STEREOLOGIC ULTRASTRUCTURAL INVESTIGATION OF MYOCARDIAL ATROPHY IN HYPOKINESIA

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The prolonged limitation of motor activity on the cardiovascular system is a problem which is engaging the attention of many specialists. Structural changes reflecting reduction of the functional reserves of the heart are found in hypokinesia [2, 5, 12]. However, few quantitative morphological investigations have so far been undertaken [6, 9, 15].

The object of the present investigation was a quantitative stereologic study of the ultrastructural organization of the rat myocardium in the course of 30 days of hypokinesia.

EXPERIMENTAL METHOD

Experiments were carried out on 18 male Wistar rats weighing 250-300 g. Hypokinesia was produced by keeping the animals in special constraining cages, the bottom of which was made of transparent plastic, the top of a piece of fine metal gauze (the size of the cage corresponded to that of the animal). The experiment was planned so that the animals were sacrificed at the same age, namely 6 months. The animals were decapitated after 5, 15, and 30 days of hypokinesia. After removal from the thorax, the heart was quickly cooled in finely crushed ice until it stopped beating, after which its weight and that of the left ventricle were determined. Specimens of papillary muscle from the left ventricle were fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide solution, and after dehydration with propylene oxide, they were embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on the LKB-III Ultrotome. The diameter of the cardiomyocytes was measured in semithin sections by means of an MOV-15 ocular micrometer.

The ultrastructural stereologic analysis was conducted on electron micrographs obtained in the JEM 100B electron microscope. The bulk and surface density of the myofibrils, mitochondria, T system, and sarcoplasmic reticulum and the relative volume of the remaining structures of the cardiomyocyte were estimated. Secondary calculated parameters were determined: the ratio of the bulk density of the mitochondria, sarcoplasmic reticulum, and T system to the bulk density of the myofibrils and the surface/volume ratio of the principal organelles of the cardiomyocyte. The technique of stereologic analysis and also the test systems used were described in detail by the writers previously [7, 10]. The significance of differences was determined by Student's t test at the $P < 0.05$ level.

EXPERIMENTAL RESULTS

After hypokinesia for 30 days the body weight of the rats was considerably reduced (Table 1). The absolute weight of the heart of the experimental animals also fell during the experiment: The decrease was significant on the 15th and 30th days of hypokinesia. Separate weighing revealed a decrease in absolute mass of the left ventricle by 11, 16, and 19% after 5, 15, and 30 days of hypokinesia respectively, compared with its value in the control ani-

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TABLE 1. Results of Morphometric and Stereologic Investigation of the Rat Heart in Hypokinesia

Parameter studied	Control	Hypokinesia		
		5 days	15 days	30 days
Morphometric characteristics of heart and muscle fibers				
Body weight, g	320,0±11,6	250,0±7,1***	245,0±5,0***	210,0±5,8**
Absolute weight of heart, mg	916,7±44,1	812,5±37,5	787,5±23,9*	733,3±16,7*
Relative weight of heart, mg/g body weight	2,87±0,10	3,25±0,09*	3,22±0,05*	3,50±0,16**
Absolute weight of left ventricle, mg	689,7±38,7	613,3±29,2	579,0±25,7	558,0±13,6*
Relative weight of left ventricle, mg/g body weight	2,15±0,08	2,45±0,06**	2,36±0,07	2,66±0,14*
Diameter of cardiomyocytes, μ	16,10±0,15	15,02±0,21**	14,81±0,09**	13,50±0,10***
Stereologic ultrastructural characteristics of cardiomyocytes				
Relative volume (V_{V_i} cyt), mm^3/cm^3, of:				
myofibrils	513,6±23,8	532,1±15,0	587,6±13,8	591,3±7,5*
mitochondria	312,0±12,6	318,4±14,1	280,9±9,9	257,9±8,4*
sarcoplasmic reticulum	10,0±1,1	11,5±1,0	10,7±0,4	14,0±0,9*
T system	17,7±2,0	19,9±1,7	19,3±2,9	20,9±0,8
remaining structures of cytoplasm	146,7±11,2	119,1±1,3	101,5±15,5*	115,9±2,6*
Relative surface area (S_{V_i} cyt), m^2/cm^3, of:				
myofibrils	0,849±0,023	0,819±0,040	0,782±0,056	0,695±0,032*
mitochondria	1,519±0,103	1,511±0,119	1,236±0,094	1,081±0,087*
sarcoplasmic reticulum	0,323±0,033	0,336±0,012	0,321±0,017	0,415±0,025*
T system	0,327±0,024	0,393±0,006	0,368±0,020	0,388±0,021
Surface/volume ratio (S_{V_i}/V_{V_i}), m^2/cm^3, of:				
myofibrils	1,65±0,03	1,54±0,09	1,33±0,07	1,18±0,07**
mitochondria	4,86±0,14	4,74±0,29	4,39±0,19	4,18±0,19*
sarcoplasmic reticulum	32,40±1,37	29,62±1,79	30,19±2,45	29,88±2,72
T system	21,30±1,18	21,24±2,39	19,85±2,57	18,59±1,33
Ratio of bulk density ($V_{V_i}/V_{V_{mf}}$), cm/cm (number) of:				
mitochondria and myofibrils	0,613±0,052	0,601±0,044	0,479±0,024	0,437±0,019*
sarcoplasmic reticulum and myofibrils	0,020±0,004	0,022±0,002	0,018±0,0004	0,024±0,002
T-system and myofibrils	0,035±0,005	0,036±0,004	0,033±0,004	0,036±0,002
remaining structure of cytoplasm and myofibrils	0,289±0,033	0,224±0,008	0,174±0,029	0,196±0,002*

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

mals. The relative weight of the heart and left ventricle increased during hypokinesia, due to the slower regression of the weight of the heart than the body weight. The diameter of the cardiomyocyte was reduced by 8 and 16% after 15 and 30 days, respectively. The decrease in absolute weight of the heart and in the diameter of the cardiomyocytes is evidence of the development of myocardial atrophy.

The most marked ultrastructural changes were found in muscle cells of the rat heart after hypokinesia for 15 and 30 days. The lytic apparatus of the cardiomyocytes was appreciably activated, as was manifested by an increase in the size and number of lysosomes (Fig. 1a, b). Small foci of lysis of the myofibrils were located both in the region of the Z disks and between them (Fig. 1c, d). The mitochondrial compartment was highly polymorphic, but most organelles were large (1-2 sarcomeres in length) with tightly packed cristae (Fig. 2a). The cisterns of the sarcoplasmic reticulum and the T-systems were dilated (Fig. 2b).

Stereologic analysis of the cardiomyocytes (Table 1, Fig. 3) after 15 days of hypokinesia revealed a significant increase in the relative volume of myofibrils (by 14.4% compared with the control), and also a significant decrease in the bulk density of the cytoplasmic matrix (by 30.8%), due to tissue dehydration [4]. No significant changes were found in the surface to volume ratios of the mitochondria, T-system, and sarcoplasmic reticulum at this stage of the experiment. However, there was a tendency for the ratio of volume of mitochondria to volume of myofibrils to decrease (Fig. 4).

A more fundamental ultrastructural reorganization of the cardiomyocytes took place after 30 days of hypokinesia. The relative volume of the myofibrils continued to increase and reached 591.3 ± 7.5 mm^3/cm^3 (513.6 ± 23.8 mm^3/cm^3 in the control; $P < 0.05$). The surface density of the myofibrils decreased by 18.1%. This disproportionate change in the surface/

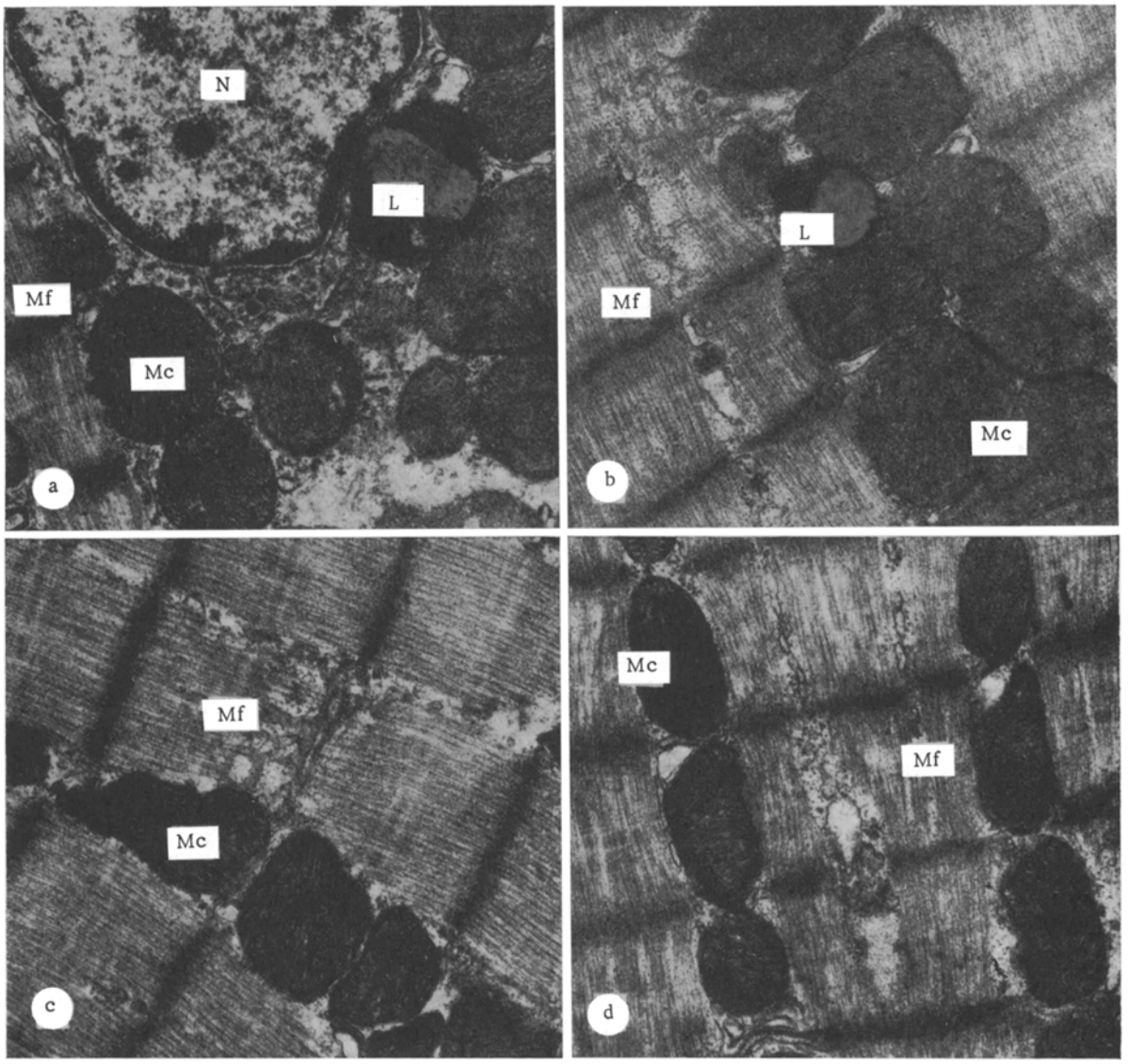

Fig. 1. Lytic processes in cardiomyocytes of rats after 30 days of hypokinesia: a) lysosomes (L) in perinuclear zone; b) lysosomes in myofibrillary zone; c, d) different locations of foci of lysis of myofibrils (Mf). N) Nucleus; Mc) mitochondria. Here and in Fig. 2, 8300×.

volume characteristics led to a decrease in the surface/volume ratio by 28.5%, evidence of considerable thickening of the myofibrils (Fig. 2c, d). A phenomenon of thickening of myofibrils is observed in myocardial hypertrophy of varied genesis [11, 14], and also in cardiac atrophy due to starvation [8]. During hypertrophy of the cardiomyocytes thickening of the myofibrils may be the result of increased protein synthesis in response to an increase in load. The mechanism of this phenomenon in myocardial atrophy when the protein-synthesizing function of the cells is depressed or disturbed has not yet been finally explained. Thickening of the myofibrils evidently takes place as a result of fusion of neighboring myofibrils after lysis of the band of mitochondria located between them, and also partly because of incomplete relaxation of the myofibrils. Thickening of the myofibrils is probably aimed at compensating for their reduced contractility, arising as a result of focal lysis of protofibrils.

The bulk and surface density of the sarcoplasmic reticulum were significantly increased (by 40.0 and 28.5%, respectively), after immobilization of the animals for 30 days. There was a tendency for the bulk and surface density of the T system to increase. The surface to volume ratio of the sarcoplasmic reticulum and T system showed no significant change during 30 days of hypokinesia. The relatively balanced change in bulk and surface densities of the

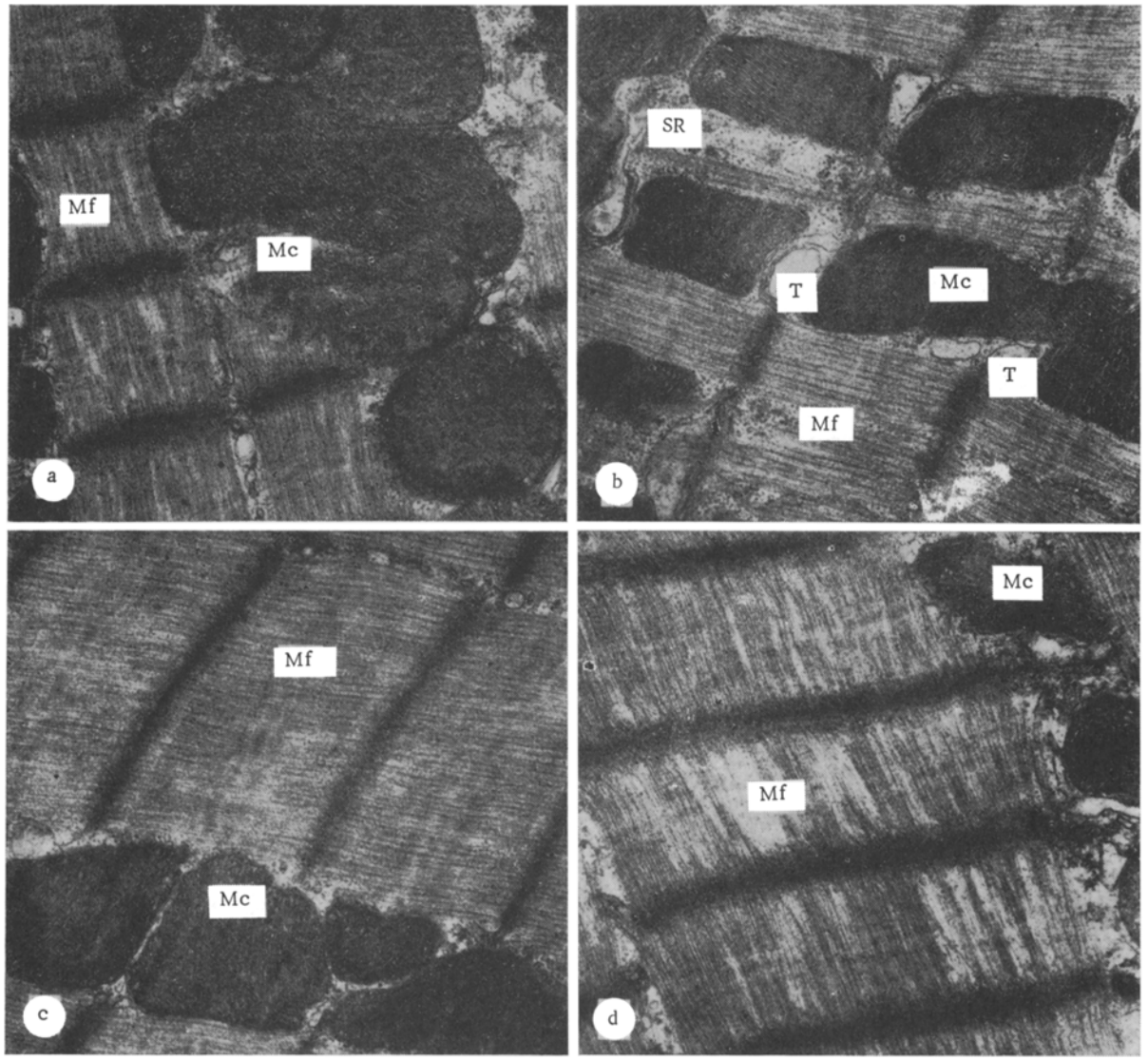

Fig. 2. Changes in organelles of energy-producing and transport systems and of contractile apparatus of cardiomyocytes in rats after 30 days of hypokinesia: a) increase in number of large mitochondria (Mc) with dense packing of cristae; b) dilatation of tubules of T system (T) and of sarcoplasmic reticulum (SR); c) thickening of myofibrils (Mf); d) small foci of liquefaction of protofibrils in thickness of myofibrils.

sarcoplasmic reticulum and relative volume of the myofibrils will be noted. The volume ratios of the apparatus were maintained under these circumstances at the same level as in the control. The compensatory enlargement of the sarcoplasmic reticulum may be due not only to an increase in the heart rate during hypokinesia, but also to disturbances of the electrolyte balance and, in particular, of the calcium metabolism.

The energy apparatus of the cardiomyocyte underwent considerable changes during 30 days of hypokinesia. The bulk density of the mitochondria decreased by 17.3% compared with the control and the surface density decreased by 28.8%. The surface to volume ratio of the mitochondria also decreased from 4.86 ± 0.14 m^2/cm^3 in the control to 4.18 ± 0.19 m^2/cm^3 ($P < 0.05$). These changes reflect a tendency for the above-mentioned organelles to increase in size.

The ratio of bulk density of the mitochondria to that of the myofibrils decreased significantly after 30 days of hypokinesia from 0.613 ± 0.052 in the control to 0.437 ± 0.019 ($P < 0.05$) on account of a predominant increase in the relative volume of the contractile system. According to the results of other investigations [13] this parameter is increased at the above times of hypokinesia, despite a decrease in the number of mitochondria.

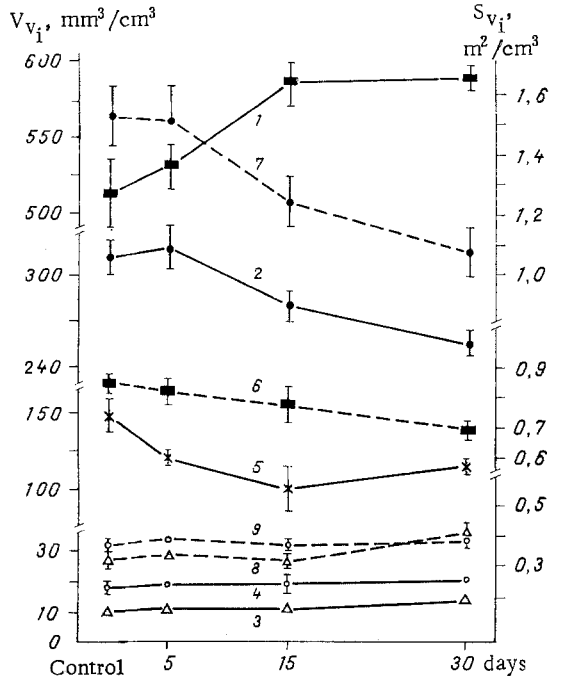

Fig. 3

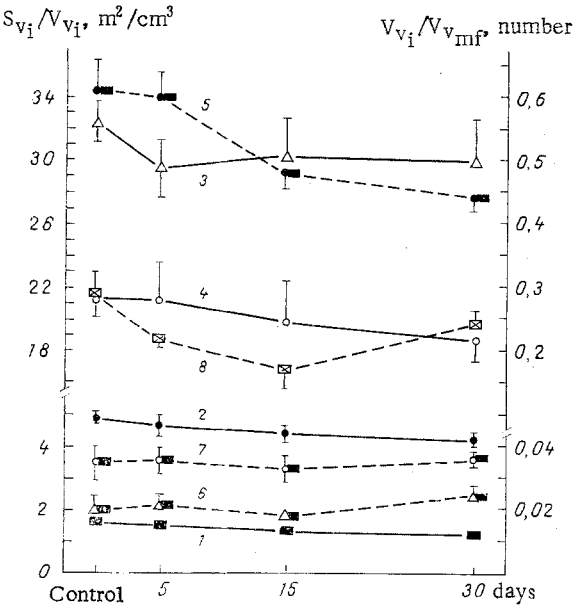

Fig. 4

Fig. 3. Primary stereologic parameters of organelles of work cardiomyocytes during hypokinesia. Abscissa, duration of hypokinesia (in days); ordinate: on left — bulk density, on right — surface density. 1, 6) Myofibrils; 2, 7) mitochondria; 3, 8) sarcoplasmic reticulum; 4, 9) T system; 5) remaining structures of cytoplasm.

Fig. 4. Secondary stereologic parameters of organelles of rat cardiomyocytes during hypokinesia. Abscissa, duration of hypokinesia (in days); ordinate: on left — surface/volume ratio of organelles, on right — ratio of relative volume of various organelles to relative volume of myofibrils. 1) Myofibrils; 2) mitochondria; 3) sarcoplasmic reticulum; 4) T system; 5) mitochondria/myofibrils; 6) sarocplasmic reticulum/myofibrils; 7) T system of myofibrils; 8) remaining structures of cytoplasm/myofibrils.

After 30 days of hypokinesia the relative volume of the cytoplasmic matrix was significantly reduced, as also was the ratio of its bulk density to the relative volume of the myofibrils, probably on account of cell dehydration.

This ultrastructural stereologic investigation thus showed that during 30 days of hypokinesia the structural organization of the cardiomyocyte was significantly modified. The character of this compensatory-adaptive modification is evidence of strain on the function of the intracellular energy-providing system, of membrane ion transport, and of the contractile system, and together with depression of protein synthesis [1, 16], this may lead to a reduction of the functional reserves of the heart and insufficiency of structural metabolism of the myocardium.

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IMMUNOMORPHOLOGICAL INVESTIGATION OF DISTRIBUTION OF COLLAGEN OF TYPES I, III, IV, AND V IN PRIMARY CULTURE OF HUMAN AORTIC CELLS

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Fibrosis is the most important manifestation of atherosclerosis of human arteries. This process is associated with the accumulation of connective-tissue proteins, namely collagen, in the vessel wall. The main part of the connective-tissue matrix in the intact vessel wall consists of type III collagen, whereas in the atherosclerotic intima it consists of type I collagen [5]. Collagens of types IV and V also are present in blood vessels [2, 6, 14] as components of basement membranes. The principal cells which produce collagen in blood vessels are smooth-muscle cells. It has been shown that these cells can synthesize collagen both in the blood vessel *(in vivo)* and also in culture *(in vitro)* [1, 11].

Cultures of vascular smooth-muscle cells are widely used at the present time to study mechanisms of atherogenesis and, in particular, mechanisms of fibrosis [9]. A particularly promising model for such investigations is evidently a primary culture, in which the particular features of vascular cells are preserved to the greatest degree [1]. A primary culture of human aortic cells has recently been obtained and characterized [4, 8] and it can be hoped that it will serve as a useful tool with which to study mechanisms of fibrosis in atherosclerosis in man.

The object of this investigation was the immunocytochemical detection of different types of collagen in such a culture.

EXPERIMENTAL METHOD

Experiments were carried out on 7-day cultures of cells isolated with the aid of enzymes from human aorta. The cells were isolated and cultured as described previously [4, 8]. The morphological characteristics of the cultures were described in [4]. The cells were seeded in Lab Tek chambers (Miles Laboratories, USA) with a density of 10^4 cells/cm^2. On the 7th day of culture the cells were washed with isotonic phosphate buffer, pH 7.4, and fixed with

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