CORRELATION BETWEEN THE NUMBER OF LYSOSOMES IN THE MYOCARDIUM WITH THE INTENSITY OF DIVISION OF THE MITOCHONDRIA AND THEIR ENERGY EFFICIENCY

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An electron-microscopic investigation of the myocardium of the left and right ventricles of rabbits was carried out during the winter, spring, summer, and fall. A parallel was established between the number of lysosomes in the myocardium and some parameters of the mitochondrial bioenergetics. A direct and strong correlation was found between the number of lysosomes and the intensity of division of the mitochondria in both the left and the right ventricle. Strong inverse correlation was found between the number of lysosomes and the energy efficiency of the mitochondria in the left ventricle, but in the right ventricle no significant correlation could be found. It is postulated that lysosomes may regulate bioenergetic processes in the myocardial cell.

It was noted in an earlier investigation [5] that the number of lysosomes rises sharply in the myocardium in a state of increased function, and intensive division of the mitochondria takes place; contact is also observed between the mitochondria and lysosomes, and at the points of contact the outer layer of the mitochondrial membrane is broken. It was postulated that damage to the outer mitochondrial membrane by the lysosomes, leading to the liberation of mitochondrial DNA and its entry into the cytoplasm, is the starting point for the synthesis of these organelles.

The object of this investigation was to develop this hypothesis further by studying correlations between the number of lysosomes in the myocardium and certain parameters characterizing the level of mitochondrial function.

EXPERIMENTAL METHOD

Experiments were carried out on 20 chinchilla rabbits weighing 2.5-3.5 kg. The animals were divided into 4 groups (5 rabbits in each group) and were used in the experiments at the following times of year: January, April, July, and October. The animals were killed by thoracotomy, the heart was removed, and pieces of the left and right ventricles were excised for electron-microscopic investigation. The material was fixed in 1% buffered solution of osmic acid, pH 7.2-7.4, and embedded in Araldite. Sections were cut on the LKB-8800 ultratome, stained with lead hydroxide and uranyl acetate, and examined in the UEMV-100 electron microscope under a magnification of 10,000 to 50,000 times.

A quantitative analysis of the state of the ultrastructure of the myocardial cell was carried out by the writer's method [2] in 100 electron micrographs from the left and 100 from the right ventricle under a magnification of 20,000 times: the mean number of mitochondria per electron micrograph and the mean number of cristae per mitochondrion and per electron micrograph were counted after which the mean area of 1 mitochondrion, the mean total area of the mitochondria in 1 electron micrograph, and the efficiency of the

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TABLE 1. Seasonal Changes in Number of Lysosomes and in Values of CIMD and EM (for 100 electron micrographs in each group)

Season	Left ventricle			Right ventricle		
	no. of lysosomes	CIMD	EM (in %)	no. of lysosomes	CIMD	EM (in %)
Winter	47*	0,044*	28,1	68	0,058	35,1
Spring	43*	0,041*	35,2*	60	0,03	98,2*
Summer	13	0,013	100,0	5	0,018	100,0*
Fall	35*	0,029	33,5*	20	0,029	69,8

*For values marked with an asterisk the difference between the means (for 1 electron micrograph) is not significant.

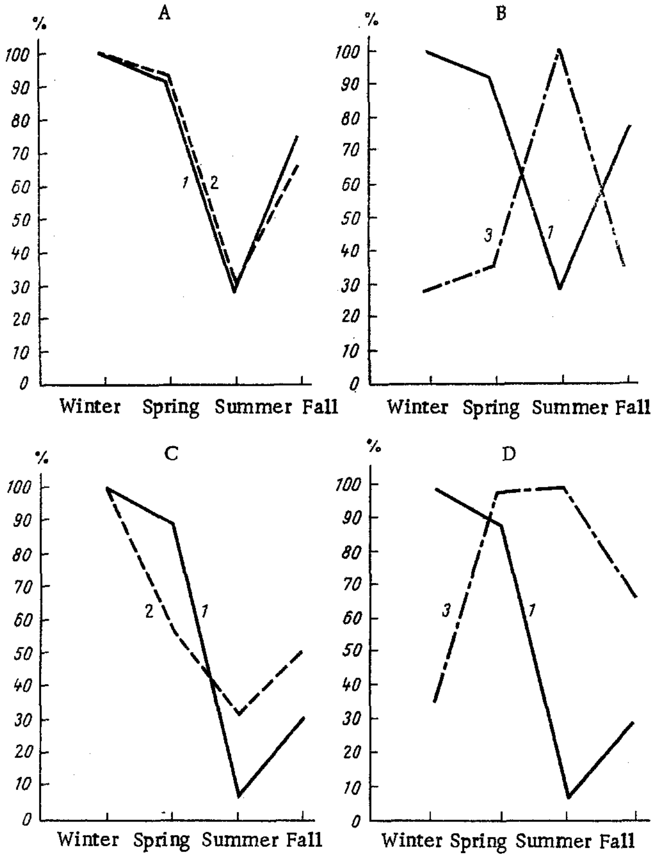

Fig. 1. Changes in number of lysosomes (1), in CIMD (2), and in EM (3) by seasons in the left (A, B) and right (C, D) ventricles of an intact rabbit.

mitochondria (EM) were calculated. Besides these parameters, the method of determination of which is fully described elsewhere [2], the total number for the season and the mean number of lysosomes per electron micrograph also were counted, and the coefficient of intensity of mitochondrial division (CIMD) was determined (for the season as a whole and on the average for 1 electron micrograph). The CIMD was the ratio between the number of dividing mitochondria and their total number.

The significance of the difference between the means was determined by Student's method. Differences between means were taken as significant for which $P \leq 0.05$. The presence or absence of correlation between the various parameters of the state of the cell ultrastructure was determined by correlation analysis [1]. Correlation with regard to their significance for which $r > 0.78$ ($P < 0.05$) and the strength of correlation was estimated by the value of the coefficient r (under 0.4 weak, from 0.4 to 0.7 average, and over 0.7 strong correlation).

EXPERIMENTAL RESULTS

The results of the electron-microscopic analysis showed that the ultrastructure of the myocardium of both ventricles underwent marked seasonal changes. The mitochondrial apparatus functioned most intensively in the winter and least in the summer. The spring and fall were intermediate in this respect. A distinct dynamics of variation in the number of lysosomes and the values of EM and CIMD also was observed for the various seasons (Table 1).

Changes in the number of lysosomes and in the values of CIMD and EM for the various seasons are illustrated in Fig. 1 (A and B left ventricle, C and D right ventricle). For convenience of comparison all values are given as percentages (the largest absolute value was taken as 100% for each parameter).

Correlation analysis carried out for the absolute values of each parameter showed strong significant negative correlation between the number of lysosomes and the value of EM for the different seasons for the left ventricle ($r = -0.84$; $P < 0.05$); correlation for the right ventricle was not significant.

Strong significant positive correlation was found between the number of lysosomes and CIMD for both the left and right ventricles (for the left ventricle: $r = +0.97$, $P < 0.01$; for the right ventricle: $r = +0.8$, $P < 0.05$). No correlation was found between the number of lysosomes and the total number of mitochondria, between the number of lysosomes and the number of mitochondrial cristae, between the number of lysosomes and the volume of the mitochondria, or between CIMD and EM.

These experiments thus showed that significant correlation exists between the number of lysosomes in the myocardium and other parameters reflecting the intensity of function of the mitochondrial apparatus of the cell (CIMD and EM). Statistically this correlation is twofold, for correlation analysis does not answer the question of which is the cause and which is the effect in this case. However, logical analysis provides a different interpretation of the functional connection between these values. Since experimental physiology has shown that in winter the contractile power of the myocardium is at its highest and in summer at its lowest, the decrease in the value of EM in the intact heart can be interpreted as an increase in energy formation in the mitochondria. Evidence in support of this view is given by the moderate swelling of these organelles, destruction of the cristae, clarification of the matrix, and a decrease in the number of cell granules – all changes characteristic of the myocardium of "winter" animals. An increase in energy formation in the myocardium must inevitably lead also to intensification of structural processes, for with an increase in the output of energy, its proportion utilized in the supply of structural materials must also increase. Consequently, the decrease in EM, reflecting hyperfunction of the mitochondria, can be linked with the subsequent increase in the number of lysosomes (as a result of intensification of their synthesis). The increase in the number of lysosomes in contact with the mitochondria leads to an increase in CIMD. As was shown above, correlation does not exist between CIMD and EM. This is evidently because the value of EM depends not only on the intensity of division of the mitochondria, but also on the degree of their swelling, and so on, i.e., on several factors not considered in the present investigation. However, since the number of mitochondria is bound to affect the bioenergetics of the cell, it can be postulated that some form of connection (even if not correlation) must nevertheless exist between CIMD and EM. In the myocardium, in a state of increased functional activity, a circular connection of cause and effect must thus exist between the individual components of the bioenergetic processes; this mechanism may intensify intracellular regeneration, one form of which is known [3] to be an increase in the number of cell ultrastructures and an increase in the rate of their renewal.

These arguments are valid for both the left and the right ventricle. The fact that no significant correlation could be demonstrated between the number of lysosomes and the value of EM in the right ventricle depends on the less marked dynamics of EM in this part of the heart, for it has a higher initial level of function [4], and the change in its parameters reflecting its bioenergetics is not accompanied by such marked fluctuations as in the left ventricle.

LITERATURE CITED

1. E. V. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medical and Biological Research [in Russian], Moscow (1973).
2. V. S. Paukov, T. A. Kazanskaya, and V. A. Frolov, Byull. Éksperim. Biol. i Med., No. 4, 122 (1971).
3. D. S. Sarkisov and B. V. Vtyurin, Electron-Microscopic Analysis of Increased Tolerance of the Heart [in Russian], Moscow (1969).

4. V. A. Frolov, V. S. Paukov, and T. A. Kazanskaya, Arkh. Pat., No. 6, 33 (1972).
5. V. A. Frolov, in: Proceedings of a Scientific Conference of the Medical Faculty of the P. Lumumba Peoples' Friendship University [in Russian], Moscow (1972), p. 25.