ROLE OF MITOCHONDRIAL SWELLING IN ENERGY EFFICIENCY OF THE INTACT MYOCARDIAL CELL (ELECTRON-MICROSCOPIC INVESTIGATION)

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In experiments on 20 rabbits the myocardial ultrastructure was studied in the left and right ventricles at different times of the year. A definite seasonal dynamics was found in several quantitative electron-microscopic parameters. In particular, correlation was found between the efficiency of the mitochondrion (an integral value, the product of the area of the mitochondrion and the number of mitochondrial cristae) and the area of the mitochondrion. With an increase in the latter to 0.85 μ^2, correlation was positive and linear in character. On further swelling of the mitochondria this relationship no longer held good.

Previous experiments [3, 4] showed marked seasonal variations in the ultrastructure of the intact myocardium. It was decided to examine the dynamics of energy formation in mitochondria of the cardiac myocytes in relation to season and to examine the possible mechanisms controlling it.

EXPERIMENTAL METHOD

Twenty Chinchilla rabbits weighing 2.5-3.5 kg were used. The animals were divided into four groups (five rabbits in each group) in accordance with the time of year. The animals were killed at the middle of January, April, July, and October and an electron-microscopic study made of the myocardium of the left and right ventricles. Pieces of heart muscle were fixed in 1% buffered osmium tetroxide solution, 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 UÉMV-100 and Simmens electron microscopes under magnifications of between 10,000 and 50,000 times. One hundred electron micrographs each of the left and right ventricles (for each season) were subjected to quantitative analysis under a magnification of 20,000 times by the method described previously [1] and, in addition, the mean efficiency of one mitochondrion (EM) was calculated as the product of the area of the mitochondrion, in square microns, and the number of cristae in that mitochondrion. This parameter, including both the number of mitochondrial cristae and the area of the organelle, enabled the size of the energy-forming surface of the mitochondrion to be judged and its energy production defined. The numerical results were subjected to statistical analysis with, in particular, correlation analysis, so that the presence or absence of statistical correlation between the individual parameters of the electron micrograph could be established.

EXPERIMENTAL RESULTS

The seasonal dynamics of EM, the area of the mitochondrion, and the number of cristae are shown in Fig. 1. For convenience of comparison all three parameters were expressed as percentages (the maximal value of each parameter was taken as 100%). The graph shows similarity between the dynamics of EM and of the area of the mitochondrion, but the change in the number of cristae did not correspond to the dynamics of these two parameters. Correlation analysis showed that in both ventricles correlation was significant between EM and the area of the mitochondrion (coefficient of correlation for these parameters +0.93

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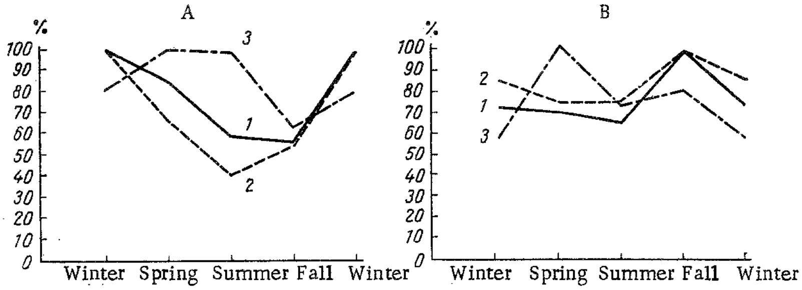

Fig. 1. Dynamics of EM (1), area (2), and number of cristae (3) in one mitochondrion at different seasons in left (A) and right (B) ventricles.

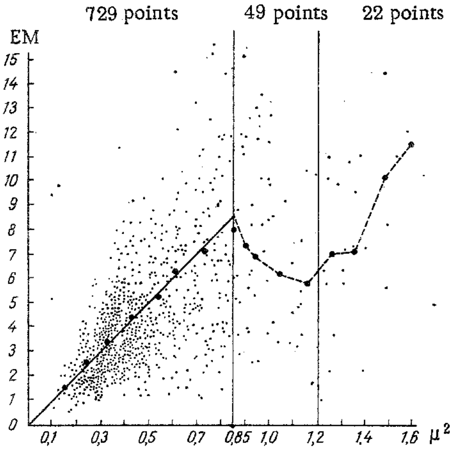

Fig. 2. EM as a function of area of mitochondrion (explanation in text). Abscissa, area of mitochondrion (in μ^2); ordinate, EM.

for the left and +0.95 for the right ventricle). The coefficients of correlation between EM and the number of cristae in the mitochondrion were +0.19 and −0.12 for the left and right ventricles, respectively, i.e., correlation was weak and not significant.

The analysis of the relationship between EM and the area of the mitochondrion, allowing for the normal distribution for all variants (Fig. 2), showed that the function was not the same for different degrees of swelling of the mitochondria. Over the region to 0.85 μ^2 the function was linear: $S = a \cdot EM$, where $a = 0.94$. In the region from 0.85 to 1.2 μ^2, with an increase in area of the mitochondrion EM fell, but after 1.2 μ^2 the scatter was so great and the number of points so small that it was impossible to speak of any definite relationship, for the difference between the means in this range of values was not significant.

Two principles emerge from the results described above: 1) the value of EM is determined by the area of the mitochondrion, and the number of mitochondrial cristae does not significantly affect this parameter; 2) 0.85 μ^2 is the limiting area of the mitochondrion after which any further increase is not associated with an increase in energy output.

The following explanation can be suggested for the results.

A change in the number of cristae per mitochondrion cannot significantly affect the output of energy if, at the same time, the volume of the organelle changes since swelling or contraction of the mitochondrion leads not only to a change in area of its internal surface but also to a change in the area of the cristae, which "expand" during swelling and reduce their surface area during contraction [5]. The principal regulator of the output of energy from the mitochondria can thus be assumed to be a change in their volume. The change in volume of the mitochondria is a much faster process than destruction or synthesis of mitochondrial cristae. By virtue of this mechanism, the level of energy formation can thus be regulated to allow for the demands presented to the heart by external environmental factors. During the process of intracellular regeneration there is known [2] a rapid destruction and formation of subcellular organelles, especially mitochondria. The writers' investigations demonstrated an almost universal destruction of the outer mitochondrial membranes in the intensively functioning heart, with liberation of fatty acids from their lipid components. These acids are agents uncoupling respiration and phosphorylation and causing swelling of the mitochondria [7], as a result of which the energy production of these organelles increases and the intensively functioning cell receives an additional supply of energy. The process of swelling and contraction of the mitochondria is thus a regulator of cellular energy formation.

However, the role of regulator of mitochondrial swelling can be played only up to a certain degree, because of the structure and arrangement of the respiratory groups on the surface of the inner mitochondrial membrane and the mitochondrial cristae. In the modern view [6], respiratory groups of mitochondria are analogous to the elementary particles located on their membranes. Definite spatial relations between the elementary particles are necessary for the normal function of the respiratory chain. The suggestion has been made that every such particle contains molecules of one type of electron carrier and that neighboring particles interact with each other by means of oscillatory movements limited by the length and flexibility of their pedicle [5]. During marked swelling of the mitochondria and "overstretching" of their membranes the distance between the individual particles may exceed the amplitude of oscillations of the respiratory groups, as a result of which a particular portion of the respiratory chain is inactivated.

During quantitative analysis of electron micrographs of both the normal and the altered heart it is thus essential not only to calculate the area of one "average" mitochondrion but also to determine the relative numbers of mitochondria with normal volume and those with an area exceeding 0.85 μ^2.

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