive reactions in the myocardium in arterial hypertension at the ultrastructural level is important not only for diagnosis and prognosis, but also for an understanding of general, stereotyped reactions, and also of reactions developing in the cardiomyocyte when the functional load on the heart is increased [3, 6-8]. A study of the dynamics of changes in intracellular structures and the character of their interrelations during increasing hypertension, by means of quantitative morphological methods, is of great interest in this respect.

This paper describes a study of the ultrastructure of rat cardiomyocytes hypertrophied as a result of experimental arterial (renal) hypertension, by the use of morphometric and stereologic analysis.

EXPERIMENTAL METHOD

Experiments were carried out on 35 male Wistar rats weighing initially 200.9 ± 20.3 g. To produce arterial hypertension in normotensive rats the animals were anesthetized with ether, laparotomy was performed, the region of the aorta giving rise to the renal arteries

Laboratory of Pathological Anatomy and Histocytometry, Department of Pathomorphology and Morphometry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Laboratory of Histochemistry and Autoradiography, Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 366-370, March, 1984. Original article submitted May 14, 1983.

0007-4888/84/9703-0364$08.50

TABLE 1. Results of Morphometric and Stereologic Analysis of Heart of Wistar Rats with Arterial Hypertension (M ± m)

Parameter	Control	Time after operation, days		
		5	12	35
Morphometric characteristics of heart and muscle fibers:				
Body weight, g	205,0±25,0	212,3±7,9	216,7±8,8	250,0±11,5
absolute weight of heart, mg	970,0±70,0	1083,2±52,4	1230,0±30,6*	1356,7±56,7*
relative weight of heart, mg/g body weight	4,76±0,24	5,12±0,21	5,97±0,23*	5,47±0,48
diameter of cardiomyocytes, μ	15,9±0,2	16,5±0,4	24,5±0,7‡	24,4±0,5‡
Stereologic ultrastructural characteristics of cardiomyocytes:				
Relative volume, V_{v_i}, mm^3/cm^3				
of myofibrils	508,1±18,2	523,6±11,5	542,5±15,1	617,0±10,0*
of mitochondria	355,1±32,6	318,7±10,2	276,5±6,5	250,7±5,9*
of sarcoplasmic reticulum	14,5±1,9	17,2±2,1	18,4±2,6	21,9±1,2
of T-system	13,5±1,9	13,8±1,5	13,1±1,0	13,0±0,5
of remaining structures of cytoplasm	108,8±10,6	112,9±12,1	149,5±23,9	97,4±6,4
Relative surface area, S_{v_i}, m^2/cm^3:				
of myofibrils	1,165±0,042	1,178±0,053	1,252±0,074	1,416±0,076
of mitochondria	1,146±0,083	1,150±0,062	1,193±0,032	1,097±0,059
of sarcoplasmic reticulum	0,272±0,010	0,302±0,015	0,409±0,061	0,496±0,008†
of T-system	0,145±0,018	0,143±0,016	0,134±0,010	0,159±0,020
Surface/volume ratios, S_{v_i}/V_{v_i}, m^2/cm^3				
of myofibrils	2,30±0,17	2,25±0,12	2,30±0,10	2,29±0,09
of mitochondria	3,24±0,07	3,61±0,10	4,32±0,18*	4,37±0,21*
of sarcoplasmic reticulum	18,99±1,81	17,56±0,83	22,16±0,28	23,10±0,22
of T-system	10,78±0,02	12,65±0,10	10,18±0,03†	12,18±0,09†
Volume ratios of principal organelles of cardiomyocyte and myofibrils, $V_{v_i}/F_{v_{mf}}$ (number):				
of mitochondria and myofibrils	0,702±0,089	0,609±0,011	0,510±0,005	0,407±0,016*
of sarcoplasmic reticulum and myofibrils	0,029±0,003	0,033±0,002	0,034±0,004	0,036±0,004
of T-system and myofibrils	0,027±0,003	0,026±0,002	0,024±0,001	0,21±0,001
of remaining organelles and myofibrils	0,214±0,013	0,216±0,025	0,278±0,053	0,158±0,012

Legend. *P < 0.05, †P < 0.01, ‡P < 0.001.

was exposed, and a titanium coil was applied, constricting the lumen of the aorta by two-thirds of its diameter. The arterial pressure was measured by an indirect method. A transducer with rubber cuff, applied to the animal's tail, was used. The signal from the transducer was recorded on a Mingograph-34 apparatus (Elema-Schönander, Sweden). The animals were decapitated 5, 12, 15, 20, and 35 days after the operation. The hearts from rats undergoing a mock operation served as the control.

After decapitation of the animals the heart was removed from the thorax and placed in a cold chamber until it completely stopped beating. The absolute and relative weight of each heart was then determined. Samples of tissue from the left papillary muscles were fixed in a 4% solution of paraformaldehyde, postfixed in 2% osmium tetroxide solution, dehydrated with alcohol and propylene oxide, and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on the LKB III Ultrotome. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-100B electron microscope.

Morphometric and stereologic analysis of the heart muscle cells was carried out 5, 12, and 35 days after constriction of the abdominal aorta. The mean diameter of the cardiomyocytes for each group of animals was measured in semithin sections, stained with azure II, by means of an MOV-1-15× ocular micrometer. Ultrastructural stereologic analysis was carried out on electron micrographs under a final magnification of 18,000. Methods described previously [3] were used to estimate the relative volume of the myofibrils, mitochondria, smooth sarcoplasmic reticulum (SSR), T-system and cytoplasmic matrix (total of ribosomes, glycogen, lipid droplets, lysosomes, lamellar complex, and cytoplasm proper), and the relative surface area of the organelles. From these primary data secondary parameters were calculated: surface–volume ratios for ultrastructures and volume ratios for the principal organelles of the cell relative to volume of myofibrils. The results of the quantitative measurements were subjected to statistical analysis [1, 4] by Student's t test and to correlation analysis.

EXPERIMENTAL RESULTS

The systolic blood pressure of intact Wistar rats was 108 ± 5 mm Hg, rising 5 days after constriction of the abdominal aorta to 138 ± 4 mm Hg, 12 days after to 150 ± 5 mm Hg, 20 days to 160 ± 8 mm Hg, and 35 days after the operation to 205 ± 32 mm Hg. The rise of arterial pressure in normotensive rats in response to constriction of the abdominal aorta during the first 2 weeks after the operation was due to an increase in cardiac output and simultaneous increase in heart rate. Later in the course of the investigation the peripheral vascular resistance was raised, and this was evidently due to the high arterial pressure level.

The development of persistent hypertension in normotensive Wistar rats after constriction of the abdominal aorta was also connected with the development of hypertrophy of the heart. The absolute weight of the heart in the animals 35 days after constriction of the abdominal aorta increased by 40% and its relative weight by 15%; the mean diameter of the cardiomyocytes increased (Table 1).

In the early stages after the operation (12-15 days) widening of the intercellular spaces was observed (Fig. 1a). The number of micropinocytotic vesicles in the subsarcolemmal zone was increased. In the late stages (35 days) vacuoles and myelin-like structures were often found in the sarcoplasm. These same structures also appeared in the intercellular space and capillary lumen. Ultrastructural changes took place in the region of the intercalated disks, which acquired a zigzag external appearance; gap junctions of the intercalated disks were often widened (Fig. 1b). Myofibrils in the zone of the intercalated disks sometimes showed lysis and lost their cross-striation (Fig. 1c).

The relative volume of the myofibrils in hypertrophied cardiomyocytes was significantly increased from 508.1 ± 18.2 mm^3/cm^3 in animals of the control group to 617.0 ± 10.0 mm^3/cm^3 35 days after the operation (Table 1, Fig. 2). The bulk density of the myofibrils under these circumstances showed an increase of 21%. This was due primarily to increased synthesis of myofilaments, as shown by the appearance of elements of rough sarcoplasmic reticulum and numerous free ribosomes in certain areas of the cardiomyocytes (Fig. 1d). Parallel with the increase in relative volume of the myofibrils their relative surface area also increased, with the result that the surface/volume ratio of the myofibrils was unchanged (Fig. 3).

Hypertrophy of the cardiomyocytes was accompanied by a significant decrease in bulk density of the mitochondria from 355.1 ± 32.6 mm^3/cm^3 in the control to 250.7 ± 5.9 mm^3/cm^3 after 35 days of the experiment. The surface density of the mitochondria showed no significant

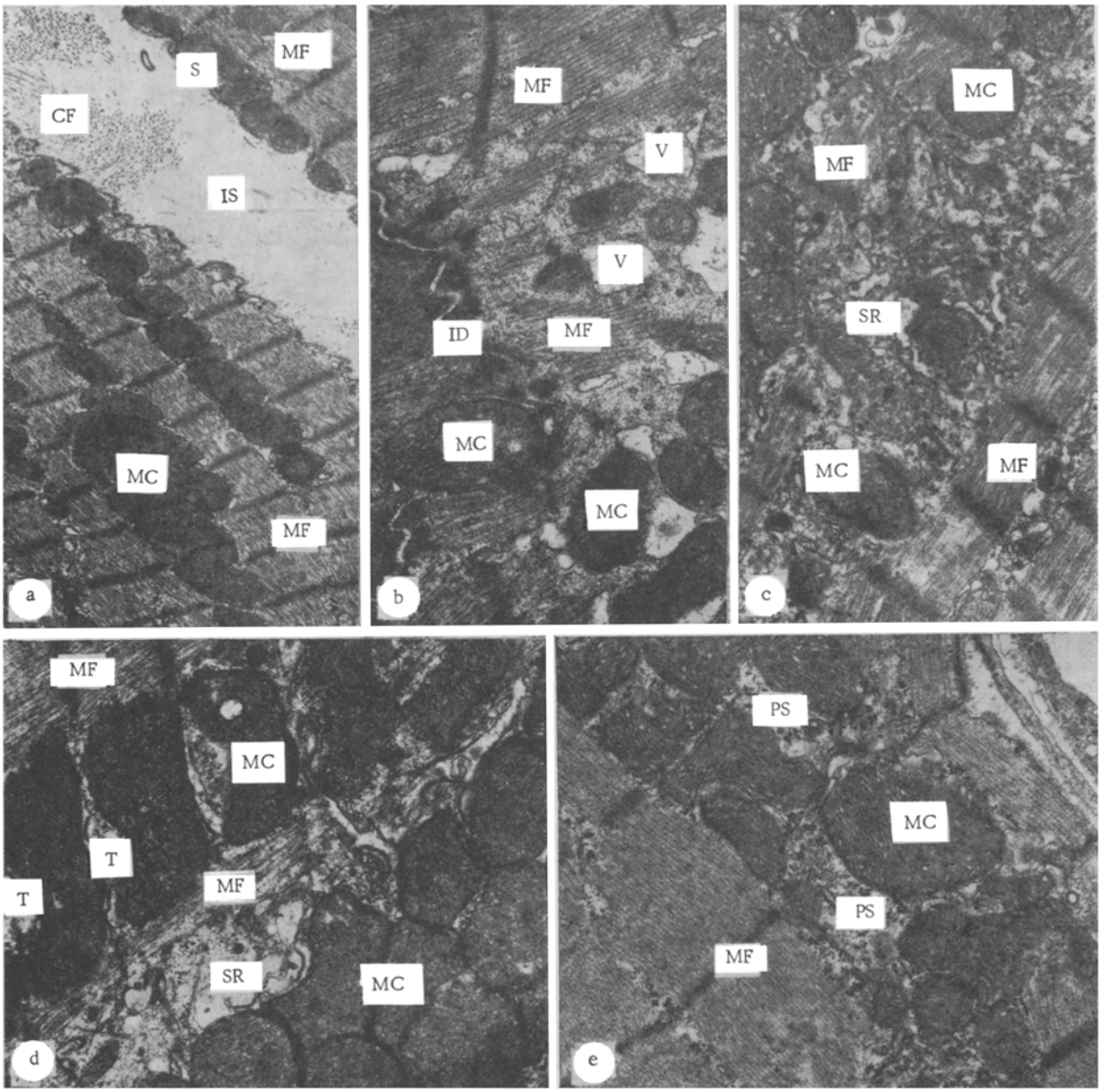

Fig. 1. Ultrastructure of cardiomyocytes of Wistar rats after constriction of abdominal aorta. a) Widening of intercellular spaces (IS), contractures of myofibrils (MF), festooning of sarcolemma (S). MC) Mitochondria, CF) collagen fibers; b) zigzag appearance of intercalated disk (ID), widening of zone of contact between two muscle cells, formation of vacuoles (V), foci of lysis of myofibrils; c) lysis of myofibrils, denudation of sarcoplasmic reticulum (SR) and dilatation of its tubules, partial vacuolation of cristae and disturbance of their parallel arrangement in mitochondria; d) fragments of rough sarcoplasmic reticulum and numerous polysomes (PS) in sarcoplasm; e) lysis of myofibrils, dilatation of tubules of sarcoplasmic reticulum and T-system (T), focal destruction of cristae in mitochondria with formation of vacuoles bounded by membrane. b, c) 12 days, a, e) 15 days, d) 35 days after operation. Magnification: a) 3000 ×; b-e) 5000 ×.

change, so that there was a significant increase in the surface/volume ratio of these organelles by 35% by the end of the experiment. Polymorphism of the mitochondria was increased, and in individual mitochondria signs of destruction were visible, and inclusions (including those of glycogen), surrounded by a membrane, appeared (Fig. 1e).

These different trends of changes in the mitochondria and myofibrils led to a decrease in the volume ratio of these structures by 42% 35 days after the operation.

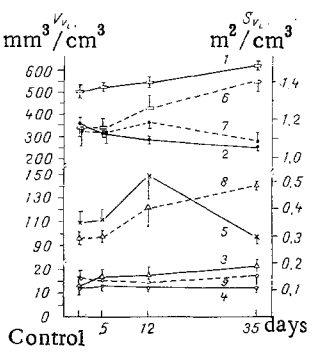

Fig. 2

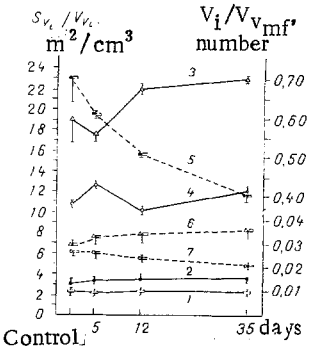

Fig. 3

Fig. 2. Results of measurements of primary stereologic parameters of cardiomyocyte organelles in Wistar rats with arterial hypertension. Abscissa, time after operation (in days); ordinate: on left — bulk density, on right — surface density. 1, 6) Myofibrils; 2, 7) mitochondria; 3, 8) SSR; 4, 9) T-system; 5) remaining structures of cytoplasm.

Fig. 3. Results of calculation of secondary stereologic parameters of cardiomyocyte organelles in Wistar rats with arterial hypertension. Abscissa, time after operation (in days); ordinate: on left — surface/volume ratio of organelles, on right — ratio of relative volumes of organelles to relative volume of myofibrils. 1) Myofibrils; 2) mitochondria; 3) SSR; 4) T-system; 5) mitochondria/myofibrils; 6) SSR/myofibrils; 7) T-system/myofibrils.

Many workers [2, 9, 11, 13] have described a decrease in the ratio of volumes of mitochondria to myofibrils in hypertrophied cardiomyocytes [5, 12] in arterial hypertension. It is probably a response of adaptive importance.

During hypertrophy of heart muscle fibers considerable changes took place in SSR, the dilated tubules of which were clearly visible in areas of lysis of myofibrils in the early stages of the experiment. The relative volume of this organelle was increased by 51%. The surface density of SSR increased even more: from 0.272 ± 0.010 m^2/cm^3 in control animals to 0.496 ± 0.008 m^2/cm^3 in rats on the 35th day after the operation. Postiive correlation was found between the diameter of the cardiomyocytes and the relative surface area of SSR ($r = +0.795$; $P < 0.05$), whereas the increase in relative volume of this compartment correlated only weakly with the increase in size of the cardiomyocytes ($r = +0.246$; $P < 0.05$). The substantial increase in area of the SSR membranes led to an increase in surface/volume ratio of this structure ($P < 0.05$). Since the increase in relative volume of SSR during hypertrophy, expressed as a percentage, was almost twice the increase in bulk density of the myofibrils, a distinct tendency was observed for the ratio between volume of SSR and volume of myofibrils to increase on the 35th day after constriction of the abdominal aorta.

To understand the functional state of the cardiomyocytes it is important to estimate the ratio of the surface density of the SSR membranes to volume of myofibrils. This ratio was significantly ($P < 0.001$) increased in the hypertensive rats toward the end of the experiment: 0.535 ± 0.0002 m^2/cm^3 in the control; 0.751 ± 0.098 and 0.805 ± 0.005 m^2/cm^3 in rats on the 12th and 35th days, respectively, after the operation.

The increase in volume and surface/volume ratio of sarcoplasmic reticulum to myofibrils is evidence that growth of this compartment precedes growth of the myofibrils. A similar enlargement of SSR has been found in many models of hypertrophy of the heart [10, 14, 16] and it is of evident adaptive importance, reflecting the increased demand for Ca^{++} ions, required for the coupled process of contraction and relaxation. However, there is also a different point of view, according to which the increase in area of SSR membranes is connected with the reduced ability of this structure to bind and release Ca^{++} [15]. The latter is evidently characteristic of the stage of subcompensation or even decompensation of the heart, when the morphological features of cardiac hypertrophy are completely preserved.

The bulk density of the T-system was unchanged during hypertrophy of the cardiomyocytes, whereas the relative surface area at first showed a very small decrease on the 12th day after the operation, followed by an increase again (by 9% relative to the control). This led to a

significant decrease in the surface/volume ratio of the T-system on the 12th day after the operation and to a significant increase by the end of the experiment. There was practically no change in the ratio of volume of T-system to volume of myofibrils.

The relative volume of the cytoplasmic matrix relative to the control did not change significantly, although on the 12th day after the operation an increase, and on the 35th day, a decrease in this parameter were observed. A similar change occurred in the volume of the cytoplasmic matrix relative to the volume of the myofibrils. Hyperplasia of the lamellar complex, especially of its vesicular part, was observed in the cytoplasm of the cardiomyocytes.

On the whole, hypertrophy of the cardiomyocytes in rats with arterial hypertension is characterized at the ultrastructural level by a decrease in the ratio of total bulk density of the mitochondria, sarcoplasmic reticulum, and T-system to volume of the myofibrils from 0.754 ± 0.084 in the control group to 0.463 ± 0.017 in the animals on the 35th day after constriction of the abdominal aorta ($P < 0.05$). Negative correlation ($r = -0.863$; $P < 0.05$) was found between the diameter of the cardiomyocytes and the ratio of the total volume of the principal organelles to volume of myofibrils.

Intensification of metabolism and hyperplasia of ultrastructures [7] during hypertensive myocardial hypertrophy are manifested primarily as growth of elements of the sarcoplasmic reticulum before growth of myofibrils and other cell compartments, whereas the relative volume of the mitochondria falls considerably during the development of hypertrophy.

LITERATURE CITED

1. G. G. Avtandilov, Introduction to Quantitative Pathological Morphology [in Russian], Moscow (1980).
2. E. L. Lushnikova, G. I. Nepomnyashchikh, V. P. Tumanov, et al., Byull. Éksp. Biol. Med., No. 1, 9 (1983).
3. L. M. Nepomnyashchikh, Pathological Anatomy and Ultrastructure of the Heart [in Russian], Novosibirsk (1981).
4. L. M. Nepomnyashchikh, E. L. Lushnikova, and L. V. Kolesnikova, Arkh. Anat., No. 10, 94 (1981).
5. L. M. Nepomnyashchikh, E. L. Lushnikova, and M. G. Chernokalova, Byull. Éksp. Biol. Med., No. 7, 101 (1981).
6. V. S. Paukov, and V. A. Frolov, Elements of a Theory of Pathology of the Heart [in Russian], Moscow (1982).
7. D. S. Sarkisov, Outlines of Structural Principles of Homeostasis [in Russian], Moscow (1977).
8. V. A. Frolov and G. A. Drozdova, Arkh. Patol., No. 5, 35 (1982).
9. Yu. G. Tsellarius and N. K. Eriskovskaya, Byull. Éksp. Biol. Med., No. 6, 627 (1979).
10. P. Anversa, A. V. Loud, F. Giacomelli, et al., Lab. Invest., 38, 597 (1978).
11. P. Anversa, G. Olivetti, H. Melissari, et al., Lab. Invest., 40, 341 (1979).
12. E. A. Breisch, A. A. Bove, and S. J. Phillips, Cardiovasc. Res., 14, 161 (1980).
13. K. Imamura, Jpn. Circ. J., 42, 979 (1978).
14. M. L. Lazarus, J. A. Colgan, and H. G. Sachs, J. Mol. Cell. Cardiol., 8, 431 (1976).
15. W. B. McCollum, C. Crow, S. Harigaya, et al., J. Molec. Cell. Cardiol., 1, 445 (1970).
16. S. Singh, F. C. White, and C. M. Bloor, Arch. Path. Anat., Abt. B, Zellpathol., 39, 293 (1982).