Electron Microscopic Investigation of Endomyocardial Biopsy Samples in Hypertrophy and Cardiomyopathy

A Semiquantitative Study in 48 Patients

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AND E. G. J. OLSEN, M.D.

SUMMARY

Electron microscopic and statistical analyses of 66 right ventricular biopsies from 48 patients were undertaken to investigate whether quantitative differences exist between those patients with "ordinary" myocardial hypertrophy and those suffering from a form of cardiomyopathy. The electron microscopic changes were scored and correlated with hemodynamic variables such as ejection fraction (EF), left ventricular end-diastolic pressure (LVEDP) and length of history. The patients were followed for an average of 22.5 months, permitting an assessment of prognosis. The results show that the three diagnostic groups—"ordinary" hypertrophy, hypertrophic cardiomyopathy (HOCM) and congestive cardiomyopathy (COCM)—overlap, but crossovers of sarcomeres is more frequent in patients in whom HOCM is diagnosed. Except for a tenuous relationship between EF, and the electron microscopy (EM) (r = -0.46, p < 0.1) and between LVEDP and EM score (r = 0.61, p < 0.01), in the COCM group, no correlation could be established between EF, LVEDP and length of history when the patients were grouped according to histologic or clinical diagnosis.

This study shows that the various claims regarding relationships between morphologic changes and the functional status of patients or prognosis cannot be confirmed.

ENDOMYOCARDIAL biopsy is performed for diagnostic purposes, for follow-up of treatment and for research.1-5 Indications for this procedure have been described in detail by Mason et al.,6 and cardiomyopathy is one of the most important. Endomyocardial biopsy samples have been studied by means of electron microscopy to determine whether information additional to that provided by light microscopy can be gained. The results of such studies conflict in regard not only to differentiation of the forms of cardiomyopathy, but also to assessment of prognosis by correlation of ultrastructural changes with hemodynamic and clinical variables.6-8 There is considerable evidence that changes at the ultrastructural level are nonpathognomonic.9-11

In this study, we evaluated whether biopsy tissue from patients with "ordinary" myocardial hypertrophy differs from biopsy tissue from patients with cardiomyopathy. We also undertook detailed statistical analysis of our data to ascertain whether electron microscopic changes and their relation to hemodynamic variables and length of history can be used to evaluate prognosis.

Material and Methods

Biopsy Material

Three hundred sixty-one biopsies from 201 patients with a primary clinical diagnosis of cardiomyopathy formed the basis of this morphologic and morphometric study of endomyocardial biopsies in cardiomyopathy. Cardiomyopathy was defined and diagnosed according to Goodwin and Oakley.12,13 The initial diagnosis of cardiomyopathy could not be maintained in all patients during the follow-up period.

Biopsies were obtained by means of the Konno-Sakakibara14,15 or King's bioprome,16 usually from the right ventricle. Samples of sufficient size were bisected, one portion for light microscopy and the other for electron microscopy.

Fifty-six cases were selected according to the following criteria. (1) An established histologic description of "ordinary" hypertrophy or cardiomyopathy. Cardiomyopathy includes hypertrophic cardiomyopathy (HOCM) or a condition in which the morphology is compatible with clinical congestive cardiomyopathy (COCM), i.e., hypertrophy of muscle fibers with foci of attenuation and increased prominence of smooth muscle cells within the endocardium. (2) An established clinical diagnosis with information on the subsequent course of the patient. (3) Suitable tissue for electron microscopic investigation evaluated by semithin sections (1 μm).

After selection, the specimens were relabeled to
avoid bias in the subsequent analysis of the material. Eight patients were excluded because the technical quality of the micrographs did not permit proper examination. The material available for analysis consisted of 66 right ventricular tissue blocks from 48 patients — 38 males with a mean age of 45.6 years (range 17–64 years) and 10 females with a mean age of 43 years (range 35–64 years).

Fixation, Embedding and Impregnation

Immediately after removal from the heart, the sample was placed in a drop of fixative, 3% sodium cacodylate-buffered glutaraldehyde at a temperature of 4°C. Cubes less than 1 mm³ were chopped and the available material was collected into a Pasteur pipette and expelled into a sealable jar containing fixative. The fixative was replaced by buffered sucrose washing solution after 1 hour (4°C, pH 7.4). The material was then postfixed in 1% osmium tetroxide, processed by standard techniques and embedded in epon. Sections were cut on a Porter Blum MT1 ultramicrotome; 1-μm-thick sections were stained with alkaline toluidine blue for selection purposes. Ultrathin sections approximately 50 mm thick were mounted on copper grids of 300 mesh and stained with uranyl acetate and lead citrate. We attempted to cut sections with a predominantly longitudinal arrangement of myocardial fibers.

Electron Microscopy

The examination was carried out on a Philips EM 301 electron microscope. Every section from each case was viewed and four or more areas were photographed. Randomization in photography was not complete, as areas predominantly affected by artifactual changes were excluded. We decided that an area should be excluded if three observers were in agreement. The entire area was photographed at a magnification of × 2200 and serial micrographs were subsequently taken at a magnification of × 5900–9800; higher magnification was used for specific details. On average, 4.9 fields per patient and 4.3 micrographs per field were photographed. Kodak electron image film 4463 (plates) was used, and printing was performed on Kodak Verifilm paper, 20.3 × 25.4 cm. A printing magnification of × 2.5 was used.

Semiquantitative Assessment

The structures and changes that were assessed are summarized in table 1. Fiber diameter was measured from 1-μm-thick sections stained with alkaline toluidine blue, and the results were compared with the corresponding sections stained with hematoxylin and eosin for light microscopy. No statistically significant difference (matched-pairs Wilcoxon signed-ranks test) was found. The severity of fibrosis was evaluated by determining the volume fraction of collagen in sections stained with elastic van Gieson stain for light microscopy. The electron microscopy (EM) score, including points 1–5 (table 1), proposed by Kuhn et al., was used throughout this study. Kuhn et al. did not use half scores, which we allowed in this investigation when it was impossible to decide upon a score of 1 or 2. By convention, but also by principles of general biological behavior, grading is easier when it is possible to score by three grades. Optimal separation of the patients was achieved in this study by assigning patients with scores less than 4 points to group 1, and those with scores greater than or equal to 4 to group 2.

Catheterization, Hemodynamic Measurements and Follow-up of Patients

The Seldinger technique or venous cutdown was used for right- or left-sided catheterization. Information on left ventricular end-diastolic pressure (LVEDP) and selective coronary angiography were obtained from catheterization reports. LVEDP from patients with significant valvular gradients was excluded. No patient had anatomic or significant pathologic abnormalities of the coronary arteries at the time of biopsy.

Left ventricular angiography was performed in the 35° right anterior oblique projection, and angiograms were used to calculate ejection fraction (EF) by the area-length method of Sandler and Dodge.

Follow-up information was collected by reviewing the notes and interviewing the physician who performed the biopsies and was responsible for follow-up of the patients. In general, the patients were not grouped according to New York Heart Association functional classification.

Statistical Analysis

The statistical tests used in this analysis included the chi-square test, one-way analysis of variance, two-way analysis of variance, multivariate analysis (multilinear regression or cluster type of analysis), and matched-pairs Wilcoxon signed-ranks test. A p value less than 0.05 was considered significant. Because of the null hypothesis that there is no difference between the samples or results in question, two-tailed tests were used.

Results

Morphology

The frequency of the ultrastructural changes (figs. 1–4) assessed in this study is summarized in figure 5. The three groups overlapped considerably. The crossover of sarcomeres was the only finding that differentiated HOCM from COCM and “ordinary” hypertrophy, but was not confined to HOCM and was not always present in this condition. The average score was 2 (two to four foci per section) in four patients with HOCM in whom crossover was present, com-
<table>
<thead>
<tr>
<th>Table 1. Semiquantitative Assessment</th>
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</table>

1. **Degenerative changes**
   - 0 = not present
   - 1 = rare-mild
   - 2 = frequent-severe
   - Myofibrillar lysis, cristolysis of mitochondria, lysosomal changes, myelin figures, membrane-bound vacuoles, increase of lipofuscin, lipid droplets

2. **Alteration of mitochondria**
   - 0 = normal
   - 1 = rare
   - 2 = frequent
   - Variation of size (normal range 0.3-1.7 μm), abnormal configuration, mitochondriosis

3. **Myofibrillar changes**
   - 0 = normal
   - 1 = rare
   - 2 = frequent
   - Abnormal arrangement (disarray, crossover), irregular Z bands, thickening of Z bands

4. **Interstitial fibrosis**
   - 0 = normal
   - 1 = mild
   - 2 = severe

5. **Hypertrophy of muscle fibers**
   - 0 = normal
   - 1 = mild
   - 2 = moderate
   - 3 = severe
   - Based on light microscopic investigation

6. **Crossover of sarcomeres**
   - 0 = not present
   - 1 = a few per section (≤ 1 focus)
   - 2 = some per section (2-4 foci)
   - 3 = many per section (≥ 5 foci)
   - Normal < 50 nm

7. **Membrane-bound vacuoles**
   - 0 = not present
   - 1 = a few per section (≤ 1)
   - 2 = some per section (3-6)
   - 3 = many per section (≥ 7)
   - Increased amount of matrix

8. **Widening of intercalated discs**
   - 0 = not present
   - 1 = a few per section (≤ 1)
   - 2 = some per section (2-4)
   - 3 = many per section (≥ 5)
   - Normal wall thickness < 500 nm

9. **Cellular edema**
   - 0 = not present
   - 1 = mild (focal and/or minor "floating" of organelles)
   - Increased amount of matrix

10. **Capillary edema**
    - 0 = not present
    - 1 = mild (focal and/or minor swelling)
    - 2 = moderate
    - 3 = severe (widespread and pronounced swelling)
    - Clumping of chromatin, crenation of nuclear membrane, coarse nucleoli, changes (if present) are usually widespread, irrespective of score

11. **Nuclear changes**
    - 0 = normal
    - 1 = mild
    - 2 = moderate
    - 3 = severe
    - Clumping of chromatin, crenation of nuclear membrane, coarse nucleoli, changes (if present) are usually widespread, irrespective of score

12. **Contraction bands**
    - 0 = not present
    - 1 = few areas, only minor disturbance of the myofibrillar integrity
    - 2 = some areas
    - 3 = many areas, severe disturbance of the myofibrillar integrity
    - Two to four transverse segments of intercalated discs lying along the same myofibrils each two of which are separated by one to 10 sarcomeres (the usual number is 25-64)

13. **Multiple intercalated discs**
    - Counted on the photomicrographs but are not part of the scoring system
pared with an average score of 1 in eight patients without HOCM. Crossover was not found in three patients with clinical hypertrophic cardiomyopathy in whom this diagnosis was not established histologically.

Morphology Compared with Clinical and Hemodynamic Data

Table 2 presents morphologic and hemodynamic data subdivided according to the histologic description, scoring level (group 1 or 2) and subsequent clinical course. The separation by histologic grouping (dead vs alive) showed no significant differences (table 3). Separation according to EM score (table 4) suggested, at first glance, that patients in group 1 had a better prognosis than patients in group 2. However, the chi-square test showed no significant difference (chi square = 2.86).

EF, LVEDP and length of history were compared with the EM score (figs. 6–8). Except for a tenuous relationship between EF and EM score ($r = -0.46$, $p < 0.1$) and LVEDP and EM score ($r = 0.61$, $p < 0.01$), no correlations were established. Using one-way analysis of variance, EF, LVEDP, length of history and EM score were analyzed in terms of the subsequent course of the disease to determine whether prognosis could be predicted. In patients with a stable course, EF is significantly different ($p < 0.01$) from EM in the other three categories (dead, deteriorated, improved) (table 5). The same result was obtained when patients with HOCM were omitted, which may be reasonable because these patients usually have normal (or better than normal) EF until the very last stage of the disease. No difference between the groups was found when LVEDP, length of history or EM score was related to the subsequent course.

Multivariate analysis of EF, LVEDP, length of history and EM score was undertaken to see if this test better distinguished among dead, deteriorated, stable and improved patients. Apart from the very small number of deteriorated and improved patients, it is not possible to distinguish mathematically even between the two larger groups of dead and stable patients.

Reproducibility of Electron Microscopic Evaluation

The semiquantitative scoring of the electron microscopic appearance was analyzed by comparisons between investigators. The specimens were assessed by three observers using the electron microscope. Weeks later, two observers first scored the micrographs independently and then evaluated the micrographs together and produced a common score. When these results were subjected to two-way analysis of variance, no significant difference was found.

Patients Who Died

Twelve patients died. Many of the patients with suspected cardiomyopathy were referred from other
areas and only five of the 12 patients were autopsied. Two patients had a clinical diagnosis of COCM and one patient had a clinical diagnosis of HOCM; these diagnoses were confirmed histologically by biopsy and at autopsy. In another patient with clinical HOCM, the autopsy showed subvalvular aortic stenosis and the biopsy showed ordinary hypertrophy. In a 19-year-old male with clinical COCM and long-standing cardiac insufficiency and failure, the biopsy showed HOCM, as evaluated from the histologic HOCM index. The heart was severely dilated, but HOCM was confirmed at autopsy. This patient was one of the small number of patients with HOCM who progress to chronic cardiac failure.

**Discussion**

Several investigators have concluded that right ventricular biopsies are representative of the state of the myocardium in conditions such as cardiomyopathy and secondary hypertrophy. (Hiroe M, Sekiguchi M, Hirosawa K, Imai M; unpublished results). Further, the ratio of left and right ventricular biopsies was shown to be 1:4 in more than 7800 biopsies from 3364 patients. This study confirmed that a considerable number of ultrastructural changes are present in various heart muscle disorders and that none of these changes are pathognomonic; all can be attributed to hypertrophy. Combining the observed changes does not produce specific patterns at this level of investigation and it has not been possible to separate the various conditions.

Sekiguchi et al. reported that HOCM and COCM could be distinguished by assessing fragmentation of myofibrils, swelling of mitochondria with cristolysis, widening of the intercalated discs, cellular edema, capillary edema, and deposition of degenerative substances. This score showed a good inverse correlation with EF. In the present study, these characteristics did not distinguish between COCM and HOCM or between these two groups and ordinary hypertrophy; nor could we confirm any correlation with EF. This is not surprising, as these six variables are nonspecific.

The frequent crossover of individual sarcomeres or whole groups of sarcomeres was first described by Ferrans et al., who claimed that the changes were pathognomonic of hypertrophic cardiomyopathy and provided a morphologic clue to diastolic compliance failure, the underlying mechanism of this disorder. Although subsequent studies have shown that crossover is more common in HOCM, these studies have also shown that it is not confined to HOCM and that it

**Figure 2. Alteration of mitochondria. (A) Mitochondriosis, score 2 (magnification × 5060). (B) Pronounced variation in mitochondrial shape and size. Different plane of section plays a role, but cannot be responsible for the observed extent of variation. The cristae are condensed and, in the giant mitochondrion, coarse and haphazardly arranged (magnification × 3700).**
is not always present in this condition. In our study, crossover was more frequent and more extensive in HOCM, but was not limited to and not always observed in this disorder.

Multiple intercalated discs are considered projections from the myocardial cell surface and probably represent cell growth and reinforcement during the process of hypertrophy. Multiple intercalated discs

Figure 3. Cellular edema (myofibrillar lysis?) and rather small, condensed mitochondria. Score 2 (magnification × 6050).

Figure 4. Nuclear hypertrophy. (A) Score 1 (magnification × 10,300). (B) Score 2 (magnification × 10,300). (C) Score 3 (magnification × 10,300).
Figure 5. The occurrence of various ultrastructural changes (x-axis) in percentage of biopsies from patients with different histologic diagnoses (y-axis). HY = ordinary hypertrophy; HOCM = hypertrophic cardiomyopathy; COCM = congestive cardiomyopathy; MIT = alteration of mitochondria; NUC = nuclear changes; DEG = degenerative changes; MBV = membrane-bound vacuoles; MYO = myofibrillar changes; CRO = fiber crossover; MID = multiple intercalated discs; CEL = cellular edema; CAP = capillary edema.

Table 2. Morphologic and Hemodynamic Data

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<td>Improved</td>
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<td>27-42</td>
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<td>26.0</td>
<td>22-32</td>
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Table 3. Subsequent Course of the Disease in the Three Histologic Categories

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<th>Alive</th>
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<tr>
<td>Total</td>
<td>12</td>
<td>36</td>
<td>48</td>
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</table>

Chi-square = 0.48, no statistically significant difference.

Abbreviations: COCM = congestive cardiomyopathy; HOCM = hypertrophic obstructive cardiomyopathy.

were found in all three diagnostic categories (in 27–50% of the cases), but were more frequent in HOCM, although no specific relationship to this disorder is present.

All features in table 1 have been reported, and either descriptively as single structures or features or as part of scoring systems. Semiquantitative scoring is arbitrary and subjective impressions are inevitable. To apply the scoring as closely as possible to the original reports, we contacted the authors of these reports. Information from the literature is usually vague regard-
Table 4. Subsequent Course of the Disease in Groups 1 and 2 Based on Electron Microscopy Score

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<tr>
<td>Total</td>
<td>12</td>
<td>36</td>
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Chi-square = 2.86 (no statistically significant difference).

Table 5. Mean Ejection Fraction in Patients with Different Subsequent Course of Clinical Status

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<tr>
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<th>Mean EF (%)</th>
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<td>Dead</td>
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<td>Deteriorated</td>
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<td>&lt; 0.01</td>
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<tr>
<td>Stable</td>
<td>81</td>
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</table>

Abbreviations: NS = no statistically significant difference (one-way analysis of variance); EF = ejection fraction.

Figure 6. Ejection fraction (EF) plotted against electron microscopy (EM) score. See figure 5 for other abbreviations.

Figure 7. Left ventricular end-diastolic pressure (LVEDP) plotted against electron microscopy (EM) score. See figure 5 for other abbreviations.

Figure 8. Length of history plotted against electron microscopy (EM) score. See figure 5 for other abbreviations.

Subsequent Course

4. **TABLE**

Improved 32

Stable 81

5a. **TABLE**


dimensions: 579.8x766.6

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[48x413]100o

[49x578]Subsequent Course

4. **TABLE**

Improved 32

Stable 81

5a. **TABLE**

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<tr>
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</tr>
<tr>
<td>Deteriorated</td>
<td>50</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stable</td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NS = no statistically significant difference (one-way analysis of variance); EF = ejection fraction.

Figure 6. Ejection fraction (EF) plotted against electron microscopy (EM) score. See figure 5 for other abbreviations.

Figure 7. Left ventricular end-diastolic pressure (LVEDP) plotted against electron microscopy (EM) score. See figure 5 for other abbreviations.

Figure 8. Length of history plotted against electron microscopy (EM) score. See figure 5 for other abbreviations.

Subsequent Course

4. **TABLE**

Improved 32

Stable 81

5a. **TABLE**

<table>
<thead>
<tr>
<th></th>
<th>Dead</th>
<th>Alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Group 2</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>36</td>
</tr>
</tbody>
</table>

Chi-square = 2.86 (no statistically significant difference).

Table 5. Mean Ejection Fraction in Patients with Different Subsequent Course of Clinical Status

<table>
<thead>
<tr>
<th></th>
<th>Mean EF (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved</td>
<td>32</td>
<td>NS</td>
</tr>
<tr>
<td>Dead</td>
<td>49</td>
<td>NS</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>50</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stable</td>
<td>81</td>
<td></td>
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</tbody>
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Figure 8. Length of history plotted against electron microscopy (EM) score. See figure 5 for other abbreviations.

subsequent course of the disease of the patients (dead, deteriorated, stable, improved status). The scoring of 12 points, however, does not improve differentiation, probably because factors such as edema are included that are much more prone to fluctuate or be artificial.

A good relationship between the degree of ultrastructural changes (EM score) and functional status expressed by EF and LVEDP in patients with COCM has not been substantiated in this study; nor has the study permitted any prognostication, which Kuhn et al. claimed is possible with this type of investigation.

Only three patients had an improved clinical course and therefore are not representative. These three patients — two with alcoholic heart disease and one
with systemic lupus erythematous — are noteworthy because therapy seems to have had a beneficial effect (stopped drinking and corticosteroids). These three patients were initially characterized by very low EF and high LVEDP and EM score. The hemodynamics have improved considerably. So far, the patients have not been rebiopsied, so we do not know if the observed light microscopic and ultrastructural changes have altered (decreased).

The analytic methods in the present study were identical to those used by Kuhn et al. The minor change in the score defining groups 1 and 2 was made to improve separation of patients, and a comparison of the two studies is therefore justified. If the originally suggested levels (group 1 \leq 4 points, group 2 \geq 5 points) are used, the difference between the groups is even smaller.

Kuhn et al. recently warned that the prognostic value of the EM score is reduced in the individual patient because the data overlap. The same view has been taken by Bouhour et al. and Petitier et al. As the individual patient is the target in human studies, the importance of the EM score is drastically reduced and the discrepancy between our results and those of Kuhn et al. is diminished.

A light microscopy study showed that one or two biopsies are not always representative of the state of the rest of the myocardium and that at least five biopsies are necessary. We have shown that the same condition applies to ultrastructural changes. It may be that the observed differences are fortuitous, as the studies cited were all based on fewer than five biopsies per patient, usually varying between one and three.

So far, we have discussed the results of histologic grouping of the patients. We also grouped the patients according to final clinical diagnosis (i.e., HOCM, COCM and “noncardiomyopathy”). Statistical analysis showed that the results were similar to those obtained by histologic grouping.

Investigations of endomyocardial biopsies have shown that the problem of artifacts is very real. Some years ago, Sonnenblick interpreted contraction bands as a pathologic process of the heart muscle. We found focal contraction bands in more than 80% of the patients. This common finding, which is also found in biopsies from normal hearts, strongly suggests that trauma is the underlying cause in the majority of cases.

Several proposals to overcome contraction banding have been made; bench rest of the tissue and relaxation of the sample in KCl or procainamide solutions (Petitier H: unpublished data) have been tried without success. MacKay et al. reported excellent effects of a 5-minute prefixation period in ice-cold 1% paraformaldehyde/0.2 M sucrose in phosphate buffer, pH 7.4.

Cristolysis of mitochondria, denoting degenerative changes of these organelles and, consequently, the cell, is often reported. One can argue, however, that swelling and apparent loss of cristae represent normal stages in the life cycle of normal mitochondria, and that degeneration is only present when the membrane is dissolved. Cristolysis was present at least focally in every biopsy sample we studied. This mechanism and toxic (hypoxic) changes inflicted during and after biopsy are the more likely causes of these changes.

Artificial changes cannot be eliminated, and awareness of their occurrence is essential. If samples are subjected to identical procedures and features not likely to be affected artificially, such as mitochondrial, fiber crossover and the degree of hypertrophy, are given higher priority, the effect of these changes can be reduced to a tolerable minimum. Our biopsy material was rarely spoiled by artifacts. We therefore do not share the pessimism of Eckner et al. and Olmedahl et al. regarding the problem of biotome-induced artifacts in the interpretation of endomyocardial biopsy.

No relationship between morphologic changes and functional status or prognosis has been convincingly demonstrated. Therefore, electron microscopic examination should be used only as an adjunct to other morphologic studies.

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