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Clinicopathologic Correlations in Congestive Cardiomyopathy

A Study on Endomyocardial Biopsies

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Summary. Left ventricular endomyocardial biopsies were studied in 57 patients with primary myocardial disease. The patients were divided into a group with low ejection fraction (group A, 21 patients: severe congestive cardiomyopathy), with medium ejection fraction (group B, 17 patients: slight congestive cardiomyopathy) and with high (normal) ejection fraction (group C, 19 patients: suspection of early cardiomyopathy or past myocarditis). Patients with hypertrophic or restrictive cardiomyopathy were excluded from the study.

The biopsies were investigated by light and electron microscopy. Fibre diameters, volume fractions of interstitial fibrous tissue and volume fractions of myofibrils were evaluated by light microscopic morphometry. The volume fraction of myofibrils has not previously been measured with the light microscope, and we have therefore provided formal validation of the method by calculating linear regression between light (y) and electron microscopic (x) values ($y = 1.01 + 2.25$ (vol%)).

The fibre diameters were higher in the patient groups with low ejection fraction ($P < 0.01$), the volume fraction of interstitial fibrous tissue was higher ($P < 0.05$) and the volume fraction of myofibrils was lower ($P < 0.001$). In a preliminary study of 72 patients, we found a higher correlation between the volume fraction of myofibrils and ejection fraction ($r = 0.55$; $P < 0.001$) than between fibre diameters (hypertrophy) and ejection fraction ($r = -0.32$, $P < 0.01$). This indicates that myocardial degeneration is more closely related to the clinical stage of congestive cardiomyopathy than is fibre hypertrophy.

Nine patients of group A died within 2 years after biopsy. They had significantly higher fibre diameters, a higher volume fraction of fibrous tissue and a lower volume fraction of myofibrils ($P < 0.05$) than survivors of group A.

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The occurrence of 12 qualitative morphological findings has been investigated and it was found that small mitochondria of variable size and shape, loss of myofibrils, glycogen accumulations and changes of heart muscle nuclei were significantly associated with a low ejection fraction.

Key words: Congestive cardiomyopathy – Endomyocardial biopsy – Morphometry – Clinicopathologic correlation

Introduction

The bioptome technique for obtaining fresh endomyocardial biopsy samples was introduced by Sakakibara and Konno in 1962. A long series of morphological biopsy studies concerning congestive cardiomyopathy (COCM) has been published over the last decade (Mösslacher et al. 1971; Bulloch et al. 1972; Ferrans et al. 1973; Backwinkel et al. 1974; Roberts and Ferrans 1975; Doerr et al. 1976; Knieriem 1978; Noda 1980). Now it is widely accepted that the histological and ultrastructural changes which have been observed in COCM are nonspecific (Ferrans and Roberts 1978). On the other hand, the severity of morphological alterations correlates with the ejection fraction (EF) and with the prognosis (Kuhn et al. 1978; Kunkel et al. 1978; Sekiguchi et al. 1978; Baandrup et al. 1981). Most authors have evaluated the morphological alterations in a semiquantitative score; in addition Kunkel et al. (1978) measured fibre diameters and the volume fraction of interstitial fibrous tissue.

The objective of the following study was to determine which morphological alterations are significantly associated with the clinical severity of COCM. The methods we used were qualitative light and electron microscopy and light microscopic morphometry.

Our investigation differs from similar studies in two respects:

1. Both the fibre diameters and the volume fraction of fibrous tissue were measured, together with the volume fraction of myofibrils.

2. The qualitative findings were not combined in a semiquantitative score but were tested separately in order to determine the morphological pattern of COCM which is associated with clinical severity of the disease.

Methods

Patients

The study comprised 57 patients with primary myocardial disease (49 men and 8 women, age between 23 and 55 years). At the time of catheterization each patient revealed two or more of the following features: 1. symptoms of heart failure; 2. episodes of chest discomfort; 3. roentgenographic evidence of cardiomegaly; 4. an electrocardiographic abnormality in form of intraventricular conduction defects, atrial arrhythmias, LV hypertrophy or ST segment and T wave abnormalities.

Patients with coronary heart disease, cor pulmonale or systemic disease and patients suspected of having hypertrophic or restrictive cardiomyopathy were excluded from this study.

As a criterion for the definition of patient groups the level of the angiocardiographically determined ejection fraction (EF) was used:

Group A (EF<45%). Twenty-one patients with severe congestive cardiomyopathy (17 men, 4 women; mean age (±SD): 42.9±8.0 yr).

Group B (45%≦EF<60%). Seventeen patients with slight congestive cardiomyopathy (16 men, 1 woman; mean age (±SD): 44.8±6.4 yr).

Group C (EF≧60%). Nineteen patients suspected of having early COCM or past myocarditis (16 men, 3 women; mean age (±SD): 42.3±11.0 yr).

Within 2 years after biopsy all patients were re-evaluated. 9 patients of group A have died, the patients of group B and C were all alive.

Biopsy

Left ventricular endomyocardial biopsies were obtained with the Kings College bioptome using the retrograde transarterial route and a PVC sheath (Richardson 1974). Immediately after biopsy tissue samples were fixed by immersion with 1.5% glutaraldehyde- 1.5% formaldehyde mixture in 0.2 mol phosphate buffer. The specimens were postfixed in OsO_4 and embedded in Epon-Araldite.

The light microscopic investigations were performed on very thin sections (6–12 per patient) which were stained with alkaline toluidine blue and 3% paraphenylenediamine. For electron microscopy ultrathin sections were stained with lead citrate and uranylacetate according to standard techniques.

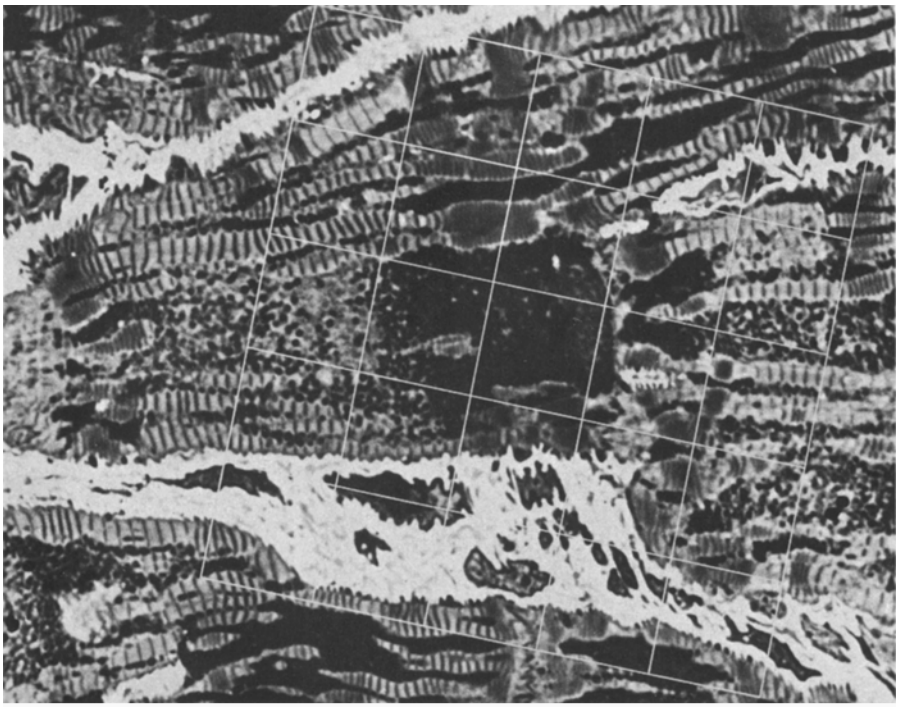
Morphometry

At a magnification of 160:1 the volume fraction of fibrous tissue was determined. For this purpose a random test area with 36 test points was projected on each section. The volume fraction was measured according to well established point counting methods (Weibel 1979). We tried carefully to exclude measurements of endocardial fibrous tissue. If the total section area of all biopsy samples of a patient was smaller than 1.2 mm^2, the biopsy was eliminated from the study.

At a magnification of 420:1 fibre diameters were determined from 80 to 300 cross or oblique sections of myocardial cells. When oblique sections were counted the short diameter of the section profile was measured. If less than 80 fibre profiles were counted, the patient was excluded from the study.

At a magnification of 1,000:1 the volume fraction of myofibrils was evaluated with oil immersion and phase contrast. 4 randomly chosen sections of each patient and at least 3 random test areas with 36 test points per area were used (Figs. 1 and 2).

The volume fraction of myofibrils has not been measured with the light microscope to date. For the quantification of myofibrils we preferred light microscopy, since in endomyocardial biopsies of patients with COCM there is evidently a considerable variation in the morphological findings. It would therefore be useful to analyse the larger test areas which can be obtained with the light microscope. But we considered it necessary to give formal validation of our light microscopic measurements and for this purpose, 12 tissue blocks were chosen from 12 of the 57 patients. One semithin and one ultrathin section of each block were analysed. In the light microscope 6 test fields per block were evaluated with 216 test points (total test area 15,000 μ^2). 40 random test fields per block were evaluated with 3,200 test points in the electron microscope (total test area 12,000 μ^2, EM magnification 10,000:1). Figure 3 illustrates the strong correlation of light and electron microscopic values, but the linear regression slope indicates a slight overestimation of the light microscopic values (+2.5 vol% on average).



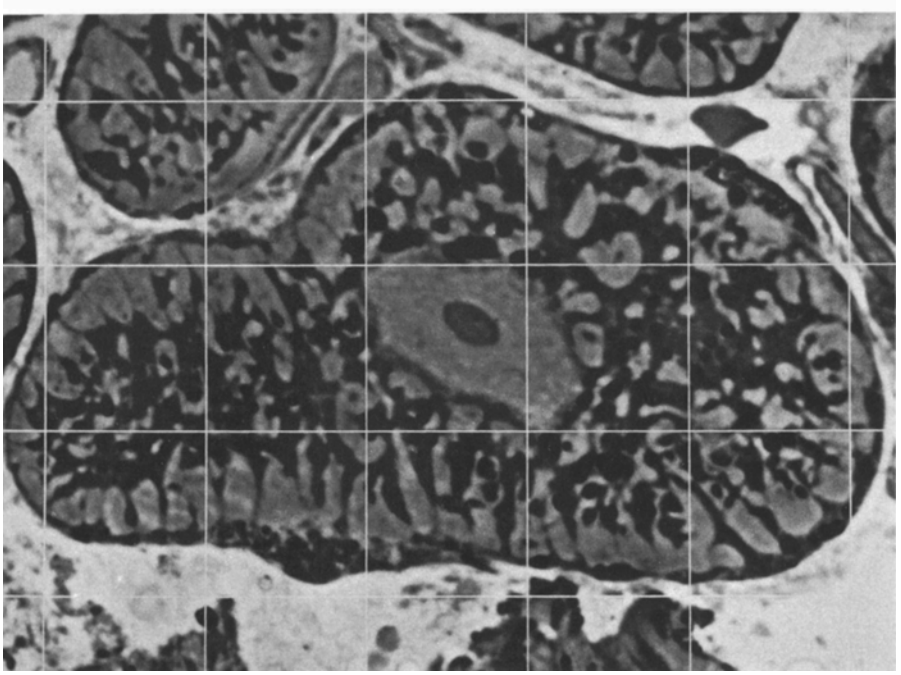


Fig. 1. Magnification 960:1, light microscopy. The test lattice we used is projected on a longitudinal section of muscle fibres

Fig. 2. Magnification 1,470:1, light microscopy. The test lattice we used projected on a cross section of muscle fibres. The light areas correspond to the myofibrils

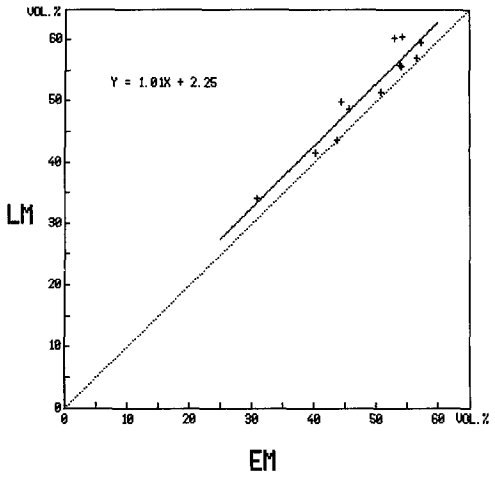


Fig. 3. Figure 3 illustrates that light microscopic values of the volume fraction of myofibrils (*LM*) are closely correlated with electron microscopic values (*EM*). The regression slope indicates a slight over-estimation with light microscopic morphometry

Sampling Variability

In order to establish the variability in the measurements of different biopsy samples from the same heart, we determined the reproducibility in terms of sampling variability of endomyocardial biopsies. The variability was determined in 26 patients in whom 2 ($n=14$) or 3 ($n=12$) separate biopsy specimens were obtained. Variability was determined by the coefficient of variation, defined as the standard deviation of all repeated measurements in a given case divided by the mean of those measurements. Two standard deviations on either side of the mean were calculated from the estimates of reproducibility, and the 95% confidence interval was determined for each measurement; then the actual value by which each measurement can vary was calculated.

Qualitative Evaluation

The occurrence of the following qualitative morphological findings ("variables") was investigated without prior knowledge of clinical data (Figs. 4–10):

1. Bizarre nuclei of muscle cells (variable 1)
2. Enlarged nucleoli (variable 2)
3. Disarray of myofibrils (variable 3)
4. Loss of myofibrils (variable 4)
5. Increased glycogen content (variable 5)
6. Increased number of lipid droplets (variable 6)
7. Increased number of lipofuscin granules (variable 7)
8. Proliferation of interstitial cells (variable 8)

These variables were examined by light microscopy.

The following variables require electron microscopic investigation (2 sections per patient):

1. Swelling of mitochondria (variable 9)
2. Small mitochondria of variable size and shape (variable 10)
3. Small myelin figures (variable 11)
4. Dilatation of sarcoplasmic reticulum (variable 12)

Each variable could be either positive or negative.

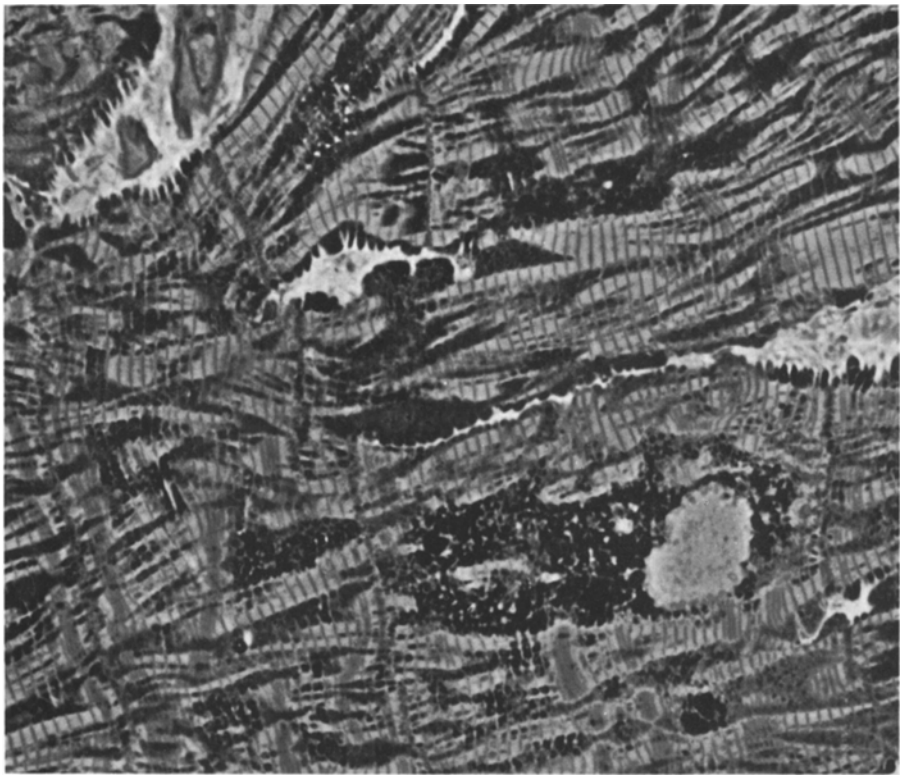


Fig. 4. Magnification 1,150:1, light microscopy. Longitudinal section of myocardial cells without apparent degenerative alterations

Fig. 5. Magnification 960:1, light microscopy. Reduced numbers of myofibrils (variable 4) and perinuclear glycogen accumulations (variable 5) are often seen in severe COCM

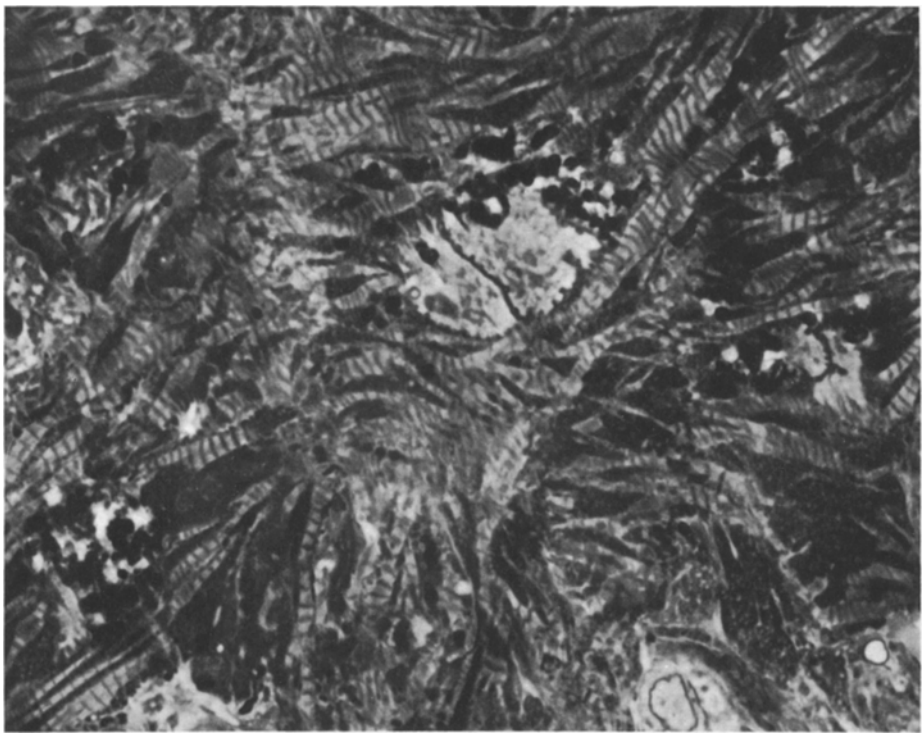


Fig. 6. Magnification 1,190:1, light microscopy. Myofibrillar disarray (variable 3) is not associated with the clinical stage of COCM

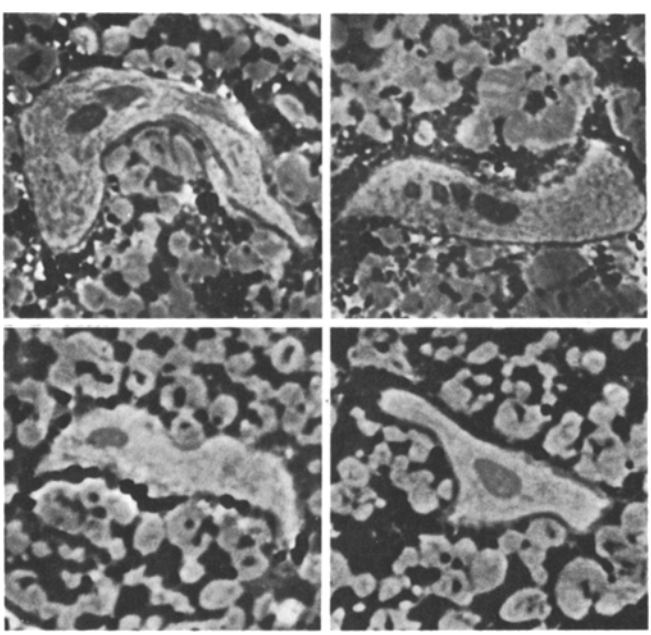


Fig. 7. Magnification 3,500:1, light microscopy. Extremely bizarre nuclei with enlarged nucleoli (variables 1, 2) are regularly found in severe COCM

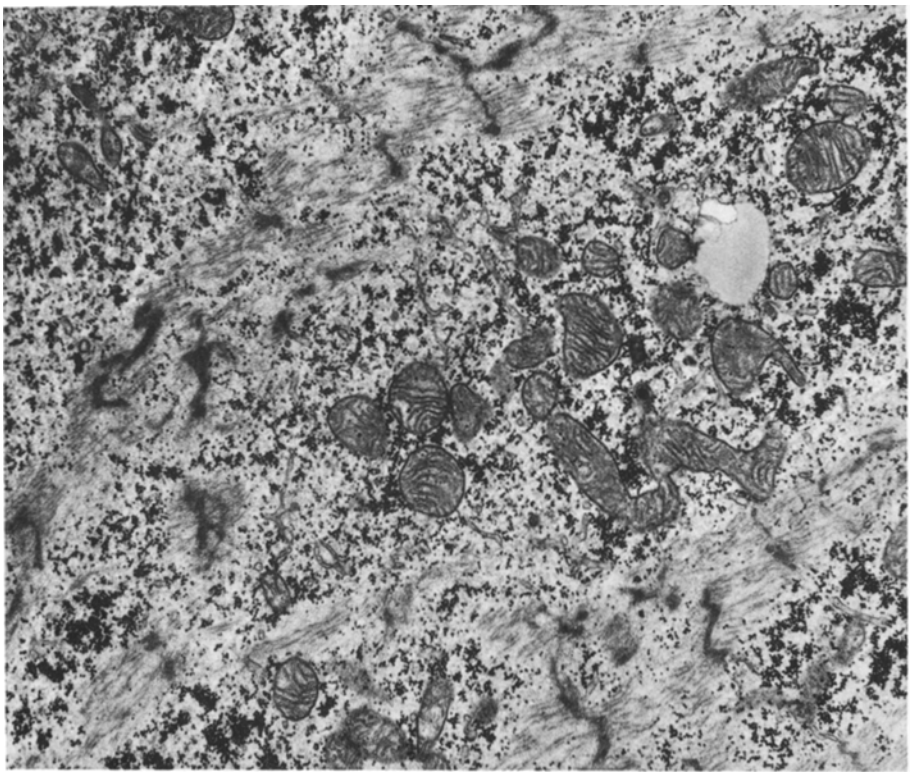


Fig. 8. Magnification 15,300:1, electron microscopy. Loss of myofibrils (variable 4), Z band disturbances and glycogen accumulations (variable 5) are often found in patients suffering from severe COCM

Statistics

The morphometric variables of group A, B and C were compared by the rank test according to Kruskal and Wallis (Immich 1974).

The morphometric variables of the survivors and nonsurvivors were compared by the rank test for unpaired data according to Wilcoxon (Immich 1974).

The qualitative variables of group A, B and C were compared by the chi^2 test (Sachs 1972).

Results

Sampling Variability. Data concerning sampling variability of morphometric analysis are presented in Table 1. The greatest scatter of data is found for the determination of interstitial fibrous tissue, while the evaluation of fibre diameters and volume fraction of myofibrils reveals less variability.

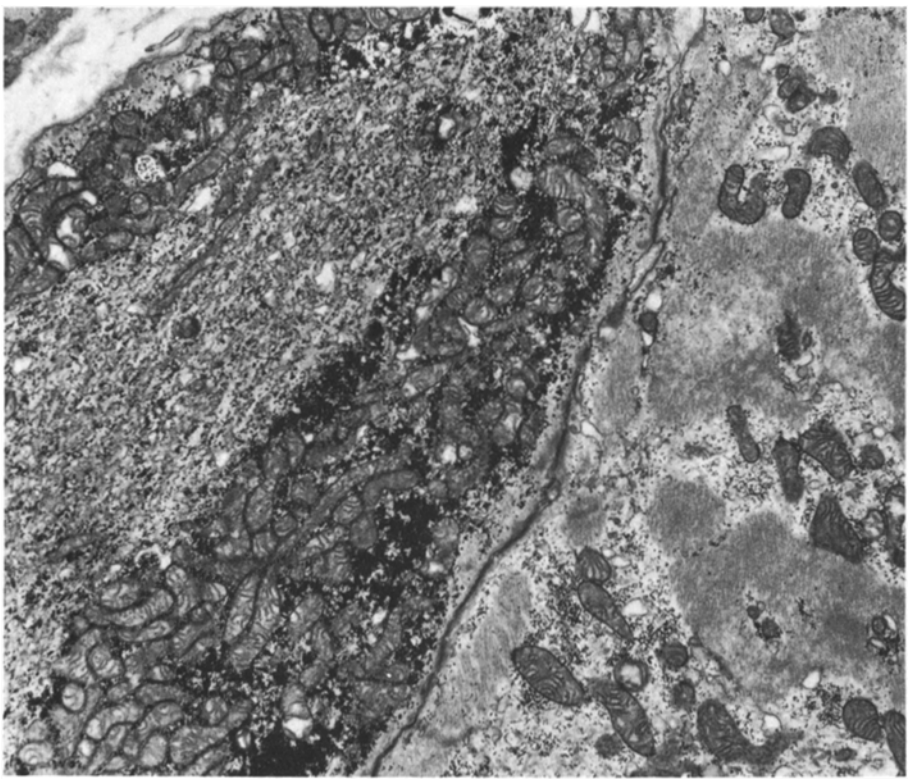


Fig. 9. Magnification 7,200:1, electron microscopy. Severely degenerated cardiac muscle cell with loss of myofibrils (variable 4), glycogen accumulations (variable 5) and mitochondria of variable size and shape (variable 10). Patient of group A who died 3 months after biopsy

Clinicopathologic Correlation. The fibre diameters ($P<0.01$), the volume fraction of interstitial fibrous tissue ($P<0.05$) and the volume fraction of myofibrils ($P<0.001$) differ significantly in the 3 groups (Table 2, Figs. 11–13).

The fibre diameters, the volume fraction of interstitial fibrous tissue and the volume fraction of myofibrils differ significantly between survivors and nonsurvivors in group A ($P<0.05$; Table 3).

The qualitative variables 1, 2, 4, 5, 7 and 10 were significantly different in the 3 groups (Table 4).

Discussion

Methods

We used light microscopy to obtain the volume fraction of myofibrils in order to reduce the sampling error that is naturally high in electron micros-

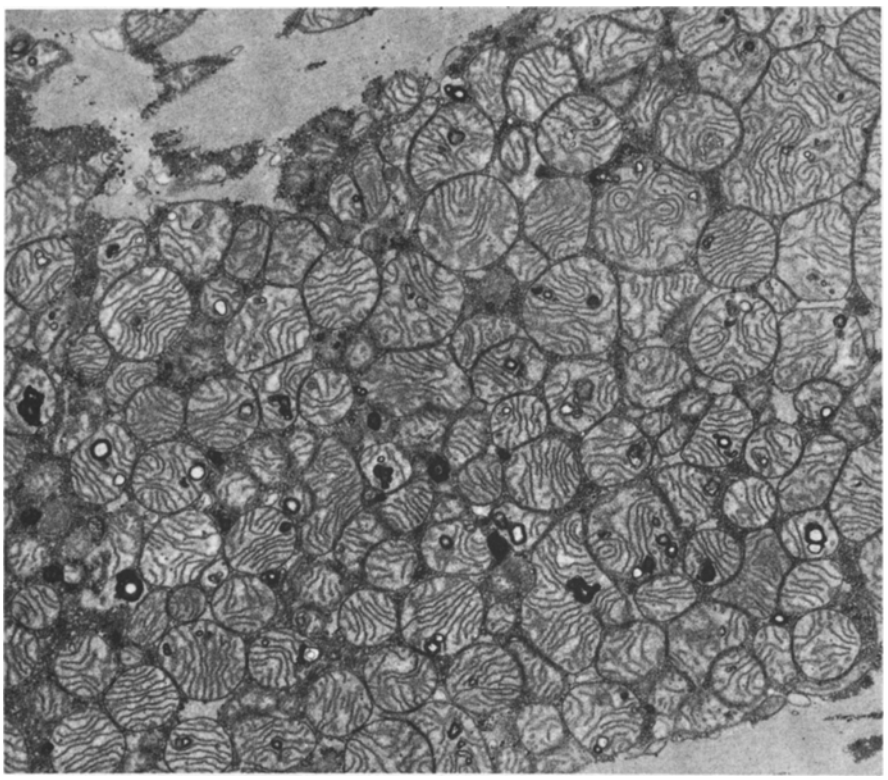


Fig. 10. Magnification 22,100:1, electron microscopy. Small myelin figures (variable 11) are not associated with the clinical stage of COCM

Table 1. Sampling variability of endomyocardial biopsies ($n=26$ patients)

	Average of measured data (±SD)	Coefficient of variation (%)	95% confidence interval (±SD) of each measurement
Fibre diameter (μ)	24.5±4.0	6	±2.9
Volume fraction of fibrous tissue (%)	10.7±9.7	43	±9.2
Volume fraction of myofibrils (%)	55.9±4.7	3	±3.4

copy. As shown above, there is a strong correlation between light and electron microscopic values. However, if myofibrils are measured with the light microscope, three prerequisites have to be fulfilled: 1. use of thin sections (0.5 μ) 2. combined staining with toluidine blue and paraphenylenediamine and 3. use of phase contrast microscopy with oil immersion. As it turned out, our light microscopic measurements show some overestimation when compared with electron microscopic values (+2–3 vol%). Fleischer et al. (1980) measured the volume fraction of myofibrils in normal human left ventricles and found 52 vol%, Schwarz et al. (1980) found 59 vol%. Our

Table 2. Morphometric characteristics of the patient groups (mean ± SD)

	Group A ($n=21$)	Group B ($n=17$)	Group C ($n=19$)	Level of statistical significance[b]
Fibre diameter (μ)	25.7 ± 3.5	23.6 ± 4.0	21.3 ± 3.1	$P<0.01$
Volume fraction of fibrous tissue (%)	13.5 ± 15.4	8.8 ± 11.4	3.7 ± 3.8	$P<0.05$
Volume fraction[a] of myofibrils (%)	53.3 ± 5.7	56.6 ± 5.1	60.1 ± 3.7	$P<0.001$

[a] Volume fraction of myofibrils: Volume of myofibrils per volume of myocardial cells in percent

[b] Significant difference between the 3 groups with the rank test according to Kruskal and Wallis

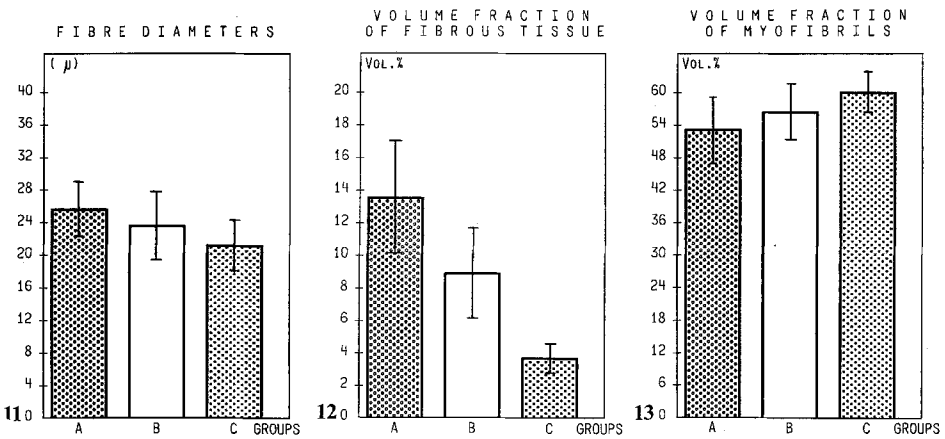


Fig. 11. Means (± standard deviations) of the fibre diameters. Group A: Patients with low ejection fraction (EF < 45%). Group B: Patients with slightly reduced ejection fraction (45% ≦ EF < 60%). Group C: Patients with normal ejection fraction (EF ≧ 60%)

Fig. 12. Means (± standard errors) of the volume fractions of interstitial fibrous tissue. Group A: Patients with low ejection fraction (EF < 45%). Group B: Patients with slightly reduced ejection fraction (45% ≦ EF < 60%). Group C: Patients with normal ejection fraction (EF ≧ 60%)

Fig. 13. Means (± standard deviations) of the volume fractions of myofibrils (volume of myofibrils per volume of myocardial cells in %). Group A: Patients with low ejection fraction (EF < 45%). Group B: Patients with slightly reduced ejection fraction (45% ≦ EF < 60%). Group C: Patients with normal ejection fraction (EF ≧ 60%)

light microscopic value of group C amounts to 60 vol% and – corrected for electron microscopy – to 57–58 vol%.

Reproducibility

The sampling variability of the morphometric variables was low in the case of fibre diameters and volume fraction of myofibrils when compared with

Table 3. Morphometric variables of survivors and nonsurvivors of group A (mean ± SD)

	Nonsurvivors ($n=9$)	Survivors ($n=12$)	Level of statistical significance
Fibre diameter (μ)	27.3 ± 3.3	23.7 ± 3.3	$P<0.01$
Volume fraction of fibrous tissue (%)	20.7 ± 17.3	8.1 ± 11.8	$P<0.05$
Volume fraction[a] of myofibrils (%)	50.6 ± 7.8	55.3 ± 2.2	$P<0.05$

[a] Volume fraction of myofibrils: Volume of myofibrils per volume of myocardial cells in percent

Table 4. Absolute frequency of qualitative morphological findings in the 3 patient groups

	Group A ($n=21$)	Group B ($n=17$)	Group C ($n=19$)	Level of statistical significance[a]
1. Bizarre nuclei	16	11	5	$P<0.01$
2. Enlarged nucleoli	18	13	3	$P<0.001$
3. Disarray of myofibrils	9	5	6	–
4. Loss of myofibrils	12	5	2	$P<0.01$
5. Increased glycogen content	13	4	5	$P<0.05$
6. Increased number of lipid droplets	10	10	11	–
7. Increased number of lipofuscin granules	5	11	12	$P<0.05$
8. Proliferation of interstitial cells	7	8	4	–
9. Swelling of mitochondria	9	4	6	–
10. Small mitochondria of variable size and shape	12	3	2	$P<0.01$
11. Small myelin figures	9	8	11	–
12. Dilatation of sarcoplasmic reticulum	9	8	12	–

[a] Significant difference between the 3 groups (chi^2-test)

the volume fraction of interstitial fibrous tissue. This means that the small endomyocardial biopsy samples are highly representative in respect of fibre diameters and myofibrillar volume fraction and less representative of the degree of interstitial fibrosis. It should be noted that Noda (1980) established a low but significant correlation between the histological findings of right and left ventricular biopsy samples using a semiquantitative score ($r=0.43$; $P<0.05$).

Quantitative Results

Our measurements of the mean fibre diameters in COCM (group A:25.7 μ; group B:23.6 μ) are compatible with the results of other authors. Hess et al. (1981) found 23.6 μ on average, Noda (1980) 22.7 μ and Kunkel et al. (1978) in 65% of the patients with severe COCM more than 21 μ. From right ventricular biopsies lower values have been reported (Baandrup et al. 1981: 17 μ; Noda 1980: 18 μ).

The volume fraction of interstitial fibrous tissue ("degree of interstitial fibrosis") was 8.8% in group B and 13.5% in group A. Hess et al. (1981) found 28%, Kunkel et al. (1978) in 65% of the patients with severe COCM more than 11%, Baandrup et al. (1981) reported very low values obtained from right ventricular myocardium (less than 5%). The differences may be explained by the high sampling variability and different staining techniques (Baandrup et al. 1981).

The volume fraction of myofibrils has been determined in cardiac valve disease. Schwarz et al. (1981) described a decreased volume fraction of myofibrils in patients with aortic valve disease, which correlates with a low ejection fraction. Analogous to these findings our results indicate that low ejection fractions are associated with low volume fractions of myofibrils even in COCM.

Each morphometric variable (fibre diameters, volume fractions of myofibrils and fibrous tissue) shows a significant relationship to the prognosis of the disease. Kuhn et al. (1978) and Bouhour et al. (1976) found the prognosis dependend on the severity of morphological alterations, which was estimated on a semiquantitative score.

Qualitative Results

Nuclear changes (variables 1, 2), loss of myofibrils (variable 4), increased glycogen content (variable 5) and small mitochondria of variable size and shape (variable 10) are associated with a low ejection fraction.

No association between the clinical stage and the morphological alterations could be found in myofibrillar disarray, lipid droplets, proliferation of interstitial cells, myelin figures and dilatation of sarcoplasmic reticulum (variables 3, 6, 8, 9, 11, 12), an increased number of lipofuscin granules (variable 7) was significantly less frequent in patients with a low ejection fraction.

The 12 variables we investigated have been observed in COCM by several groups (Kunkel et al. 1978; Knieriem 1978; Roberts and Ferrans 1975; Bulloch et al. 1972), but our results indicate that some of the variables are either not closely enough or absolutely not related to the severity of primary myocardial disease.

Nuclear changes are well known to occur in cardiac hypertrophy (Adler et al. 1977). Loss of myofibrils, mitochondrial changes and glycogen accumulations correspond to severe degeneration of hypertrophic muscle fibres of human hearts as described by Maron and Ferrans (1978).

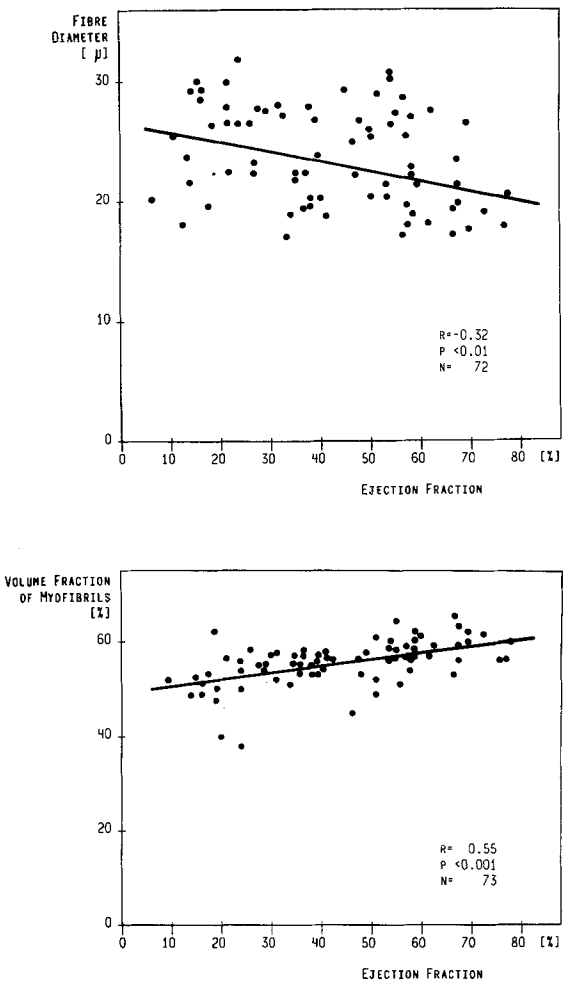


Fig. 14. Correlations between ejection fraction (EF) in % and fibre diameters and the volume fraction of myofibrils

Final Comments

The highest significance of all the morphometric variables ($P<0.001$) was obtained by testing the volume fraction of myofibrils. In a preliminary study on more than 70 patients we found that the volume fraction of myofibrils correlates better with the ejection fraction than the fibre diameters (Fig. 14). Loss of myofibrils is a common finding in degenerated cardiac muscle cells under various conditions, such as cardiac valve disease (Maron et al. 1975), congenital heart disease (Jones and Ferrans 1977), and congestive cardiomyopathy (Ferrans et al. 1973). Cardiac hypertrophy is not necessarily associated with degenerative changes of the cell organelles but if these occur, they are considered to be representative of Meerson's third stage of hypertrophy (Meerson 1969), i.e., the stage of cellular exhaustion (Maron and Ferrans 1978). We believe that the volume fraction of myofibrils is likely to be a measure of the progression of myocardial degeneration. Our

data indicate that the severity of cardiac degeneration is probably better correlated with cardiac impairment in COCM than is the degree of hypertrophy.

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