

KEY WORDS: myocardium; ultrastructure; biorhythms; coarctation of the aorta.

Numerous investigations [1-3, 8, 9] have demonstrated the important role of the seasonal factor in changes in various parameters of the cardiovascular system and the onset of heart diseases. However, the great majority of these investigations have been clinical studies. Experimental data on the effect of seasonal factors on the heart are scanty and there have been virtually no studies of changes in heart muscle ultrastructure in the course of the year.

It was accordingly decided to make a parallel study of some parameters of cardiac function and ultrastructure at different seasons of the year and to compare them with the corresponding parameters of cardiac activity under pathological conditions. Data described in this paper were obtained jointly with V. M. Derevyanko, L. V. Efimova, and S. M. Chibisov.*

EXPERIMENTAL METHOD

Experiments were carried out on 80 male Chinchilla rabbits weighing 2.5-3.5 kg. The diastolic arterial pressure (BP_d), the maximal systolic pressure in the left and right ventricles during occlusion of the ascending aorta and pulmonary artery, respectively, for 5 sec (BP_s), the blood level of free fatty acids (FFA), the thickness of the myocardial fibers, and the state of myocardial ultrastructure were studied in the experiments of series I in intact animals during the middle month of each season of the year (January, April, July, October). In series II BP_s in the left ventricle and myocardial ultrastructure were studied in animals 6 days after constriction of the ascending aorta by one third or after injection of 1 MLD diphtheria toxin. BP_d and BP_s were recorded by an electromanometer, the FFA concentration was determined titrimetrically, and the thickness of the muscle fibers was measured in the MBI-15 light microscope. Material for electron microscopy was obtained under perfusion conditions, fixed in 2.5% glutaraldehyde solution, and postfixed in 1% buffered OsO_4 solution at pH 7.2-7.4, and embedded in Araldite. Sections were cut on a Reichert-Jung Ultracut ultramicrotome, stained with lead hydroxide and uranyl acetate, and studied in the Tesla-BS540 electron microscope under a magnification of 20,000-50,000. Electron micrographs were subjected to quantitative analysis by the method in [5], modified as follows: In addition to the coefficient of energy efficiency of the mitochondria for the electron micrograph as a whole ($CEEM_{em}$), the corresponding parameter also was calculated on average for one mitochondrion ($CEEM_{mc}$). When electron micrographs from intact rabbits were studied, the maximal absolute value of these parameters in the corresponding season were taken as 100 for both $CEEM_{em}$ and $CEEM_{mc}$. The numerical data were subjected to statistical analysis by Student's t test. The level of significance of differences was $P \leq 0.05$.

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TABLE 1. Effect of the Seasonal Factor on Some Physiological Parameters in Rabbits

Parameter	Winter	Spring	Summer	Fall
FFA, μ moles/ml	$0,689 \pm 0,037$	$0,738 \pm 0,056$	$0,378 \pm 0,027$	$0,894 \pm 0,037$
BP_d , mm Hg	46 ± 5	77 ± 5	75 ± 10	59 ± 7
Thickness of fibers of left ventricular myocardium, μ m	$20,7 \pm 1,7$	$18 \pm 0,95$	$19,3 \pm 0,9$	$24,2 \pm 1,1$
BP_s of left ventricle, mm Hg	242 ± 7	$252 \pm 8,6$	184 ± 7	220 ± 5
BP_s of right ventricle, mm Hg	53 ± 2	$70 \pm 3,2$	78 ± 4	$48 \pm 3,6$

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Fig. 1

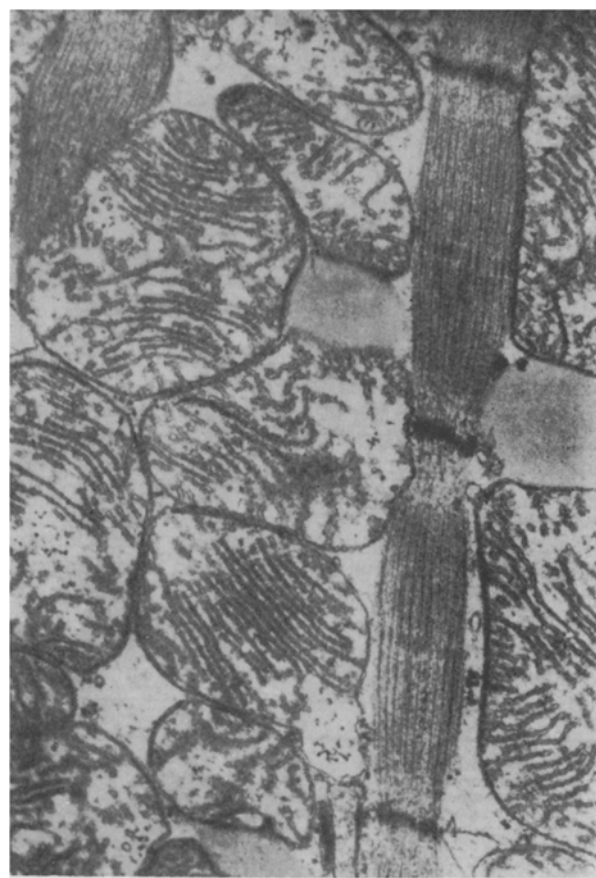


Fig. 2

Fig. 1. **Electron micrograph** of left ventricle of intact rabbit in winter: swelling of mitochondria, destruction of cristae, translucency of matrix, and appearance of lipid inclusions (20,000 ×).

Fig. 2. **Electron micrograph** of left ventricle of intact rabbit in summer: mitochondria small, with intact cristae, and with dark, dense matrix (20,000 ×).

TABLE 2. Quantitative Analysis of Electron Micrographs of Left and Right Ventricles at Different Seasons

Parameter	Left ventricle				Right ventricle			
	winter	spring	summer	fall	winter	spring	summer	fall
No. of mitochondria in electron micrograph	7 ± 0,31	9,2 ± 0,5	20,3 ± 0,7	12,6 ± 0,45	8,5 ± 0,43	11,3 ± 0,5	13 ± 0,56	10 ± 0,43
No. of cristae per mitochondrion	6 ± 0,18	7,4 ± 0,2	7,4 ± 0,26	4,7 ± 0,2	5 ± 0,19	8,8 ± 0,22	6,4 ± 0,1	7 ± 0,3
Total No. of cristae in electron micrograph	45 ± 2,1	67,6 ± 5,2	150,6 ± 6	59,1 ± 2,6	47 ± 2	99 ± 5	84 ± 3,8	71 ± 3
Area of mitochondria, μm^2	0,79 ± 0,02	0,54 ± 0,03	0,32 ± 0,003	0,44 ± 0,015	0,48 ± 0,01	0,42 ± 0,01	0,42 ± 0,02	0,56 ± 0,02
Total area of mitochondria in electron micrograph, μm^2	5,89 ± 0,22	4,94 ± 0,2	6,47 ± 0,37	5,48 ± 0,2	4,06 ± 0,17	4,73 ± 0,21	5,85 ± 0,24	5,54 ± 0,19
CEEMem, %	70,9	66,9	100	52,7	49,9	96,5	83,3	100
CEEMmc, %	100	85,4	60	57,2	73,1	69,6	66,4	100

EXPERIMENTAL RESULTS

Data on seasonal differences in some parameters of cardiac function, the thickness of the myocardial fibers, and blood FFA levels are given in Table 1.

The results of quantitative analysis of electron micrographs of the left and right ventricles are given in Table 2.

Analysis of the data in Tables 1 and 2 shows that all parameters used exhibit a distinct seasonal dynamics, and each seasonal range of variation has a considerable amplitude. For example, in the fall there was a significant increase in the blood FFA level compared with the other seasons, but in summer there was a significant and abrupt fall in the level of this parameter. In winter the blood pressure was significantly lower than in spring and summer. The thickness of the left ventricular myocardial muscle fibers was significantly greater in the fall than in all other seasons. BP_s of the left ventricle in summer was significantly lower than at all other seasons, and reached its highest value in winter and spring (the difference between the values of this parameter in winter and spring was not significant). BP_s of the right ventricle was significantly higher in spring and summer than in winter and fall.

Marked seasonal differences also were observed on quantitative analysis of electron micrographs of the myocardium. In both right and left ventricles the number of mitochondria in the electron micrograph reached a maximum in summer and a minimum in winter (difference significant). Marked swelling of the mitochondria was observed in the left ventricle in winter, and they were smallest in summer (difference significant). The trend of this parameter in the right ventricle was rather different: a maximum in the fall and a minimum in spring and summer (difference significant). There was also a marked seasonal dynamics of other parameters of the electron micrograph, including such important characteristics of bioenergetics of the cardiomyocyte as $CEEM_{em}$ and $CEEM_{mc}$.

Other seasonal differences also were demonstrated electron microscopically. Electron micrographs of the left ventricular myocardium of a rabbit in winter and summer, respectively, are illustrated in Figs. 1 and 2. Marked swelling of the mitochondria, translucency and indistinctness of the matrix, and the appearance of lipid inclusions were observed in winter. In summer the mitochondria were smaller, with well-defined cristae, and with a dense and dark matrix. These differences were less marked in the right ventricle.

The results described above are evidence of marked differences in function and ultrastructure of the myocardium in different seasons and confirm views on the intermittent activity of functioning structures [4] and that the main manifestation of the cyclic character of biological processes at the cell, organ, tissue, system, and whole body level is alternation of rising and falling intensity, as a result of which every functioning structure can restore its trophic-plastic potential actually while working [6, 7].

Comparison of some of the parameters characterizing cardiac function and structure in winter and summer during the development of pathological processes in the myocardium revealed the following results. During coarctation of the aorta in winter BP_s of the left ventricle was 177 ± 7 mm Hg, not significantly different from the "summer" norm (184 ± 7 mm Hg), but 27% below the "winter" control. In summer, during coarctation of the aorta, BP_s increased by 22% compared with the "summer" control and practically reached the level of the "winter" control (225 ± 15 mm Hg in the case of "summer" coarctation, 242 ± 7 mm Hg in the winter control; difference not significant). After injection of diphtheria toxin, BP_s of the left ventricle in winter fell to the level of the "summer" control (170 ± 17 mm Hg in winter after diphtheria toxin and 184 ± 7 mm Hg in the summer control; difference not significant). A similar situation also was observed on analysis of cardiomyocyte ultrastructure. In summer, for example, both after coarctation of the aorta and after injection of diphtheria toxin, significant swelling of the mitochondria took place (the area of one mitochondrion after coarctation of the aorta was $0.66 \pm 0.03 \mu m^2$, after injection of diphtheria toxin $0.44 \pm 0.03 \mu m^2$, and in the control $0.32 \pm 0.003 \mu m^2$; the difference from the control is significant). However, in both cases the area of the swollen mitochondria in pathological states was significantly smaller than in intact animals in winter ($0.79 \pm 0.02 \mu m^2$).

It must thus be noted that when any experiment is envisaged and any quantitative investigations carried out (including morphometric), the results must be compared with an appropriate seasonal control, for seasonal differences in parameters characterizing both function and structure of the heart can "overlap" the amplitude of changes taking place as a result of the pathological process.

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