FUNCTIONAL AND MORPHOLOGICAL INVESTIGATION OF MYOCARDIAL MITOCHONDRIA

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An electron-microscopic investigation of ultrathin sections of the myocardial cell and of pure fragments of mitochondria isolated from the myocardium, together with the biochemical analysis of these fractions, demonstrated the heterogeneity of the organoids, which could be subdivided into mitochondria of "muscular type," with numerous strictly oriented cristae, and circular mitochondria of "general type" with short cristae. The mitochondria of muscular type have a higher level of respiration coupled with phosphorylation and a higher respiratory control. Their specific activity of incorporation of C^{14}-labeled amino acids into their proteins is twice as high.

Previous investigations led to the conclusion that the mitochondrial fraction isolated from the dog's myocardium contains two types of organoids described as mitochondria of "muscular" and "general" type, differing from each other in their ultrastructure and some biochemical properties [2]. However, the following problems remained unsolved: a) do both types of mitochondria belong to the myocardial cell or are the mitochondria of one type organoids of the connective tissue which is inevitably present in the homogenate from which the mitochondria are isolated?; b) is the ultrastructural heterogeneity of the investigated mitochondria the result of the method of differential centrifugation used?; c) is there a connection between the ultrastructural differences of the subfractions of myocardial mitochondria and the activity of their functions?

To answer these questions an electron-microscopic study was made of ultrathin sections of myocardial tissue, mitochondria (after differential centrifugation) were investigated in ultrathin sections by the negative staining technique, and the degree of respiratory and phosphorylating activity and of respiratory control was compared with protein synthesis in the morphologically different types of myocardial mitochondria.

EXPERIMENTAL METHOD

Preparation of the subfractions of myocardial mitochondria (in vitro), incorporation of radioactive acids into their proteins, and the methods of electron-microscopic examination have been described previously [2-4]. The intensity of oxygen absorption was determined polarographically by means of a platinum electrode [1]. The following incubation medium was used (in μM): sucrose 120; KH_2PO_4 23; $MgCl_2$ 515; KCl 11.0. The phosphate acceptor was ADP (0.24 μM). Pyruvate, glutamate, succinate, and α-ketoglutarate were used as substrates.

EXPERIMENTAL RESULTS AND DISCUSSION

Electron-microscopic examination of the ultrathin sections of the myocardial cell showed heterogeneity of the mitochondria contained in it. At the periphery of the cell there were as a rule round mitochon-

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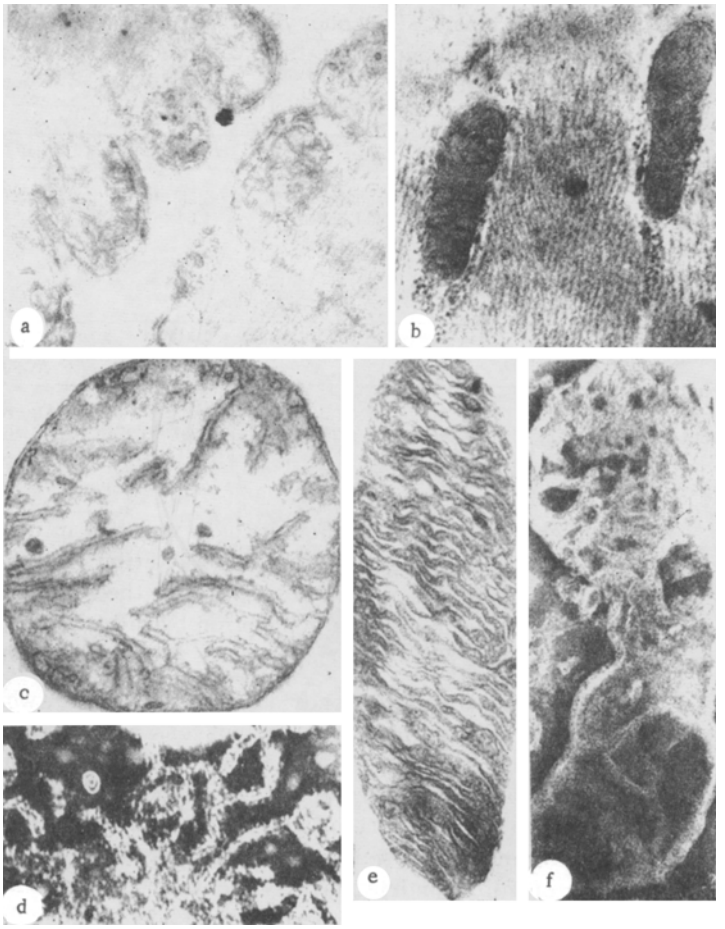

Fig. 1. Mitochondria of the myocardium of dogs: a) mitochondria of general type, located at the periphery of a myocardial cell, 25,000×; b) mitochondria of muscular type, located among myofibrils, 25,000×; c) section through a mitochondrion of general type, 65,000×; d) cristae of mitochondria of general type with fungoid subunits; negative staining with phosphotungstic acid, 65,000×; e) section through mitochondrion of muscular type, 65,000×; f) mitochondria of muscular type, negatively stained with phosphotungstic acid, 65,000×.

dria of general type (Fig. 1a) with short cristae, giving off few branches, and a transparent matrix. Mitochondria of muscular type with tightly packed cristae, lying parallel to one another, reaching the opposite side of the organoid and with a dense matrix (Fig. 1b) were located in the sarcoplasm between the myofibrils. This picture is probably the morphological expression of differences in the functional state of the mitochondria [10].

When isolated at 3000 g in a pure fraction, the mitochondria of general type also had a circular shape, relatively short but regularly oriented cristae, and a transparent matrix occupying a comparatively large volume (Fig. 1c). In negatively stained specimens, because the membranes of these mitochondria are easily broken, free-lying cristae were found. The fungoid structures typical of these organoids, with heads 80 Å in diameter and with pedicles 50 Å in length, were found on their surface (Fig. 1d).

The mitochondria isolated at 12,000 g differed considerably in their ultrastructural organization from those described above and consisted of mitochondria of muscular type (Fig. 1e). Because of the high resistance of their outer membrane to hypotonic media these organoids remained in the native state in negatively stained specimens (Fig. 1f). As the result, the macromolecular organization of their cristae could not be studied.

TABLE 1. Rate of Oxidative Phosphorylation and Respiratory Control in Myocardial Mitochondria (rate of oxygen absorption in μA O_2/sec. g mitochondrial protein)

Mitochondria	Pyruvate + malate			
	V_1	V_2	resp. cont.	ADP/O
General type	2,76±0,094	1,13±0,157	2,55±0,168	2,52±0,139
Muscular type	6,18±0,142	1,55±0,114	4,04±0,79	2,75±0,11

Table 1. (continued)

Mitochondria	Glutamate + malate			
	V_1	V_2	resp. cont.	ADP/O
General type	3,38±0,34	1,0±0,139	2,85±0,184	3,0±0,2
Muscular type	4,4±0,146	1,26±0,125	4,07±0,276	2,7±0,342

TABLE 2. Incorporation of C^{14} -Amino Acids into Myocardial Mitochondrial Preteins (mean results of three parallel determinations given)

Determination	Spec. act. (in pulses/min per milligram mitochondrial protein)	
	mitochondria of gen. type	mitochondria of musc. type
1	121,5	244,9
2	124,7	284,8
3	118,4	263,3
4	118,7	200,9
5	88,3	298,7

Biochemical investigation of these types of mitochondria showed that oxidation of pyruvate and glutamate on the addition of phosphate acceptor (ADP) stimulated the rate of oxygen absorption by the mitochondria of general type by 2.5 times, but stimulated its absorption by mitochondria of "muscular type" by 4 times (Table 1). When other substrates were used a simular picture was oberved.

Consequently, mitochondria of muscular type, which are the more specialized organoids in the myocardium, possess a higher level of respiration coupled with phosphorylation and a much higher respiratory control. Since the biosynthesis of mitochondrial protein is linked with oxidative phosphorylation [5-9], the incorporation of a mixture of C^{14} -labeled amino acids* into the proteins of the investigated mitochondrial fractions was studied. The results showed that both fractions of mitochondria actively incorporate the radioactive amino acids, but that the specific activity of the mitochondria of muscular type was twice as high as that of the general type (Table 2).

The results show that a single myocardial cell contains mitochondria of two morphologically different types. The electron-microscopic picture of these types of mitochondria in vivo corresponds exactly to that obtained in vitro. The mitochondria of muscular type possess higher functional activity. The existence of two types of mitochondria with different morphology and function within the same myocardial cell and the fact that their structural organization is related to their functional activity, may reflect the ability of the cell, developed in the course of evolution, to regulate its energy metabolism and its biosynthetic activity.

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*Proline, valine, arginine, threonine, and serine.