BIOCHEMICAL AND ELECTRON-MICROSCOPIC CHARACTERISTICS OF MITOCHONDRIA FROM DIFFERENT PARTS OF THE DOG'S HEART IN EXPERIMENTAL HYPERTROPHY

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Seven days after stenosis of the aorta causing cardiac hypertrophy a marked increase was observed in the RNA concentration in the mitochondria, accompanied by their increased ability to incorporate C^{14}-labeled amino acids. Development of hypertrophy leads to a gradual decrease in the RNA concentration in the mitochondria and to gradual loss of their ability to synthesize protein. An electron-microscopic study of mitochondria of the left and right ventricles revealed marked morphological changes, which increased in intensity as the hypertrophy developed and were manifested not only as gradual destruction of the mitochondria and changes in their cristae, but also, and this is particularly important, as disappearance of mitochondria of muscular type.

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It has previously been shown that two types of mitochondria, differing in their morphological structure and in their ability to incorporate radioactive amino acids, exist in heart muscle [1]. These types of mitochondria are unequally distributed in the ventricles of the dog's heart [2].

The object of the investigation described below was to compare changes in the concentration and composition of high-polymer RNA and ability to incorporate radioactive amino acids in vitro in an isolated fraction of mitochondria from the myocardium of the left and right ventricles with their morphological structure at various stages of experimental cardiac hypertrophy in dogs.

EXPERIMENTAL METHOD

Experiments were carried out on mongrel dogs. Hypertrophy of the myocardium was produced by creating stenosis of the ascending aorta, reducing the cross section of the aorta by 60% of its normal value. The degree of hypertrophy was judged from the increase in relative weight of the heart, using the ratio between the weight of the lateral wall of the left ventricle and the animal's body weight for this purpose: for intact animals its value is 0.0054±0.00024.

Differential centrifugation to obtain a pure mitochondrial fraction, isolation of high-polymer RNA from the fraction, determination of its concentration and composition, and the electron-microscopic control were described previously [1, 2].

Incorporation of C^{14}-labeled amino acids* into proteins of the mitochondrial fraction of the myocardium in vitro was carried out (with some modifications) by the method of Simpson and McLean [6].

*Proline, valine, arginine, serine, threonine.

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TABLE 1. Concentration of High-Polymer RNA in Mitochondria from Ventricles of Hypertrophied Heart Following Aortic Stenosis (M + m)

Group of animals	High-polymer RNA (in μg/kg mitochondria protein)	
	left ventricle	right ventricle
Intact	5.3±0.1	3.69±0.2
After mock operation	4.94±0.07	3.24±0.27
After stenosis of aorta:		
7 days	7.13±0.1	4.48±0.23
21 days	4.96±0.07	3.04±0.33
90 days	2.07±0.3	2.83±0.38

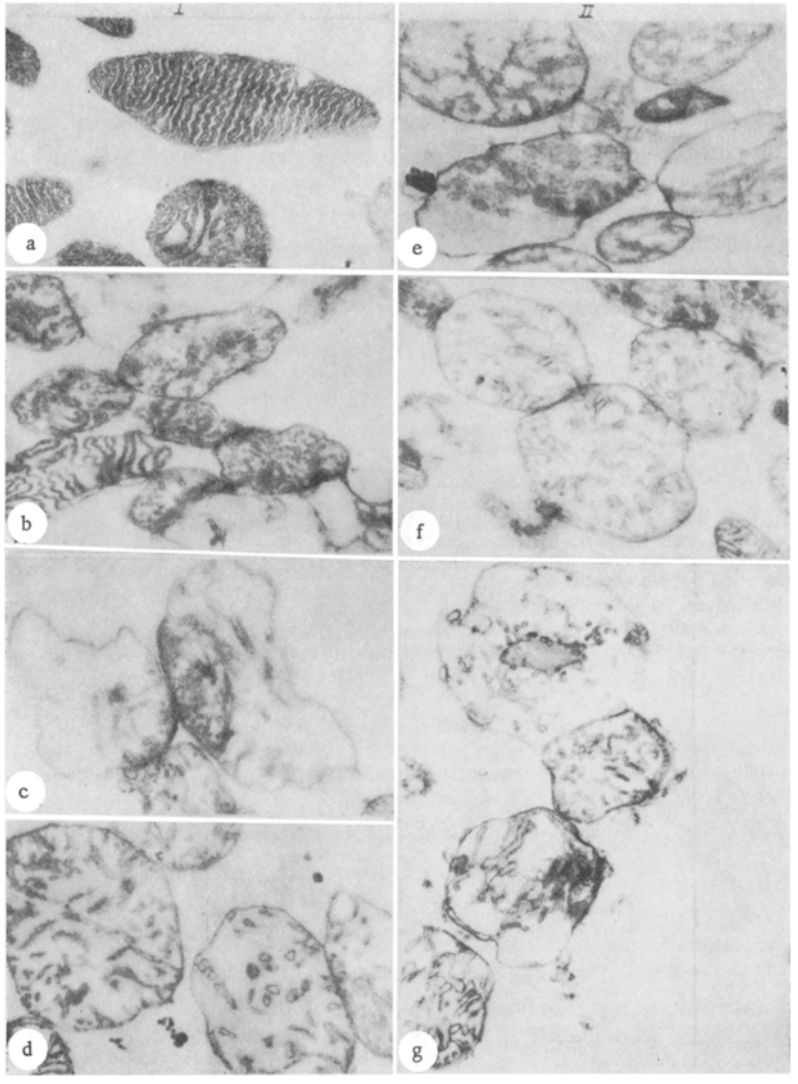

Fig. 1. Mitochondria isolated from muscle of left (I) and right (II) ventricle of dogs' heart. In I: a) Intact dogs; b) 7 days; c) 21 days; d) 90 days after aortic stenosis; in II: e) Intact dogs; f) 7 days; g) 90 days after aortic stenosis, 25,000×.

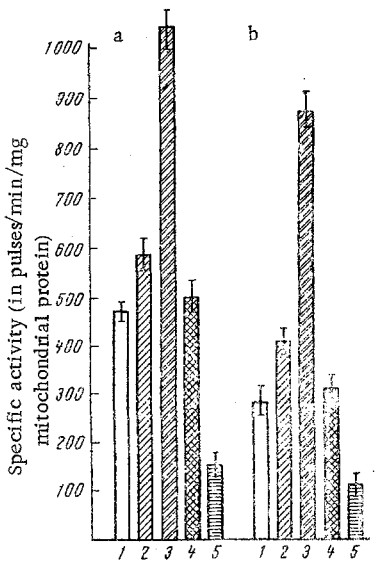

Fig. 2. Dynamics of incorporation of C^{14}-labeled amino acids in vitro into myocardial mitochondria of left (a) and right (b) ventricles of the heart in intact animals (1), animals undergoing a mock operation (2), and 7 (3), 21 (4), and 90 (5) days after stenosis of the aorta.

EXPERIMENTAL RESULTS

Seven days after creation of aortic stenosis the relative weight of the heart was increased by 12.2-12.5% compared with its value in intact animals and in animals undergoing the mock operation. The concentration of high-polymer RNA was increased by 29.5% in the mitochondria of the left ventricle and by 18.2% in mitochondria of the right ventricle (Table 1). Incorporation of C^{14}-labeled amino acids into mitochondrial proteins of the left and right ventricles was twice as high in the experimental groups as in both control groups. The $(G+C)/(A+U)$ ratio, characterizing the high-polymer RNA of the myocardial mitochondria as RNA of the GC-type, was 1.25 ± 0.07 and 1.28 ± 0.02 for RNA of mitochondria of the left and right ventricles of the intact animals, respectively. The slight decrease in the base ratio of the high-polymer RNA of mitochondria of the left ventricle to 1.16 ± 0.057 seven days after creation of aortic stenosis followed by the same trend as the total RNA of the hypertrophied heart [3], but it was less marked.

Seven days after stenosis of the aorta, electron-microscopic examination of the left ventricle (Figs. 1b, c, d) revealed a sharp decrease in the number of mitochondria of muscular type. The remaining mitochondria showed a tendency to swell, their matrix became clearer, and the regular arrangement of their cristae was disturbed, so that most mitochondria in the sections had short, tubular cristae, some of which were considerably swollen, their matrix was saturated with water, and the cristae were fragmented. In sections of the right ventricle (Fig. 1f, g) mitochondria of muscular type were much less common than in the left ventricle. The sections consisted mainly of mitochondria with short, tubular cristae, while many mitochondria were swollen and contained vacuolated cirstae, appearing in the sections as vesicles (Fig. 1f).

On the 21st day after aortic stenosis the relative weight of the heart was increased by 15.2-16.7% compared with the control groups. The concentration of high-polymer RNA in the mitochondria of the left ventricle was nearly normal, while in the right it was slightly below normal. The base ratio of high-polymer RNA was within normal limits for both ventricles. The ability of the mitochondria to incorporate radioactive amino acids was sharply diminished (Fig. 2).

The electron-microscopic picture of sections through the mitochondria 21 days after stenosis of the aorta (Fig. 1c) differed considerably from that observed after 7 days. Only single small muscular mitochondria with lamellar cristae, arranged in the typical manner, were found in the mitochondria of the left ventricle, and most of the isolated structures were mitochondria with short, tubular cristae, in various stages of degradation. However, a very few intact organoids resembling the ordinary mitochondria could still be seen in the sections. Sections through the right ventricle revealed mitochondria with short, tubular cristae at various stages of disintegration.

On the 90th day after stenosis of the aorta the relative weight of the left and right ventricles was increased by 21.6 and 24.2%, respectively. The concentration of high-polymer RNA in the mitochondria of the left and right ventricles was increased by 61.3 and 30%, respectively. The base ratio was within normal limits. Specific activity of the mitochondria continued to decrease (Fig. 2) and reached very low values. The electron-microscopic picture of the myocardium of the left and right ventricles (Fig. 1) was close to that observed 21 days after stenosis of the aorta. Mitochondria of muscular type were almost completely absent, the number of disintegrated forms was considerably increased, and the mitochondria of the right ventricle showed more severe changes, being swollen to a greater degree, while their cristae were adherent to each other and were split up into layers with the formation of vacuoles; of many mitochondria in both the left and the right ventricle, only the boundary membrane remained.

Hence, during a progressive increase in hypertrophy of the heart, a gradual degradation and destruction of the mitochondria were observed, the morphological changes in the structure of the mitochondria bearing a very close relationship to disturbance of their metabolism. This "wearing out" of the hypertrophied heart led not only to disturbance of the ultrastructure of the mitochondria, but also to a gradual decrease in the

number of mitochondria, especially those of muscular type, in the hypertrophied muscular fibers, evidently because of loss of their ability to reproduce themselves. The decrease in the specific activity of the mitochondria 90 days after aortic stenosis can evidently be attributed to the marked decrease in their number in the myocardial tissue at this time. Intensive incorporation of labeled amino acids into the mitochondria and an increase in the RNA concentration 7 days after aortic stenosis could evidently be attributed to an increase in the number of mitochondria (which was clearly observed in the early stages of hypertrophy) and to an increase in the content of mitochondrial ribosomes. This hypothesis is in agreement with investigations [4, 5] showing that the principal factor determining the increase in protein synthesis leading to hypertrophy of the heart is an early increase in the content of ribosomes in the hypertrophied myocardium. In the present case both these factors may play a role.

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