BIOCHEMICAL AND ELECTRON-MICROSCOPIC CHARACTERISTICS OF DIFFERENT TYPES OF CARDIAC MITOCHONDRIA

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Electron-microscopic studies show that three fractions of mitochondria isolated from dog heart homogenate by centrifugation consist of two types of mitochondria. The mitochondria of the first type have a high sedimentation coefficient and separate as fraction I, while fractions II and III consist of sarcosomes and mitochondria of the other type, differing in their size and their sedimentation rate in a centrifugal field. The concentration of ribosomal RNA (calculated per mitochondrial protein) in fractions II and III is 56 and 27% higher respectively than in the mitochondria of fraction I. The coefficient of specificity of RNA for mitochondria of the first type is 1.33±0.0082, and for mitochondria of the second type 1.18±0.022.

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It has previously been shown that dog heart muscle tissue contains mitochondria which are heterogeneous with respect both to their morphology and to their concentration of ribosomal RNA. It has also been shown that the ratio between these two types of mitochondria is not the same in the left and right ventricles [3]. Bearing in mind the special functional properties of the left ventricle and the relationship between function and the synthesis of nucleic acids and proteins [4, 8, 10], it was decided to investigate, first, whether any relationship exists between the RNA concentration in the different types of mitochondria and their ultrastructure and, second, how the RNA concentration in these types of mitochondria is related to their distribution in the left and right ventricles.

To examine these problems, a total mitochondrial suspension was subjected to graded separation into subfractions on the basis of their sedimentation in a centrifugal field [5, 7, 9, 17, 20]. Ribosomal RNA was isolated from the mitochondrial subfractions thus obtained, and its concentration and composition were determined.

EXPERIMENTAL METHOD

Mitochondria were isolated from the walls of the left and right ventricles of the dog's heart. Isolation was carried out in the cold by differential centrifugation in 0.44 M sucrose solution. The original material thus obtained was examined in the electron microscope before fractionation to verify the purity of isolation.

The washed initial mitochondrial residue was suspended in 9 volumes of 0.44 M sucrose solution and again fractionated in a refrigerator centrifuge at 3000 g for 5 min (residue—fraction I). The supernatant was collected and centrifuged at 6000 g under the same conditions (residue—fraction II), the supernatant was again collected and centrifuged at 12,000 g (residue—fraction III), and fraction IV was obtained by centrifugation of the last supernatant at 18,000 g. The final residue was used only for electron microscopy.

The mitochondrial residues obtained after fractionation were suspended in an ice-cold 0.14 M solution of NaCl, and homogenized in a glass homogenizer with a teflon pestle for 20 sec. A sample was withdrawn for testing for protein [18], and the remaining homogenate of the mitochondrial fractions was subjected to phenol deproteinization and salt fractionation [1, 13, 14]. The concentration of ribosomal RNA was determined by Spirin's method [6]. The results were expressed in μg RNA/mg mitochondrial protein. The nucleotide composition was determined by means of Dowex 5 OH^+ cation-exchange columns [12],

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TABLE 1. Concentration of Ribosomal RNA in Subfractions of Mitochondria (M ± m)

Fraction	Conc. of ribosomal RNA	P
I	2,27±0,130	$P_{II-I}<0,001$
II	5,20±0,450	$P_{II-III}<0,001$
III	3,10±0,349	$P_{I-III}<0,01$

followed by spectrophotometry. To study the structure of the mitochondria the material was fixed in 2% OsO_4 solution; subsequent treatment was as described previously [3].

EXPERIMENTAL RESULTS AND DISCUSSION

Electron-microscopic control examination of the initial mitochondrial fraction showed that it was in fact a pure fraction of these organoids consisting of two types of mitochondria. Further separation of the initial mitochondrial fraction into three subfractions showed that fraction I contained only mitochondria consisting of circular double-membrane structures measuring from 1 to 1.5 × 0.5 μ (Fig. 1a). The inner membrane of the mitochondria formed a number of short cristae, their matrix occupying a considerable volume and possessing low density, while the concentration of ribosomal RNA in this fraction was 2.27 ± 0.13 (Table 1). Mitochondria with densely packed parallel cristae, forming complete partitions (Fig. 1b) were isolated in fraction II. Their matrix was less dense than the space between the outer and inner membranes. The mitochondria of this fraction were usually long—from 2.5 to 2.8 μ in length, and their width varied from 0.5 to 1 μ. The concentration of ribosomal RNA was 5.2 ± 0.45, or 56.2% higher than its level in the mitochondria in fraction I. Fraction III contained small, dense mitochondria, mainly circular in shape and measuring from 0.5 × 1.5 to 1.5 × 1 μ (Fig. 1c). The cristae forming partitions, just as in the mitochondria of fraction II, were densely packed in the matrix and completely divided the organoid into compartments. The concentration of ribosomal RNA in the mitochondria of fraction III was 3.1 ± 0.349. In fraction IV, swollen and severely damaged mitochondria from fractions I and II were found. No other cell structures could be detected in the material examined, demonstrating the high degree of purity of the original mitochondrial material.

The study of the nucleotide composition of the ribosomal RNA obtained from all three fractions showed that the coefficient of specificity of fractions II and III was much higher than that of fraction I (Table 2).

These results show that the mitochondria of dog heart muscle are composed of two populations: fraction I consists of mitochondria of only one type and fractions II and III contain mitochondria which differ in their size and structure. The concentration of ribosomal RNA and its coefficient of specificity in the mitochondria of fractions II and III are higher than those of the mitochondria of fraction I. Bearing in mind that the mitochondria of fractions II and III are distributed mainly in the tissues of the left ventricle, with a higher level of physiological activity, and comparing this with the fact that their RNA concentration is considerably higher than that in the mitochondria of fraction I, it can only be concluded that the metabolic processes taking place in them, notably RNA metabolism, do so most intensively. The differences between the mitochondria of fractions II and III in size and sedimentation rate in a centrifugal field can perhaps be explained as follows. Starting from the fact that the mitochondria possess the ability to synthesize protein independently [2, 11, 12, 15, 16, 19], self-reproduction of these organelles must take place in the cell, in which case the mitochondria in the cell must be subdivided into immature and mature forms, differing from each other in their size and, correspondingly, in their sedimentation rate. From this point of view the small mitochondria of fraction II are an intermediate stage in the formation of the large mitochondria isolated in fraction II.

TABLE 2. Nucleotide Composition of Ribosomal RNA of Submitochondrial Fractions Isolated from Dog Heart Muscle (M ± m)

Fraction of mitochondria	Conc. of nucleotides (in moles %)				Purines / Pyrimidines	G + C / A + U	P
	A	G	C	U			
I	22,4±0,35	27,2±0,445	26,3±1,21	23,8±1,39	0,10±0,014	1,18±0,022	$P_{II-I}=0,001$
II	21,4±0,485	28,0±1,06	29,1±1,04	21,6±0,53	0,98±0,039	1,33±0,0082	$P_{II-III}=0,001$
III	22,5±1,20	26,6±0,775	28,7±0,94	22,2±0,91	0,97±0,036	1,23±0,065	$P_{III-I}=0,01$

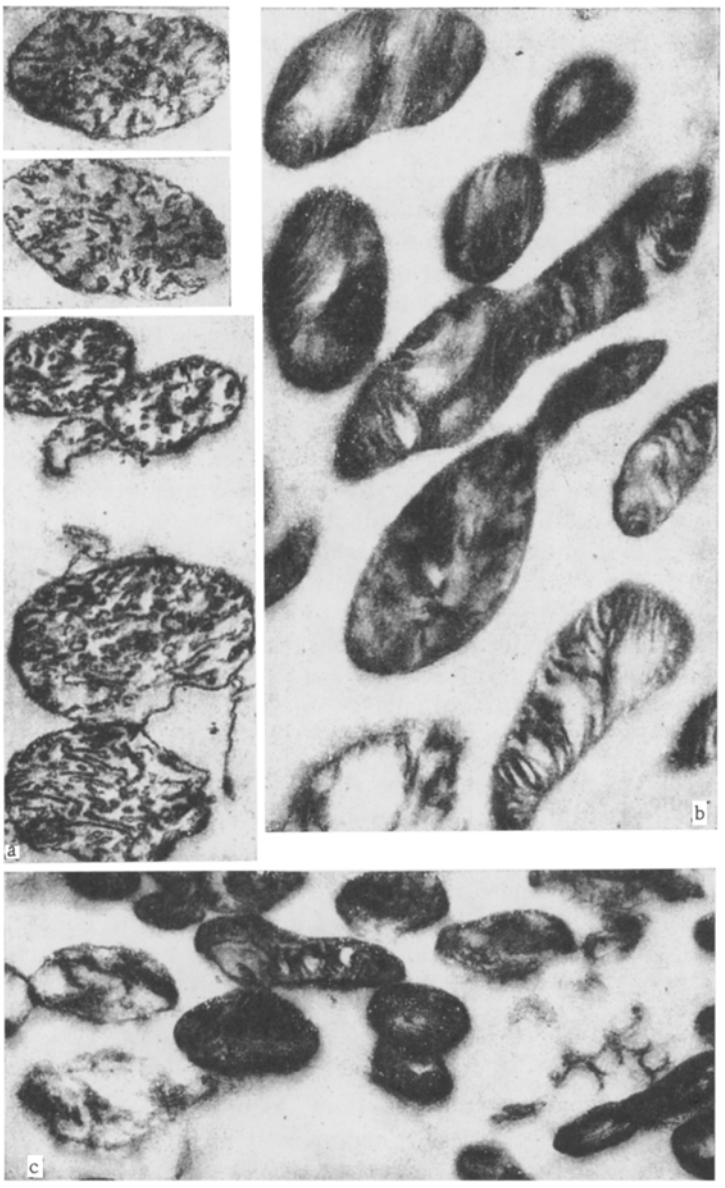

Fig. 1. Mitochondria of dog's heart muscle. a) First type, fraction I; b) second type, fraction II; c) second type, fraction III, 30,000 ×.

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