MITOCHONDRIAL ALTERATIONS IN THE MYOCARDIUM OF DOGS WITH AORTIC STENOSIS

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Light microscope studies have produced no evidence for morphological changes in the myocardium indicative of chronic cardiac failure as it occurs in the vast majority of cases (1). Using the electron microscope, Kisch and coworkers (2) detected osmiophilic remains of mitochondria in the hearts of patients who had died of this disease. According to these authors, the observed degenerative change may partly have been the result of protracted agony, but it is conceivable that it was due entirely to that cause. Mölbert and Iijima (3) noted that the mitochondria in hypertrophied heart muscle of hypertensive rabbits were poor in inner structure and suggested that this might constitute the anatomic substratum for heart failure. No information was given, however, about the functional state of the heart.

The present report describes preliminary observations on the fine structure of the myocardium of dogs with experimentally produced hypertrophy and chronic congestive failure of the heart. In this species, cardiac performance and reserve power are more readily assessed than in most other laboratory animals and there are no limitations, as in the case of cardiac patients, for removing specimens of heart muscle during life.

The ascending aorta of young adult shepherd-dogs was exposed and a perlon band 2 cm in width was placed around the vessel about 2 to 4 cm above its origin. The band was tightened so as to reduce the aortic lumen by approximately 50 per cent. Eleven out of 12 dogs subjected to this procedure survived. Non-operated dogs and thoracotomized animals without aortic constriction served as controls.

Four to 12 months after the operation, specimens of the central layer of the outer left and right ventricular walls, situated 3 to 4 cm from the apex, were taken from the beating hearts under pentobarbital anesthesia and positive pressure breathing. They were fixed in osmium tetroxide and embedded in methacrylate. Thin sections were cut and examined in the electron microscope, using a Siemens and Halske Elmiskop I model.

Nine of the dogs with aortic stenosis developed left ventricular hypertrophy of varying degree without displaying overt manifestations of cardiac failure up to the time of sacrifice. Moderately severe exercise was well tolerated and normal atrial and end-diastolic left ventricular pressures were recorded. In the left, but not in the right ventricle of some of these animals, empty-looking mitochondria of the type described by Mölbert and Iijima (3), possessing few cristae with little osmiophilic matrix between them, were found in abundant numbers. As emphasized by these authors, such loss in inner mitochondrial structure is not a phenomenon restricted to cardiac hypertrophy and occurs also in non-hypertrophic myocardium as a result of anoxia and other conditions of stress.

In two of the dogs with damaged aorta, severe cardiac failure eventually involving both sides of the heart developed about 1 month following the operation, as evidenced by a progressively decreasing exercise tolerance, dyspnea on mild and later even on slight exertion, edema, ascites, and signs of pulmonary and hepatic congestion. The condition of the animals was improved by administration of cardiac glycosides, but treatment with these drugs was abandoned several weeks prior to sacrifice. Although roentgenograms had shown markedly enlarged cardiac silhouettes, the hearts were not found to be exceptionally heavy.

Electron micrographs of ventricular muscle sections of these dogs revealed the presence of mitochondria of unusual size. The familiar mitochondria (sarcosomes) of adult dog myocardium, which do not often exceed 2 μ in length and which in longitudinal sections are frequently seen lying

in single file alongside the myofibrils, one mitochondrion per sarcomere, are partly replaced by elongated, rod-shaped bodies extending over the distance of several sarcomeres (Fig. 1) and approaching or exceeding in some instances 10 μ in length. Bulky forms of greater than usual width were less common. In not a single instance were structures such as the one depicted in Fig. 1 encountered in the heart muscle of control dogs or of stenosis dogs with unimpaired cardiac contractility.

All elongated mitochondria were bounded by a double membrane and possessed the characteristic details of inner mitochondrial structure. In many cases, however, their cristae did not show the regularity of disposition usually found in cardiac mitochondria and exhibited a more or less disordered arrangement (see especially Fig. 3). This was, in general, not true of the smaller mitochondria in the failing hearts.

Data on the frequency of occurrence of elongated mitochondria in the failing and in the control hearts and on their share of the total mitochondrial space (as reflected by the percentage of the total area (in μ^2) within the mitochondrial contours occupied by them on the electron micrographs) are presented in Table I. Only micrographs of longitudinal sections were used, care being taken to select not more than one section from among those cut in a series. The mitochondria are grouped in the table according to whether or not they exceeded 1, 2, or 3 sarcomeres of adjacent myofibrils in length. Large differences between failing and normal heart muscle are revealed. In the sections of failing myocardium, mitochondria measuring more than one sarcomere in length amounted to 14.4 per cent of the mitochondrial population and covered 40.3 per cent of the total mitochondrial area, as contrasted to corresponding control figures of 2.8 and 9.2 per cent, respectively. While not a single control mitochondrion had grown to be longer than 3 sarcomeres, more than 10 per cent of the mitochondrial area in the failing heart muscle is accounted for by organelles of this length.

In addition to taking these measurements, the area occupied by the myofibrils was determined and compared to that taken up by the mitochondria. Longitudinal sections showing myofibrils of sarcomere length 1.8 to 2.0 μ were used exclusively. No difference was found between normal and failing ventricles, the ratio of mitochondrial to myofibrillar area being in both cases 1:2.3. There is thus no indication that the appearance of "hypertrophic" mitochondria in the muscle cells of the failing ventricles resulted in a

FIGURE 1

Electron micrograph of longitudinally sectioned ventricular heart muscle of dog with cardiac failure. A rod-shaped mitochondrion runs parallel to the course of the myofibrils through at least 5 or 6 sarcomeres. The arrow points at one of a number of small vesicular structures of about 300 A diameter, located on or very near the mitochondrial surface. The muscle is in the contracted state. × 31,000.

FIGURE 2

Two mitochondria in the left ventricle of dog with heart failure, meeting with their ends opposite a *Z* band. At the site marked by the arrow the 2 organelles seem to be connected by 2 channels or cristae about 200 A in width. One may speculate that this represents an initial step in mitochondrial fusion leading to the elongated forms shown in Figs. 3 and 1. × 60,000.

FIGURE 3

Elongated mitochondrion in another part of the same tissue specimen with indentures opposite the *Z* bands. This might be the shape assumed after completion of the end-to-end type of union indicated in Fig. 3. Notice the irregular course of the cristae in the indented mitochondrion, as compared to their more orderly arrangement in the mitochondria at the right-hand border of the micrograph. × 42,000.

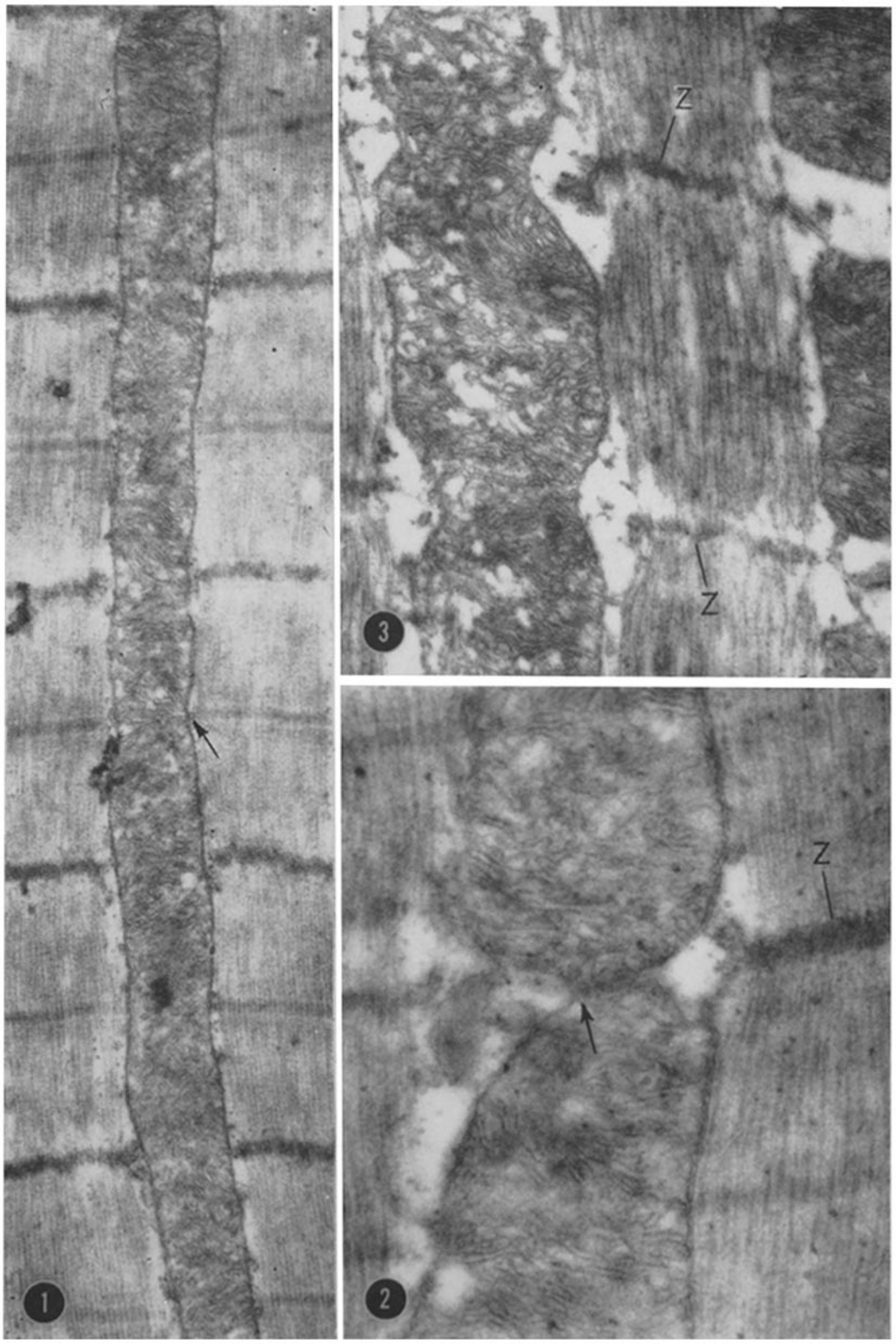

TABLE I

Area Covered on Longitudinal Sections of Normal and Failing Heart Muscle by Mitochondria of Differing Lengths

Column A: Per cent of total mitochondrial population
Column B: Per cent of total mitochondrial area

Mitochondrial length	9 control hearts (1763 mitochondria covering 867.9 μ^2)		2 failing hearts (514 mitochondria covering 374.1 μ^2)	
	A	B	A	B
< 1 sarcomere	97.2	90.8	85.7	59.7
1–2 sarcomeres	2.5	7.3	9.2	19.4
2–3 sarcomeres	0.3	1.9	2.7	10.3
> 3 sarcomeres	0.0	0.0	2.5[1]	10.6[1]

[1] Since several of these mitochondria came only partly into view on the micrographs, the area occupied per mitochondrion is actually greater than is apparent from the figures.

selective expansion of the mitochondrial cell compartment.

Mitochondrial "giantism" was recently observed by Duncan and Hild (4) in cultures of central nervous tissue. As pointed out by these authors, one way in which large forms might arise is by fusion of mitochondria of normal size. Fig. 2 shows a picture of what possibly might be an early stage of this process. Two narrow channels or bridges are seen extending across the border of two adjoining mitochondria, seemingly forming connections between the interior parts of the two organelles. This kind of growth could well give rise to "pinched" forms such as that depicted in Fig. 3. Mitochondria of this shape were indeed more common in the failing than in normal heart muscle.

A second series of experiments, using dogs with gradually developing but ultimately more marked aortic stenosis, is now being initiated with the intent of establishing the time relationship between the expected changes in cardiac size, contractility, and microstructure and of studying their biochemical correlates. As to the latter, it has been reported that mitochondria isolated from "chronically" failing hearts of animals with aortic constriction consume less oxygen in the presence of α-ketoglutarate plus malonate (5) and give lower P:O ratios (6) than mitochondria from normal hearts. Data on mitochondrial morphology were not presented in these reports. The present data make one wonder whether such lowering of metabolic activity might not, at least to some extent, be due to disruption of elongated mitochondria during tissue homogenization.

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