Quantitative Analysis of Cardiac Muscle Cell Disorganization in the Ventricular Septum of Patients with Hypertrophic Cardiomyopathy

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SUMMARY The presence of numerous abnormally arranged cardiac muscle cells in the ventricular septum has been considered a characteristic anatomic feature of patients with hypertrophic cardiomyopathy. To determine the specificity of this histologic marker for patients with hypertrophic cardiomyopathy, we used a quantitative method to determine the area of myocardium occupied by disorganized cells. In hypertrophic cardiomyopathy, septal disorganization was present in 94% of the 54 patients studied at necropsy. Furthermore, disorganization was extensive in most of these patients, involving 5% or more of the transverse plane tissue section in 89% of the patients and 25% or more of the section in 56% of the patients. Septal disorganization was best identified in tissue sections cut perpendicular to the long axis of the left ventricle. Septal disorganization was present in only 26% of the 144 control patients with other heart diseases or normal hearts. Most important, when present in these patients, disorganization was usually limited in extent. In only 7% of the controls did abnormally arranged cells occupy 5% or more of the tissue section. The average area of septum disorganized was 31 ± 3% (mean ± SEM) in patients with hypertrophic cardiomyopathy and only 1.5 ± 0.6% in the controls (p < 0.001). Hence, while the presence of ventricular septal disorganization is not pathognomonic of hypertrophic cardiomyopathy, widespread distribution of this abnormality is a very sensitive and specific histologic marker for this disease.

IN THE INITIAL PATHOLOGIC REPORT of hypertrophic cardiomyopathy published in 1958, Teare described a bizarre and unique arrangement of cardiac muscle cells in the asymmetrically hypertrophied ventricular septum.1 Several other investigators2-11 have made similar observations regarding the histologic appearance of ventricular septal myocardium in this disease. However, the presence of disorganized septal architecture per se is not pathognomonic of hypertrophic cardiomyopathy, i.e., areas of disorganization, although usually small, may be present in the ventricular septum of some patients with various acquired or congenital heart diseases,12-15 as well as in normal embryos and fetuses,16 normal infants17,18 and infants who die suddenly without evidence of structural cardiac disease.17

Some investigators18-21 have questioned the specificity of disordered septal architecture for patients with hypertrophic cardiomyopathy, based primarily on qualitative or semiquantitative histologic analysis of myocardium obtained from patients with heart diseases other than hypertrophic cardiomyopathy. Hence, the present study was undertaken to: 1) establish detailed morphologic criteria for cardiac muscle cell disorganization; and 2) determine, for the first time in quantitative terms, the extent of cardiac muscle cell disorganization in the ventricular sep-

tum of patients with hypertrophic cardiomyopathy compared with patients who have other forms of cardiac hypertrophy.

Selection of Case Material

Patients with Hypertrophic Cardiomyopathy

The cardiovascular registry of the Pathology Branch, National Heart, Lung, and Blood Institute, was reviewed and 64 hearts with hypertrophic cardiomyopathy were considered for inclusion in the study. Ten of these specimens were excluded because we could not obtain ventricular septal tissue suitable for histologic analysis, including two specimens that were in poor condition and five that had extensive septal scarring. Three other hearts that were particularly good examples of hypertrophic cardiomyopathy were excluded so that the pathologic specimens could be preserved intact. The remaining 54 hearts comprised the study group.

In 48 (89%) of the 54 patients the diagnosis of hypertrophic cardiomyopathy was based on the presence of asymmetric septal hypertrophy (defined as a septal-free wall ratio of 1.3 or greater), identified either at necropsy6,22 or by echocardiography,23-25 in the absence of an associated valvular or congenital heart defect which itself could have produced left ventricular hypertrophy. In 37 of these 48 patients the typical clinical, hemodynamic, angiographic and operative26-30 findings of hypertrophic cardiomyopathy were also present; the 11 other patients had no cardiac evaluation during life, and sudden death was the first definitive manifestation of cardiac disease.31

In the other six patients with hypertrophic cardiomyopathy, concentric ventricular wall thickening
Table 1. Clinical and Hemodynamic Findings in 54 Patients with Hypertrophic Cardiomyopathy

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>LVOT - PSG (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>Mode of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>≥30 at rest</td>
<td>&lt;30 at rest</td>
<td>Provocative*</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>38</td>
<td>16</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>70</td>
<td>30</td>
<td>63</td>
<td>10</td>
</tr>
</tbody>
</table>

* <30 mm Hg at rest; ≥30 mm Hg with provocation (Valsalva maneuver or isoproterenol infusion).
† Includes one patient with severe (i.e., >18 mm Hg PSG) obstruction to right ventricular outflow but no LVOT obstruction.
‡ Provocative maneuvers not performed in five other patients with <30 mm Hg LVOT gradient at rest.

Abbreviations: Cath = cardiac catheterization; CHF = congestive heart failure; CVA = cerebrovascular accident; LVOT = left ventricular outflow tract; Op = cardiac operation; PSG = peak systolic gradient; + = present; 0 = absent.

Table 2. Cardiac Diseases in 144 Control Patients

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients</th>
<th>No. with marked septal disorganization§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic valve disease</td>
<td>39*</td>
<td>5†</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>Systemic hypertension</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Isolated mitral valve disease</td>
<td>13†</td>
<td>1**</td>
</tr>
<tr>
<td>Dilated (congenital) cardiomyopathy</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Primary pulmonary hypertension</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Combined mitral and aortic valve disease</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonic valve stenosis</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Mitral valve stenosis</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>3‡</td>
<td>0</td>
</tr>
<tr>
<td>Discrete (membranous) subaortic stenosis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Supravalvular aortic stenosis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>144</td>
<td>11 (7%)</td>
</tr>
</tbody>
</table>

* Includes 28 patients with predominant aortic stenosis, eight with pure aortic regurgitation and three with combined aortic stenosis and regurgitation.
† Includes three patients with predominant mitral stenosis, seven with predominant mitral regurgitation and three with combined mitral stenosis and regurgitation.
‡ Includes one patient with associated valvular pulmonic stenosis.
§ Disorganized cardiac muscle cells involving ≥5% of the relevant areas of the tissue section.
** Includes three patients with pure aortic regurgitation, one patient with predominant aortic stenosis and one with combined aortic stenosis and regurgitation.

Hypertrophy and the presence of an endocardial contact plaque on the ventricular septum in apposition to a thickened anterior mitral leaflet.

Table 3. Comparison of Clinical and Morphologic Features in Patients with Hypertrophic Cardiomyopathy and in Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypertrophic cardiomyopathy</th>
<th>Other heart diseases and normals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>54</td>
<td>144</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean (range)</td>
<td>(11-70)</td>
<td>(5-91)</td>
</tr>
<tr>
<td>Female: male (%)</td>
<td>61:39</td>
<td>40:60</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>Mean (range)</td>
<td>535‡</td>
<td>532§</td>
</tr>
<tr>
<td>Septal thickness (mm)</td>
<td>Mean (range)</td>
<td>26</td>
<td>(15-44)</td>
</tr>
<tr>
<td>Septal-free wall ratio</td>
<td>Mean (range)</td>
<td>1.7</td>
<td>(1.0-2.6)</td>
</tr>
<tr>
<td>Septal-free wall ratio ≥ 1.3</td>
<td>78†</td>
<td>16‡</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Ratio of maximal ventricular septal thickness (usually present one-third to one-half the distance from base to apex) to posterior left ventricular wall thickness measured at the level of the inferior margins of the mitral leaflets.
† Echocardiograms were recorded during life in seven of 12 patients with concentric hypertrophy (septal-free wall ratio <1.3) at necropsy; asymmetric septal hypertrophy was present in each of these studies. Since septal-free wall ratios during life usually exceed those obtained at necropsy, it is likely that at least 40 (91%) of the 54 patients with hypertrophic cardiomyopathy would have had asymmetric septal hypertrophy during life by echocardiography.
‡ Septal-free wall ratio was <1.3 in each of the 11 normal hearts; one patient with coronary heart disease had a ratio of >1.3, due primarily to scarring, loss of myocardium, and hence decreased posterior left ventricular wall thickness (in the area of the wall through which the ultrasound beam would have passed in life); this patient was considered to have concentric ventricular wall thickening for the purposes of data analysis.
§ Heart weight exceeded normal values in all 54 patients.
¶ Heart weight exceeded normal values in each of 133 patients with congenital or acquired heart disease and was within normal limits in each of the 11 subjects without cardiac disease.

Abbreviation: NS = not significant.
LONGITUDINAL PLANE

Associated coronary artery disease (> 50% narrowing by atherosclerosis of the cross-sectional lumen of at least one major coronary artery) was present in seven of the 54 patients with hypertrophic cardiomyopathy; one of these patients also had a secundum atrial septal defect. Clinical and hemodynamic data in the 54 patients with hypertrophic cardiomyopathy are summarized in table 1.

Patients with Cardiac Diseases Other Than Hypertrophic Cardiomyopathy and Normal Hearts

The hearts of 144 patients were selected for study as controls. This group included 133 patients with a variety of congenital or acquired heart diseases and 11 subjects with structurally normal hearts who died of trauma (table 2). The 133 diseased control hearts had greater weights (mean ± SEM 555 ± 17 g vs 295 ± 12 g; p < 0.001) and ventricular septal thicknesses (16 ± 0.4 mm vs 12 ± 0.6 mm; p < 0.02) than did the 11 normal hearts; these two groups did not differ with regard to age, sex distribution or septal-free wall ratio. The normal and diseased controls were combined for convenience in data analysis. The 144 control patients and 54 patients with hypertrophic cardiomyopathy are compared with regard to clinical and anatomic parameters in table 3.

Materials and Methods

Measurement of Ventricular Wall Thicknesses

Measurements of ventricular wall thicknesses were made in the following two areas: 1) ventricular septum, at the point of maximum thickness, usually about one-half the distance between the base of the aortic valve and the apex of left ventricle; and 2) posterobasal left ventricular wall, behind the midpoint of the posterior mitral leaflet, at a level corresponding to the tips of the mitral leaflets. In making measurements of ventricular wall thicknesses, we avoided including trabeculae, papillary muscles or crista supraventricularis.

Preparation of Tissue

In each of the 198 hearts in this study (54 with hypertrophic cardiomyopathy and 144 controls) tissue blocks were taken from the full thickness of the ventricular septum, at the point of maximal thickness, and in a plane perpendicular to the long axis of the left ventricle (i.e., transverse plane) (fig. 1); in the anteroposterior axis these sections extended to the point of junction of the ventricular septum with the left and right ventricular free walls. In 25 of the 54 patients with hypertrophic cardiomyopathy and in eight control patients, additional tissue blocks (about 3 mm thick) were taken from the ventricular septum in a plane parallel to the long axis of the left ventricle (i.e., longitudinal plane) (fig. 1); these sections extended from the most cephalad aspect of the muscular septum to a point approximately three-fourths the distance from base and apex. Each tissue block was embedded in paraffin, sectioned at 6 μ and stained with hematoxylin and eosin.

Definition and Classification of Cardiac Muscle Cell Arrangement

Abnormally Arranged Cardiac Muscle Cells

In this study, cardiac muscle cell disorganization was classified into four major types (figs. 2 and 3). Type I-A disorganization was the most common, and consisted of areas of myocardium in which adjacent cardiac muscle cells were aligned perpendicularly or obliquely to each other, usually forming tangled masses or "pinwheel" configurations. Although most of these lesions were small, the size of individual foci of type I-A disorganization varied greatly. Disorganization of this type assumed a wide spectrum of morphologic appearances (fig. 4). In Type I-B disorganization relatively broad bundles of muscle cells were oriented at oblique or perpendicular angles to each other; cells within these bundles were, however, normally arranged. Both types I-A and I-B exclusively involved areas of septum in which cardiac muscle cells were cut longitudinally, i.e., appeared to be rectangularly shaped.

Type II-A disorganization consisted of relatively narrow (usually one or two cells wide), longitudinally cut bundles of cells that were interlaced in various directions among larger groups of transversely cut cells (which appeared circular). This type of disorganization gave the myocardium a swirled

FIGURE 1. Orientations in which sections of ventricular septum were taken. Longitudinal sections were obtained in a plane parallel to the long axis of the left ventricle; transverse sections were obtained in a plane perpendicular to the long axis of the left ventricle.
appearance. Type II-B was similar to type II-A disorganization, except that the narrow, longitudinally cut bundles of cells were more linear. A fifth type of cardiac muscle cell disorganization (type II-C) was identified in only three control patients; none had hypertrophic cardiomyopathy. This pattern of cardiac muscle cell malalignment showed relatively small "islands" of disorganized, longitudinally cut cells within much larger areas of transversely cut cells (fig. 5). Hence, type II-A, II-B or II-C disorganization involved both longitudinally and transversely cut cardiac muscle cells.

Abnormally arranged cardiac muscle cells were not considered to represent true disorganization if present in the following areas where cells normally converge at acute angles: 1) at or near the junction of ventricular septum with left and right ventricular free walls; 2) in trabeculations; 3) in or at the edges of areas of fibrosis; 4) at points of convergence of major muscle bundles; or 5) adjacent to interstitial spaces containing blood vessels.

**Normally Arranged Cardiac Muscle Cells**

A definition of cardiac muscle cell disorganization also requires a description of cardiac muscle cell arrangements not considered to constitute true abnormalities (fig. 6). Examples of such arrangements include, in addition to cardiac muscle cells in rigidly parallel relation to each other (fig. 6A), 1) minor deviations in alignment from the rigid parallelism shown in figure 6A, which often give cells the appearance of "branching" (fig. 6B); 2) small "fascicles" of cells in areas in which two muscle bundles converge (figs. 6C and D); and 3) artifacts of tissue preparation such as "waviness" (fig. 6E) or "buckling" (fig. 6F). The histologic appearance of "buckled" tissue is probably due to the phenomenon illustrated in figure 7.

**Quantitation of Cardiac Muscle Cell Arrangement**

We used a technique to assess quantitatively the extent to which cardiac muscle cell disorganization was present in tissue sections of ventricular septum from the 198 hearts. Hematoxylin and eosin-stained tissue sections were photographed and the images were enlarged to occupy 30° × 40° positive prints; this resulted in an average magnification of 1,200 times the original tissue section (range in magnification of × 200–5,300) (fig. 8). A transparent cellulose overlay was then placed over the print. Using a marking pen, areas of myocardium occupied by disorganized cardiac muscle cells were outlined on the transparent overlay. Although we could often make this assessment exclusively from direct examination of the print, we frequently had to reexamine the original tissue section with a light microscope to aid in localizing the disorganized area. In addition, areas in which cardiac muscle cells were cut either longitudinally or transversely were demarcated. Large areas of fibrosis (replacement or interstitial), artifacts of tissue preparation or large interstitial spaces with blood vessels also were outlined and ultimately excluded from the analysis. The transparent overlay was then removed from the print, photographed and the image reproduced (with substantial reduction) as a 5 × 7 inch positive print. Each area into which the tissue section had been divided was outlined separately with a fine-point marking pen on ordinary tablet paper. The area of each outlined silhouette was then quantitated using a video planimetry system.34,35 We analyzed print enlargements, usually without knowledge of whether the tissue section was from a patient with hypertrophic cardiomyopathy. Often, however, recognition of the patient's disease was unavoidable because a particularly large tissue section strongly suggested the presence of hypertrophic cardiomyopathy.

**Figure 2.** Classification of four major types of cardiac muscle cell disorganization present in ventricular septal myocardium. Types I-A and I-B exclusively involve cardiac muscle cells cut longitudinally. Types II-A and II-B involve cardiac muscle cells cut either longitudinally or transversely. Magnifications: I-A, × 130; I-B and II-A, × 40, II-B, × 80. These and all tissue sections shown subsequently were stained with hematoxylin and eosin.
Formulas Used in Quantitative Calculations of Cardiac Muscle Cell Arrangement

The formulas used to calculate the percent area of ventricular septum occupied by disorganized cardiac muscle cells are expressed below; the area of septum "at risk" for disorganization is in the denominator of both equations:

\[
\% \text{ area of type I (I-A + I-B) disorganization} = \frac{D_I}{L + D_I} \times 100;
\]

\[
\% \text{ area of type II (II-A + II-B + II-C) disorganization} = \frac{D_{II}}{L + T + D_{II}} \times 100,
\]

where \( L \) = area occupied by longitudinally cut but normally arranged cells, \( T \) = area occupied by transversely cut cells (excluding those incorporated into areas of type II disorganization), \( D_I \) = area occupied by type I disorganization, and \( D_{II} \) = area occupied by type II disorganization. The percent septal disorganization plotted for each patient was the value for type I or type II disorganization, whichever figure was greater.
We could not combine values for types I and II disorganization in those patients who had both types, since this calculation often resulted in a marked underestimation of the overall extent of disorganization present in a tissue section. We did not attempt to assess the degree to which cells were malaligned in a given area of tissue. Rather, septal disorganization was judged only to be present or absent, and its severity was expressed in terms of extent.

Two other formulas were used to calculate the percent area of longitudinally or transversely cut cardiac muscle cells:

\[
\text{\% area of longitudinally cut cells} = \frac{L + D_l}{T_o} \times 100;
\]

\[
\text{\% area of transversely cut cells} = \frac{T + D_{II}}{T_o} \times 100,
\]

where \(L\) = area occupied by longitudinally cut cells, \(T\) = area occupied by transversely cut cells, \(T_o\) = total area of tissue section, \(D_l\) = area occupied by type I disorganization (if present), and \(D_{II}\) = area occupied by type II disorganization (if present).

Results

Ventricular Wall Thicknesses

Ventricular septal thicknesses and septal-free wall ratios were significantly greater in the patients with hypertrophic cardiomyopathy than in control patients (table 3). In addition, asymmetric septal hypertrophy was present in 42 (78%) of the 54 patients with hypertrophic cardiomyopathy compared with 23 (16%) of the 144 controls (table 3). This latter prevalence figure is similar to that previously reported in necropsy studies of similar groups of hearts.\(^{19, 15}\)

Quantitative Histologic Findings

in Transverse Plane Tissue Sections

The architecture of large muscle bundles was relatively uniform in transverse plane tissue sections. Usually, a narrow bundle of longitudinally cut cardiac muscle cells was present in the center of the tissue section extending from the anterior to the posterior limits of the ventricular septum (fig. 9); this band usually comprised one-third of the overall tissue section area in both patients with hypertrophic cardiomyopathy and controls (table 4). Usually, transversely cut cells were present in the areas on either side of the central band (adjacent to the left and right ventricular cavities); each of these two areas comprised about one-third of the tissue section.

\*Obliquely cut cells were classified as either longitudinally or transversely cut, depending on the degree of obliquity. Such cells were most commonly present in areas where large bundles of cells came into apposition at acute angles.

Figure 4. Several examples of type I-A disorganization which illustrate the wide morphologic spectrum of this lesion. A) "Chaotic" pattern in which adjacent cells are arranged at perpendicular and oblique angles to each other (also, see upper left panel in figure 2 for similar example); magnification \(\times 55\); B) "Swiss cheese" appearance that results when adjacent cells are oriented at particularly acute angles; magnification \(\times 350\); C) Tangled arrangement of cardiac muscle cells in whorled configurations; magnification \(\times 350\). D) Whorl pattern in which some cells are oriented circumferentially to other cells; magnification \(\times 130\); E) and F) Patterns in which small groups of cells are oriented at extremely acute angles to larger groups of cells; magnification \(\times 130\).
FIGURE 5. Uncommon fifth type of cardiac muscle cell disorganization (type II-C). Consists of small "islands" of disorganized, longitudinally cut cardiac muscle cells, present within much larger areas of transversely cut cells; magnification × 80.

Patients with hypertrophic cardiomyopathy and controls differed markedly in the extent of ventricular septal disorganization (fig. 10, table 4). In patients with hypertrophic cardiomyopathy, the percent area of septum disorganized ranged from 0–94% (mean ± sem 31 ± 3%). In contrast, 109 (76%) of the 144 control patients showed no disorganization. While there was some overlap between control patients and patients with hypertrophic cardiomyopathy and controls differed markedly in the extent of ventricular septal disorganization (fig. 10, table 4). In patients with hypertrophic cardiomyopathy, the percent area of septum disorganized ranged from 0–94% (mean ± sem 31 ± 3%). In contrast, 109 (76%) of the 144 control patients showed no disorganization. While there was some overlap between control patients and 

<table>
<thead>
<tr>
<th>Table 4. Quantitative Histologic Findings</th>
</tr>
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<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Size of septal tissue section (cm²)</td>
</tr>
<tr>
<td>Transverse plane</td>
</tr>
<tr>
<td>Longitudinal plane</td>
</tr>
<tr>
<td>% area longitudinally cut cells in septum</td>
</tr>
<tr>
<td>Transverse plane</td>
</tr>
<tr>
<td>Longitudinal plane</td>
</tr>
<tr>
<td>% area of septum disorganized (transverse plane)</td>
</tr>
<tr>
<td>Type I-A</td>
</tr>
<tr>
<td>Type I-B</td>
</tr>
<tr>
<td>Total type I</td>
</tr>
<tr>
<td>Type II-A</td>
</tr>
<tr>
<td>Type II-B</td>
</tr>
<tr>
<td>Type II-C</td>
</tr>
<tr>
<td>Total type II</td>
</tr>
<tr>
<td>Total type I or II§</td>
</tr>
<tr>
<td>% area of septum disorganized (longitudinal plane)</td>
</tr>
<tr>
<td>Type I-A</td>
</tr>
<tr>
<td>Type I-B</td>
</tr>
<tr>
<td>Total type I</td>
</tr>
<tr>
<td>Type II-A</td>
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<tr>
<td>Type II-B</td>
</tr>
<tr>
<td>Total type II</td>
</tr>
<tr>
<td>Total type I or II§</td>
</tr>
</tbody>
</table>

*p < 0.001 when these two variables are compared.
†p < 0.005 when these two variables are compared.
‡p < 0.001 when these two variables are compared.
§Value for % septal disorganization used for each patient was that of either type I or type II disorganization, whichever figure was greater.
Abbreviation: NS = not significant.
those with hypertrophic cardiomyopathy, the mean area of septum disorganized in controls (1.5 ± 0.6%) was significantly less than in patients with hypertrophic cardiomyopathy (p < 0.001).

The extent of cardiac muscle cell disorganization in patients with hypertrophic cardiomyopathy compared to patients with other cardiac diseases is illustrated in figures 11–13. Of the 54 patients with hypertrophic cardiomyopathy, 51 (94%) had some disorganization, 48 (89%) had disorganization comprising 5% or more of the relevant part of the tissue section, 30 (56%) had disorganization comprising 25% or more of the section, and 14 (26%) had particularly extensive disorganization involving 50% or more of the section. In contrast, cardiac muscle cell disorganization in the 144 control patients was much less extensive; only 7% of these patients had areas of disorganization comprising 5% or more of the tissue section, and just 1% (two patients) had 25% or more of the section involved (fig. 11). In the control group, significant septal disorganization was most common in patients with aortic valve disease (table 2). However, the percent area of septum disorganized did not differ significantly among control patients with acquired (mean 1.9 ± 0.7%) or congenital heart disease (mean 0.5 ± 0.4%) or normal hearts (mean 1.3 ± 1.2%).

At relatively high magnification (fig. 13A), the small areas of disorganization in some control patients appeared qualitatively identical to the widespread lesions in patients with hypertrophic cardiomyopathy; however, at lower magnification such areas of disorganization were barely discernible and obviously constituted only a small part of the tissue section (fig. 13B).

Each of the 51 patients with hypertrophic cardiomyopathy and septal disorganization had type I (I-A, I-B or both) lesions. Ten of these 51 patients also had type II (II-A, II-B or both) lesions, although in only one patient did the extent of type II disorganization exceed that of type I (17% vs 10%). The percent area of ventricular septum occupied by either type I or type II lesions was significantly greater in patients with hypertrophic cardiomyopathy than in controls (table 4). Furthermore, while individual foci of type I and type II disorganization varied considerably in size, they were usually larger in patients with hypertrophic cardiomyopathy than in controls. Type I-A disorganization generally manifested the greater number of individual foci of disorganization per tissue block; these foci of disordered cells were interspersed among areas of myocardium in which the cells were more normally arranged (fig. 14).

In transverse plane sections from patients with hypertrophic cardiomyopathy or controls, abnormally arranged cells were most common in the middle one-third of the septum, although there was no particular localization of disorganized cells within this area (e.g., anteriorly or posteriorly). However, it is in the middle one-third of the septum that longitudinally cut cells are usually present (fig. 9). Hence, we would expect that disorganization (i.e., the common type I which can only be identified in areas of longitudinally cut cells) would be observed most frequently in the middle one-third of sections taken perpendicular to the long axis of the left ventricle. It was difficult to determine the extent to which disorganization was present in the areas of septum adjacent to the ventricular cavities. Cells in these regions were usually cut transversely, prohibiting identification of type I disorganization. However, analysis of sections taken parallel to the long axis of the left ventricle showed that relatively small foci of disorganization were frequently present (in 14 of 23 patients studied; table 4) in areas adjacent to the ventricular cavities.

Quantitative Histologic Findings in Longitudinal Plane Tissue Sections

In tissue sections obtained parallel to the long axis of the left ventricle, longitudinally cut cardiac muscle cells were located primarily in narrow strips on or near the periphery of the septum, adjacent to the ventricular cavities (fig. 15). These areas occupy a relatively small segment of the overall tissue section (about 15% in patients with hypertrophic cardiomyopathy and in controls; table 4).

The extent of septal disorganization was significantly greater in transverse plane sections (mean 32 ± 4%) than in longitudinal plane sections (6 ± 2%; p < 0.001) (fig. 16); in 23 of the 25 patients so analyzed, septal disorganization was more marked in the transverse plane sections. Analysis of only the longitudinal plane sections showed that 16 of the 25 patients had no or minimal disorganization (< 5% of the tissue section); hence, the diagnosis of hypertrophic cardiomyopathy could not have been made histologically in the majority of these patients from analysis of these sections alone. However, in 14 of these 16 patients, the transverse plane sections showed extensive disorganization (≥ 5%) compatible with the histologic diagnosis of hypertrophic cardiomyopathy.

Correlation of Extent of Septal Disorganization with Other Parameters

Various clinical, hemodynamic and anatomic parameters identified in the 54 patients with hypertrophic cardiomyopathy were analyzed to determine whether there was a correlation with the extent of septal disorganization (in transverse plane sections). There was no relation between extent of septal disorganization and sex, presence or absence of symptoms, duration of symptoms, presence of coronary heart disease or paroxysmal atrial fibrillation, mode of death (sudden vs chronic decompensation), magnitude of left ventricular outflow tract obstruction or left ventricular end-diastolic pressure, heart weight, ventricular septal thickness, septal-free wall ratio, or whether septal myotomy-myectomy had been performed.

However, three variables did show a statistically significant relation with the extent of septal disorganization (table 5). Septal disorganization was significantly greater in patients younger than 18 years of age at death compared with those older than 18.
years, although a linear relation did not exist between percent septal disorganization and increasing age. In addition, septal disorganization was more marked in patients who were members of families with "malignant" hypertrophic cardiomyopathy, but less extensive in patients with a documented history of systemic hypertension. Furthermore, no clinical or anatomic parameter appeared to be characteristic of either the six patients with hypertrophic cardiomyopathy who showed no or minimal (≤ 3%) septal disorganization or the five patients with marked (> 70%) disorganization.

**Discussion**

The findings of this quantitative morphologic study indicate that extensive cardiac muscle cell disorganization in the ventricular septum is a highly specific histologic marker of hypertrophic cardiomyopathy; hence, these data confirm our previous impression, based upon qualitative analysis of septal myocardium from patients with hypertrophic cardiomyopathy or other heart diseases. Our data also emphasize that three considerations are most important in assessing the specificity of disorganized septal architecture for patients with hypertrophic cardiomyopathy: 1) well-defined, descriptive morphologic criteria for abnormal cardiac muscle cell arrangement, based on analysis of septal tissue from a wide variety of cardiac diseases, including hypertrophic cardiomyopathy; 2) assessment of the extent of cardiac muscle cell disorganization in quantitative terms; and 3) selection of the optimal tissue section for analysis.
First, since there has been no specific, generally accepted definition of what constitutes abnormal cardiac muscle cell arrangement, confusion has arisen among investigators in this field. Perhaps the classification proposed in this study will minimize such problems. Our definition of disorganization is confined to more obvious abnormalities of cellular arrangement, and therefore excludes minimal deviations from parallel alignment. Several recent morphologic studies have concluded that septal disorganization is not a particularly sensitive or reliable marker of hypertrophic cardiomyopathy. However, these studies were based primarily on analysis of patients with heart diseases other than hypertrophic cardiomyopathy.\textsuperscript{18-21} Such an experimental design creates a more limited view of what constitutes cardiac muscle cell disorganization than would an analysis that also included a large number of patients with hypertrophic cardiomyopathy, many of whom demonstrate the most extensive and striking examples of cellular disorganization we have observed in septal myocardium.

Second, the quantitative method we used demonstrated that cardiac muscle cell disorganization is characteristically widespread in patients with hypertrophic cardiomyopathy. Almost 90\% of our patients had disorganization occupying 5\% or more of the relevant part of the tissue section, over 50\% of the

<table>
<thead>
<tr>
<th>Table 5. Clinical Parameters Showing Positive Correlation with Percent Septal Disorganization</th>
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<tbody>
<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>1. Age (years)</td>
</tr>
<tr>
<td>&lt;18</td>
</tr>
<tr>
<td>&gt;18</td>
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<tr>
<td>2. Premature death in &gt;1 family member</td>
</tr>
<tr>
<td>&quot;malignant&quot; HCM*</td>
</tr>
<tr>
<td>in 0 or 1 family member</td>
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<tr>
<td>3. Systemic hypertension*</td>
</tr>
<tr>
<td>Absent</td>
</tr>
<tr>
<td>Present</td>
</tr>
</tbody>
</table>

\*In eight patients the available history was inadequate to determine reliably whether or not systemic hypertension had been present.

Abbreviations: HCM = hypertrophic cardiomyopathy.
FIGURE 7. Schematic representation of tissue section “buckling” phenomenon that results in histologic pattern shown in figure 6F. A) View of “buckled” tissue section from the side; solid line denotes the plane of section; B) Lower half of tissue section viewed from above after removal of the upper half. Hence, sectioning this tissue results in the appearance of alternating layers of transversely and longitudinally cut cells.

FIGURE 8. Photographic enlargement (with transparent overlay in place) of a section of ventricular septum from a patient with hypertrophic cardiomyopathy. Actual size of tissue section is shown at lower left (arrow) for comparison. Magnification of the print relative to the original tissue section was 2,000 times.

FIGURE 9. “Map” of transverse plane tissue section from a 16-year-old girl with mitral regurgitation and concentric ventricular hypertrophy. Longitudinally cut cardiac muscle cells, all of which are normally arranged, are present in the relatively narrow striped area in the center of the section.
patients had ≥ 25% of the section involved and over 25% of the patients had more than 50% involved. Nevertheless, the presence per se of cellular disorganization in the ventricular septum is not pathognomonic of hypertrophic cardiomyopathy. About 25% of patients with cardiac diseases other than hypertrophic cardiomyopathy or normal subjects showed some disorganization in ventricular septal tissue.

Figure 10. Percent area of ventricular septum occupied by disorganized cardiac muscle cells in 54 patients with hypertrophic cardiomyopathy and 144 control patients. Control subjects with normal hearts are shown by open circles, those with congenital heart disease by triangles, and those with acquired heart disease by solid circles. \( \bar{x} \) = mean value.
However, in almost 70% of these patients the areas of disorganization were very small (<5% of the tissue section analyzed). Hence, it is not the presence or absence of septal disorganization that distinguishes patients with hypertrophic cardiomyopathy from those with other heart diseases histologically, but rather the extent to which disorganization is present.

Without comprehensive echocardiographic studies in first-degree relatives, we cannot definitively exclude the possibility that some of the control patients in this study with extensive septal disorganization also had genetically transmitted hypertrophic cardiomyopathy. It is possible that the sole control patient in this study with >35% septal disorganization actually had hypertrophic cardiomyopathy, particularly since, in addition to her isolated, severe mitral regurgitation, she had marked septal thickening of 25 mm and a septal-free wall ratio of 1.5.

The cut point that appeared to best distinguish patients with hypertrophic cardiomyopathy from patients with other cardiac diseases, histologically, was septal disorganization involving 5% or more of the relevant part of the tissue section. Therefore, septal disorganization of ≥5% is consistent with the histologic diagnosis of hypertrophic cardiomyopathy, while septal disorganization of <5% makes this diagnosis unlikely. Furthermore, if a patient with congenital, valvular, or coronary heart disease had <5% septal disorganization and a septal-free wall ratio...
< 1.3, the chance of hypertrophic cardiomyopathy being present would be virtually nil; only 2% of our control group had both disproportionate septal thickening and ≥ 5% septal disorganization.

Third, our data indicate that optimal identification of cellular disorganization depends greatly on selection of the most appropriate tissue section for analysis — i.e., that taken perpendicular to the long axis of the left ventricle. Disorganization was present both more frequently and to a greater extent in transverse plane sections than in longitudinal plane sections. This is apparently related to the fact that longitudinally cut cardiac muscle cells comprise a relatively greater area of tissue sections taken perpendicular to the long axis of the left ventricle than of sections taken parallel to the long axis; hence, the more common type I disorganization, which can only be identified in longitudinally cut cardiac muscle cells, is most likely to be observed in septal tissue sections taken perpendicular to the long axis of the left ventricle.

The present investigation was confined to the study of malalignment between cardiac muscle cells in the ventricular septum. Disarray of myofibrils and myofilaments in septal muscle cells has also been described in light and electron microscopic studies of plastic embedded tissue from patients with hypertrophic cardiomyopathy. These abnormalities are most pronounced in branched, stellate-shaped and dis-

**Figure 13.** Photomicrographs of the same area of ventricular septal myocardium from a 52-year-old woman with severe valvular aortic stenosis. A) At a relatively high magnification of ×350 an abnormal arrangement of cardiac muscle cells typical of type I-A disorganization is shown. B) Same area of myocardium shown at a much lower magnification of ×55; the area of disorganization (outlined by broken line), so impressive at high magnification, is barely discernible here and obviously constitutes an extremely small part of the overall tissue section.
organized cells, but are also found occasionally in cells with more normal shapes and orientation. However, disarray of myofibrils and myofilaments occurs in a variety of cardiac conditions in both man and animals\textsuperscript{2,38-43} and hence, appears to be a relatively nonspecific morphologic alteration. For this reason, and because the histologic techniques we used did not permit reliable identification of myofibrillar and

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig14}
\caption{Ventricular septum from a 14-year-old boy with hypertrophic cardiomyopathy. Broken line outlines focus of cardiac muscle cell disorganization. Note that myocardium surrounding this area is normally arranged. Magnification $\times 40$.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig15}
\caption{Map of section of ventricular septum obtained parallel to the long axis of the left ventricle superimposed upon actual tissue section. Cardiac muscle cells viewed in longitudinal orientation occupy only the relatively small (striped) areas adjacent to the right ventricular (RV) and left ventricular (LV) cavities (compare with fig. 9). The section is from a 56-year-old woman with hypertrophic cardiomyopathy.}
\end{figure}
cells in the ventricular septum. Although true cellular disorganization has also been reported in areas of diseased hearts other than the septum, including crista supraventricularis\(^{40,44}\) and the left and right ventricular free walls,\(^8\) we did not study this subject in the present investigation.

The potential bias introduced into our data by tissue sample selection must also be considered. All tissue sections from patients with hypertrophic cardiomyopathy and patients with other cardiac diseases were obtained in a standardized fashion and location. However, because of practical considerations related to preservation of necropsy specimens, our data analysis was essentially limited to a single section of ventricular septum from each patient. Because the distribution of cardiac muscle cell disorganization was often "patchy" in the tissue sections analyzed, the theoretical possibility arises that the extent of disorganization might differ significantly in other areas of the ventricular septum not analyzed as part of this study. However, some of our findings suggest that significant variations in disorganization in different areas of the septum do not occur. For example, in four patients with hypertrophic cardiomyopathy and zero or \(\leq 3\%\) disorganization, a second transverse plane tissue section (taken 2 cm below the first section) showed an almost identical extent of septal disorganization as observed in the first section.

In conclusion, our data suggest that marked cellular disorganization in septal myocardium links patients within the varied clinical and pathological spectrum of hypertrophic cardiomyopathy; disorganization was present and did not differ significantly in extent in patients with or without symptoms before death, patients with asymmetric or symmetric (concentric) ventricular wall thickening at necropsy, or patients with or without obstruction to left ventricular outflow. Furthermore, the presence or extent of septal disorganization was not related to absolute heart weight; hence, disorganization cannot be considered simply a manifestation of particularly marked cardiac hypertrophy, as occurs in hypertrophic cardiomyopathy. These findings support our hypothesis\(^8\) that extensive cardiac muscle cell disorganization is probably a morphologic manifestation of the underlying genetic defect present in hypertrophic cardiomyopathy.

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Quantitative analysis of cardiac muscle cell disorganization in the ventricular septum of patients with hypertrophic cardiomyopathy.

B J Maron and W C Roberts

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