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Morphological and Stereological Characteristics of Myocardial Remodeling in Aged Spontaneously Hypertensive SHR Rats

E. L. Lushnikova and L. M. Nepomnyashchikh

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Myocardial remodeling in SHR rats with age-related hypertrophy was characterized by elimination of cardiomyocytes, their hypertrophy, and marked increase in the volume of the connective tissue. The count of cardiomyocytes with contracture injuries and subsegmental contractures increased, and pronounced perivascular and interstitial sclerosis developed in the hypertrophic myocardium of SHR rats. Damage to the microcirculatory bed manifested in degenerative changes and destruction of some endotheliocytes. Signs of atypical intracellular regeneration in myofibrils and impairment of their longitudinal orientation were revealed in cardiomyocytes in the late stage of compensatory hypertrophy.

Key Words: *SHR rats; myocardial remodeling ; hypertrophy; cardiomyocyte elimination; stereology*

Spontaneously hypertensive SHR rats are a convenient model for studies of not only essential hypertension, but also genetically determined heart hypertrophy [3]. In SHR rats this disorder develops over the first 2 months of life when blood pressure is not increased [5,12,15]. Recent studies showed that arterial hypertension and cardiac hypertrophy have different genetic determinants [7,11]. Proliferation, growth, and death of cardiomyocytes (CMC) and parenchymal cells in other organs and tissues of hypertensive animals are regulated independently on the level of blood pressure [13]. Rats of various strains with genetically determined hypertension are characterized by high proliferative activity of cells in early postnatal ontogeny during inhibition of apoptosis [9,10]. It was hypothesized that the pathogenesis of essential hypertension is associated with a genetically determined increase in proliferative activity of parenchymal cells in the heart and kidneys and smooth muscle cells in vessels [6]. The pathogenetic mechanisms of arterial hypertension and hypertrophy of organs and tissues (particularly heart and vessels) were extensively studied. However, the relationships and regulation of hyperplastic and hypertrophic processes in the myocardium remain unclear.

Here we perform a complex morphological study of main tissue and cellular determinants underlying myocardial remodeling in SHR rats with age-related compensatory hypertrophy.

MATERIALS AND METHODS

A complex morphological study of the myocardium was performed on male inbred SHR rats aged 1 (n=5), 4 (n=5), and 11 months (n=4). Inbred male Wistar rats of the same age served as the control. Blood pressure was measured using a sensor device equipped with a robber cuff and fixed on the tail in animals under light ether anesthesia. Signals were recorded on a Mingograf-34 device (Elema-Schonander).

Laboratory of Cytology and Cell Biology, Laboratory of General Pathological Anatomy, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** pathol@ceber.ma.nsk.ru. Lushnikova E. L.

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The rats were decapitated under narcosis, and the hearts were weighted. Specimens for light microscopy were fixed in 10% neutral formalin and treated by routine techniques. The tissue was embedded into paraffin. The staining procedure for paraffin sections included hematoxylin-eosin, method of van Gieson, and periodic acid-Schiff (PAS) reaction. For an electron microscopy study samples of the left ventricle and papillary muscles were fixed in 4% paraformaldehyde, postfixed in 2% OsO_4, and embedded in Epon—araldite mixture. Semithin sections were stained with azure II and examined under a Docuval universal light microscope. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under JEM-100B and JEM 1010 electron microscopes.

The volume and surface density of main tissue and intracellular compartments in semithin and ultrathin sections was evaluated by morphometric and stereologic assays [1,4]. Secondary stereological parameters (volume and surface-volume relationships between structures) were evaluated after the analysis of primary characteristics. The absolute volume and area of tissue and intracellular structures in the left ventricle were calculated from the relative stereological parameters. The total count of CMC in the left ventricle was estimated after stereological examination. Mathematical and statistical processing of the results was performed as described elsewhere [4].

RESULTS

Quantitative morphological examination showed that 1-month-old male SHR rats had a lower body weight than Wistar rats of the same age (Table 1). However, in SHR rats the relative weight of the heart and volume of individual CMC surpassed those in Wistar rats by 49 ($p<0.001$) and 11%, respectively. Systolic blood pressure in SHR rats aged 1 month was 118±10 mm Hg.

The weight of the heart in 4-month-old SHR rats increased by 21%. These changes did not produce an increase in the cardiac index, which was associated with a greater increase in body weight (by 28%). It should be emphasized that the volume of individual CMC in these animals was 194% higher than in 4-month-old Wistar rats ($p<0.01$, Table 1). These data illustrate severe hypertrophy of CMC in 4-month-old SHR rats. Four-month-old SHR rats were characterized by stable arterial hypertension. Blood pressure in these animals reached 158±4 mm Hg (*vs*. 111±6 mm Hg in the control). In SHR rats aged 11 months the weight and index of the heart and volume of individual CM were higher than in Wistar rats by 34, 85, and 184%, respectively ($p<0.01$, Table 1). Hypertrophy of CMC in SHR rats was primarily related to an increase in their diameter by 68-69%. The length of CMC in these animals remained practically unchanged. Blood pressure in 11-month-old SHR rats was 179±6 mm Hg. In Wistar rats of the same age blood pressure remained unchanged.

Cardiac hypertrophy in SHR rats with stable arterial hypertension (4-11 months) was primarily related to hypertrophy of the left ventricle. The weight and volume of the myocardium in the left ventricle of SHR rats aged 4 and 11 moths increased by 24 and 45%, respectively, compared to the control. In these animals we revealed an increase in the absolute total volume of CMC in the left ventricle (by 25 and 36%, respectively) and connective tissue (by 56 and 66%, respectively). The connective tissue/CMC volume ratio in SHR rats aged 4 and 11 months increased by 22 and 35%, respectively.

Remodeling of hypertrophic hearts in SHR rats aged 4 and 11 months was associated with a decrease in the count of CMC in the left ventricle by 58 ($p<0.01$) and 56% ($p<0.001$), respectively, compared to the control. Elimination of CMC was primarily related to apoptosis, since focuses of coagulating and colliquative necroses of parenchymal cells were not revealed in the myocardium of SHR rats. Intensive apoptosis of CMC in hypertrophic myocardium in SHR rats serves as a marker for decompensation and developing cardiac insufficiency [8]. Pronounced elimination of apoptotic CMC was observed in 4-month-old SHR rats. However, in the period from 4 to 11 months a loss of CMC was compensated by hypertrophy of preserved cells. Intensification of apoptosis in the myocardium of SHR rats induced hypertrophy of parenchymal cells and contributed to remodeling in the heart. Intensive apoptosis in CMC was observed in other models of cardiac hypertrophy (*e.g.*, over the first days after ligation of the aorta in Wistar rats) [14].

Light and electron microscopy confirmed the results of morphometric examination. The myocardium in 1-month-old SHR rats retained normal composition (Fig. 1, *a*). Polarization microscopy revealed contracture changes of myofibrils in individual muscle segments. The myocardial stroma contained single collagen fibers and considerable number of fibroblasts and mast cells. We observed spasm or secondary paresis of intramural arteries. Endotheliocytes swelled into the lumen of these vessels. Smooth muscle cells in the intermediate layer of arteries were irregularly contracted and vacuolized.

Structural and functional changes in the myocardium of SHR rats aged 4 and 11 months were determined by the development of arterial hypertension and manifested in severe hypertrophy of CMC (Fig. 1, *b*, *c*). The number of eosinophilic muscle segments increased, particularly in the intermediate layer of the myocardium in the left and right ventricles. These changes

TABLE 1. Quantitative Morphological Characteristics of the Heart and Cardiomyocytes in Aged SHR Rats with Genetically Determined Spontaneous Hypertension (*M*±*m*)

Parameter	Age, months					
	1		4		11	
	Wistar	SHR	Wistar	SHR	Wistar	SHR
Body weight, g	113.0±4.4	62.0±1.2*	213.3±8.8	273.3±23.3	370.0±22.7	265.0±14.9***
Weight of the heart, mg	525.0±5.0	431.0±19.5**	933.3±44.2	1126.7±148.6	1112.3±68.6	1485.0±84.6***
Relative weight of the heart, mg/g	4.66±0.15	6.96±0.22*	4.37±0.08	4.09±0.22	3.02±0.16	5.60±0.01*
Weight of the left ventricle, mg	0.370±0.003	0.294±0.019**	0.649±0.030	0.808±0.108	0.736±0.023	1.069±0.060**
Myocardial volume in the left ventricle, cm^3	0.349±0.003	0.277±0.018	0.612±0.028	0.762±0.102	0.694±0.022	1.008±0.057**
Diameter of cardiomyocytes, m	12.9±0.2	13.0±0.6	15.2±0.9	25.7±1.4***	15.5±0.4	26.0±0.9*
Length of cardiomyocytes, m	68.9±1.7	75.7±0.4	77.5±0.7	80.3±1.68	0.9±3.8	81.1±4.9
Volume of individual cardiomyocyte, m^3	9086.5±436.8	10 090.8±992.1	14 231.1±1745.5	41 855.1±3776.1**	15 201.7±178.4	43 247.6±4894.6**
Absolute volume (left ventricle), cm^3:						
cardiomyocytes	0.293±0.003	0.232±0.017	0.518±0.022	0.649±0.083	0.602±0.003	0.828±0.023**
connective tissue	0.031±0.003	0.026±0.002	0.052±0.003	0.081±0.015	0.068±0.008	0.113±0.018
microvessels	0.024±0.002	0.019±0.002	0.042±0.006	0.032±0.004	0.045±0.005	0.052±0.007
Connective tissue/cardiomyocyte volume ratio	0.104±0.010	0.113±0.010	0.101±0.002	0.123±0.006***	0.112±0.043	0.151±0.018
Cardiomyocyte count in the left ventricle, $\times 10^6$	32.516±1.697	23.754±4.180	37.181±3.205	15.625±1.864*	39.588±0.443	17.368±0.158*

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to Wistar rats of the same age.

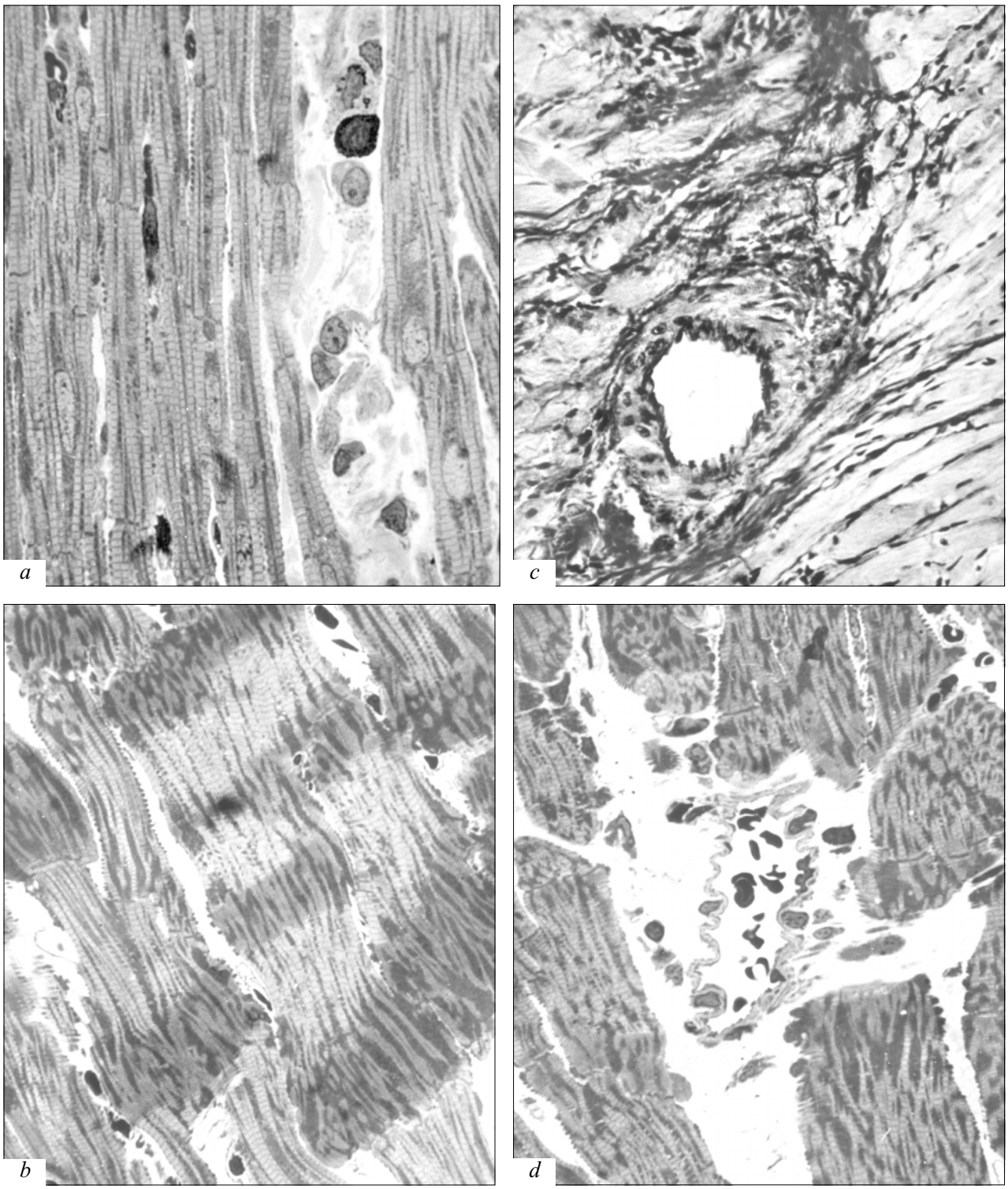

Fig. 1. Morphological characteristics of the myocardium in aged SHR rats: normal composition of the myocardium in 1-month-old animals (*a*); hypertrophy of muscle fibers and subsegmental contractures in 4-month-old animals (*b*); hypertrophy of the muscle layer and perivascular sclerosis of intramural arteries spreading to the interstitial tissue in 4-month-old animals (*c*); hypertrophy of cardiomyocytes and perivascular sclerosis in 11-month-old animals (*d*). Semithin sections stained with azure II (*a*, *b*, *d*, ×1000). Van Gieson staining (*c*, ×400).

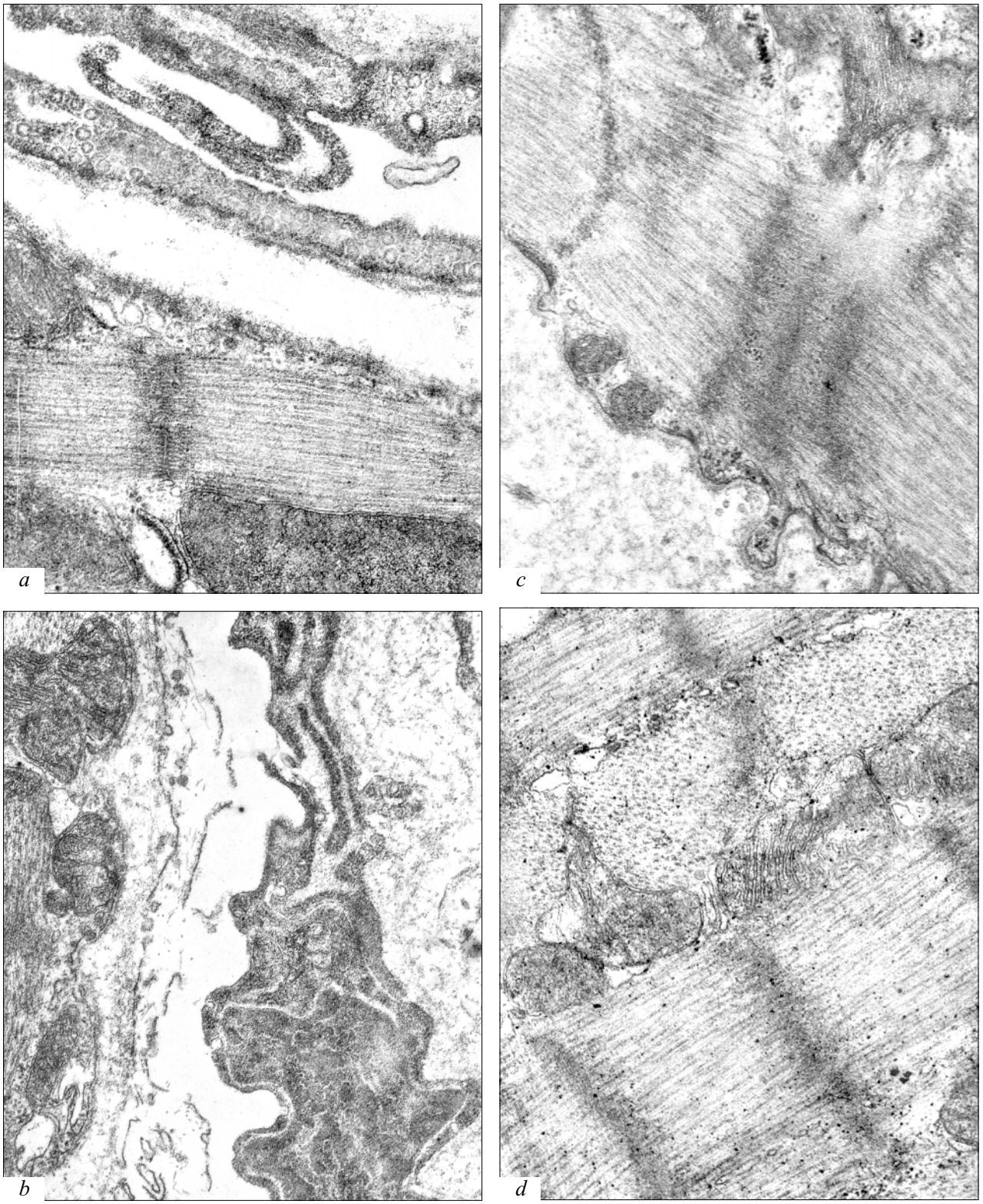

Fig. 2. Ultrastructural characteristics of the myocardium in aged SHR rats: plasma membrane processes in the capillary of 1-month-old animals (*a*, ×10,000); heterogeneous microvascular endotheliocytes in 1-month-old animals (*b*, ×7000); subsegmental contractures in 4-month-old animals (*c*, ×13,300); disorientation of myofibrillar bundles during their regeneration in 11-month-old animals (*d*, ×8000).

were associated with contracture injuries of different severity and subsegmental contractures (Fig. 1, *b*). Intramural vessels underwent most pronounced changes. The wall of arteries was thickened, and their lumen was narrowed due to severe hypertrophy and hyperplasia of smooth muscle cells (Fig. 1, *c*). Sclerosis in various layers of the wall in intramural arteries spread to the perivascular and interstitial connective tissue. We observed multiplication of elastic fibers. SHR rats aged 11 months were characterized by most pronounced intervascular and perivascular sclerosis. Various layers of the arterial wall and perivascular and interstitial connective tissue were characterized by moderate edema (Fig. 1, *d*).

Some ultrastructural characteristics of CMC were revealed in 1-month-old SHR rats. We observed pronounced polymorphism of mitochondria and hyperplasia of the Golgi complex. Bundles of myofibrils had a regular sarcomeric composition and lay along the longitudinal axis of cells. Vacuolar and myelin figures in the subsarcolemmal region of CMC in SHR rats were more often found than in normotensive Wistar rats of the same age.

Capillary endotheliocytes had a similar composition, but differed in a great variety of morphological characteristics. Numerous process with unusual shape were formed on the luminal surface of most endotheliocytes (Fig. 2, *a*). Endotheliocytes with dystrophic and necrobiotic changes were often found (Fig. 2, *b*). Severe damage to the plasma membrane of these cells was revealed in the capillary lumen. The cytoplasm was electron-transparent. Sometimes these cells were completely destructed.

The number of CMC with contracture injuries increased in SHR rats aged 4 and 11 months. In individual cells some myofibrillar bundles were contracted, while others were relaxed. Subsegmental contractures were revealed (Fig. 2, *c*). Myofibrillar bundles in individual CMC of 11-month-old SHR rats were positioned perpendicular to each other (Fig. 2, *d*). The data indicate that intracellular regeneration was accompanied by spatial disorientation of myofibrils. These changes were observed in CMC of Wistar rats with anthracycline-induced cardiomyopathy and progressive regenerative processes [2]. The number of destructed mitochondria increased in SHR rats aged 4 and 11 months. In 4-month-old animals we revealed vacuolization of vesicles in the smooth sarcoplasmic reticulum. The Golgi complex was hyperplastic in SHR rats of both groups.

Capillary endotheliocytes in the myocardium of SHR rats aged 4 and 11 months underwent more pronounced destructive changes. The count of cells with the electron-transparent cytoplasm and the number of thickened collagen fibers in the myocardial stroma increased.

Our results suggest that myocardial remodeling in SHR rats with cardiac hypertrophy is characterized by massive elimination of CMC (by 56-58% after the 4th month of life), severe hypertrophy (increase in the individual volume by 184-194%), and considerable increase in the volume of the connective tissue (by 66% in 11-month-old animals). These changes reflect myocardial remodeling in aged SHR rats with severe genetically determined arterial hypertension. The individual volume of CMC, cell count, and connective tissue/parenchyma volume ratio remained unchanged in Wistar rats of the same age. Our results and published data [10,15] suggest that apoptosis is the main processes regulating the number of CMC during remodeling of the heart. The inhibition of apoptosis is accompanied by hyperplasia of CMC, while activation of this process produces hypertrophic changes.

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