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Morphological Characteristics of Myocardial Remodeling during Compensatory Hypertrophy in Aging Wistar Rats

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Remodeling of the myocardium in Wistar rats during aging is characterized by cardiomyocyte hypertrophy and increase of their total volume in the left ventricle; the total volume of the connective tissue also increases without marked changes in cardiomyocyte count and total volume of blood capillaries. The number of cardiomyocytes with contracture injuries of different severity in the myocardium of aging animals increases against the background of hemodynamic disorders. At the cellular level, compensatory hypertrophy of cardiomyocytes during aging is characterized by a pronounced increase in the myofibril bulk paralleled by relative deficiency of the mitochondria and pronounced deficiency of agranular sarcoplasmic reticulum and T system, involved in the regulation of intracellular Ca^{2+}.

Key Words: *aging Wistar rats; myocardium; compensatory hypertrophy; cardiomyocyte ultrastructure; stereology*

Heart remodeling, *i. e.* rearrangement of the normal structural composition and architectonics of the organ, now attracts special attention because of search for integral morphofunctional criteria characterizing heart failure of different origin and deciphering of the molecular genetic mechanisms underlying this phenomenon [2,9,10,15]. Remodeling of the myocardium (RM) is a complex process of adaptive reorganization of cardiomyocytes (CMC), vascular network, and connective tissue components caused by hemodynamic changes, etiological, epidemiological, neurohormonal, and other factors [15]. One of the most important RM determinants is cell death, because it leads to the loss of contractile cells, compensatory hypertrophy, and reparative fibrosis [12, 16]. It is now accepted that RM characterizes pathological states and is related to aging.

According to epidemiological data, RM is the main cause of death in humans over 65 years. Changes in heart architectonics, its phenotypical modifications in cardiomyopathies of different genesis, myocardial infarction or myocarditis differ from its structural and functional rearrangement during aging [15]. However, many aspects of RM induced by pathological processes and associated with aging remain little studied, which does not allow us to define the difference between changes caused by pathological factors and fundamental process of biological aging.

We investigated the main tissue and cell determinants of RM in the course of gerontogenesis in Wistar rats.

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MATERIALS AND METHODS

Tissue and cell reorganization of the myocardium during gerontogenesis was studied in 106 male Wistar rats aged 4-33 months. The animals were kept under standard vivarium conditions with free access to water. The animals were regularly weighed. The rats were sacrificed by decapitation under chloroform narcosis, the heart and its compartments were weighed.

General histological study was carried out on paraffin sections (histotopograms) including myocardium of the right and left ventricles (LV) and interventricular septum. The sections were stained with hematoxylin and eosin with Perls reaction, by Van-Gieson's method, and PAS test was carried out. For special histological study, semithin sections were stained with Azur II and examined under a Docuval universal optic microscope.

For electron microscopy, specimens of the left papillary muscles and LV were fixed for 30 min and cut into small pieces retaining fiber orientation. Tissue specimens were fixed in a fresh portion of paraformaldehyde for 2.5-3.0 h at 4°C and postfixed in 1% OsO_4 for 2 h. After standard processing tissue specimens were embedded in epon-araldite. Ultrathin sections were examined under JEM 100B and JEM 1010 electron microscopes at accelerating voltage 60 kV.

Volume and surface density of the main tissue and cellular components was evaluated by morphometric and stereological methods on semithin and ultrathin sections. Secondary parameters (volume and surface-volume ratios of structures) were calculated on the basis of the primary stereological parameters. Based on the relative stereological parameters, the absolute volumes and areas of LV tissue and cell structures were calculated [1]. The total count of CMC in the heart LV was evaluated by stereological methods [5,14].

The data were processed by mathematical and statistical methods as described previously [3].

RESULTS

RM in Wistar rats during gerontogenesis was characterized by cardiac hypertrophy, which was determined primarily by CMC hypertrophy (Table 1). Heart weight in 24-month-old rats increased by 66%, the weight and volume of LV increased by 37%. In 33-month-old animals heart weight increased by 55% ($p<0.001$) and LV weight and volume by 44% ($p<0.001$). Myocardial hypertrophy and increase in the total volume of CMC and LV (by 35 and 44% at the age of 24 and 33 months, respectively, $p<0.01$) were mainly due to increased volume of CMC (by 41 and 45% at the age of 24 and 33 months, respectively, $p<0.05$), because CMC count was virtually the same during different periods of ontogeny (Table 1).

The fundamental structure of the myocardium was similar in rats of all ages (4-33 months). However, the parenchyma-stroma and intracellular interactions were different at different age. In 4- and 11-month-old rats, CMC in all myocardial layers were evenly stained, transverse striation of muscle fibers was clearly seen (Fig. 1, *a*). Myocardial stroma pre-

TABLE 1. Quantitative Characteristics of Myocardium in Wistar Rat in the Course of Gerontogenesis ($M\pm m$)

Parameter	Age, months		
	4	24	33
Body weight, g	213.3±8.8	240.0±25.2	337.5±5.0*
Heart weight, mg	933.3±44.2	1550.0±0.4*	1450.0±86.5*
Relative heart weight, mg/g	4.37±0.08	6.20±0.85***	4.31±0.30
Cardiac LV weight, mg	0.649±0.030	0.892±0.002**	0.935±0.024*
Cardiac LV volume, cm^3	0.612±0.028	0.842±0.002*	0.884±0.022*
CMC diameter, μ	15.2±0.9	17.8±0.2***	18.0±0.6***
CMC length, μ	77.5±0.7	80.5±0.5	79.5±0.8
Individual CMC volume, μ^3	14 231.1±1745.5	20 041.8±617.2***	20 670.4±1750.9***
Absolute volume (for LV), cm^3:			
CMC	0.518±0.022	0.699±0.004**	0.744±0.017**
connective tissue	0.052±0.003	0.091±0.009***	0.098±0.012***
capillaries	0.042±0.006	0.051±0.006	0.051±0.003
CMC count in LV, 10^6	37.181±3.205	39.699±5.596	36.405±2.158

Note. Here and in Table 2: *$p<0.001$, **$p<0.01$, ***$p<0.05$ *vs.* 4-month-old animals.

TABLE 2. Quantitative Characteristics of CMC in Wistar Rat in the Course of Gerontogenesis ($M \pm m$)

Parameter	Age, months		
	4	24	33
Volume density of, mm^3/cm^3			
myofibrils	475.7±14.7	558.0±23.5***	565.3±11.6**
mitochondria	312.3±10.5	256.5±15.7***	286.9±10.0
ASR	24.2±0.9	14.0±1.4*	10.5±0.6*
T system	22.6±0.6	16.5±0.3*	9.2±0.8*
cytoplasmatic matrix	165.2±1.3	154.9±15.5	128.1±12.3
Surface density, m^2/cm^3			
myofibrils	1.673±0.082	1.637±0.037	1.466±0.053
mitochondria	1.706±0.055	1.581±0.112	1.266±0.036
ASR	0.455±0.043	0.267±0.033	0.258±0.028
T system	0.341±0.032	0.212±0.014	0.121±0.032
Absolute volume (for LV), cm^3			
myofibrils	0.243±0.009	0.386±0.016**	0.417±0.012*
mitochondria	0.160±0.012	0.177±0.012	0.211±0.004**
ASR	0.012±0.001	0.0100±0.0007	0.0080±0.0004***
T system	0.011±0.001	0.0110±0.0004	0.0070±0.0006***
Surface area (for LV), m^2			
myofibrils	0.853±0.022	1.133±0.032**	1.081±0.051***
mitochondria	0.873±0.054	1.096±0.083	0.933±0.035
ASR	0.233±0.028	0.185±0.022	0.189±0.018
T system	0.174±0.016	0.147±0.009	0.096±0.008**

sented as individual bundles of collagen fibers and solitary fibroblasts. Irregular venous and capillary plethora was found in practically all animals.

In 24-and 33-month animals we found hypertrophic muscle fibers (Fig. 1, *b*) and increased number of CMC with contracture injuries of different severity (Fig. 1, *c, d*). Hemodynamic disorders included vascular plethora and moderate interstitial edema. The majority of intramural arteries were in the state of spasm or secondary paresis. Moderate perivascular sclerosis involved the adjacent interstitial connective tissue. In some 33-month animals we observed pronounced sclerotic changes in the epicardium: thickened bundles of collagen fibers, increased number of connective tissue cells, and accumulation of elastic fibers. Quantitative morphological analysis showed that in aging rats remodeling of hypertrophied myocardium was associated with increased total volume of connective tissue in LV (by 75 and 88% at the age of 24 and 33 months, respectively, $p<0.05$), while the total volume of capillaries remained virtually unchanged in late ontogeny in comparison with that at the age of 4 months (Table 1).

The general ultrastructure of CMC was similar in all age groups. Myofibrils were oriented parallel to the longitudinal cell axis (Fig. 2, *a, b*), A and I bands and Z lines in I bands were clearly seen. Myofibrillar bundles thickened starting from the age of 20 months. Starting from the age of 33 months, fragmentation and blurring of Z lines with impairment of the sarcomer structure was observed in the insertion disks. Partial lysis of myofilaments in the Z lines was observed in some CMC, which led to visualization of the structure of agranular sarcoplasmic reticulum (ASR) (Fig. 2, *c*).

Densely packed mitochondria (mainly oval) were seen between myofibrils. Destructive changes in the mitochondria (decreased number of cristae and their disorganization, focal lysis of mitochondrial matrix) were observed only in senescent animals. CMC ultrastructure in senescent rats was also characterized by the appearance of giant mitochondria (several sarcomers long). The Golgi complex in both young and senescent animals was moderately developed and located mainly in the perinuclear zone. A specific feature of ultrastructural organization of CMC in old rats was the appearance of numerous secondary lysosomes and autophagosomes in the sarcoplasm (Fig. 2, *d*). These structures were sometimes giant and located in various parts of the cell. Secondary lysosomes

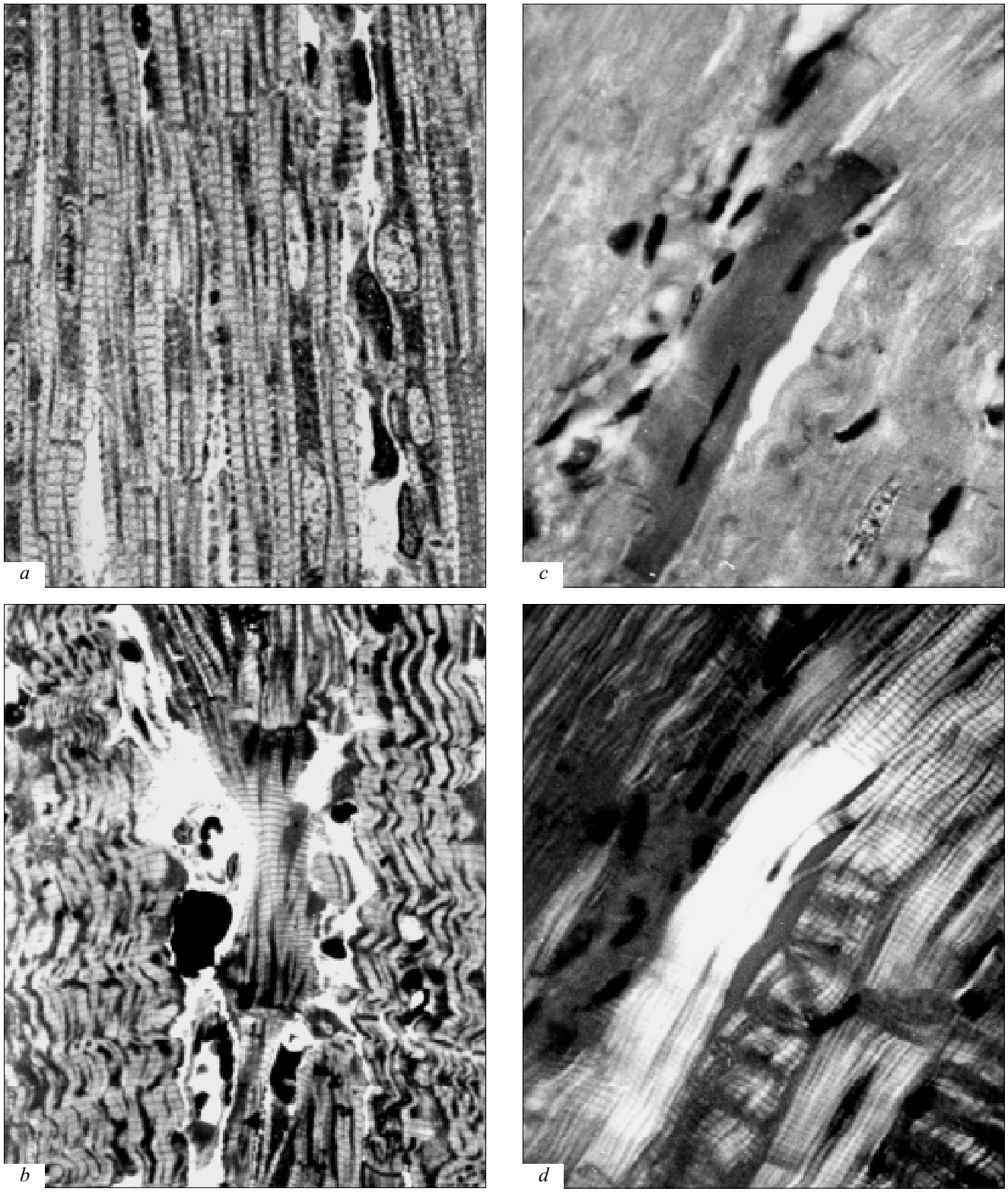

Fig. 1. Morphological changes in the myocardium of Wistar rats with compensatory hypertrophy during aging. *a*) normal myocardium of a 4-month-old rat, ×1000; *b*) cardiomyocyte hypertrophy and myofibril contractures at the age of 33 months, ×1200; *c*) eosinophilia of a muscle segment due to contracture injury at the age of 24 months, ×800; *d*) the same in polarized light. *a, b*) semithin sections, Azur II staining; *c, d*) hematoxylin and eosin staining.

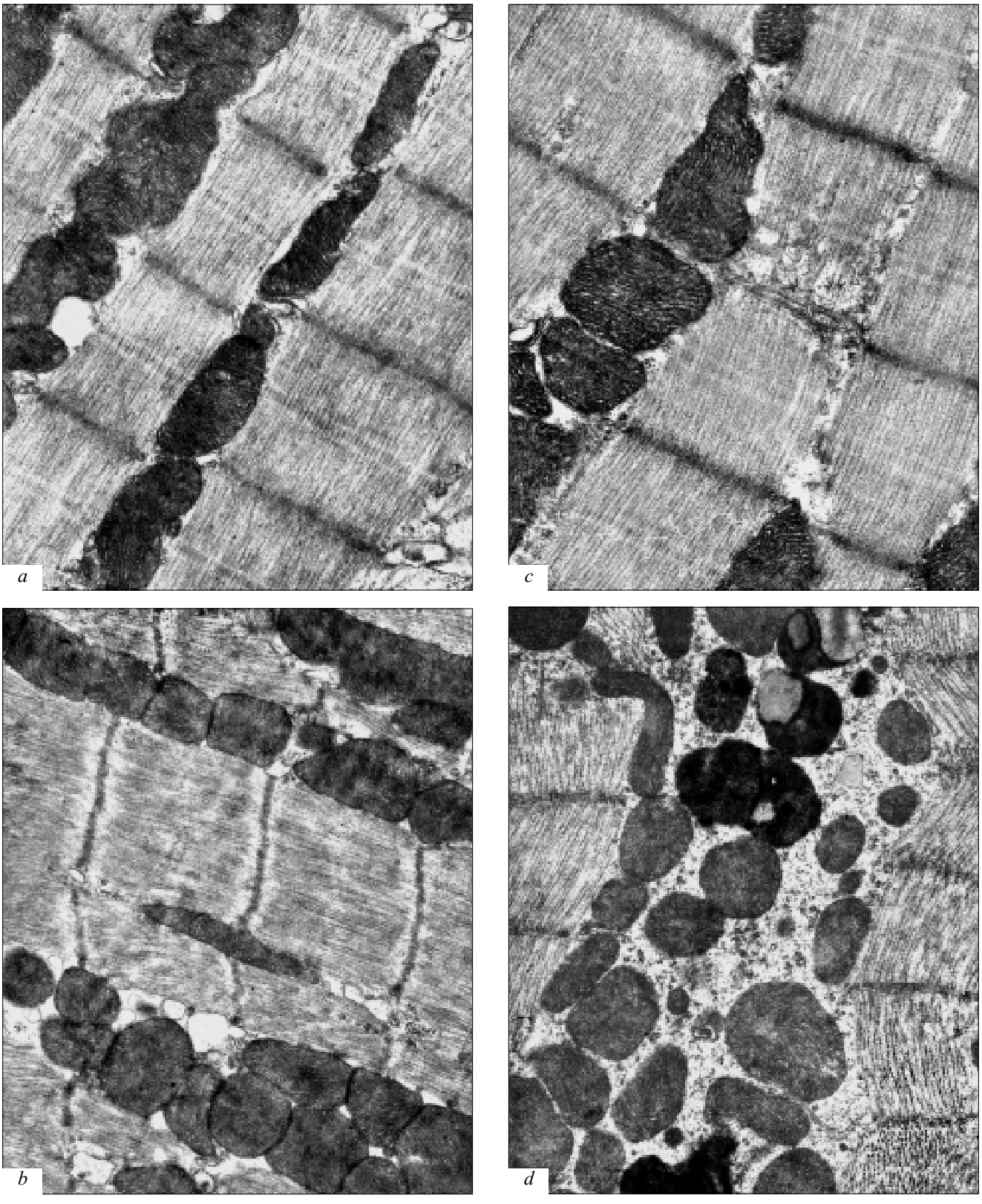

Fig. 2. Cardiomyocyte ultrastructure in Wistar rats with compensatory hypertrophy during aging. *a*) intact cardiomyocyte of a 4-month-old rat; *b*) thickened myofibrillar bundles in a 11-month-old rat; *c*) visualization of agranular sarcoplasmic reticulum as a result of focal lysis of myofilaments at the age of 24 months; *d*) secondary lysosomes with heterogeneous contents at the age of 24 months.

contained lipofuchsine, myelin-like structures, and glycogen sequestra.

Spatial reorganization in CMC was characterized by a higher volume density of myofibrils (by 17 and 19% in animals aged 24 and 33 months, respectively) and decreased volume density of mitochondria (by 18 and 8%, respectively), ASR (by 42 and 57%), and T system (by 37 and 59%, Table 2). Disproportionate changes in the volume density of the main organelles are typical for intracellular reorganization of CMC in the presence of compensatory hypertrophy [1]. This was paralleled by a decrease in the surface density of the main CMC organelles. Estimation of the absolute volume and surface area of cytoplasmatic structures in LV myocardium showed a significant increase in the volume (by 58 and 72% in animals aged 24 and 33 months, respectively) and surface area (by 33 and 27% in animals aged 24 and 33 months, respectively) of myofibrils and of the volume (by 11 and 32%, respectively) and surface area (by 26 and 7%, respectively) of the mitochondria. The absolute volume of ASR and T system decreased significantly in 33-month-old rats by 33 and 36% and the surface areas of these structures decreased by 19 and 45%, respectively (Table 2).

These data indicate that compensatory hypertrophy of CMC during aging is characterized by pronounced increase in the myofibril bulk associated with relative deficiency of mitochondria and pronounced deficiency of ASR and T system involved in the regulation of intracellular Ca^{2+} level.

Changes in aging heart are caused by three basic factors: general aging, aging of the endocrine system, and aging of vessels (specifically, increase of the aortic impedance created LV loading) [15]. Even in the absence of arterial hypertension and coronary insufficiency the aging heart was characterized by the same phenotypical modification of contractile and membrane proteins as the heart under conditions of high blood pressure, which results in more economic contractions [7]. Fibrosis and membrane modifications in aging heart, modifying the duration of action potential and calcium transport facilitate arrhythmias and ectopic contractions, and decreased variability of the heart rate [8]. The density of β-adrenergic and muscarinic receptors in CMC decreases during aging [11,13]. These functional changes are paralleled by a decrease in CMC count due to apoptosis and necrosis [4]. CMC proliferate at some stages of late ontogeny (in 12-29-month-old rats), which leads to incomplete restoration of their population [6].

We detected no manifest changes in CMC count in LV of aging rats, which can be attributed to proliferation of CMC at certain stages of ontogeny. However, the proliferative potential of CMC is insufficient for compensating for their death, which results in CMC hypertrophy. It is noteworthy that CMC hypertrophy during aging is less pronounced than during states characterized by LV overload [1].

Hence, RM in aging Wistar rats is determined by general adaptation to changing conditions of functioning (CMC and heart hypertrophy), intensification of sclerotic processes, and relative stabilization of CMC count in late ontogeny.

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