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PARENCHYMAL–STROMAL INTERACTION IN HEART MUSCLE DURING AGING

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

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Although the concept of interrelations between parenchyma and stroma is well established [1-3, 10, 11], there are very few concrete facts on parenchymal–stromal interaction under normal and pathological conditions [5, 6]. In research already undertaken the connective tissue as a rule has been studied in isolation from the parenchyma – the structure with which it constitutes a functional and dynamic, indissoluble unity. Investigations of the parenchyma and stroma of the heart by quantitative morphological methods are rare [7, 12-14]. From the age aspect the problem has been studied only at the ultrastructural level [8, 15].

This paper describes a morphometric and stereologic study of the muscle and connective tissue of the rat myocardium during aging.

EXPERIMENTAL METHOD

A quantitative study of tissue interrelation in the myocardium was undertaken on 10 intact male Wistar rats aged 4 months (three animals) and weighing 213.3 ± 8.8 g, aged 24 months (three animals) and weighing 240.0 ± 25.2 g, and aged 33 months (four animals) and weighing 337.5 ±5.0 g. After decapitation of the animals the heart was removed from the chest and placed in a cold chamber until it completely stopped beating. The relative weight of each heart was then determined. Samples of tissue from the left ventricle were fixed in 4% paraformaldehyde, postfixed in 2% OsO_4 solution, dehydrated in propylene oxide, and embedded in a mixture of Epon and Araldite. Semithin sections through cardiomyocytes were obtained on an LKB (Sweden) Ultrotome, stained with 1% azure II solution, and examined in a universal electron microscope. For each group of animals the mean diameter of the cardiomyocytes was determined in semithin sections by means of the MOV-15^{x} ocular micrometer with a magnification of 639. The same sections were used for stereologic analysis, under a magnification of 1000. A test system of short segments was used (n = 36, P = 72, L = 7.05 μ). By the stereologic methods described previously [6] the following primary parameters were assessed: relative volume (V_V) of the cardiomyocytes, of the cardiomyocyte nuclei, and of the lumen and endothelial cells of the capillaries, cells, and ground substance of the connective tissue, and also the relative surface area (S_V) of the cell structures. These data were used to calculate values of surface–volume ratios of the tissue structures, ratios of volumes of capillaries and stroma of the myocardium to cardiomyocytes and the ratio of the surface density of the capillaries to the bulk density of the cardiomyocytes. Statistical significance of differences between means was determined by Student's t test.

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 Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 6, pp. 119-122, June, 1983. Original article submitted November 18, 1982.

TABLE 1. Quantitative Morphological Characteristics of Parenchymal–Stromal Interrelations for Rat Myocardium during Aging (M ± m)

Parameters	Age of animals, months			P_{1-2}	P_{1-3}	P_{2-3}
	$4_{(1)}$	$24_{(2)}$	$33_{(3)}$			
Morphometric data						
Body weight, g	213,3±8,8	240,0±25,2	337,5±5,0		0,001	0,01
Absolute weight of heart, mg	933,3±44,2	1550,0±0,0	1450,0±86,5	0,001	0,01	
Relative weight of heart, mg/g body weight	4,37±0,08	6,20±0,85	4,31±0,30	0,05		0,05
Diameter of cardiomyocytes, μ	15,2±0,9	17,8±0,2	17,9±0,6	0,05	0,05	
Stereologic data						
Relative volume ($V_{V_1}^{mn}$) mm^3/cm^3						
of cardiomyocytes	835,7±7,5	823,7±5,1	833,6±11,3			
of cardiomyocyte nuclei	10,6±0,2	7,6±0,8	6,3±1,1	0,05	0,05	
of capillaries	48,8±5,6	45,0±7,1	40,8±4,7			
of endothelial cells	19,4±2,1	15,2±1,4	16,7±2,0			
of connective-tissue cells	12,0±1,4	11,9±1,2	13,8±1,8			
of ground substance of of connective tissue	73,5±1,4	96,6±9,6	88,8±9,2			
Relative surface area ($S_{V_1}^{mk}$), m^2/cm^2:						
of cardiomyocytes	0,1072±0,0062	0,0690±0,0032	0,0639±0,0039	0,01	0,01	
of cardiomyocyte nuclei	0,0069±0,0003	0,0050±0,0006	0,0045±0,0008			
of capillaries	0,0358±0,0016	0,0267±0,0021	0,0237±0,0009	0,05	0,01	
of connective-tissue cells	0,0126±0,0014	0,0128±0,0026	0,0129±0,0018			
Surface–volume ratios (S_{V_1}/V_{V_1}) m^2/cm^3:						
of cardiomyocytes	0,128±0,009	0,084±0,004	0,077±0,005	0,01	0,01	
of cardiomyocyte nuclei	0,650±0,031	0,657±0,087	0,705±0,023			
of capillaries	0,745±0,049	0,610±0,066	0,607±0,079			
of connective-tissue cells	1,056±0,031	1,072±0,165	0,934±0,049			
Ratio of bulk density of stroma to bulk density of parenchyma ($V_{V_{str}}/V_{V_{cmc}}$)	0,182±0,011	0,203±0,008	0,191±0,018			
Ratio of bulk density of capillaries to bulk density of cardiomyocytes ($V_{V_{cap}}/V_{V_{cmc}}$)	0,059±0,007	0,055±0,009	0,049±0,006			
Ratio of surface density of capillaries to bulk density of cardiomyocytes, m^2/cm^3	0,043±0,002	0,032±0,002	0,028±0,001	0,05	0,01	

EXPERIMENTAL RESULTS

During aging in rats hypertrophy of the heart develops: the absolute weight of the organ increased from 933.3 ± 44.2 to 1550.0 ± 0.0 mg (on average by 66%), and the diameter of the muscle fiber increased from 15.2 ± 0.9 to 17.9 ± 0.6 μ ($P < 0.05$; Table 1). A characteristic feature of hypertrophy of the heart during aging (Fig. 1) was preservation of the relative volume of the cardiomyocytes: 835.7 ± 7.5, 823.7 ± 5.1, and 833.6 ± 11.3 mm^3/cm^3 in rats aged 4, 24, and 33 months respectively (Fig. 1). The relative volume of the cardiomyocyte nuclei decreased with age by about 40%. The surface density of the cardiomyocytes and their nuclei fell considerably during aging — by 40 and 35% respectively. The decrease in surface density of the cardiomyocytes led to a decrease in their surface–volume ratio with age from 0.128 ± 0.09 m^2/cm^3 in animals aged 4 months to 0.084 ± 0.004 and 0.077 ± 0.005 m^2/cm^3 in rats aged 24 and 33 months respectively ($P < 0.01$). The increase in the surface–volume ratio of the cardiomyocyte nuclei in rats aged 33 months was not significant (Table 1). The significant decrease in surface density of the cardiomyocytes and in their surface–volume ratio is evidence of thickening of these cells during aging.

During aging a tendency was noted for the capillarization of the myocardial tissue to decrease, as shown by a decrease in the relative volume of the capillary lumen with age. In

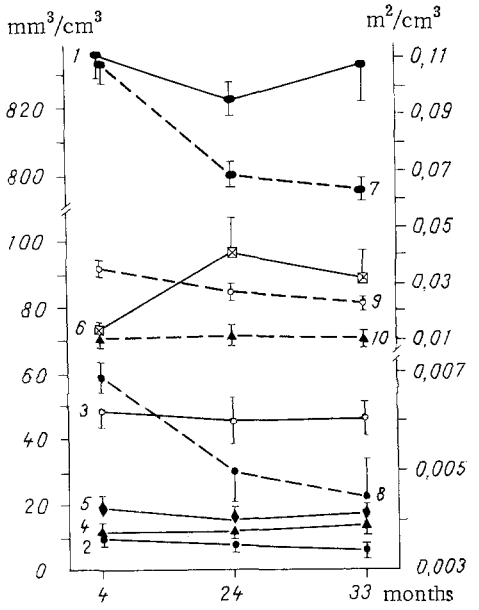

Fig. 1. Results of measurements of primary stereologic parameters on myocardial tissue structures in rats during aging. Abscissa, animals' age (in months); ordinate: on left — bulk density, on right — surface density. 1, 7) Cardiomyocytes; 2, 8) cardiomyocyte nuclei; 3, 9) capillaries; 4, 10) connective-tissue cells; 5) endothelial cells; 6) ground substance of connective tissue.

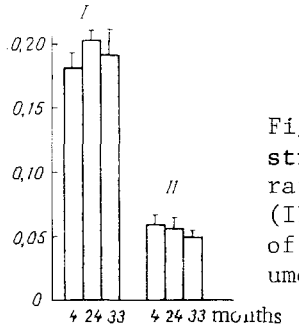

Fig. 2. Volume ratio of myocardial stroma to parenchyma (I) and volume ratio of capillaries to cardiomyocytes (II) of aging animals. Abscissa, age of animals (in months); ordinate, volume ratio of tissue structures.

rats aged 24 and 33 months the relative surface area of the capillaries fell significantly — 0.0267 ± 0.0021 and 0.0237 ± 0.0002 m^2/cm^3 respectively compared with 0.0358 ± 0.006 m^2/cm^3 in rats aged 4 months. The surface—volume ratio of the capillaries did not change significantly with age, although there was a tendency for this paramenter to decrease. In the course of senile hypertropy of the heart the relative volume of the capillaries in the stroma as a whole showed a decrease. For instance, it was 32% of the total volume of the stromal tissues in rats aged 4 months, but 26 and 25% at the ages of 24 and 33 months. This indicates that growth of the capillaries relative to the stroma decreased more than relative to the myocardial tissue as a whole. Interrelations between capillaries and cardiomyocytes make a basic contribution to the understanding of the structural and functional state of the myocardial tissue. Quantitative parameters describing these interrelations are the ratios of surface and bulk densities of the capillaries to bulk density of the cardiomyocytes. With age there was a significant decrease in the ratio of the surface density of the capillaries to the volume of the cardiomyocytes from 0.043 ± 0.002 m^2/cm^3 in rats aged 12 months to 0.0267 ± 0.0021 and 0.028 ± 0.001 m^2/cm^3 in animals aged 24 and 33 months ($P < 0.05$). Meanwhile the ratio of bulk density of the capillaries to volume of cardiomyocytes did not change significantly (Fig. 2).

The time course of the changes in quantitative parameters of the capillaries was parallel to that of the endothelial cells. The relative volume of these structures decreased but not significantly. The relative content of endothelial cells in the myocardial stroma fell with age. In animals aged 4 months the volume of the endothelial cells was 13% of the volume of the stroma, at the age of 24 months it was 9%, and 33 months — 10%.

Hypertrophy of the heart during aging was reflected in a very small increase in the relative volume of connective tissue: from 85.5 ± 1.3 mm^3/cm^3 in animals aged 4 months to 108.5 ± 10.5 and 102.6 ± 10.9 mm^3/cm^3 in rats aged 24 and 33 months. The relative volume of both cellular and noncellular components of the connective tissue increased. The surface density of the cellular components of the connective tissue and their surface—volume ratio showed no significant change. Connective tissue accounted for 56% of the myocardial stroma in rats aged 4 months. The percentage content of connective tissue in the stroma of the heart increased with age to 64% in rats 24 months old, and thereafter became stabilized at this level. In animals aged 33 months connective tissue also accounted for 64% of the volume of the stroma.

On the whole the quantitative parameters of parenchymal—stromal interaction at the tissue level of organization of the myocardium did not change significantly with age [4]. The relative volume of the myocardial stroma increased, but not significantly, during aging from 153.8 ± 7.7 mm^3/cm^3 in rats aged 4 months to 168.7 ± 5.7 and 160 ± 12.4 mm^3/cm^3 in animals aged 24 and 33 months. The ratio of bulk density of the stroma to bulk density of the parenchyma also increased a little (Fig. 2), more so in rats aged 24 months, but the differences here were not significant.

The bulk density of the parenchyma and stroma was thus not significantly changed in the myocardium of old rats. Within muscle tissue and connective tissue, interacting with each other, quantitative structural changes caused by chronic hypertrophic growth of the heart took place. The results of this investigation show that during age hypertrophy of the myocardium, just as during induced growth of the heart [12], the increase in weight of the myocardial parenchyma was accomplished purely by hypertrophy of preexisting cardiomyocytes and hyperplasia of cells of the interstitial connective tissue. This quantitative stereologic investigation of cardiomyocytes and their nuclei at the tissue level, and also an ultrastructural stereologic analysis of organelles of cardiomyocytes undertaken by the writers previously during aging [8] show that the views expressed on intracellular regeneration are characteristic also of heart muscle in old age [9].

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