Virchows Archiv A Pathol Anat (1990) 416:473–478

Virchows Archiv A
Pathological Anatomy and Histopathology

Morphometric comparison of mitochondria and myofibrils of cardiomyocytes between hypertrophic and dilated cardiomyopathies

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Received October 4, 1989 / Received after revision December 18, 1989 / Accepted December 19, 1989

Summary. We performed an ultrastructural, morphometric comparison of mitochondria and myofibrils of cardiomyocytes using endomyocardial biopsy specimens in hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). Biopsies came from the right ventricular side of the interventricular septum in nine patients with HCM, nine with DCM, and nine controls with arrhythmia and/or ST depression. Morphometric analysis was carried out using electron microscopic photographs and an image analyser. Mitochondria were significantly greater in number and smaller in size in HCM than in the control group. In DCM, the size of mitochondria was also significantly smaller than in the control group, although their number was similar to that of the control group. No statistically significant difference was found regarding the size of mitochondria between HCM and DCM. The percentages of both mitochondrial and myofibrillar areas in cytoplasm were smaller in the DCM than the HCM and control groups, though no difference was seen between the latter two. The ratio of mitochondrial area to myofibrillar area was almost the same in each group. These results suggest increased mitochondrial function to match hypertrophic cardiomyocytes in HCM, and decreased mitochondrial function and cardiomyocytic contractility in DCM.

Key words: Cardiomyopathies – Ultrastructure – Morphometry – Mitochondria – Myofibrils

Introduction

Cardiomyopathies are defined as heart muscle diseases of unknown cause (WHO/ISFC task force 1980) and are usually classified into three groups: hypertrophic (HCM); dilated, formerly called congestive (DCM); and restrictive (RCM) cardiomyopathy. HCM and DCM have the highest incidence and thus constitute the main groups (Goodwin and Oaklay 1972). Ventricular hypertrophy occurs disproportionately in HCM, and its characteristic functional abnormality is not systolic but diastolic (WHO/ISFC task force 1980). The major histological findings of HCM are hypertrophy and disarray of cardiomyocytes (Roberts and Ferrans 1975). DCM is characterized by ventricular dilation with systolic dysfunction (WHO/ISFC task force 1980) and its major histological findings are cardiomyocytic degeneration and fibrosis of the myocardium (Roberts and Ferrans 1975; Dick et al. 1982).

With respect to the ultrastructure of cardiomyocytes in cardiomyopathies, mitochondrial changes such as swelling, destruction of cristae, presence of concentric lamellae, and numerical increase of mitochondria have been described in the literature (Noorden et al. 1971; Ferrans et al. 1972; Sekiguchi 1974; Maron et al. 1975; Olsen 1980; Baandrup et al. 1981). However, there are very few reports of ultrastructural morphometric analysis of biopsy specimens of DCM and none for HCM. Measurement of mitochondrial size in DCM before and after administration of dobutamine (an inotropic agent) by Unverferth et al. (1980) showed that mitochondria became small in the patients who had a good clinical response. The myofibrillar volume fraction and the ventricular ejection fraction decreased in parallel in DCM (Schwarz et al. 1983) and the volume density of mitochondria was significantly smaller in DCM than in arrhythmia, neurocirculatory asthenia, and secondary cardiac hypertrophy due to hypertension or valvular heart failure (Katagiri et al. 1987). The area of mitochondria was also significantly smaller in DCM than in disease-free transplant donor hearts (Rowan et al. 1988). Thus, the differences in cardiac function between HCM and DCM are presumably caused by mitochondrial as well as by myofibrillar changes. Until now, however, there has been no investigation of the comparative ultrastructure of mitochondrial and myofibrillar changes in HCM and DCM. In this report, ultrastructural morphometric comparison of endomyocardial biopsy specimens taken

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from patients with HCM and DCM were performed to assess the number and size of mitochondria as well as the myofibrillar area in cardiomyocytes.

Materials and methods

Endomyocardial biopsy was performed by cardiac catheterization on the right ventricular side of the interventricular septum in nine patients with HCM, nine with DCM, and nine controls with arrhythmias and/or ST depression on ECG. Cardiomyopathies were diagnosed clinically according to the definition and classification of the WHO/ISFC task force (1980). Of nine controls with arrhythmias and/or ST depression we found single cases of both second and third degree atrioventricular block, as well as sick sinus syndrome, ventricular tachycardia, ventricular extrasystole, supraventricular tachycardia, and atrial fibrillation. ST depression was seen in two. No significant light microscopic changes were observed in the control patients. The sex and age of the patients in each group were as follows: HCM, seven males and two females between 15 and 63 years (42.9 ± 17.4, mean $\pm$ SD); DCM, nine males between 28 and 48 years (39.1 ± 8.8); and control, five males and four females between 16 and 53 years (42.0 ± 12.6).

Two to three tissue specimens were obtained from each patient and the specimens were immersed in fixation solutions within 30 s. For light microscopy the specimens were fixed in 15% buffered formalin, dehydrated in graded alcohol, and embedded in paraffin. Sections were cut at 3 μm and stained with haematoxylin-eosin, as well as by the azan-Mallory and the combined Weigert and van Gieson methods. For electron microscopy the specimens were fixed in 2.5% glutaraldehyde for 2 h, postfixed in 1% osmium tetroxide for 1.5 h, dehydrated in graded alcohol, and embedded in Epon 812. Ultrathin sections were doubly stained with uranyl acetate and lead citrate. We took six to ten electron micrographs of longitudinally cut cardiomyocytes at a magnification of 5000 times in each case and printed these at an enlargement of 2.5 times. Thus, we measured at a final magnification of 12500 times. The total area of measurement ranged from 1,244 μm^2 to 2,074 μm^2 in each case. The profiles of mitochondria and myofibrils were traced on transparent sheets, and these sheets were used for measurement by an image analyser (IBAS-2000, Zeiss, Oberkochen, FRG).

The myofibrillar area, the number of mitochondria and their area, and the long and short axes of each mitochondrion were measured. The number of measured mitochondria ranged from 538 to 1668 in each case. Subsequently, we computed the number of mitochondria in unit area (100 μm^2), the percentage of mitochondrial and myofibrillar areas in cytoplasm, and the ratio of mitochondrial area to myofibrillar area. These values were compared statistically for the HCM, DCM, and control groups by the Student's *t*-test.

Results

By light microscopy cardiomyocytes were hypertrophic and disarrayed in all the patients with HCM (Fig. 1). In DCM, however, cardiomyocytes were degenerative and showed nuclear deformity and/or cytoplasmic vacuoles (Fig. 2). A few hypertrophic cardiomyocytes were occasionally intermingled with degenerative ones in slight fibrosis of the myocardium in two of nine patients with DCM.

On electron microscopy myofibrillar disarray was frequently observed in the disarrayed cardiomyocytes of HCM (Fig. 3). No abnormalities were observed on myofilaments. In DCM, myofilaments were occasionally arranged loosely in myofibrils (Fig. 4) and the sarcoplasmic reticulum was frequently dilated (Fig. 4), though myofibrillar disarray was virtually non-existent. Disruption of cristae in mitochondria was found more frequent-

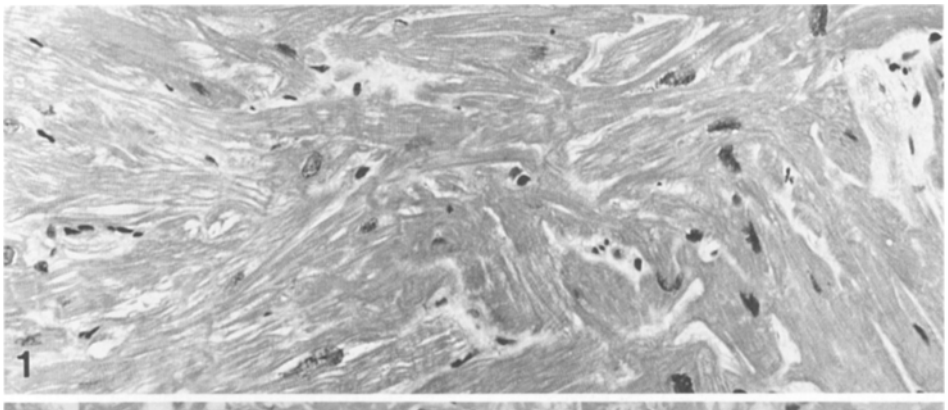

Fig. 1. Hypertrophic cardiomyopathy (HCM). Hypertrophy and disarray of cardiomyocytes. Female, 62 years old. H & E ×260

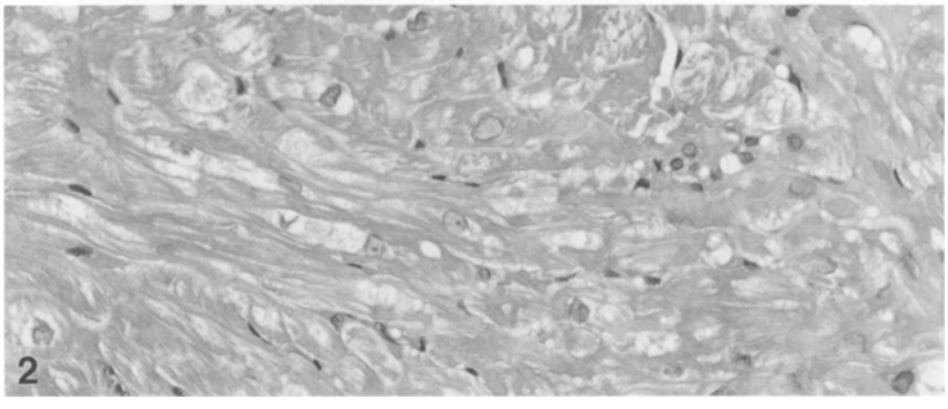

Fig. 2. Dilated cardiomyopathy (DCM). Cytoplasmic vacuoles of cardiomyocytes. Male, 37 years old. H & E ×260

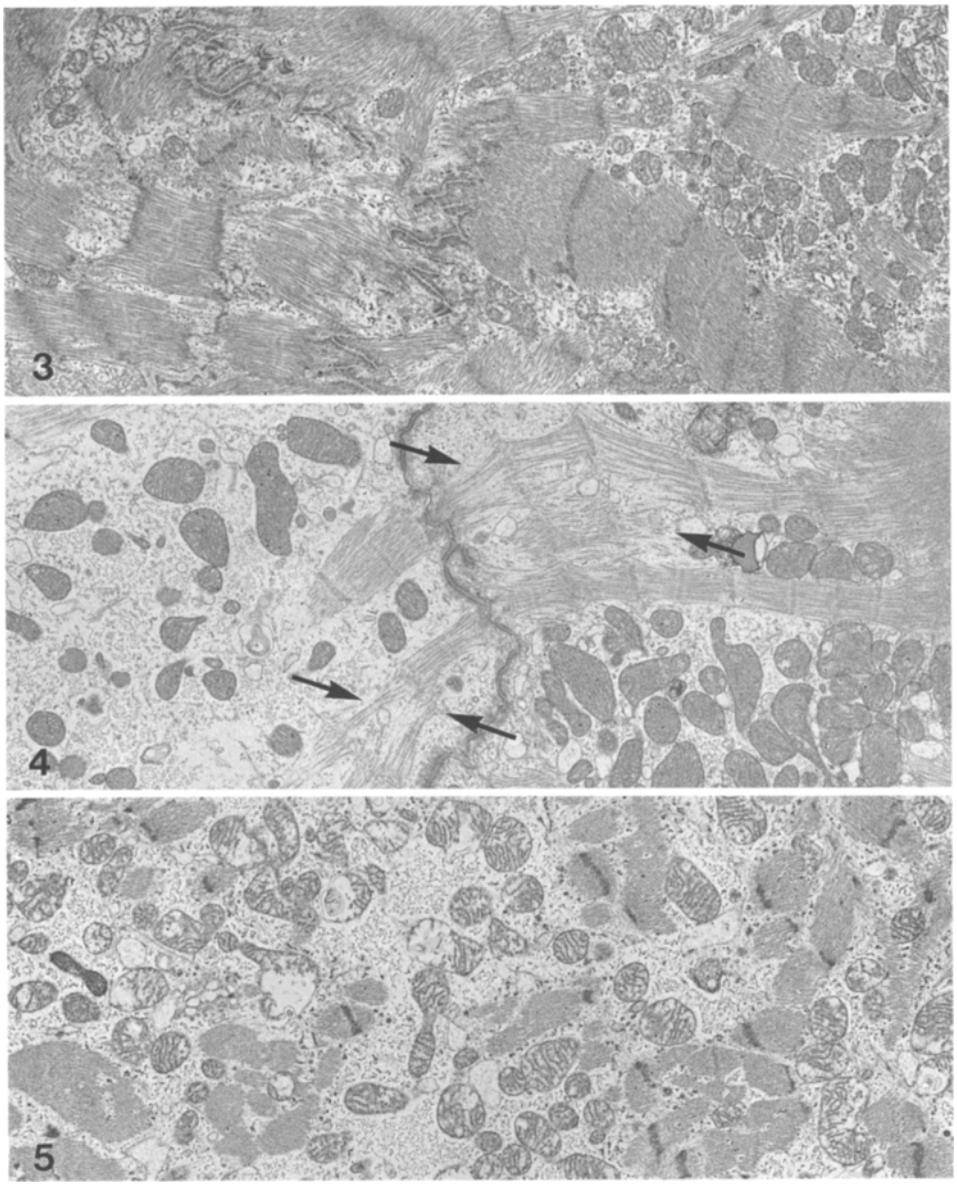

Fig. 3. HCM. Myofibrils are frequently disoriented (myofibrillar disarray) in cardiomyocytes. Male, 33 years old. ×9200

Fig. 4. DCM. Myofilaments of myofibrils are loose in cardiomyocytes. Male, 40 years old. ×9200

Fig. 5. DCM. Disruption of cristae in mitochondria is frequently found in cardiomyocytes of DCM. In addition, myofibrillar arrangement is loose. The same patient as shown in Fig. 4. ×9200

ly in the DCM than the HCM or control group (Fig. 5).

The mean number of mitochondria per unit area (100 μm^2) in each group is shown in Table 1. The number of mitochondria was significantly greater in the HCM than in the DCM and control groups (Figs. 6a–c), but did not differ between the latter two.

The area and the long and short axes of mitochondria are summarized in Table 2. The means for area and long and short axes were significantly smaller in the HCM and DCM groups than in the control. The mean and standard deviation for the percentage of mitochondrial area in the cytoplasm were 16.9% and 2.9% for HCM, 15.6% and 3.9% for DCM, and 17.9% and 3.5% for the control group, respectively. No significant differences were found between the groups.

Concerning the percentage of myofibrillar area in the cytoplasm, the mean percentage was 40.4% for HCM, 35.2% for DCM, and 40.9% for the control group (Table 3). The difference between the DCM and control groups was signficant, but not so for the HCM and DCM groups.

The mean and standard deviation of the ratio of mitochondrial area to myofibrillar area were almost the same in the groups, i.e., 0.43 and 0.09 for HCM, 0.44 and 0.12 for DCM, and 0.45 and 0.10 for the control group.

Table 1. Number of mitochondria in unit area (100 μm^2)

Types of cardiomyopathy	n	Min–Max	Mean ± SD	t-test	
Hypertrophic	9	68.4–95.9	80.5 ± 10.3	$p<0.01$	$p<0.01$
Dilated	9	53.9–85.9	66.0 ± 10.5	NS	
Control	9	54.7–73.1	63.4 ± 7.1		

n = Number of patients; Min = minimal value; Max = maximal value; SD = standard deviation; NS = not significant

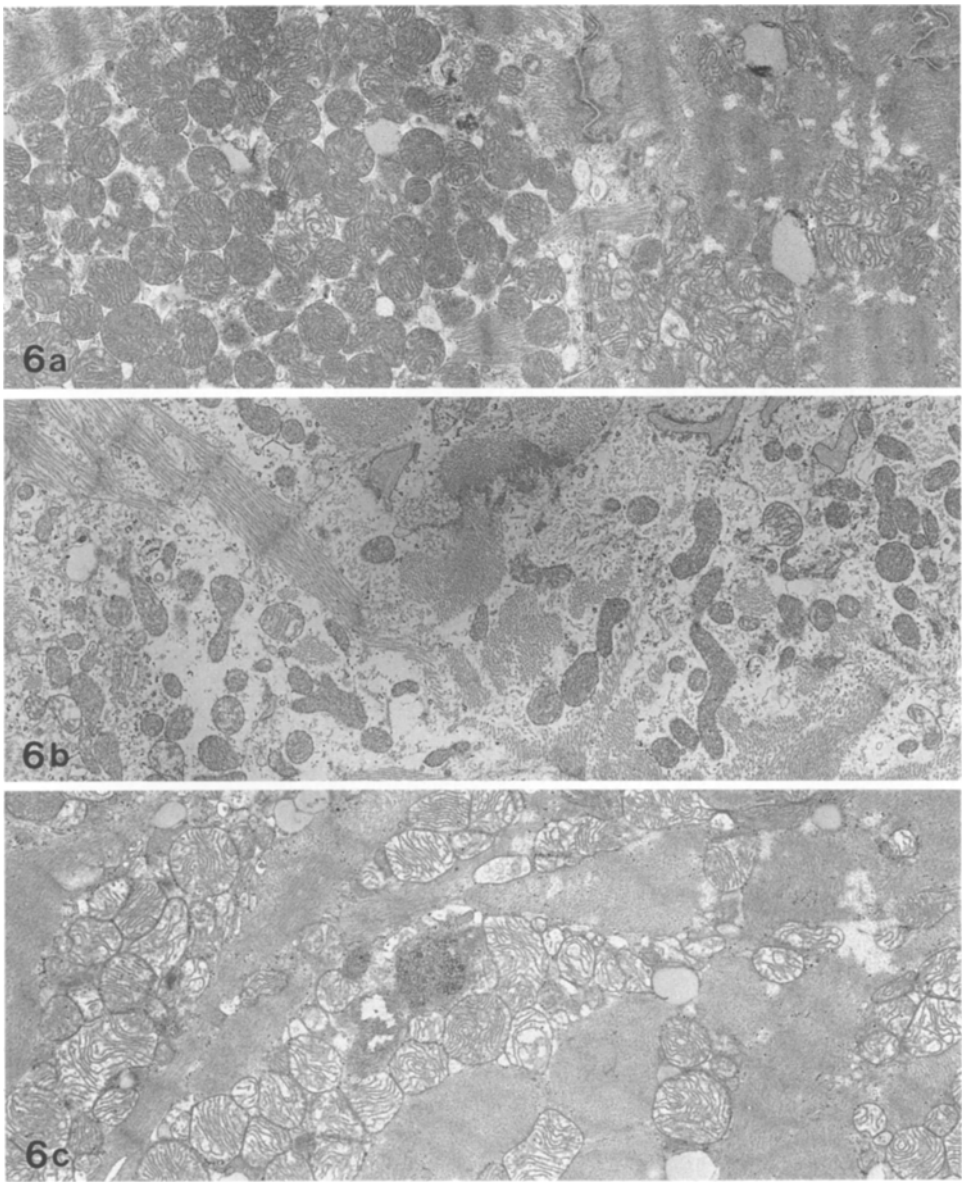

Fig. 6a–c. Comparison of mitochondrial size among HCM, DCM, and control. **a** HCM. Numerous and small mitochondria are in cardiomyocytes. Male, 56 years old. ×9200. **b** DCM. Small mitochondria are shown in cardiomyocytes. However, mitochondria are decreased in number compared to those in HCM. Male, 28 years old. ×9200. **c** Control. Mitochondria are larger in control than in HCM and DCM. ×9200

Discussion

In HCM Noorden et al. (1971), Ferrans et al. (1970), and Maron et al. (1975) have reported swelling of mitochondria, disruption of cristae, formation of intramitochondrial concentric lamellae, and accumulation of mitochondria in surgical biopsy specimens obtained from the interventricular septum of the heart. However, since such mitochondrial changes were also seen in endomyocardial biopsy specimens of DCM and other heart diseases they were considered non-specific findings (Sekiguchi 1974; Baandrup et al. 1981). Nonetheless, in the present study, disruption of cristae was observed less frequently in the HCM than DCM group. In our morphometric comparison of mitochondria, the number of mitochondria in unit cytoplasmic area (100 μm^2) was significantly greater in the HCM than in the DCM and control groups, and their area as well as their long and short axes were significantly smaller in the HCM than control group. As for the percentage of mitochondrial area, no significant differences were found between the HCM, DCM, and control groups. Mitochondria increase in number and decrease in size in hypertrophic cardiomyocytes of dog and rabbit hearts overloaded by stenosis of the main pulmonary artery induced by an inflatable rubber cuff (Bishop and Cole 1969). Increased number and decreased size of mitochondria have also been considered characteristic of cardiomyocytic hypertrophy in rabbit hearts experimentally pressure-overloaded by binding the pulmonary artery (Legato et al. 1984) as well as pressure- and volume-overloaded rat hearts (Hatt 1977). Hatt (1977) suggested that numerous small mitochondria are probably more active than a small number of larger ones, if total volume is the same. The increased number and decreased size of mitochondria in HCM increase the total area of their surface, where ATP and energy-producing enzymes are rich (Anversa et al. 1971). Increased surface area is more efficient for mitochondri-

Table 2. Size of mitochondria

Types of cardiomyopathy	n	Area in μm^2			
		Min–Max	Mean ± SD	t-test	
Hypertrophic	9	0.16–0.25	0.18 ± 0.03	NS	$p<0.01$
Dilated	9	0.15–0.25	0.20 ± 0.04	$p<0.01$	
Control	9	0.20–0.30	0.25 ± 0.03		
Types of cardiomyopathy	n	Long axis (μm)			
		Min–Max	Mean ± SD	t-test	
Hypertrophic	9	0.55–0.75	0.62 ± 0.06	NS	$p<0.01$
Dilated	9	0.57–0.73	0.65 ± 0.05	$p<0.05$	
Control	9	0.63–0.78	0.70 ± 0.05		
Types of cardiomyopathy	n	Short axis (μm)			
		Min–Max	Mean ± SD	t-test	
Hypertrophic	9	0.36–0.44	0.38 ± 0.02	NS	$p<0.01$
Dilated	9	0.33–0.45	0.40 ± 0.04	$p<0.01$	
Control	9	0.42–0.51	0.46 ± 0.03		

Table 3. Percentage of myofibrillar area in cytoplasm

Types of cardiomyopathy	n	Min–Max	Mean ± SD	t-test	
Hypertrophic	9	31.9–51.4	40.4 ± 5.9	NS	NS
Dilated	9	27.3–40.8	35.2 ± 4.5	$p<0.05$	
Control	9	32.8–49.4	40.9 ± 5.8		

al functions, resulting in increased energy supplies for hypertrophic cardiomyocytes. These findings suggest the adaptation of mitochondria to hypertrophy or increased cardiomyocytic function in HCM.

In DCM, mitochondria can either increase or decrease in number within cardiomyocytes and frequent disruption of cristae of mitochondria is seen (Sekiguchi 1974; Baandrup et al. 1981; Baker 1985). Mitochondria in most DCM patients showed a wide size range and were frequently smaller than normal (Roberts and Ferrans 1975). According to Unverferth et al. (1980), the mean size of mitochondria in DCM was 0.26 μm^2 by the point count method. Rowan et al. (1988) made a comparative investigation of mitochondria using a computer-assisted system of morphometry, and observed that the mitochondria of DCM patients were significantly smaller than those in disease-free transplant donor hearts. However, the number of mitochondria in DCM does not increase when compared with a control group, though the area and the long and short axes of mitochondria are smaller in such cases. Katagiri et al. (1987) reported that the volume density of mitochondria was significantly smaller in DCM than in arrhythmia, neurocirculatory asthenia, and secondary cardiac hypertrophy due to hypertensive or valvular heart diseases. In our study, the percentage of mitochondrial area in cytoplasm was smaller in the DCM than control group, but the result was not statistically significant. Our results suggest that mitochondrial function decreases in the cardiomyocytes of DCM when compared with the control group and the frequent presence of disrupted cristae in DCM also supports this view. Peters et al. (1977) reported reduced activity of mitochondria by enzymatic analysis of biopsy specimens obtained from DCM, a finding that supports this suggestion.

With respect to myofibrillar changes in HCM, disarray or abnormal orientation has frequently been described (Roberts and Ferrans 1975; Ferrans 1972). In DCM, myofibrillar loss is frequently found and the myofibrillar volume fraction decreased, though disarray is not marked (Katagiri 1987; Baker 1985). Ventricular function is related to the volume fraction of myofibrils in various cardiac diseases (Schwarz 1983; Schaper and Schaper 1983).

In the present study, no statistically significant difference was observed between the percentages of myofibrillar area in the cytoplasm of cardiomyocytes of the HCM and control groups. However, the percentage of myofibrillar area was significantly smaller in the DCM than control group. It was also smaller in the DCM than HCM group, although the difference was insignificant. In addition, a loose arrangement of myofilaments in myofibrils was frequently found in DCM. These findings suggest that ventricular contractility is decreased in DCM in relation to the decrease in myofibrils and myofilaments.

In conclusion, mitochondrial function appears to parallel both the increase in myofibrillar area in cardiomyocytes in HCM, and the decrease in myofibrils in DCM. A combined increase in number and decrease in size of mitochondria suggests increased mitochondrial function since the membrane area of cristae with energy-producing activities efficiently increases under such a condition. The decreased mitochondrial function of cardiomyocytes in DCM may result from a decrease in mitochondrial size without a concomitant increase in number. Moreover, though contractility of individual cardiomyocytes is increased in HCM, that of the ventricular myocardium is disturbed due to the disarray of the cardiomyocytes. In DCM, the contractility of the ventricular myocaridum is probably disturbed by the degeneration of individual cardiomyocytes.

Acknowledgements. The authors are grateful to Professors M. Kato (Department of Medicine) and R. Satodate (Department of Pathology) for their support of this work.

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