tional load on the myocardium, the increase in weight of the contractile substance takes place mainly on account of an increase in the number of myofibrils in each muscle cell, i.e., as a result of intracellular hyperplasia [3].

When these differences in the mechanisms of myocardial hypertrophy in early postnatal ontogeny of rats under high altitude conditions are assessed, guidance must be taken not so much from the anatomical manifestations of this process, as from the nature of the regenerative-hyperplastic processes which lie at its basis.

LITERATURE CITED

1. I. K. Esipova and O. Ya. Kaufman, Postnatal Reorganization of the Pulmonary Circulation and Atelectasis of the Newborn [in Russian], Leningrad (1968).
2. V. A. Kononova, Arkh. Patol., No. 2, 66 (1981).
3. D. S. Sarkisov, Outlines of the Structural Principles of Homeostasis [in Russian], Moscow (1977).
4. S. P. Bishop, Circulation, 44, Suppl. 2, 111 (1974).
5. J. F. Neffgen and B. Koresky, Circ. Res., 30, 104 (1972).
6. M. Hollenberg, Am. J. Physiol., 231, 1445 (1976).
7. R. Sasaki, T. Morishite, and S. Vamagata, Tohoku J. Exp. Med., 96, 405 (1968).
8. R. Zak, Circ. Res., 35, No. 2, Suppl. 2, 11 (1974).

TISSUE STEREOLOGIC ANALYSIS OF MYOCARDIAL ATROPHY DURING HYPOKINESIA

G. I. Nepomnyashchikh, V. P. Tumanov,
L. V. Kolesnikova, and L. M. Nepomnyashchikh

UDC 612.766.2–08:616.127–007.23

KEY WORDS: hypokinesia, atrophy of the heart, myocardial parenchyma and stroma, morphometry and stereology

Prolonged limitation of movement (hypokinesia) disturbs activity of the cardiovascular system and considerably reduces the functional reserves of the heart [3, 10]. An excessive reduction of energy expenditure on active surmounting of body weight can be used as a model with which to study structural manifestations of adaptation and disadaptation processes [1]. For a more complete understanding of these processes, quantitative studies of intracellular and tissue changes in the myocardium during hypokinesia are necessary. However, there have been few investigations into this problem, and these have been conducted mainly at the ultrastructural level [5, 7, 13].

The aim of this investigation was a quantitative stereologic study of the tissue organization of the rat myocardium in hypokinesia due to immobilization of the animals.

EXPERIMENTAL METHOD

Experiments were carried out on 48 male Wistar rats weighing 250–300 g. The motor activity of the rats was restricted by confining them in special restraining cages, the size of which corresponded to that of the animal. For morphometric and stereologic investigation 18 rats aged 6 months were used (four rats in each experimental group and six in the control). The animals were decapitated after 5, 15, and 30 days of hypokinesia. The heart was removed from the thorax and cooled in a cold chamber until it stopped beating, when the

Laboratory of Connective Tissue Pathology, 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 Mèdical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 6, pp. 743-748, June, 1984. Original article submitted August 26, 1983.

TABLE 1. Results of Stereologic Investigation of Myocardial Tissue Components in Wistar Rats after Hypokinesia (M ± m)

Parameter	Control	Duration of hypokinesia, days		
		5	15	30
Relative volume ($V_{v_i}^{mk}$), mm³/cm³:				
parenchyma	868,1±3,5	843,9±14,8	872,4±7,1	798,8±5,6***
cytoplasm of cardiomyocytes	856,1±2,9	830,4±15,3	859,9±6,6	789,1±5,9***
nuclei of cardiomyocytes	12,0±1,2	13,5±0,6	12,5±1,0	9,7±0,4
stroma	131,9±3,6	156,1±14,8	127,6±7,1	201,2±5,6***
capillaries	51,4±2,9	68,8±4,9*	49,4±2,9	78,1±1,3**
endotheliocytes	11,5±1,3	10,6±0,6	11,2±0,6	9,7±0,8
connective-tissue cells	5,0±0,3	5,4±1,9	4,9±0,9	6,2±0,9
fibers and ground substance	64,0±4,3	71,3±11,4	62,1±10,3	107,2±7,4**
Relative surface area ($S_{v_i}^{mk}$), m²/cm³:				
cardiomyocytes	0,1135±0,0022	0,1189±0,0041	0,1206±0,0033	0,1440±0,0067*
nuclei of cardiomyocytes	0,0066±0,0005	0,0073±0,0002	0,0074±0,0005	0,0060±0,00004
capillaries	0,0314±0,0008	0,0385±0,0014*	0,0348±0,0024	0,0430±0,0015**
connective-tissue cells	0,0035±0,0001	0,0040±0,0004	0,0035±0,0001	0,0052±0,0009
Surface to volume ratio (S_{v_i}/V_{v_i}), m²/cm³, of				
cardiomyocytes	0,131±0,004	0,141±0,003	0,138±0,005	0,180±0,007**
nuclei of cardiomyocytes	0,572±0,101	0,545±0,024	0,593±0,019	0,624±0,023
capillaries	0,614±0,021	0,571±0,056	0,703±0,015*	0,550±0,011*
connective-tissue cells	0,717±0,039	0,790±0,089	0,775±0,105	0,832±0,052
Ratio of bulk density (V_{v_i}/V_{v_i}), number, of				
nuclei to cytoplasm of cardiomyocytes	0,0140±0,0014	0,0163±0,0009	0,0145±0,0012	0,0123±0,0005
stroma to parenchyma	0,152±0,004	0,185±0,021	0,146±0,009	0,252±0,008***
capillaries to cardiomyocytes	0,059±0,003	0,082±0,007	0,057±0,003	0,098±0,001**
connective-tissue cells to cardiomyocytes	0,0058±0,0004	0,0064±0,0011	0,0056±0,0011	0,0078±0,0011
fibers and ground substance to cardiomyocytes	0,0737±0,0086	0,0845±0,0152	0,0712±0,0125	0,1342±0,0101*
Ratio of surface density of capillaries: to bulk density of cardiomyocytes, m²/cm³	0,0360±0,0009	0,0454±0,0017*	0,0396±0,0026	0,0536±0,0018**
to surface density of cardiomyocytes, number	0,277±0,008	0,325±0,017	0,290±0,024	0,293±0,009

Legend. *P < 0.05, **P < 0.01, ***P < 0.001 compared with control.

weight of each heart and the left ventricle was determined. Samples from the left papillary muscle were fixed in a 4% solution of paraformaldehyde, postfixed in a 1% solution of osmium tetroxide and, after dehydration in alcohols and propylene oxide, they were embedded in Epon and Araldite. Semithin (1 μ) sections were cut on the LKB III Ultratome, stained with azure II, and examined in the "Docuval" universal biological light microscope (Carl Zeiss, West Germany).

The morphometric analysis was carried out on semithin sections, transverse and longitudinal, through muscle fibers; the diameter of the cardiomyocytes was measured with a MOV-1-15× ocular micrometer. Stereologic analysis of the tissue organization of the myocardium was undertaken on longitudinal semithin sections of short segments of muscle fibers by means of an ocular test grid (n = 36, P = 72), with objective 100 and ocular 10. The bulk and surface density of the muscle fibers and their nuclei, capillaries, and connective-tissue cells and the relative volume of the fibers and ground substance of the interstitial connective tissue together were estimated. Secondary numerical parameters were calculated: surface/volume ratio of the cardiomyocytes and their nuclei, capillaries, and connective-tissue cells and the ratio of the bulk density of the stromal components of the myocardium to the relative volume of the cardiomyocytes. The technique of morphometric and stereologic analysis was described fully by the writers previously [4, 6, 8]. The significance of differences between these was determined by Student's test, at a $P < 0.05$ level of significance. The coefficient of correlation was calculated between the weight of the heart and the diameter of the cardiomyocytes. Parameters of entropy, relative entropy, and excess of structures of the myocardial parenchyma and stroma were determined by informational analysis.

EXPERIMENTAL RESULTS

Limitation of motor activity for 30 days led to a considerable decrease in the body weight of the rats (Table 1). The absolute weight of the heart and left ventricale also was reduced in the course of the experiment by 20 and 19.1% respectively ($P < 0.05$). The relative weight of the heart and left ventricle increased during hypokinesia, due to the more rapid regression of body weight than of weight of the heart. The diameter of the cardiomyocyte decreased from 16.10 ± 0.15μ in the control to 13.50 ± 0.10μ after 30 days of hypokinesia ($P < 0.001$). A decrease in the weight of the heart and diameter of the cardiomyocytes in experimental animals during prolonged restriction of motor activity has been observed in other investigations [2, 3, 12].

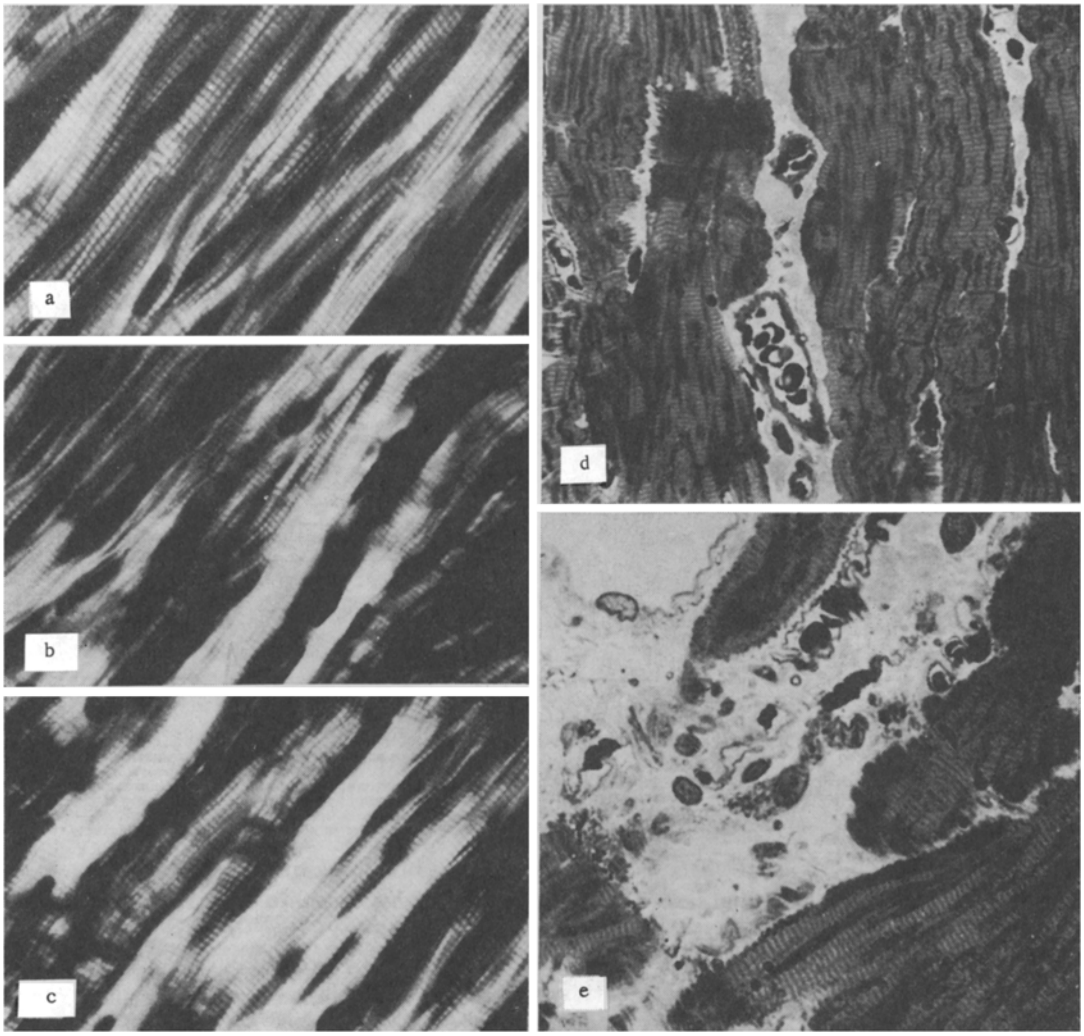

Fig. 1. Morphological changes in myocardium of rats after hypokinesia for 30 days (1250 ×). a) Decrease in thickness of muscle fibers; contractures of myofibrils of I degree: intensification of anisotropy of A disks; b) contractures of myofibrils of II degree: shortening of isotropic disks; c) contractures of myofibrils of III degree: formation of anisotropic conglomerate, disappearance of isotropic disks; d) collagen fibers in pericapillary and intercellular region; e) perivascular collagenization of stroma. a, b, c) Paraffin sections, stained with hematoxylin and eosin, photographed in polarized light; d, e) semithin sections, stained with azure II.

Correlation analysis showed strong positive correlation between the change in weight of the heart and the diameter of the cardiomyocytes during myocardial atrophy induced by hypokinesia ($r = 0.621$). This can serve as direct evidence that the decrease in weight of the heart takes place on account of a decrease in size and not number of the muscle cells. However, qualitative morphological investigation of photomicrographs and electron-micrographs of the myocardium of animals in the late stages of the experiment revealed cardiomyocytes with signs of structural damage of contracture type (Fig. 1a-c) and signs of an insufficiency of structural materials [9]. Consequently the possibility of a reduction in the number of heart muscle cells in the process of atrophy cannot be completely ruled out.

Morphometric and stereologic investigations (Table 1) showed that the decrease in weight of the heart was accompanied by a decrease in the relative volume of muscle tissue (Fig. 2) from 868.1 ± 3.5 mm^3/cm^3 in the control to 798.8 ± 5.6 mm^3/cm^3 after 30 days of hypokinesia ($P < 0.001$). The surface density and surface to volume ratio of the cardiomyocytes (Fig. 3) increased under these circumstances by 26.9 and 37.4% respectively. The increase in surface to volume ratio, whereas the diameter of the muscle cell decreased, can be interpreted as a short-term adaptive reaction, for the metabolic pathway and conduction of electrical impulses

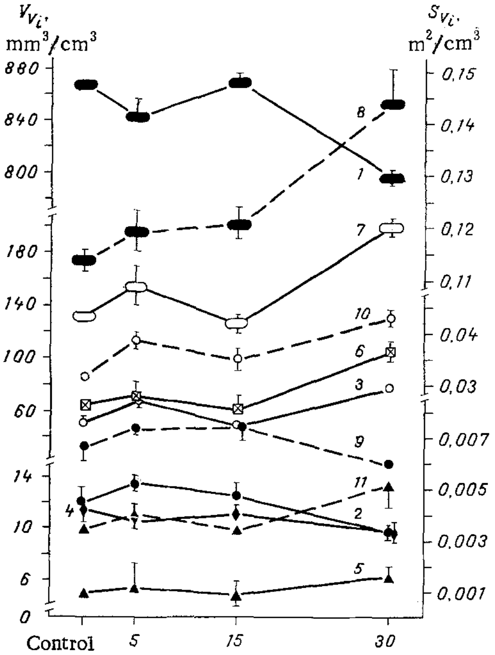

Fig. 2

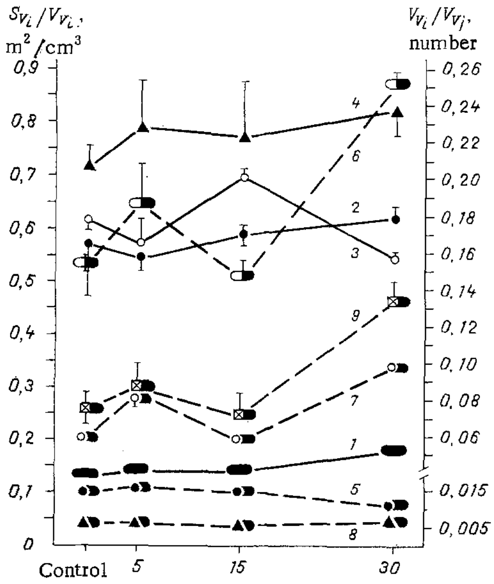

Fig. 3

Fig. 2. Results of measurements of primary stereologic parameters of myocardial tissue organization of rats after hypokinesia. Abscissa, duration of hypokinesia (in days); ordinate: left – bulk density, right – surface density. 1, 8) Cardiomyocytes, 2, 9) cardiomyocyte nuclei, 3, 10) capillaries, 4) endothelial cells, 5, 11) connective-tissue cells, 6) fibers and ground substance of connective tissue, 7) stromal component of tissue.

Fig. 3. Results of calculations of secondary stereologic parameters of myocardial tissue organization of rats after hypokinesia. Abscissa, duration of hypokinesia (in days); ordinate: left – surface to volume ratio of principal tissue components, right – ratio of bulk density of principal tissue components to bulk density of cardiomyocytes. 1) Cardiomyocytes, 2) cardiomyocyte nuclei, 3) capillaries, 4) connective-tissue cells, 5) nuclei/cytoplasm of cardiomyocytes, 6) stroma/parenchyma, 7) capillaries/cardiomyocytes, 8) connective-tissue cells/cardiomyocytes, 9) fibers and ground substance of connective tissue/cardiomyocytes.

TABLE 2. Parameters of Information Analysis for Stereologic Investigation of Myocardium of Wistar Rats after 30 Days of Hypokinesia

Duration of hypokinesia, days	Test object	Entropy, binary units	Relative entropy	Excess, %
Control	Parenchyma and stroma	0,855	0,331	66,9
	Stroma	1,522	0,761	23,9
5	Parenchyma and stroma	0,954	0,369	63,1
	Stroma	1,470	0,735	26,5
15	Parenchyma and stroma	0,840	0,325	67,5
	Stroma	1,523	0,762	23,8
30	Parenchyma and stroma	1,078	0,417	58,3
	Stroma	1,380	0,690	31,0

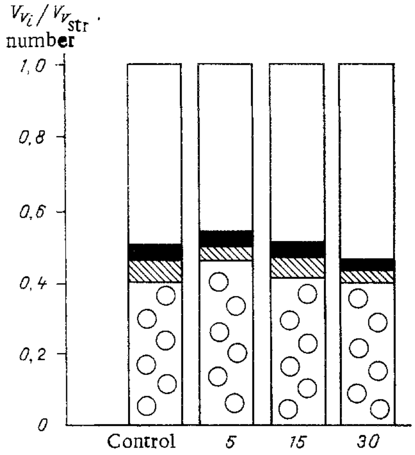

Fig. 4. Ratio of bulk density of components of interstitial connective tissue to relative volume of myocardial stroma of rats after hypokinesia. Abscissa, duration of hypokinesia (in days); ordinate, volume ratio. Unshaded part of column denotes fibers and ground substance of connective tissue/ stroma; part shaded black denotes connective-tissue cells/ stroma; obliquely shaded part, endothelial cells/ stroma; circles denote capillaries/ stroma.

from the surface to the center of the fiber were considerably shortened. The surface to volume characteristics of the cardiomyocyte nuclei and also the nucleo—cytoplasmic volume ratio did not change significantly in the course of the experiment; only a tendency was observed for these parameters to decrease after 30 days of restricted movement.

The bulk density of the stroma (overall relative volume of capillaries, cells, fibers, and ground substance at the interstitial connective tissue) increased significantly to 201.2 ± 5.6 mm^3/cm^3 on the 30th day of hypokinesia (131.9 ± 3.6 mm^3/cm^3 in the control; $P < 0.001$). This increase was due mainly to an increase in the relative volume of the microcirculation and intercellular substance. In the early stages of the experiment the increase in relative volume and surface density of the capillaries (by 33.9 and 22.6% respectively) was associated with a marked stress reaction [14]. The surface to volume ratio of the microcirculation was unchanged on the 5th day of the experiment. After 15 days of restriction of motor activity the bulk and surface density of the capillaries was indistinguishable from the control, but the surface to volume ratio was increased by 14.5% ($P < 0.05$). On the 30th day of hypokinesia, evidence of congestion in the myocardium was well marked, and under these circumstances the relative volume and relative surface area of the microcirculation were considerably increased by 51.9 and 36.9% respectively; $P < 0.01$) and the surface to volume ratio decreased by 10.4% compared with the control.

The considerable increase in the relative total volume of collagen fibers and ground substance (to 107.2 ± 7.4 mm^3/cm^3) toward the end of the experiment (64.0 ± 4.3 mm^3/cm^3 in the control) took place chiefly on account of an increase in the content of collagen bundles (Fig. 1d, e). Collagenization of the myocardial stroma during hypokinesia has been observed by a number of investigators [2, 11]. Incidentally, no significant changes were found in the relative proportions of stromal components (Fig. 4), but a tendency was discovered for the relative percentage of ground substance in the myocardial stroma of the rats to increase after 30 days of hypokinesia, together with a decrease in the number of capillaries, endothelial cells, and connective-tissue cells.

As a result of the structural changes described above, relations between parenchyma and stroma in the tissue organization of the myocardium were considerably altered after 30 days of hypokinesia. The ratio of bulk density of the stroma to the relative volume of the parenchyma rose by 65.8% after 30 days of hypokinesia ($P < 0.001$). An increase was observed in the volume ratio both of capillaries to cardiomyocytes (by 66.1%) and of fibers and ground substance to cardiomyocytes (by 82.1%). The ratio of bulk density of connective-tissue cells to the relative volume of the cardiomyocytes did not change significantly during the experiment.

Information analysis revealed an increase in entropy and relative entropy and a decrease in excess of the myocardial tissue in the course of its atrophy caused by hypokinesia for 30 days (Table 2). The highest value of entropy and the lowest value of excess, evidence of an increase in the indeterminancy of the system (myocardial tissue) were observed in the final stage of the experiment. Intensification of sclerosis in the myocardium during prolonged restriction of motor activity led under these circumstances to a reduction in entropy and relative entropy of the stroma.

This morphometric and stereologic investigation of the tissue organization of the rat myocardium during hypokinesia for 30 days thus showed that the reduction in absolute weight of the heart was accompanied by a decrease in diameter of the cardiomyocytes and by considerable changes in the relations between stromal and parenchymatous components of the organ. Among the most important of these changes were a significant increase in the relative volume of the stroma, collagenization of the interstitial connective tissue, and a marked tendency toward a decrease in the surface to volume ratio of the capillaries. This is evidence of the unfavorable structural changes in the tissue microregion during prolonged hypokinesia.

LITERATURE CITED

1. A. P. Avtsyn and V. A. Shakhlamov, Ultrastructural Principles of Cell Pathology [in Russian], Moscow (1979).
2. V. S. Bevzyuk, Zdravookhr. Tradzhikistana, No. 3, 47 (1975).
3. E. A. Kovalenko and N. N. Gurovskii, Hypokinesia [in Russian], Moscow (1980).
4. L. V. Kolesnikova and L. M. Nepomnyashchikh, Arkh. Anat., No. 4, 28 (1978).
5. N. A. Levkova, S. A. Kakabadze, N. P. Teplyakova, et al., Byull. Éksp. Biol. Med. No. 2, 231 (1981).
6. L. M. Nepomnyashchikh, Pathological Anatomy and Ultrastructure of the Heart [in Russian], Novosibirsk (1981).
7. L. M. Nepomnyashchikh, L. V. Kolesnikova, V. P. Tumanov, et al., Byull. Éksp. Biol. Med., No. 10, 105 (1983).
8. L. M. Nepomnyashchikh, E. L. Lushnikova, and L. V. Kolesnikova, Arkh. Anat., No. 10, 94 (1981).
9. L. M. Nepomnyashchikh, D. E. Semenov, L. A. Semenova, et al., Arkh. Patol., No. 5, 19 (1983).
10. N. E. Panferova, Hypodynamia and the Cardiovascular System [in Russian], Moscow (1977).
11. P. P. Potapov, Kosmich. Biol., No. 3, 44 (1977).
12. G. M. Pruss and V. I. Kuznetsov, Kosmich. Biol., No. 6, 45 (1974).
13. V. S. Romanov, Kosmich. Biol., No. 4, 50 (1976).
14. I. P. Chernov, Kosmich. Biol., No. 4, 57 (1980).