

RELATIONSHIP BETWEEN FUNCTIONS OF PARTS OF THE DOG'S HEART AND BIOCHEMICAL AND ULTRASTRUCTURAL PROPERTIES OF THE MITOCHONDRIA

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The content of high-polymer RNA in mitochondria isolated from tissue of the left ventricle is higher than that in mitochondria from the right ventricle. The nucleotide composition of the high-polymer RNA of the mitochondria of the left and right ventricles is practically identical. It is shown by the electron microscope that the heart muscle of dogs contains two types of mitochondria, differing in their ultrastructural organization. Mitochondria of the "sarcosome" type predominate in the left ventricle and "typical mitochondria" in the tissue of the right ventricle.

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The relationship between the increase in mass of the mitochondria and functional activity of the cell [4, 5] is associated with activation of synthesis of nucleic acids and protein [2].

Bearing in mind that the ventricles differ in the work they perform, it is interesting to determine whether the different functional loads on the ventricles give rise to biochemical and morphological differences in the mitochondria of these regions. The content and composition of high-polymer RNA of the mitochondria (isolated from the tissues of the right and left ventricles) and the ultrastructural organization of the mitochondria were studied.

EXPERIMENTAL METHOD

Mitochondria were isolated from the ventricular tissues of dogs. The heart was extracted under ether anesthesia using artificial respiration. The hearts were kept in ice-cold physiological saline and washed in 15% sucrose solution to remove traces of blood. The right and left ventricles were separated. The tissue was homogenized in 9 volumes of 0.44 M sucrose solution. The mitochondria were isolated by differential centrifugation. Samples of the isolated preparations were examined under the electron microscope. A suspension of isolated mitochondria was homogenized in 0.14 M NaCl solution. Protein was determined [9] and the residual homogenates of the mitochondria were treated by phenol deproteinization and salt fractionation [1, 7, 8]. The concentration of high-polymer RNA thus obtained was determined by acid hydrolysis and subsequent spectrophotometry [3].

The results are expressed in $\mu\text{g RNA}/\mu\text{g protein}$ of mitochondria. The nucleotide composition was determined both by chromatography of paper [10] and on Dowex 50 H^+ cation-exchange columns [6].

The ultrastructure of the mitochondria was studied under the EM-54 electron microscope. The material was fixed with 2% OsO_4 in veronal-acetate buffer with sucrose, pH 7.2, for 1 h. After fixation, the material was embedded in 2% warm agar-agar, dehydrated in a series of alcohols of increasing concentration, and mounted in a mixture of n-butyl (9 parts) and n-methyl (1 part) methacrylates. The polymerization catalyst was benzoyl peroxide. Polymerization took place for 24 h at 50°. Negative contrasting was carried out by the method of Parsons [11].

EXPERIMENTAL RESULTS AND DISCUSSION

A high degree of purity of the preparations was established (Fig. 1a). The mitochondria of the left and right ventricles of healthy dogs differed in their content of high-polymer RNA (Table 1). The ratio of

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TABLE 1. Nucleotide Composition of High-Polymer RNA of Mitochondria from Dogs' Ventricles ($M \pm m$)

Region of heart	Content of nucleotides (in molar %)					
	A	G	C	U	$\frac{\text{Pur}}{\text{Pyr}}$	$\frac{G+C}{A+Y}$
Left ventricle	$22,4 \pm 0,35$	$25,9 \pm 0,33$	$26,9 \pm 0,30$	$24,8 \pm 0,36$	$0,98 \pm 0,015$	$1,12 \pm 0,013$
Right ventricle	$22,7 \pm 0,20$	$26,1 \pm 0,28$	$26,8 \pm 0,25$	$24,4 \pm 0,41$	$0,95 \pm 0,013$	$1,12 \pm 0,013$



Fig. 1. Mitochondria isolated from myocardium of the left and right ventricles of a dog. a) Pure fraction of mitochondria isolated from myocardium; b) mitochondria isolated from muscle of right ventricle; c) mitochondria isolated from muscle of left ventricle; d) "ordinary mitochondria"; e) "typical sarcosomes."

high-polymer RNA to mitochondrial protein in the mitochondria of the left ventricle was 5.3 ± 0.101 , and in the mitochondria of the right ventricle 3.69 ± 0.209 ($P < 0.001$).

It is clear from Table 1 that the nucleotide composition of the high-polymer RNA from mitochondria of the right and left ventricles of healthy dogs was practically identical whether determined by chromatography on paper or on cation-exchange resins.

Electron-microscopic study of sections of mitochondria isolated from the left and right ventricles showed that in both parts of the heart mitochondria of two types were present, differing sharply in their ultrastructural organization. Some mitochondria ("typical sarcosomes", Fig. 1e) were structures in which the cristae joined the two opposite walls. The cristae in these mitochondria branched and twisted to form closed cells. A fairly dense substance with periodic bridges and round pores was contained between the outer and inner membranes and in the space between the cristae.

Investigation of these mitochondria by the negative contrasting method showed them to be of considerable strength, probably because of the cristae joining the walls of the "sarcosomes." The ultrastructure of the mitochondria of the second types, the "ordinary mitochondria" (Fig. 1d), differed sharply from that described above. The cristae in these mitochondria were projections of the inner layer of the membrane just as in the mitochondria of most other tissues. They were plates or, more frequently, tubes, not long enough to reach the opposite wall of the mitochondria. The space between the cristae did not contain material of high density.

The muscle tissue of the left and right ventricles of the dogs was found to differ in the relative proportion of these types of mitochondria. The left ventricle (Fig. 1c) contained many more mitochondria of the "sarcosome" type, while the right (Fig. 1b) contained more

of the "ordinary mitochondria." The larger number of mitochondria of "sarcosome" type in the tissue of the left ventricle and the higher concentration of RNA in the mitochondrial fraction obtained from this tissue suggests that mitochondria of the "sarcosome" type contained more RNA than "ordinary mitochondria." This may account for the difference between the RNA concentrations in the mitochondria of the left and right ventricles. Predominance of mitochondria of the "sarcosome" type in the wall of the left ventricle, assuming that these structures possess a more highly developed protein-synthesizing system, may evidently be explained by the fact that the left ventricle performs more intensive work throughout its life than the other parts of the heart, and this calls for increased synthesis of nucleic acids and protein. Increased synthesis of nucleic acids and protein, associated with the more intensive work of the left ventricle, is evidently characteristic not only of the whole systems responsible for the direct functional activity of the cell, but also of its individual structural components, especially the mitochondria.

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