Hypertrophic cardiomyopathy (HCM) is a primary cardiac disease with a heterogeneous clinical and morphologic profile (1–3). Recently, considerable attention has focused on several mutations in genes encoding proteins of the cardiac sarcomere, including β-myosin heavy chain, cardiac troponin-T, troponin-I, α-tropomyosin and myosin-binding protein-C (3–6). Indeed, in HCM, many prominent and characteristic structural alterations, such as left ventricular (LV) wall thickening (7) and disorganization of cardiac muscle cells (8,9), directly involve proteins of the sarcomere.

However, other common pathologic features of HCM, such as the thickened and narrowed intramural coronary arteries (1,10), elongated mitral valve leaflets (11–13), congenital malformations of the mitral apparatus (14) and myocardial fibrosis (15–20), largely involve connective tissue elements. Thus, we have examined the possibility that in HCM an expanded LV collagen matrix is involved in this process (19) and may contribute to the abnormally increased...
LV wall thickness, the sine qua non of this disease (7). Consequently, the present study analyzes the morphologic appearance and extent of the collagen matrix in patients with HCM who had died suddenly in comparison with several control groups.

METHODS

Patient population. The 16 study patients were selected from the HCM registry of the Pathology Branch of the National Heart, Lung, and Blood Institute. Each heart met the morphologic definition of HCM, that is, a hypertrophied, nondilated LV in the absence of another cardiac or systemic disease capable of producing the magnitude of hypertrophy present (7). Patients were selected by virtue of the following inclusion criteria: 1) age <35 years at the time of death; 2) absence of symptoms or evidence of cardiac dysfunction during life; 3) sudden and unexpected, out-of-hospital death as the only clinical manifestation of HCM; 4) absence of myocardial scarring on gross visual inspection; and 5) absence of cross-sectional luminal narrowing ≥75% by atherosclerosis of a major epicardial coronary artery.

The control groups consisted of: 1) 16 adults (20 to 78 years; mean 43 ± 18 years) with structurally normal hearts who died of noncardiovascular causes (all related to trauma); 2) five nondiabetic adults (39 to 55 years, mean 46 ± 7 years) with LV hypertrophy as a consequence of long-standing and severe systemic hypertension in the absence of significant atherosclerotic coronary artery narrowing, who died of noncardiovascular causes (trauma in four and cerebral hemorrhage in one); and 3) six infants with HCM who were stillborn or died of congestive heart failure (1 to 146 days of age; mean 71 ± 63 days).

Gross examination. After fixation in 10% phosphate-buffered formaldehyde, each heart was sectioned transversely (i.e., perpendicular to the long axis of the LV), from apex to base, at approximately 1 cm intervals. Maximal ventricular septal and LV free wall thicknesses were measured perpendicular to the endocardial surfaces of the wall (excluding papillary muscles and trabeculae).

Preparation of specimens for histologic studies. A transmural section of ventricular septum, taken at the mid-LV cavity level in the area of maximal wall thickness, was removed for histomorphologic examination, taking care to exclude the LV outflow tract fibrous plaque (Fig. 1). Each specimen was embedded in paraffin, sectioned at 5-µm thickness and stained with hematoxylin and eosin and picrosirius red (Pfaltz and Bauer, Stamford, Connecticut). Stained sections were utilized for quantitative analysis of collagen content and the extent of cardiac muscle cell disorganization.

Qualitative and quantitative assessment of collagen. Morphology of interstitial (matrix) and perivascular (adventitial) collagen was assessed from the picrosirius red-stained sections with polarization microscopy. Picrosirius red is a collagen-specific stain that also enhances the natural birefringence of the collagen fibrils under polarized light and permits identification of various components of the matrix collagen (perimysial coils, pericellular weaves and struts) (21,22).

Collagen volume fractions were quantified in tissue sections using a semi-automated computerized image analysis system (Quantimet 520; Cambridge Laboratories, Inc., Cambridge, Massachusetts), similar to the technique described by Hoyt et al. (23). Analysis was performed with a standard light microscope and a mounted video camera system connected to a host computer and a digitizer. First, by systematically scanning each microscopic slide (at a magnification of 7.8×), a composite digitized image was obtained. The volume fraction of interstitial collagen was then calculated as the area occupied by connective tissue divided by the sum of the areas occupied by connective tissue and cardiac muscle cells. Intramural vessels, perivascular collagen, endocardium and trabeculae were excluded from this particular analysis.

Perivascular collagen was defined as that collagen in the adventitia of those intramural coronary arteries identified by picrosirius red staining. This volume fraction was calculated by dividing the sum of all perivascular collagen by the total area of the tissue section.

Quantitation of cardiac muscle cell disorganization. The area of ventricular septum occupied by disorganized cardiac muscle cells was measured using the method described by Maron and Roberts (9). Briefly, hematoxylin and eosin-stained sections were magnified to occupy a 30 × 40 in. positive print, over which a transparent cellulose overlay was
LV free wall thicknesses were 10 to 15 mm (mean 12.6 ± 3.5 mm in women; 17 to 40 mm (mean 27 ± 17 mm) and within normal limits in each subject (<350 g in women; <400 g in men). Ventricular septal and LV free wall thicknesses were 10 to 15 mm (mean 12 ± 2 mm). Scattered small foci of disorganized cardiac muscle cells, present in each subject, occupied 2 ± 1% of septal tissue sections.

**Table 1.** Demographic and Morphologic Findings in Infants, Children and Young Adults With Hypertrophic Cardiomyopathy: Patients With Systemic Hypertension and Normal Controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HCM</th>
<th>Hypertension</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>16</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Age, years (mean, range)</td>
<td>20 ± 7 (11–31)</td>
<td>0.2 ± 0.2</td>
<td>46 ± 7 (39–55)</td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>10 (62%)</td>
<td>4 (67%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>518 ± 156 (320–970)</td>
<td>438 ± 38 (387–489)</td>
<td>305 ± 56 (195–390)</td>
</tr>
<tr>
<td>Ventricular septal thickness, mm</td>
<td>27 ± 7 (17–40)</td>
<td>17 ± 7 (8–30)</td>
<td>16 ± 1 (15–17)</td>
</tr>
<tr>
<td>LV free wall thickness, mm</td>
<td>15 ± 3 (9–20)</td>
<td>8 ± 3 (3–13)</td>
<td>16 ± 1 (14–17)</td>
</tr>
<tr>
<td>Septum/free wall ratio</td>
<td>1.9 ± 0.5 (0.9–2.7)</td>
<td>2.1 ± 0.5 (1.4–2.7)</td>
<td>1.0 ± 0.1 (0.9–1.1)</td>
</tr>
<tr>
<td>Area of septum examined, cm²</td>
<td>5.2 ± 2.0 (2.1–9.1)</td>
<td>2.3 ± 0.5 (1.4–3.0)</td>
<td>4.4 ± 0.4 (3.9–4.9)</td>
</tr>
<tr>
<td>Percent area of disorganized cells</td>
<td>53 ± 20 (12–88)</td>
<td>25 ± 8 (17–34)</td>
<td>2 ± 1 (0–3.9)</td>
</tr>
</tbody>
</table>

**Statistical analyses.** Data are expressed as mean ± standard deviation. Differences between continuous variables were assessed with the unpaired Student t test. Chi-square or Fisher exact tests compared proportions. Selected variables were compared using linear regression analysis. Univariate regression analysis assessed the degree to which collagen amount was associated with particular clinical, demographic and morphologic disease parameters.

**RESULTS**

**Normal control subjects.** GROSS AND HISTOLOGIC FINDINGS (TABLE 1). Heart weights were 195 to 390 g (mean 305 ± 56 g) and within normal limits in each subject (<350 g in women; <400 g in men). Ventricular septal and LV free wall thicknesses were 10 to 15 mm (mean 12 ± 2 mm). Scattered small foci of disorganized cardiac muscle cells, present in each subject, occupied 2 ± 1% of septal tissue sections.

**Assessment of Collagen Fiber Morphology.** The collagen matrix in normal controls consisted of an orderly arrangement of thin “spring-like” perimysial coils (Fig. 2A and B) that coursed parallel to the long axis of the cardiac muscle cells and were connected through transversely oriented struts to a fine network of pericellular weaves (Fig. 2A and B) encircling the sarcolemma of individual muscle cells. In addition, lateral surfaces of adjacent muscle cells were also interconnected by thin, transversely oriented struts (Fig. 2A and B).

**Collagen Volume Fraction (Table 1; Fig. 3).** Total collagen volume in septal tissue sections was 4.0 ± 1.6%. Volume fractions of interstitial (matrix) and perivascular collagen were 1.8 ± 1.0% and 2.2 ± 1.0%, respectively, and interstitial/perivascular collagen ratio was 0.81. Interstitial collagen was evenly distributed throughout the middle, inner (adjacent to LV) and outer (adjacent to right ventricle) one-thirds of the septal tissue sections (35 ± 3%, 35 ± 3% and 31 ± 4%, respectively).

**Children and young adults with HCM.** GROSS AND HISTOLOGIC FINDINGS (TABLE 1). Heart weights were 320 to 970 g (mean 518 ± 156 g), and increased relative to age and gender in each patient. Ventricular septal and free wall thicknesses were 17 to 40 mm (mean 27 ± 7 mm) and 9 to 20 mm (mean 15 ± 3 mm), respectively. Extensive cardiac muscle cell disorganization was present in each patient, occupying 53 ± 20% of the septal tissue section. Cellular disorganization involved areas of myocardium of varying size in which adjacent longitudinally sectioned cells (or bundles of cells) were aligned perpendicularly and obliquely, or in which longitudinally sectioned cells were interlaced among transversely oriented cells. Microscopic areas of replacement fibrosis were not observed.
Assessment of Collagen Morphology (Table 2). Although variation was evident among patients, in sharp contrast to normal controls, HCM hearts showed a marked increase in the number and thickness of all fiber components of the collagen matrix (Figs. 2C–F and 4). The perimysial fibers appeared particularly thickened and straighter than normal with a loss of their normal “spring-like” coiled configuration (Fig. 2C and D). Pericellular weaves formed dense networks of thickened fibers encasing cardiac muscle cells in substantial portions of ventricular septum and were particularly evident in association with cardiac muscle cells sectioned transversely (Fig. 4). Fiber components of the matrix showed a disorganized arrangement compared with normals, particularly in areas of cardiac muscle cell disarray. Therefore, when compared with normal controls as well as to hypertensives, the appearance of these collagen fiber types was markedly abnormal, both in areas of disorganized myocytes (Fig. 2E and F) and normal myocardial architecture (Fig. 2C and D).

In addition, dense perivascular collagen encased numerous abnormal intramural coronary arteries with apparently narrowed lumen and thickened walls (with media containing abundant collagen) (Figs. 5 and 6).

Collagen Volume Fraction (Table 1; Fig. 3). Total collagen volume in the ventricular septal tissue sections was 16.7 ± 9.1%, significantly exceeding that in the normal controls (4.0 ± 1.6%), hypertensives (8.2 ± 1.8%) and HCM infants (7.0 ± 3.0%) (p < 0.0001). Volume fraction of the matrix collagen was 14.1 ± 8.8%, also significantly exceeding normal controls (1.8 ± 1.0%), hypertensives (4.5 ± 1.3%) and HCM infants (4.0 ± 2.4%). This represented an eightfold increase in matrix collagen compared with normal controls, and a threefold increase with respect to hypertensives. Interstitial collagen was distributed disproportionately in the middle one-third of the transmural tissue sections (49 ± 9%), compared with either the inner (34 ± 13%) or outer (17 ± 12%) one-thirds (p < 0.001).

Volume fraction of perivascular collagen was 2.6 ± 1.0% in children and young adults with HCM, and did not differ significantly from that in any control group. Therefore, the highest proportion of collagen identified was in the interstitial compartment, with an interstitial/perivascular collagen ratio of 5.4.

Patients with Systemic Hypertension. Gross and Histologic Findings. Heart weights were 387 to 489 g (mean 438 ± 38 g) and were increased in each (≥350 g in women and >400 g in men). Septal and free wall thicknesses were 15 to 17 mm (mean 16 ± 1 mm) and 14 to 17 mm (mean 16 ± 1 mm), respectively. Scattered small foci of disorganized cardiac muscle cells, present in four patients, occupied 2 ± 1% of the tissue sections. Microscopic areas of replacement fibrosis were not observed.

Qualitative Assessment of Collagen. Within the interstitial space, intercellular struts, pericellular weaves and perimysial coils appeared more abundant and thicker than normal, but with ordered arrangement. Abundant perivascular collagen surrounded intramural coronary arteries, a few of which showed thickened walls with increased medial collagen.

Collagen Volume Fraction (Table 1; Fig. 3). Total and interstitial collagen volume in patients with hypertension were significantly greater than normal but less than in children and adults with HCM. Interstitial collagen appeared evenly distributed throughout the middle, inner and outer one-thirds of tissue sections. Interstitial/perivascular collagen ratio was only 1.2, and the perivascular collagen volume fraction did not differ from normals.

Infants with HCM. Gross and Histologic Findings. Ventricular septal and LV free wall thicknesses ranged 8 to 30 mm (mean 17 ± 7 mm) and 3 to 13 mm (mean 8 ±
Extensive disorganization of myocytes was present in each infant, occupying 25–68% of the tissue section. Small microscopic foci of replacement fibrosis were evident in each heart.

**QUALITATIVE ASSESSMENT OF COLLAGEN.** Alterations in collagen fiber morphology, consisting of thickening and increased numbers of struts, weaves and perimysial coils (also with stretching of the coils), were similar to that of children and adults with HCM. Collagen fibers also appeared disorganized in their arrangement, particularly in areas of abnormal myocyte arrangement. Similar to the older patients with HCM, areas of dense perivascular collagen encased numerous abnormal intramural coronary arteries with apparently narrowed lumen, thickened walls and increased medial collagen.

**COLLAGEN VOLUME FRACTION (TABLE 1; FIG. 3).** Total and interstitial collagen volume in HCM infants was significantly less than in children and adults with HCM but

![Figure 3. Volume fractions of interstitial, perivascular and total collagen compartments in ventricular septal tissue sections from children and young adults with HCM, compared with controls with structurally normal hearts, patients with systemic hypertension and secondary left ventricular hypertrophy and infants with HCM. Significant differences are present between the groups in total and interstitial, but not perivascular, collagen volume fraction.](image)

**Table 2.** Correlation Coefficients Relating Demographic and Clinical Parameters to Amount of Collagen in Tissue Sections From 16 Children and Young Adults with Hypertrophic Cardiomyopathy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Collagen Volume Fractions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.21 (0.56)</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.33 (0.22)</td>
</tr>
<tr>
<td>Septal thickness (mm)</td>
<td>0.44 (0.09)</td>
</tr>
<tr>
<td>Percent cellular disorganization</td>
<td>0.15 (0.58)</td>
</tr>
<tr>
<td>Media area/total area of intramural coronary arteries</td>
<td>0.32 (0.23)</td>
</tr>
</tbody>
</table>

Numbers shown in table are r values from univariate analyses; appropriate p values appear in parentheses.

![Figure 4. Transversely cut cardiac muscle cells in ventricular septum from a 17-year-old patient with HCM, stained with picrosirius red and viewed by light (A) and polarization (B) microscopy. The myocytes show characteristic encasement within a dense network of matrix collagen. (Magnification ×100.)](image)
greater than in normal (adult) controls (although for interstitial volume, \( p \) was only 0.07). The interstitial/perivascular collagen ratio was 1.3, and the perivascular collagen volume fraction in HCM infants did not differ from the other groups.

**Relation of amount of collagen to clinical and morphologic features (Table 2).** By univariate analysis, for hearts of the children and young adults with HCM, no statistically significant relation was identified between the total or interstitial collagen volume fractions and several clinical and morphologic parameters: age, gender, heart weight, percent of section occupied by disorganized cells and wall thickness of intramural arteries. Only the relation between total collagen and septal thickness approached statistical significance (\( r = 0.44; p = 0.09 \); Table 2).

**DISCUSSION**

**Collagen matrix in HCM.** The cardiac collagen matrix, the fibrillar collagen network comprising the interstitium of the heart, represents the structural skeletal framework of the myocardium composed of pericellular, intercellular and fascicular connective tissue (22). In the present study, we demonstrated that this collagen network in the ventricular septum of children and adults with HCM (who died suddenly as the first clinical manifestation of their disease) was substantially greater in size and differed considerably in morphologic appearance when compared with controls without evidence of cardiovascular disease as well as patients with systemic hypertension and secondary LV hypertrophy (24–27). For example, the calculated volume fraction of interstitial collagen was about eight times greater than that of normal controls (and three times greater than that of hypertensives), and comprised about 15% (up to 27%) of the transmural septal tissue sections. Consequently, thickening of the ventricular septum in HCM (7) is not solely due to increased size or number of myocytes, but is also in part a consequence of the substantial expansion of the collagen matrix.

Furthermore, in contrast to the more orderly arrangement of fine collagen fibrils in normal hearts, or in the hypertrophied hearts of patients with hypertension, the fibrillar collagen in our patients with HCM was characteristically more numerous and thickened and was frequently arranged in disorganized patterns.

**Matrix collagen expansion as a primary abnormality.** Several of our observations suggest that the excess amount and disorganized architecture of matrix collagen in HCM may represent a primary abnormality of ventricular structure, rather than a secondary consequence of cardiac muscle cell hypertrophy or other factors. First, the expanded size of the collagen matrix in HCM as compared with secondary LV hypertrophy (i.e., systemic hypertension) and normals proved to be largely independent of all potentially relevant morphologic or clinical disease variables (such as heart weight, septal thickness, age and gender). In addition, we found no consistent relationship among our patients between the amount of interstitial collagen and disorganization of myocardial architecture, suggesting that the size of the interstitium is independent of (and not determined by) disordered myocyte architecture (26). Second, infants with HCM who died <5 months of age had already showed greater than normal amounts of total and interstitial collagen. The additional finding that these infants had 3.5 times less collagen than older children and adults with HCM also suggests a remodeling process in which LV interstitial collagen compartment expands as the ventricular wall thickens during childhood with accelerated growth and development (28). Third, the morphologic appearance and architectural organization of individual collagen fibrils in HCM that constitute the complex connective tissue framework of the matrix differed distinctly from that in normal hearts and those from patients with severe hypertension and LV hypertrophy. Finally, the distribution of interstitial collagen across the transmural tissue sections in adults and young...
children with HCM was atypical by virtue of its concentration in the central portion of the wall rather than in those areas adjacent to the left and right ventricular cavities.

**Clinical significance of collagen matrix.** The expanded collagen matrix compartment in the ventricular septum can potentially explain certain clinical and functional features of HCM. While HCM is frequently associated with impaired LV relaxation and filling (1–3,29–31), there is some evidence that diastolic dysfunction is largely unrelated to the magnitude or distribution of LV hypertrophy (30), suggesting a contributory role for factors other than myocyte hypertrophy. Indeed, it is possible that the expanded and morphologically abnormal collagen matrix in HCM may affect the rate and extent of myocyte relaxation and filling of the LV chamber. Also, the increased amounts of collagen present in the perivascular compartment of these patients provide a possible structural explanation for the impaired coronary vasodilator reserve and myocardial ischemia described in HCM (in the absence of epicardial coronary artery disease) (32,33).

**Prior studies of collagen.** Previous studies have addressed the general issue of fibrosis in HCM (15–20,24). The vast majority of these were, however, largely concerned with pathologic scars (replacement fibrosis) (20), and assessed fibrosis by semiquantitative grading methods (15–18,24), utilized small tissue samples from the LV obtained by biopsy or at operation (19,24) or did not focus on the interstitial (matrix) collagen compartment with collagen-specific staining techniques (15–18,24). In contrast, in the present study, we specifically analyzed interstitial and perivascular collagen in a quantitative fashion using picrosirius staining, and in large transmural tissue sections representative of the LV (exclusive of pathologic scarring). We minimized the chances of encountering replacement fibrosis, however, by limiting our study to a relatively homogeneous group of young patients who died suddenly as the first manifestation of HCM in whom congestive symptoms and cardiac dysfunction were absent.

Of note, Factor et al. (19) described the matrix connective tissue morphology in surgically resected specimens of myocardium from patients with HCM using collagen-specific staining (silver impregnation method). However, the small tissue specimens removed at myotomy-myectomy were not of sufficient size to permit quantitation of the volume fraction of the interstitial compartment as described here, nor to be certain whether the observed collagen abnormalities were truly representative of the LV. Also, because these observations were confined to severely symptomatic patients (who had undergone operation), tissue samples included pathologic scarring as well as interstitial fibrosis.

Our observation that interstitial collagen in HCM was more extensive than in normal hearts or in patients with systemic hypertension and secondary LV hypertrophy differs from that of some other reports in which the size of the interstitial compartment in HCM was described as similar to normals (15) or hypertensives (16,24), probably due to the use of less precise methods of collagen quantitation utilizing other than collagen-specific stains. Most of these studies (15–18,20,24,34) employed staining techniques (such as Masson’s trichrome) that permit examination of pathologic fibrosis rather than the thin collagen fibers of the matrix (35). On the other hand, other authors have reported that the quantities of fibrous tissue in HCM significantly exceed that in the hearts of hypertensive patients (17,19). Also, some prior studies have shown greater than normal amounts of fibrous tissue in hearts with aortic stenosis (36).

**Methodology utilized.** Picrosirius red staining used in the present study has proved to be a reliable method for quantifying the amount of collagen in myocardial tissue. Indeed, assessment of the volume fraction of myocardial collagen by this technique has been shown to correlate with the hydroxyproline content of tissue (27). Sirius red F3BA is a strong anionic dye with an elongated molecule and six sulphonic acid groups, which at low pH binds to lysine and hydroxyproline (21), the major components of the collagen molecule. Picric acid prevents staining of noncollagenous elements, and therefore, picrosirius red selectively stains collagen. Other histologic stains (such as hematoxylin and eosin or Masson’s trichrome) do not stain matrix components selectively, and consequently may underestimate the amount of collagen in tissue sections (35). The computerized videodensitometry method we used to quantitate collagen content in tissue sections has been shown to be more accurate than other techniques, such as visual grading or point counting-grid methods (23,35).

Polarization microscopy in combination with picrosirius red staining is a powerful method for the morphologic assessment of the collagen matrix. Consequently, even very thin collagen fibers can be identified in tissue sections stained with picrosirius red and viewed by polarization microscopy. Although collagen fiber structure has also been studied by the silver impregnation technique (37) and electron microscopy (38), these techniques do not permit the quantification of myocardial collagen within tissue sections.

**Significance of findings to basic molecular defects in HCM.** HCM is a primary cardiac disease with a heterogeneous clinical and morphologic expression for which disease-causing mutations in five major genes, each encoding proteins of the cardiac sarcomere, have been identified (1,3–6). These laboratory observations have suggested a unifying principal by which HCM can be regarded as a single etiological entity and a disease of the sarcomere (3–5). However, the present findings describing an expanded and morphologically abnormal collagen matrix compartment in HCM are consistent with the view that the phenotypic expression of HCM is not limited to the abnormal structure and arrangement of cardiac muscle cells (1,9). Indeed,
several other prominent morphologic features of HCM also importantly involve connective tissue elements. For example, enlargement and elongation of the mitral valve leaflets (11–13) may be particularly marked in many patients, independent of the magnitude of LV hypertrophy. In addition, the “small vessel disease” in patients with HCM, characterized by numerous intramural coronary arteries with apparently narrowed lumen and thickened walls due to medial hypertrophy (10,29), is probably responsible, in part, for impaired coronary reserve and myocardial ischemia (30,31).

These morphologic abnormalities prominently involving connective tissue elements, which are common in HCM, suggest that the phenotypic expression of this disease may be importantly influenced by factors other than the many reported disease-causing mutations in genes encoding sarcomeric proteins (1,3–6), such as modifier genes (possibly angiotensin-I converting enzyme genotypes) (4–6), or presently undefined environmental variables.

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