Critical analysis of endomyocardial biopsies from patients suspected of having cardiomyopathy

I: Morphological and morphometric aspects

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SUMMARY Three hundred and sixty-one biopsies obtained by bioprobe from 201 patients with suspected cardiomyopathy were analysed morphologically, semiquantitatively, quantitatively, and statistically to ascertain whether or not histological examination provided a measurable pattern characterising the different forms of cardiomyopathy. In addition, we have tried to find out whether or not morphometry increases the diagnostic reliability. Hearts from 29 cases (10 female and 19 male) who died as a result of road traffic accidents or other extracardiac causes were sampled, selecting specimens from sites usually biopsied during life.

Of these biopsies, 16.1 per cent were of such poor quality that morphological categorisation was not possible. The mean size of biopsies was 0.79 mm², and on average 1.38 mm² of tissue was available per patient. The histological descriptions were grouped in eight entities; morphometric analysis of the endocardium, myocardium, and interstitium resulted in a more accurate evaluation of the morphological changes. Muscle fibre diameter showed distinct variance between several morphological groups, particularly between normal and pathological groupings and between right and left sided ventricular samples. The applied scoring system included the histological HOCM index (hypertrophic cardiomyopathy) which separated clearly this type of cardiomyopathy from other disorders.

Small foci of chronic inflammatory cells were seen in 25 per cent of the biopsies and did not indicate myocarditis. This study did not confirm the possible existence of small vessel disease.

Morphometric data permitted distinction between several histological groups but this did not apply to individual patients in view of a considerable degree of overlap of the pathological changes.

Combining the various morphological features and applying measurements failed to establish a definite pattern for congestive cardiomyopathy (COCM), and alcoholic heart muscle disease could not be separated from COCM.

It is, however, suggested that semiquantitative and morphometric methods are mandatory, not only to define more accurately the morphological changes, but also to provide the basis for additional biochemical, immunological, and virological analysis.

Since its introduction in 1962 endomyocardial biopsy by bioprobe¹ has become an established method of investigation. With the exception of tissue taken during cardiac surgery, this is a most convenient procedure permitting the study of fresh tissue samples from the heart and the follow up of

the morphological course of cardiac disease.

Most of the patients subjected to this form of investigation are thought to suffer from a form of cardiomyopathy, which is defined as heart muscle disease of unknown cause.² A number of reports and reviews have been published which concentrate on the diagnostic aspects of the morphological changes in biopsy material.³⁻⁶ With few exceptions^{7,8} (and M Sekiguchi: unpublished data) these analyses have been subjective and have not been

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submitted to semiquantitative or quantitative study.

In view of the paucity of available published reports on semiquantification and quantification we have applied these methods to the structure of endomyocardial biopsies, obtained from patients with suspected cardiomyopathy, to determine if histological examination discloses a measurable pattern characterising the different forms of cardiomyopathy and also whether or not these methods of investigation assist diagnosis.

Subjects and methods

Between 1972 and 1978 biopsies from nearly 600 patients were submitted from several centres to the Department of Histopathology of the National Heart Hospital, London. Biopsies from four hospitals for the years 1973, 75, and 77 were arbitrarily chosen for this study. There were 361 biopsies from 201 patients with suspected cardiomyopathy, clinically defined and diagnosed according to the suggestions of the Hammersmith group.² Samples were examined without additional knowledge of the suspected diagnosis, symptoms, or the results of investigation.

All biopsies were obtained either with the small Konno-Sakakibara biptome^{1,9} or the King's College Hospital instrument.¹⁰ If of suitable size the biopsy was divided, one part for light, the other for electron microscopical investigation. In 163 patients (264 biopsies) the site of the biopsy was the septal aspect of the right ventricle, in 20 patients (48 biopsies) the left ventricle, and in 18 patients (49 biopsies) both ventricles were biopsied. A female to male ratio of 1 : 2.5 was present with a mean age of 43.7 years (range 4 to 72 years). The mean age for females was 41.1 and for males 44.7 years.

CONTROL GROUP

Hearts from 10 females and 19 males (mean age 43.3 years, range 7 to 67 years) were sampled at necropsy. Two right and two left ventricular specimens were obtained from the interventricular septum with the King's biptome within 10 hours after death. All patients died either from road traffic accidents or from other extracardiac causes. These hearts were found to be normal at necropsy, allowing, of course, for some variation related to the different ages of the patients.

FIXATION, CUTTING, AND STAINING

Samples were processed immediately after removal

from the heart. Two hundred and eighty-four biopsies (135 patients) were frozen in melting "Freon" (BOC), precooled in liquid nitrogen, and stored at -70°C ; 5 μm thick sections were cut on a cryostat (Bright) with a cabinet temperature of -20°C .

Routine staining procedures included haematoxylin-eosin (HE), Miller's elastic van Gieson (EVG) stain,¹¹ and a reaction for PAS positive material.¹² Reticulin-silver impregnation counterstained with Galego's acetic fuchsin was undertaken in a number of cases to compare measurements of cell diameters in this stain with those in the HE stain (reticulin-silver impregnation is thought to delineate the muscle fibre outline more accurately).⁷

In several cases special stains were included when they were thought to be relevant, such as stains for micro-organisms, fibrin, amyloid, or iron deposition.

Seventy-seven biopsies (66 patients) were placed in 3 per cent cacodylate buffered glutaraldehyde for one hour at 4°C . The fixing fluid was then replaced by buffered sucrose washing solution (4°C , pH 7.4). This tissue was paraffin-embedded, and 5 μm thick sections were cut on a base sledge microtome (Jung). The staining reactions already mentioned were applied, except for the PAS reaction.

These procedures were carried out by the same medical laboratory scientist in more than 90 per cent of cases.

Table 1 *Semiquantitative assessment*

Myocardium	
Normal	Yes/No
Hypertrophy	0-3
Attenuation	0-3
Regular	Yes/No
Irregular	Yes/No
Short runs	0-3
Whorls	0-3
Perinuclear halo	0-3
Bizarre nuclei	0-3
Oedema	0-3
Necrosis/degeneration	Present/not present
Endocardium	
Normal	Yes/No
Thickened	0-3
	4 Patchy thickening
SMC	0-2
Interstitium	
Normal	Yes/No
Fibrosis	0-3
Oedema	0-2
Mononuclear cell infiltration	0-3
Granulocytic cell infiltration	0-3
Arterioles	Number; changes specified if present
Fibrin/clot	Present (partial, only), not present
Thrombus	Present (partial, only), not present

The material of the control group was formalin fixed, paraffin-embedded, and stained with HE and EVG.

SEMIQUANTITATIVE ASSESSMENT

The morphology was described and scored according to a set of criteria chosen after a pilot investigation of biopsies from 150 patients not part of this study. The histology was graded 0, 1, 2, or 3, 0 being normal or not present, and 2 or 3 characterising the severest degree of change or of increased frequency (Table 1).

In Table 2 some of the terms used in this analysis are detailed.

QUALITY OF BIOPSY

The quality of the biopsy was found to be "poor" if either only clot/fibrin, thrombus, or fibroelastic tissue were obtained, if the biopsy was too small for assessment, or if it was severely distorted by artefact. A biopsy was considered "reasonable" if

Table 2 Some structural definitions

<i>Myocardium</i>	
Hypertrophy	Both nuclear appearances and cell diameter are considered
Attenuation	Parallel rows of myocardial fibres of normal or diminished diameter, but by comparison nuclei which show changes of hypertrophy
Short runs	Fibres of up to 20 sarcomeres; at either end interrupted by connective tissue; can only be evaluated in sections cut longitudinally
<i>Endocardium</i>	
Smooth muscle cells of endocardium	Evaluated in EVG stain; the smooth muscle cells must be situated within the endocardium, that is between elastic laminae
<i>Interstitium</i>	
Fibrosis	EVG is considered to be specific for collagen, staining collagen fibres red ¹³ ; consequently red, extracellular material in this stain was interpreted as representing collagen
Arteriole	An arteriole is a vessel with a well defined elastic lamina surrounded by a well developed smooth muscle coat; the external diameter is between 30 and 100 μm ¹⁴

Fig. 1 Sections through cylinder at various angles. The smallest cross-section represents the diameter of the cylinder (d =diameter).
Fig. 2 Grid for assessment of cellular density.

it was of sufficient size but the endocardium missing. The biopsy was categorised under "good quality" if it included all components and was of sufficient size and good technical quality.

QUANTITATIVE MEASUREMENTS

Quantitative measurements were undertaken by means of a Projectina microscope.

Cell diameter

This was measured at a magnification of 460. The myocardial cell was considered to represent a near cylinder. Therefore measurements were restricted to the nuclear areas, and the smallest diameter was chosen as the one representing the actual diameter (Fig. 1).

In a single biopsy as many cells as possible were measured. The biopsy was rejected if less than 20 cells could be measured. The result is expressed as the mean value.

Cell density

This was defined as the number of cells intersected by a line without fixed orientation (Fig. 2). It is an indirect estimate of the amount of interstitium and/or cell diameter.

Thickness of the endocardium

The endocardium was measured at its thickest

point provided that sections had been cut at right-angles.

Arterioles

These were only counted.

Volume fraction of collagen

The simplest point counting system—which is a square lattice with fixed distance between the lines—was used. The grid consisted of 100 points per unit area and had a line density of 1 cm (Fig. 3).

The crosspoints of lines falling on collagen tissue served as markers for the point counting volumetry of collagen tissue.

The magnification was 460, and 10 or more random units were assessed in each biopsy. The mean value was calculated.

Volume fraction of interstitium

This was measured by counting crosspoints falling on tissue which was not myocardial fibres or endocardium. Volume fraction of interstitium was not measured in paraffin-embedded material as the interstitium in these biopsies was often represented by "empty" areas only (because of dehydration, lipid dissolution, etc.), thereby making differentiation from possible artefacts impossible.

Fig. 3 Grid for assessment of volume fraction or area.

Areas of the largest section

This was estimated by point counting using the grid system described above. The magnification was 78. Section and grid were turned independently at least three times and the mean value was calculated.

DESCRIPTIVE TERMS

Experience from the pilot study of 150 patients already mentioned formed the basis for descriptive terms (Table 3).

Table 3 Morphological, descriptive terms

Normal (N)
Hypertrophy (HY): hypertrophy of myocardial fibres and nuclei; no other significant changes
Hypertrophy + attenuation (HA)*
Hypertrophy + smooth muscle cell hypertrophy of endocardium (HS)*
Hypertrophy + attenuation + smooth muscle cell hypertrophy of the endocardium (HAS)*
Hypertrophic cardiomyopathy (HOCM): diagnosed when irregularity of arrangement of muscle fibres and three of five criteria included in histological HOCM index ¹⁵ were present
Myocarditis (MYO): inflammatory cell infiltration + degenerative and/or necrotic changes of some myocardial fibres present
Interstitial fibrosis (IF): amount of collagen estimated to be increased, but no other significant changes present

* Biopsies showing HA, HS, or HAS include features compatible with a clinical diagnosis of congestive cardiomyopathy (COCM)—HAS in fact includes all the features so far described on light microscopy of COCM.

STATISTICAL METHODS

Parametric tests were applied most frequently as most data were on a graded scale. The tests carried out were χ^2 test, t test, one-way analysis of variance, two-way analysis of variance, and correlation analysis.

Non-parametric statistics were used in some instances: Wilcoxon matched-pairs signed-ranks test, and Wilcoxon signed-ranks test.

Throughout the study the *null hypothesis* was used—that there was no difference between the samples or results in question; consequently, two-tailed tests were appraised. A p value of less than 0.05 was accepted as the limit of significance.

Results

ENTIRE BIOPSY

Size of biopsy

The mean area was 0.79 mm² per biopsy (range: 0.05 to 5.99 mm²). Relating the size to the number of patients a figure of 1.38 mm² per patient was found.

In 15 biopsies consisting of thrombus only the average size of a biopsy was 1.51 mm², and in 29 biopsies made up entirely of fibrin/clot the area was 0.29 mm² per biopsy.

Sixty frozen biopsies were weighed and the average result was 1.46 mg $\pm$ 1.1 SD.

Quality

The overall impression of the quality of specimens was “reasonable” to “good”, the mean score being 2.3.

Histological description

According to the proposed definitions the patients could be arranged as follows (Table 4).

In 17/201 patients (8.5%), or 61/361 biopsies (16.9%), the biopsies were of poor quality, not permitting morphological categorisation.

To avoid including a patient twice, the more important description was chosen in 27 patients with more than one histological “diagnosis” (that is, if both “hypertrophy” and “myocarditis” were present the latter was chosen).

The *histological HOCM index* (hypertrophic cardiomyopathy)¹⁵ was 40 per cent on average in 11 patients with morphological HOCM. Values less than 33 per cent were not seen in this group, and values above 33 per cent were not found in the other groups.

Biopsies from the two ventricles were compared in 15 patients to see which ventricle showed the severest changes, thereby giving most weight to the histological interpretation. In three patients right ventricular biopsies were superior, in three left ventricular biopsies were the more informative, and in the remaining nine patients the contribution of either was equal.

Table 4 Morphological description in 201 patients

	No. of patients
Normal	16
Hypertrophy	57
HA	25
HS	15
HAS	42
HOCM	11
Myocarditis	9
Interstitial fibrosis	9
Non-representative samples	17

MYOCARDIUM

Diameter

In 75 per cent of biopsies (91% of patients), 16 585

Fig. 4 Uneven distribution of fibre diameters. Insert: area of biopsy showing two cell populations with regard to fibre diameter. (Reticulin-silver. $\times 410$.)

nucleated cross-sections of muscle fibres were measured (that is 61 cells per biopsy and 90 cells per patient).

The distribution of cell diameters was normal in 195/200 samples. An example of uneven distribution as seen in the remaining 5/200 biopsies is shown in Fig. 4.

Assessment of the cell diameter on *HE* and *reticulin stain* in sections from 29 biopsies (22 patients) stained both ways showed no statistically significant difference (Wilcoxon matched-pairs ranked-signs test).

The diameter was analysed with respect to the *histological description* by one-way analysis of variance. Though a statistically significant difference was found between many groups, considerable overlap existed (Table 5).

To determine how semiquantitative scoring corresponds with exact measurements this relation was analysed by one-way analysis of variance (Table 6).

Comparison between *right and left ventricular biopsies* with respect to corresponding degrees of hypertrophy (Fig. 5) was carried out by individual *t* tests; the difference between right and left ventricular muscle fibre diameter was in accord with the result obtained in the control group (*t* test): in 53 right ventricular biopsies the mean diameter was $11.6 \mu\text{m} \pm 1.7 \text{ SD}$, and in 50 left

ventricular biopsies it was $14.4 \mu\text{m} \pm 2.2 \text{ SD}$ ($p < 0.001$).

Fibre direction

In 14 per cent of biopsies cross-section was found. In 36 per cent of cases longitudinal and in 50 per cent a mixed pattern was encountered.

Diameter of muscular fibres in relation to density of muscle fibres

This relation is best expressed by an exponential function. The line is highly statistically significant ($r = -0.964$, $p < 0.001$) (Fig. 6). In theory, few cells per line would imply that either a great amount of interstitium or a wide cell diameter was present; and many cells per line would indicate a small amount of interstitium or a small diameter. In practice, the good correlation between density and cell diameter shows that the amount of interstitium is of less significance, as is also concluded by direct measurement of the volume fraction of interstitium.

ENDOCARDIUM

Thickness

Endocardial thickness in the various histological groups was analysed by means of one-way analysis of variance. Statistically significant differences do exist, but the overlap is certainly pronounced. If

Table 5 Diameter of muscle fibres (one-way analysis of variance)

		Mean diam. (μ m)	SD	No. of biopsies
Normal (N)	RV	11.6	1.7	19
	LV	11.2	1.5	5
Hypertrophy (HY)	RV	16.5	3.2	78
	LV	17.9	3.6	28
HA	RV	14.4	3.1	31
	LV	17.7	3.9	6
HS	RV	17.9	4.3	18
	LV	20.6	5.4	4
HAS	RV	14.9	3.1	46
	LV	19.8	3.6	8
HOCM	RV	20.7	3.6	15
	LV	24.6		1
Myocarditis (MYO)	RV	16.7	3.4	15
	LV	23.5	3.3	3
Interstitial fibrosis (IF)	RV	12.4	3.2	12
	LV	16.5	2.7	3

	IF	HA	HAS	HY	MYO	HS	HOCM
N	NS	**	**	***	***	***	***
IF		NS	**	***	***	***	***
HA			NS	**	*	***	***
HAS				**	*	***	***
HY					NS	NS	***
MYO						NS	**
HS							*

NS: No statistically significant difference.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

RV: right ventricular biopsies;

LV: left ventricular biopsies.

Table 6 Comparison between estimated and measured muscle fibre diameter (one-way analysis of variance)

	Degree of hypertrophy	Mean diameter (μ m)	SD	p	No. of biopsies
RV	0	10.6	1.3		12
	1	14.2	2.3	< 0.001	155
	2	19.5	2.1	< 0.001	52
	3	24.6	1.8	< 0.001	10
LV	0	12.5	2.0		8
	1	16.7	2.7	< 0.001	29
	2	21.0	1.7	< 0.01	12
	3	23.9	2.5	< 0.01	9

RV: right ventricular biopsies;

LV: left ventricular biopsies.

Fig. 5 Histogram showing the relation between measured fibre diameter and degree of hypertrophy assessed by semiquantitative scoring. Statistically significant difference (*t* test) is present between RV and LV biopsies (grade 0 and 2: $p < 0.025$, grade 1: $p < 0.001$) except for grade 3. It is natural that the difference becomes progressively less as the scope of a semiquantitative choice narrows. RV: right ventricular biopsies. LV: left ventricular biopsies. SEM: standard error of the mean.

Fig. 6 The relation between fibre diameter and density.

the myocarditis group is corrected for one extreme value of 183 μm , the mean in this category was 25.7 μm , and there was no statistically significant difference from other groups (Table 7).

The thickness of the endocardium in the control group was the same as in the normal group of biopsies. In 50 right ventricular biopsies the mean thickness was 19.5 $\mu\text{m} \pm 6.3$ SD, and in 42 left ventricular biopsies it was 20.3 $\mu\text{m} \pm 6.5$ SD. There is no statistically significant difference (t test).

Smooth muscle cells of endocardium

Of 237 biopsies which could be evaluated for the content of smooth muscle cells in the endocardium, more were found in 64 per cent of biopsies (grade 0), some in 27 per cent (grade 1), and many in 9 per cent (grade 2).

In 7 of 11 cases of histological HOCM an increase in smooth muscle cells of the endocardium was found, thus denoting dilatation of the heart in these patients, though this was not the impression on angiography.

Table 7 Endocardial thickness (one-way analysis of variance)

		<i>Mean thickness (μm)</i>	<i>SD</i>	<i>No. of biopsies</i>			
Normal (N)	RV	21.6	12.3	17			
	LV	21.2	5.8	5			
Hypertrophy (HY)	RV	19.0	12.5	49			
	LV	27.5	16.1	20			
HA	RV	21.4	13.6	21			
HS	RV	36.1	22.9	13			
HAS	RV	39.9	32.9	36			
	LV	51.0	22.7	8			
HOCM	RV	27.3	14.3	12			
Myocarditis (MYO)	RV	45.4 (25.7)	45.4 (11.7)	8			
Interstitial fibrosis (IF)	RV	16.0	8.5	9			
IF	<i>HY</i>	<i>HA</i>	<i>N</i>	<i>HOCM</i>	<i>HS</i>	<i>HAS</i>	<i>MYO</i>
HY	NS	NS	NS	NS	NS	*	** (NS)
HA	NS	NS	NS	NS	*	***	** (NS)
N			NS	NS	NS	**	** (NS)
HOCM				NS	NS	**	* (NS)
HS					NS	NS	NS
HAS						NS	NS

NS: no statistically significant difference.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

(): calculation correlated for one extreme value of 183 μm .

RV: right ventricular biopsies;

LV: left ventricular biopsies.

INTERSTITIUM

Volume fraction of collagen

No difference of volume fraction of collagen was found between the right and left ventricle in the control groups (t test) (Table 8).

Table 8 Volume fraction of collagen in biopsies from the control series

	Volume fraction of collagen (%)	SD	p	No. of biopsies
RV	0.8	1.8		50
LV	1.1	2.0	NS	42

NS: no statistically significant difference (t test).

RV: right ventricular biopsies;

LV: left ventricular biopsies.

Table 9 Volume fraction of collagen in various histological groups (one-way analysis of variance)

	VFC (%)	SD	p	No. of biopsies
Normal	1.2	1.1		24
HA	1.5	1.2		32
I HAS	1.6	0.9		54
	1.7	1.3		96
Hypertrophy			< 0.01	
HS	2.2	1.7		19
HS*	2.5	1.6		33
II HOCM	2.7	1.6		16
	2.9	1.6		13
Interstitial fibrosis			< 0.001	
Myocarditis	5.1	7.8		13

HS*: included all biopsies with smooth muscle cell hypertrophy of the endocardium and no attenuation of the myocardial fibres, that is not only these cases where HS was the final histological diagnosis.

As a consequence, right and left ventricular biopsies were not separated in the following analysis (one-way analysis of variance) of levels of volume fraction of collagen in the various histological groups (Table 9). The results are difficult to display as the means show a long ranked series. They do, however, fall into two fairly natural groupings, with the myocarditis group clearly different from any other.

In addition, semiquantitative scoring of the volume fraction of collagen was compared with the exact morphometric measurements by one-way analysis of variance (Table 10).

Volume fraction of interstitium

Assessment was undertaken in 188 biopsies, that is in 66 per cent (188/284): in 77 biopsies the volume fraction of interstitium was not measured because

Table 10 Comparison between estimated and measured volume fraction of collagen (one-way analysis of variance)

Degree of fibrosis	Mean VFC (%)	SD	p	No. of biopsies
0	0.9	0.5		74
1	1.6	0.5	<0.001	83
2	2.6	0.7	<0.001	49
3	5.0	1.1	<0.001	17

Table 11 Volume fraction of interstitium in various histological groups

	VFI (%)	SD	p	No. of biopsies
N	17.2	1.9		5
Hypertrophy	16.9	4.0	NS	83
HA	18.3	3.4	NS	17
HS	18.8	5.5	NS	17
HS	16.9	3.3	NS	28
HOcm	18.5	2.6	NS	15
Myocarditis	18.9	7.0	NS	11
Interstitial fibrosis	19.9	4.6	NS	12

NS: no statistically significant difference (one-way analysis of variance).

these biopsies were fixed in glutaraldehyde and paraffin-embedded.

For the statistical evaluation one-way analysis of variance was applied (Table 11).

Volume fraction of interstitium in relation to volume fraction of collagen

When correlating volume fraction of interstitium with volume fraction of collagen a significant correlation was found ($r=0.8$, $p<0.02$); but the actual differences of volume fraction of interstitium in percentages were small, and a very considerable overlap was present (Fig. 7).

Diameter of muscle fibres in relation to volume fraction of collagen and interstitium

Plotting the diameter against volume fraction of collagen showed absolute scatter; no relation was present ($r=0.112$, $p<0.1$).

Chronic inflammatory cells

In biopsies from 50 patients (25% of the whole material) a few scattered chronic inflammatory cells (lymphocytes, monocytes) or small foci of such cells were found. In none of these patients was there any histological or clinical suspicion of myocarditis—and the subsequent course lent no support to a diagnosis of myocarditis.

Fig. 7 The relation between volume fraction of interstitium (VFI) and collagen (VFC) (1 bar = 1 SEM).

Arterioles

Three hundred and ten arterioles were recognised in 168 biopsies. Medial hypertrophy was found in one biopsy and thrombus was seen in two cases.

Discussion

The size of the average biopsy recorded in this study was smaller than has been previously reported.^{4 9 16-18} This remains the case even if the area of the biopsy is multiplied by two (as many samples were bisected to provide material for both light and electron microscopical investigation). This difference might be the result of the use of the larger Konno-Sakakibara biotome by some previous workers, but a more probable explanation of the occasional considerable discrepancy is that they did not measure the size of the biopsies as precisely and in as large a number as in this investigation.

The fact that those biopsies consisting of fibrin/clot were the smallest and those consisting of thrombus the largest, reflects the friability of the tissue. It implies that the biopsy is not only obtained by cutting, but also by some degree of avulsion.

The quality of the biopsy material was satisfactory. In 91.5 per cent of patients the specimens enabled a histological description or grouping to be made, according to the criteria which have been defined.

Quantitative measurements correlated well with semiquantitative assessment in most cases where direct comparison of the two was undertaken. By the application of morphometry, however, more accurate and precise assessments were obtained.

Biopsies from 25 of 201 patients were found semi-quantitatively to include foci of attenuated muscle fibres. One would expect this to be reflected in uneven distribution curves of fibre diameter. In only five of 200 random biopsies, however, where the distribution patterns were studied was this the case. This underlines the difference that can exist between a semiquantitative subjective approach, and quantitative objective measurement.

The diameter of muscle fibres showed distinct variance between several histological groups. Most importantly there was a clear difference between normal and pathological groups.

Muscle fibre diameter in the control group and patient group of normal biopsies corresponded well with other studies that have reported on normal myocardium (Table 12).

itself shows no significant change between the groups and therefore, we conclude, this measurement can be omitted. Volume fraction of collagen showed greater variation, which implies that it is a more sensitive indicator of abnormality in the myocardium than assessment of volume fraction of interstitium. Collagen tissue has the further advantage of being a less vulnerable structure than the interstitium as a whole and is therefore less prone to artefactual changes.

The level of volume fraction of collagen compares favourably with other techniques for estimating collagen tissue, such as electron microscopical quantification by point counting³² and biochemical assays.³³ Kunkel *et al.*,⁸ however, found substantially higher levels of volume fraction of collagen in COCM than we have by applying a trichrome

Table 12 Diameter (μm) of normal cardiac muscle fibres (adults)

Author	Number	RV	LV	RV/LV	Fixation
Goldenberg ¹⁰	3 hearts	12.7	12.4		Müller's fluid
Karsner <i>et al.</i> ¹¹	1 heart		17.6		10% formalin
Harrison <i>et al.</i> ¹²	12 hearts		16.2*		
Roberts <i>et al.</i> ¹³	10 hearts		14.0		10% formalin
Roberts and Wearn ¹⁴	26 hearts			13.9	10% formalin
Wearn ^{15, 16}	36 hearts			13.9	10% formalin
Ashley ¹⁷	13 hearts	16.2*	20.1*		Gelatin embedding
Lowe and Bate ¹⁸	5 hearts		13.1*		10% formalin
Ishikawa <i>et al.</i> ¹⁹	25 hearts	9.4 $\pm$ 1.6 SD*	11.4 $\pm$ 1.8 SD*		
Truex ²⁰	5 hearts		15.8*		10% neutr. formalin
Davies <i>et al.</i> ⁷	Biopsies from 48 necropsy hearts		15.9 $\pm$ 2.3 SD		10% formalin
Fuster <i>et al.</i> ²¹	22 hearts		5.8 $\pm$ 0.5 SD*		10% formalin
Hiroe (unpublished data)	10 hearts	$\sim$ 12*	$\sim$ 14*		
This study	19 biopsies from 16 patients	11.6 $\pm$ 1.7 SD*			Frozen sections
	53 RV and 51 LV biopsies from 29 patients (control series)	11.9 $\pm$ 1.9 SD*	14.4 $\pm$ 2.2 SD*		10% neutr. formalin

* In these studies it has been specified that measurements have taken place at nucleated cross-sections.

RV: Right ventricular biopsies;

LV: Left ventricular biopsies.

Contrary to Richardson *et al.*¹⁹ but in accordance with most studies of normal material (Table 12) and also a few investigations of biopsy material (M Hiroe, unpublished data) a distinct difference in diameter was found between muscle fibres from the right and left ventricle. Ontogenetically the cells of the two ventricles are identical, and the anatomical borders remain largely undefined in the adult heart; understanding of this "physiological hypertrophy" is thought to be essential for the comprehension of "pathological hypertrophy".

In the morphological groups there was a reasonably good correlation between volume fraction of interstitium and collagen though the former in

staining technique. Elastic van Gieson stain (EVG), as used in this study for estimating collagen, is a progressive staining method, which demonstrates collagen specifically¹³; by contrast, the trichrome techniques are regressive methods, which are very sensitive to pH changes around their isoelectric point. The trichrome stains also do not differentiate between the interstitial components of matrix (mucopolysaccharides), cells, capillaries, and fibres, of which collagen constitutes only one type. The variability of the trichrome methods is underlined by the findings reported by Davies *et al.*⁷ These authors also used a trichrome stain for assessing the amount of collagen and obtained results in

patients suffering from COCM which are comparable with the level measured in this study. We therefore conclude that EVG is a more suitable stain for the estimation of volume fraction of collagen than the trichrome stains.

Fibre diameter and volume fraction of collagen in the group of "hypertrophy with smooth muscle cell hypertrophy of the endocardium" were significantly higher than in the group with "hypertrophy with attenuation and smooth muscle cell hypertrophy of endocardium", though patients from both groups had frequently a well-established clinical diagnosis of COCM in common. This, to some measure, adds support to the view that hypertrophy of the muscle fibres occurs before dilatation of the heart (of which the increased number of smooth muscle cells in the endocardium is an indicator) takes place; increased amount of collagen tissue may encase the myocardial muscle fibres, thereby inhibiting stretch and attenuation.

A few scattered mononuclear cells or small clusters of such cells were a frequent finding in biopsies from patients without any other histological or clinical evidence of (viral) myocarditis.

This study did not confirm the presence of "small vessel disease"³⁴—at least pathological changes of the small vessels were not recognised in these groups of patients.

Changes of the small vessels have been found in diabetic patients³⁵ and have also been produced in experimental diabetes (U Baandrup, unpublished data); in our study biopsies from only three diabetic patients were included, none of which showed arteriolar changes.

By pooling the morphometric data, distinction between several of the proposed histological groups can be made, or a tendency indicated; this applies particularly to the very basic information of cell diameter. Regarding the individual patient, however, little is achieved because of the extensive overlap and variance of the results of the measurements.

Unfortunately, group results, as in animal experiments, are not acceptable in human beings, in whom the aim is to achieve results of which the consequences (diagnostic, therapeutic, or prognostic) are applicable to the individual.

Combining the various morphological features and measurements failed to establish a definite pattern of COCM; it neither permitted a firm histological diagnosis of COCM, nor made it possible to distinguish COCM from alcoholic heart muscle disease (in the histological group of hypertrophy with attenuation and smooth muscle cell hypertrophy, 15 patients are thought to have the latter).

The histological HOCM index is an example of the way several non-pathognomonic features can become diagnostic by their combination. Because of the rather irregular distribution of the abnormal features in the myocardium in HOCM, the diagnostic sensitivity in biopsy material was not high (50%) whereas the diagnostic specificity was excellent (99%). An average score of 40 per cent has been taken to represent a diagnostic level of HOCM, which differs from the level of 50 per cent originally suggested by the authors of the index¹⁵ by using larger samples obtained at operation and at necropsy.

The question of which ventricle to biopsy in order to obtain the best sample for a histological diagnosis cannot be answered by our study for most biopsies were from the right ventricle. In 15 patients where biopsies from both ventricles were taken, there was no difference as far as "diagnostic power" was concerned. A similar conclusion has been reached by others,^{19 36} but some³⁷ strongly favour left ventricular biopsies except in cases of HOCM.

Endomyocardial biopsy has proved its value in several fields of cardiology. Its potential as a research tool is considerable; the sphere of application is not limited to histological examination, but includes biochemical, immunological, and virological analysis—and probably other, yet uncharted areas. We firmly believe, however, that the best understanding will be achieved if these investigations are carried out in association with morphological examination—and for that purpose semiquantitative and morphometric methods are mandatory, however painstaking and tedious the collection of such data may be.

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