Collagen Remodeling of the Pressure-Overloaded, Hypertrophied Nonhuman Primate Myocardium


Cardiac muscle is tethered within a fibrillar collagen matrix that serves to maximize force generation. In the human pressure-overloaded, hypertrophied left ventricle, collagen concentration is known to be increased; however, the structural and biochemical remodeling of collagen and its relation to cell necrosis and myocardial mechanics is less clear. Accordingly, this study was undertaken in a nonhuman primate model of left ventricular hypertrophy caused by gradual onset experimental hypertension. The amount of collagen, its light microscopic features, and proportions of collagen types I, III, and V were determined together with diastolic and systolic mechanics of the intact ventricle during the evolutionary, early, and late phases of established left ventricular hypertrophy (4, 35, and 88 weeks, respectively). In comparison to controls, we found 1) increased collagen at 4 weeks, as well as a greater proportion of type III, in the absence of myocyte necrosis; 2) collagen septae were thick and dense at 35 weeks, while the proportion of types I and III had converted to control; 3) necrosis was evident at 88 weeks, and the structural remodeling and proportion of collagen types I and III reflected the extent of scar formation; and 4) unlike diastolic myocardial stiffness, which was unchanged at 4, 35, or 88 weeks, the systolic stress-strain relation of the myocardium was altered in either a beneficial or detrimental manner in accordance with structural remodeling of collagen and scar formation. Thus, early in left ventricular hypertrophy, reactive fibrosis and collagen remodeling occur in the absence of necrosis while, later on, reparative fibrosis is present. In this study, the remodeled collagen matrix appeared responsible for variations in force generation observed during various phases of left ventricular hypertrophy. (Circulation Research 1988;62:757-765)

In the adult heart, left ventricular hypertrophy (LVH) is primarily the result of myocyte growth. However, the volume of the interstitial compartment is also increased. Using either the hydroxyproline assay or morphometric analysis, it has been shown that the concentration of collagen is increased in man and rat with LVH secondary to a chronic pressure overload. At present, it is uncertain whether such enhanced collagen formation results from greater de novo synthesis, myocyte necrosis, or both. Moreover, the structural and biochemical features of the remodelled collagen matrix in LVH are largely unknown. Nevertheless, because collagen represents a relatively nonelastic element, particularly type I collagen, and others have suggested that it may account for abnormalities in myocardial mechanics that appear with LVH. To further examine this hypothesis, several issues must be systematically addressed. These include characterizing the structural and biochemical transformation of fibrillar collagen that occurs during the hypertrophic process, as well as defining the interrelation between collagen remodeling, myocyte necrosis, and abnormalities in myocardial mechanics. Accordingly, this study was undertaken in a nonhuman primate model of LVH secondary to a chronic pressure overload of gradual onset. The morphological and morphometric features of the collagen matrix and the proportion of collagen types I, III, and V were examined during the evolutionary, early, and late phases of established LVH and compared with normotensive controls. At each interval, the mechanical behavior of the left ventricular chamber and myocardium were determined in both the instrumented in vivo and in vitro heart preparations. The ultrastructural remodeling of the matrix was also examined at each interval, and these results have recently been reported.

Materials and Methods

Animal Model

An experimental model of systemic hypertension, developed by Page in dogs and characterized by Ferrario, was adapted to the long-tailed adult macaque (Macaca fascicularis) weighing 4.0 ± 1.2 kg as previously reported. In our model, one kidney was wrapped in cellophane, and the contralateral kidney was left in place. Systemic hypertension occurs over...
weeks. The animal colony was initiated in November 1983 and completed in October 1986. It consisted of 22 normotensive, unoperated controls (Group 1) and 35 animals with hypertension. Hypertensive animals were killed according to the following schedule: 1) 4 ± 2 weeks, chosen to represent the evolutionary phase of LVH (15 animals; Group 2); 2) 35 ± 6 weeks, representing the early phase of established LVH (10 animals; Group 3); and 3) 88 ± 7 weeks, representing the late phase of established LVH (10 animals; Group 4). Given the expected longevity (approximately 25 years) of these animals relative to man, the duration of hypertrophy in Groups 2, 3, and 4 would be equivalent to 0.2, 2, and 5 human years, respectively. Control (unoperated) animals were killed at equivalent time periods. In Groups 2 and 3, daily dietary sodium intake was 1 g, while in Group 4, sodium intake was increased to 10 g at week 48.

LVH in this nonhuman primate model was defined according to the ratio of left to right ventricular weights. In 94 normal human hearts, Bove et al.27 found this ratio to average 2.68 with 95% confidence limits varying from 2.1-3.3. Therefore, we selected a ratio of >3.3 as indicative of LVH. Differences in body weight (2.2-7.7 kg) and habitus made this the preferred criteria for LVH in this model. Right ventricular weight was not changed from control in any of the experimental groups (vide infra).

Evaluation of Diastolic and Systolic Mechanical Properties

The evaluation of mechanical properties was performed using in vivo and in vitro preparations.

In vivo preparation. All animals were studied as an open-chest preparation (median sternotomy) with anesthesia (ketamine) and artificial ventilation. Left ventricular pressure, central aortic pressure, and ascending aortic blood flow were measured by a needle-tip pressure transducer (SPR-230, Millar, Houston, Texas) inserted through an avascular region of the left ventricular free wall, a catheter-tip pressure transducer (SPR-249, Millar) introduced via a femoral artery, and an electromagnetic flow probe (Biotronix, Silver Spring, Maryland), respectively. Surface ECG was recorded using limb leads. Data (pressures, flow, and ECG) were recorded (paper and analog tape) under baseline condition for steady-state ejecting beats and the corresponding isovolumetric beat (i.e., zero aortic flow) obtained by manual occlusion of the ascending aorta during the previous diastole (Figure 1). The isovolumetric contraction was judged acceptable if the following criteria were met (Figure 1): zero aortic flow with continuously declining aortic pressure and no abnormal rise in ventricular pressure of the preceding ejecting beat.

In vitro preparation. At the end of the in vivo study, the heart was quickly removed and perfused via the ascending aorta at 100 mm Hg pressure with an oxygenated (95% O₂-5% CO₂) modified Krebs-Henseleit solution (37° C, pH 7.4) containing 10 U/l insulin and 1,000 U/l heparin. The left ventricle was vented and a compliant balloon, mounted on a stiff cannula, was positioned in the chamber via the mitral orifice after the chordal attachments of the mitral valve were severed. Balloon pressure, monitored by a fluid-filled catheter-transducer system (Statham P23Gb, Gould, Cleveland, Ohio), was taken to represent left ventricular chamber pressure while balloon volume (i.e., fluid volume introduced via the side-arm of the cannula plus the volume of the balloon) was taken to represent left ventricular chamber volume. Steady-state isovolumetric left ventricular pressures were recorded for different settings of balloon volumes (0.2-ml increments) within the end-diastolic pressure (EDP) range of 0-20 mm Hg. The unstressed left ventricular volume (V₀) corresponding to EDP of 0 mm Hg was measured.

Systolic mechanical properties. Maximum systolic elastance (Eₘₐₓ), a load independent index of contractility, was used to quantify the systolic mechanical property of the left ventricular chamber. Eₘₐₓ for the in vivo preparation was calculated as follows: Assuming equal end-diastolic volumes (EDV) for the ejecting and isovolumetric contractions, Eₘₐₓ is given by:

\[ E_{\text{max}} = \frac{(P_0 - P_s)}{SV} \]  

where \( P_0 \), \( P_s \), and \( SV \) are peak pressure in the isovolumetric beat, end-systolic pressure in the ejecting beat, and stroke volume (computed by integrating aortic flow), respectively. End-systole for the ejecting
beating was defined as the left-most upper corner of the ejecting pressure-volume loop and was identified automatically by computer algorithm. \( E^\text{m} \) for the in vitro preparation was computed as the slope of the peak isovolumetric pressure-volume relation.

To calculate myocardial mechanical properties, we converted pressure (P, millimeters mercury) and volume (V, milliliters) data to midwall circumferential stress (\( \sigma \), grams per centimeters squared) and Lagrangian strain (\( \varepsilon \)) assuming spherical geometry for the left ventricle.\(^{31}\)

\[
\sigma = \left[ 1.36 \cdot P \cdot V^{2/3} \right] \left( \frac{V + V_n}{V_n} \right)^2 - V^{2/3}
\]

\[
\varepsilon = \left[ \frac{V^{10} + (V + V_n)^{10}}{V_n^{10}} \right] - 1
\]

where \( V_n \) is left ventricular muscle volume (\( = 0.943 \) left ventricular weight), and \( V_o \) is the unstressed left ventricular volume measured in the in vitro preparation. For the in vitro preparation, peak isovolumetric stresses and corresponding strains were calculated by substituting peak isovolumetric pressures and chamber volumes (both directly measured) for \( P \) and \( V \) in Equations 2 and 3. The slope of peak isovolumetric stress-strain relation was used as an index of myocardial contractility and is denoted by \( E^\text{m} \). For the in vivo preparations, chamber volume was not measured; instead, it was indirectly calculated from the measured EDP and EDP-EDV relation obtained in the in vitro phase of the study (see below). End-systolic volume was then computed by subtracting measured stroke volume from EDV. Finally, \( E^\text{m} \) was calculated from Equation 1 after replacing the pressures and volumes by corresponding stresses and strains using Equations 2 and 3.

### Diastolic mechanical properties

Left ventricular end-diastolic chamber and myocardial stiffnesses were determined in the in vitro preparation. Experimentally measured EDP-EDV data were fitted to a monoeponential function using the following equation:

\[
EDP = A \left( e^{(EDV-V_o)} - 1 \right)
\]

where \( A \) and \( B \) were estimated using an iterative, nonlinear regression program (BMDP-3R). Left ventricular end-diastolic chamber stiffness (\( E^\text{ch} \)) is defined as the derivative of EDP with respect to EDV. Since the EDP-EDV relation is nonlinear, we will present three values of \( E^\text{ch} \) for each heart corresponding to EDPs of 5, 10, and 20 mm Hg, respectively. Left ventricular diastolic myocardial stiffness (\( E^\text{myo} \)) was computed in a similar manner after converting pressure-volume data to stress-strain using Equations 2 and 3. Three values of \( E^\text{myo} \) will be presented for each heart corresponding to the strains of 0%, 5%, and 10%, respectively.

### Collagen Composition of the Myocardium

The isolated hearts were removed from the perfusion apparatus, and their atria, great vessels, and all valves trimmed away. The right ventricle was separated from the left ventricle plus septum, and the right and left ventricles were then weighed. Thereafter, coronal sections taken from the equator of the left ventricle were sent for light and scanning electron microscopy. A third section was used to assess the hydroxyproline concentration of the myocardium. The remainder of the left ventricle was used to assess collagen types (vide infra). The morphological, morphometric, and biochemical evaluation of the myocardium was performed by investigators blinded as to the nature of the experimental groups.

**Morphometry.** A semiautomatic computer-assisted procedure was used for the morphometric studies. Five micron-thick paraffin sections, stained with Gomori's trichrome, were placed in a projection microscope and the image transferred to a mirror positioned at a 45° angle and onto a digitizing pad connected to a cursor-computer assembly. Segments representing connective tissue and muscle were manually traced and identified. The computer was programmed to calculate the area traced by the cursor as the volume fraction of collagen and muscle. One section was scanned from each heart. From this section, 16 fields were randomly selected and traced and the mean calculated. For each field, two determinations of collagen volume fraction were made: one determination included regions that surrounded arteries and arterioles while the other determination specifically avoided such regions.

Myocardial injury was assessed by light microscopy using the following criteria: 1) inflammatory or round cell infiltration, 2) confluent areas of collagen indicative of muscle fiber replacement, and 3) loss of muscle fiber nuclei and cross-striations.

**Biochemistry.** The coronal section provided for hydroxyproline assay was divided into three segments representing the epicardium and endocardium and remaining midwall. Each segment was dried in a 60° C oven overnight, and its fat twice removed by extraction with a 2:1 mixture of chloroform and methanol. The solvent used in each 2-hour extraction had at least 10 times the volume of myocardium. The fat-free sections were again dried at 60° C for 2 hours and their weights recorded. These dried segments were heated with 6N hydrochloric acid at 100° C overnight to hydrolyze collagen into its component amino acids. Hydroxyproline content was measured spectrophotometrically by its reaction with Ehrlich's reagent according to the method of Stegemann\(^{32}\) with the assumption that collagen contains 13.4% hydroxyproline. The collagen content of the specimen was calculated by multiplying the hydroxyproline content with the constant 7.46. The concentration of collagen was expressed as milligrams of collagen per 100 milligrams dry weight.

The myocardium sent for collagen typing was prepared as follows: sections were cut into small pieces and lyophilized. The lyophilized material was pulverized, and an aliquot was used for solubilization and subsequent determination of collagen types as previously reported.\(^{33,34}\) Salt soluble and acid soluble collagen were first obtained.\(^{35}\) In brief, samples were initially extracted with 1 M NaCl in 0.05 M Tris buffer, pH 7.4, containing protease inhibitors for 24 hours at 4° C. The supernatants were separated by centrifuga-
phoresis of pepsin-solubilized collagen from the myocardium of I and III collagens.

Solubilized collagen of the nonhuman primate myocardium was obtained from bovine skin. Lanes 1 and 2 are standard type III and I collagen from bovine skin while 70% of the collagen was solubilized by the three pepsin extractions. No differences in collagen solubility were seen between hearts obtained from control or hypertensive animals. The three pepsin extracts were pooled and collagen precipitated by adding sodium chloride and acetic acid yielded only 2% of total collagen while 70% of the collagen was solubilized by the three pepsin extractions. No differences in collagen solubility were seen between hearts obtained from control or hypertensive animals. The three pepsin extracts were pooled and collagen precipitated by adding sodium chloride to 0.8 M final concentration at 4 °C and collected by centrifugation. The collagen extracts were pooled and collagen precipitated by adding sodium chloride and acetic acid and dialyzed against 1 M NaCl in 0.05 M Tris, pH 7.4.

Extracts were next determined on these samples using the interrupted sodium dodecyl sulfate (SDS) slab gel electrophoresis procedure of Sykes et al. 37 Collagen types were next determined on these samples using sodium dodecyl sulfate (SDS) gel electrophoresis at two different concentrations to make sure a linear relation existed between the amount of collagen applied and the staining of the gels. A representative SDS gel electrophoretic pattern showing α chains of collagen types I, III, and V obtained from pepsin extracts are shown in Figure 2. The assumption made by this analysis is that only α chains are used to quantitate collagen types. The proportions of β and γ chains are assumed to be similar as most fibrillar collagens have similar crosslinks. In this connection, no differences in collagen solubility were noted between control and hypertrophied hearts.

**Table 1. Collagen Extractions From the Left Ventricle of the Long-Tailed Macaque**

<table>
<thead>
<tr>
<th>Extractant</th>
<th>Hydroxyproline* (mg)</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 M NaCl</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>0.5 M Acetic acid</td>
<td>0.02</td>
<td>1</td>
</tr>
<tr>
<td>Pepsin (1 mg/ml)</td>
<td>1.14</td>
<td>71</td>
</tr>
<tr>
<td>DTT</td>
<td>0.03</td>
<td>2</td>
</tr>
<tr>
<td>Pepsin after DTT</td>
<td>0.19</td>
<td>12</td>
</tr>
<tr>
<td>Residue</td>
<td>0.21</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>1.60</td>
<td>100</td>
</tr>
</tbody>
</table>

*Average of two extractions. DTT, dithiothreitol.

Next, and similar to the technique of Medugorac and Jacob, three successive pepsin (1 mg/ml) digestions were performed on the residues at 4 °C in 0.5 M acetic acid for a minimum of 24 hours for each digestion. Table 1 indicates the relative effectiveness of the extraction procedure. The initial extractions using sodium chloride and acetic acid yielded only 2% of total collagen while 70% of the collagen was solubilized by the three pepsin extractions. No differences in collagen solubility were seen between hearts obtained from control or hypertensive animals. The three pepsin extracts were pooled and collagen precipitated by adding sodium chloride to 0.8 M final concentration at 4 °C and collected by centrifugation. The collagen precipitate resolubilized in 0.5 M acetic acid and dialyzed against 1 M NaCl in 0.05 M Tris, pH 7.4.

Collagen types were next determined on these samples using the interrupted sodium dodecyl sulfate (SDS) slab gel electrophoresis procedure of Sykes et al. 37 Standard purified types I and III collagen were also included in the SDS gel electrophoresis. After electrophoresis, the gels were stained with Coomassie blue and subsequently destained in a solution of acetic acid and methanol. Under the conditions of electrophoresis, clear separation of α1 and α2 chains of type I, α chains of type III, and α chains of type V were obtained. The stained gels were scanned at 580 nm, and the recordings of the areas under each peak were determined using a planimeter. The areas representing α chains were used to calculate the relative percentage of types I, III, and V collagen. Aliquots of each sample were run on SDS gel electrophoresis at two different concentrations to make sure a linear relation existed between the amount of collagen applied and the staining of the gels. A representative SDS gel electrophoretic pattern showing α chains of collagen types I, III, and V obtained from pepsin extracts are shown in Figure 2. The assumption made by this analysis is that only α chains are used to quantitate collagen types. The proportions of β and γ chains are assumed to be similar as most fibrillar collagens have similar crosslinks. In this connection, no differences in collagen solubility were noted between control and hypertrophied hearts.

**Statistical Analysis**

Hypertrophied hearts (i.e., left-to-right ventricular weight ratio > 3.3) were grouped as noted earlier. Results for the control and hypertrophied hearts were averaged and presented as mean ± SD. Intergroup comparisons were made using the one-factor analysis of variance and the modified t test.

**Results**

**Hemodynamic Profile and Left Ventricular Hypertrophy (Table 2)**

In the unoperated control animals killed at the same time intervals as the hypertensive animals, intra-arterial systolic pressure was unchanged from baseline and, therefore, all control animals have been combined and considered Group 1. Systolic, diastolic, and mean arterial pressures for the hypertensive animals studied at 4, 35, and 88 weeks, rose significantly above control.

In Groups 3 and 4, systolic pressure was significantly greater than Group 2, which is in keeping with the gradual onset of the left ventricular pressure overload. No difference in intra-arterial systolic pressures was observed between animals studied at 35 and 88 weeks or in diastolic and mean arterial pressures in Groups 2, 3, or 4.

**Table 2. Hemodynamic Profile and Hypertrophy in the Hypertensive Long-Tailed Macaque**

<table>
<thead>
<tr>
<th>Weeks of hypertension</th>
<th>Control (Group 1)</th>
<th>4 (Group 2)</th>
<th>35 (Group 3)</th>
<th>88 (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>111 ± 13</td>
<td>159 ± 20*</td>
<td>183 ± 19*</td>
<td>177 ± 20*</td>
</tr>
<tr>
<td>DBP</td>
<td>75 ± 9</td>
<td>106 ± 15*</td>
<td>103 ± 10*</td>
<td>101 ± 15*</td>
</tr>
<tr>
<td>MBP</td>
<td>90 ± 9</td>
<td>128 ± 20*</td>
<td>128 ± 15*</td>
<td>132 ± 18*</td>
</tr>
<tr>
<td>LV/RV</td>
<td>2.9 ± 0.2</td>
<td>3.9 ± 0.4*</td>
<td>4.1 ± 0.4*</td>
<td>4.0 ± 0.5*</td>
</tr>
<tr>
<td>RV</td>
<td>4.3 ± 1.9</td>
<td>3.3 ± 1.3</td>
<td>4.0 ± 1.6</td>
<td>4.3 ± 1.0</td>
</tr>
</tbody>
</table>

*p<0.001, Group 1 vs. 2, 3, or 4. TP<0.05, Group 2 vs. 3 or 4. SBP, DBP, MBP, systolic, diastolic, and mean arterial blood pressure (mm Hg), respectively; LV/RV, ratio of left (plus septum) (LV) and right ventricular (RV) weight.
In control animals, the ratio of left to right ventricular weight averaged 2.9 ± 0.2; 3.3 is two standard deviations above this mean and is in keeping with our criteria for LVH selected at the outset of the study. A 30–40% increase in left ventricular mass was found in each of the hypertensive groups. The increment in ventricular weight ratio was due to an increase in left ventricular weight since no difference in right ventricular weight (3.9 ± 1.2 g) was noted between the control and experimental groups. The degree of LVH was similar in Groups 2, 3, and 4, indicating that the extent of LVH did not vary with the duration of the pressure overload.

**Left Ventricular Chamber Remodeling and Pump Function (Table 3)**

The data presented in Table 3 correspond to the baseline condition of the in vivo preparation. At 4 weeks of LVH, EDV was less than control; EDV was not statistically different from control in Groups 3 and 4. The V and EDV/left ventricle mass ratio were each significantly reduced during the evolutionary phase of LVH, and each returned to control at 35 weeks. A further enlargement of the left ventricular chamber was apparent at 88 weeks but did not reach statistical significance.

Cardiac output was significantly reduced from control in Group 2 but returned to control levels in both Groups 3 and 4. Heart rate (not shown) was not significantly different between control and any of the hypertensive groups. EDP was significantly elevated above control at 4 weeks of LVH when EDV/left ventricle mass ratio was reduced. EDP was no different from control at 35 and 88 weeks with a restoration of EDV and the EDV/left ventricle mass ratio although heterogeneity was observed in Group 4 (vide infra).

**Collagen Remodeling in LVH (Table 4)**

At 4, 35, and 88 weeks, the morphometrically determined collagen volume fraction was increased significantly in regions of the midwall myocardium devoid of arteries and arterioles. No further increase in collagen was apparent in these areas beyond 4 weeks. Moreover, there was no preferential distribution of collagen within the subendocardium. When regions that included larger blood vessels were included in the morphometric assessment of collagen, a significant increase in total collagen volume fraction was found in each group, however, there was still no additional rise in collagen observed between Group 2, 3, or 4 hearts.

In control hearts, thin collagen fibrils were found between and around closely arranged muscle fibers. During the evolutionary phase of LVH, heavier fibrils, located between normally arranged muscle bundles, were now evident while fine fibrils crossing perpendicular to the long axis of muscle fibers had disappeared. Fine fibrils were again noted during the early phase of established LVH, but the most predominant feature here was dense collagenous septa, located between more loosely arranged muscle bundles, and a dense perivascular network of collagen. In the late phase of established LVH, collagen appeared similar to Group 3 in many of the hearts. In several of these Group 4 hearts, collagen septa between muscle fibers had become quite thick, with muscle bundles appearing smaller and disorganized. In these hearts, the first evidence of patchy muscle necrosis was detected with replacement fibrosis; cell fragmentation and white cells were not observed. However, in the remaining Group 4 hearts, collagen septa were relatively smaller, and the areas of necrosis less extensive and in an earlier stage of development.

The hydroxyproline and thereby collagen concentration within the left ventricular wall was significantly increased above control in each of the experimental groups with LVH, but no difference in concentration could be detected between Groups 2, 3, or 4. The findings at 88 weeks were once again more heterogeneous, however. Collagen concentration was > 7 mg/100 mg dry wt in hearts with scar formation. Type I was the dominant fibrillar collagen in all hearts while type V represented < 5%. In controls, type I accounted for 85 ± 5% of the fibrillar collagen while type III was 11 ± 4%. At 4 weeks, the percent of type I was significantly reduced while type III had risen significantly indicative of early collagen remodeling. The proportions of collagen types I and III had returned to control levels in Groups 3 and 4. In Group 4 hearts with less advanced scarring, type III collagen was

### Table 3. Architectural Remodeling and Pump Function of the Hypertrophied Long-Tailed Macaque Left Ventricle

<table>
<thead>
<tr>
<th>Weeks of hypertension</th>
<th>Control (Group 1)</th>
<th>4 (Group 2)</th>
<th>35 (Group 3)</th>
<th>88 (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDV 4.1 ± 1.1</td>
<td>2.8 ± 1.6*</td>
<td>4.3 ± 1.5</td>
<td>5.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>V 3.0 ± 0.9</td>
<td>1.8 ± 1.0*</td>
<td>3.0 ± 0.9</td>
<td>3.5 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>EDV/M 0.4 ± 0.2</td>
<td>0.2 ± 0.1*</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>CO 536 ± 39</td>
<td>323 ± 114*</td>
<td>521 ± 201</td>
<td>503 ± 98</td>
<td></td>
</tr>
<tr>
<td>EDP 8 ± 5</td>
<td>17 ± 47</td>
<td>10 ± 5</td>
<td>11 ± 6</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05; p<0.01, Group 1 vs. 2, 3, or 4.

EDV, V, EDV/M, left ventricular end-diastolic and unstressed volumes (ml), respectively; EDV/M, end-diastolic volume to left ventricular mass ratio (ml/g); CO, cardiac output (ml/min); and EDP, left ventricular end-diastolic pressure (mm Hg).

### Table 4. Biochemical Remodeling of Collagen in the Hypertrophied Long-Tailed Macaque Left Ventricle

<table>
<thead>
<tr>
<th>Weeks of hypertension</th>
<th>Control (Group 1)</th>
<th>4 (Group 2)</th>
<th>35 (Group 3)</th>
<th>88 (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVF-NV 4.1 ± 2.4</td>
<td>7.3 ± 4.6*</td>
<td>7.9 ± 2.7</td>
<td>6.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>CVF-T 10.6 ± 3.7</td>
<td>15.0 ± 5.3*</td>
<td>19.5 ± 4.2</td>
<td>16.8 ± 2.1*</td>
<td></td>
</tr>
<tr>
<td>Coll</td>
<td>5.2 ± 0.8</td>
<td>6.7 ± 1.3</td>
<td>7.1 ± 2.4</td>
<td>6.0 ± 1.9</td>
</tr>
<tr>
<td>Type I</td>
<td>85 ± 5</td>
<td>81 ± 9</td>
<td>86 ± 7</td>
<td>84 ± 7</td>
</tr>
<tr>
<td>Type III</td>
<td>11 ± 4</td>
<td>16 ± 8*</td>
<td>10 ± 4</td>
<td>14 ± 7</td>
</tr>
<tr>
<td>Type V</td>
<td>3 ± 3</td>
<td>2 ± 4</td>
<td>4 ± 4</td>
<td>2 ± 2</td>
</tr>
</tbody>
</table>

*p<0.05, Group 1 vs. 2, 3, or 4. CVF-NV, collagen volume fraction (%) in regions without arteries and arterioles; CVF-T, total collagen volume fraction (%) including regions with larger blood vessels; Coll, midwall collagen concentration (mg/100 mg dry wt); and type, collagen type (%).

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higher than controls, representing 15-26% of the total and accordingly type I was reduced to 71-85% of the fibrillar collagen pool; alternatively, type I was 88-91%, respectively, in those with scarring.

**Systolic Mechanical Properties in Left Ventricular Hypertrophy**

In vivo $E_{\text{max}}$ for the left ventricular chamber tended to be greater than control animals during the evolutionary phase of LVH, but did not reach statistical significance ($p<0.06$). $E_{\text{max}}$ was no different from control during the early and late phases of established LVH. In vitro $E_{\text{max}}$ was significantly greater than controls at 4 weeks but not at 35 and 88 weeks.

The slope of the systolic stress-strain relation ($E_{\text{smax}}$) of the myocardium studied in vivo and in vitro was reduced at 4 weeks. Thereafter, $E_{\text{smax}}$ was restored to control values. In both the in vivo and in vitro hearts studied at 88 weeks, $E_{\text{smax}}$ tended to be less than controls, suggesting a decline in contractility, but for the group this was not a statistically significant difference. In Group 4 hearts with scarring, in vivo $E_{\text{smax}}$ was $6.9 \times 10^2$ g/cm² while in those with less advanced reparative fibrosis it was $>7.5 \times 10^2$ g/cm².

**Diastolic Stiffness in Left Ventricular Hypertrophy**

At 4 weeks, diastolic chamber stiffness in the isolated heart was significantly increased above control; at 35 and 88 weeks, chamber stiffness had returned to control. Diastolic myocardial stiffness ($E_{\text{ad}}$), determined from the stress-strain relation of the in vitro heart, was not significantly different from control for each of the experimental groups with LVH and over the entire range of strains (0-10%).

**Discussion**

The myocardium is a composite material consisting of myocytes that are tethered and supported within an extensive fibrillar connective tissue network composed largely of collagen. The elasticity of the myocardium is therefore determined by 1) the stress-strain relation of the muscular and collagenous compartments and their relative proportions, 2) the viscoelasticity and proportionality of types I and III collagen, and 3) the structural alignment of collagen fibrils to myocytes.

In man, it is well recognized that left ventricular diastolic and systolic ventricular function are impaired with LVH accompanying a chronic pressure overload. Considerable interest has been directed to the muscular compartment of the myocardium and the role it may play in altering ventricular function. The aim of the present study was to assess the role of the connective tissue compartment in modulating the mechanical properties of the hypertrophied myocardium. We sought to examine the structural and biochemical features of the collagen matrix during the evolutionary and established phases of LVH, and to ascertain their relation to the systolic and diastolic properties of the chamber and the corresponding stress-strain relations of the myocardium. A nonhuman primate (Macaca fascicularis) with experimentally induced hypertension was developed with the view that it would most closely represent the hypertrophic process seen in man. Based on the observations garnered from this study, we have shown that the heart’s collagen matrix is indeed remodeled during LVH, and this remodeling may account for alterations in the mechanical properties of the myocardium. Our findings pertaining to the ultrastructural remodeling of the collagen matrix during LVH in these animals have been reported elsewhere and will only be cited herein when they serve to reinforce structure-function relations.

Before discussing the findings of this cross-sectional study we should consider its shortcomings. We obviously could not perform a longitudinal study in these animals given the volume of tissue required for individual assays. Despite its multidisciplinary nature we did not examine the isoform ratio of the contractile protein myosin. In the human primate, myosin is predominantly V and is not transformed further with LVH. The response in muscle fiber alignment in this model of LVH was likewise not considered. Previous observations from our laboratory indicate that a realignment of muscle fibers does not occur during the

**Table 5. Systolic Mechanical Properties of the Hypertrophied Long-Tailed Macaque Left Ventricle**

<table>
<thead>
<tr>
<th>Weeks of Hypertension</th>
<th>Control (Group 1)</th>
<th>4 (Group 2)</th>
<th>35 (Group 3)</th>
<th>88 (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In Vivo Heart</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>24.0±4.7</td>
<td>39.3±17.3</td>
<td>29.6±8.1</td>
<td>29.8±16.2</td>
</tr>
<tr>
<td>$E_{\text{smax}}$</td>
<td>11.4±7.0</td>
<td>1.8±1.4*</td>
<td>10.3±10.0</td>
<td>7.3±1.1</td>
</tr>
<tr>
<td><strong>In Vitro Heart</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>17.4±7.4</td>
<td>47±34*</td>
<td>22±8</td>
<td>19±6</td>
</tr>
<tr>
<td>$E_{\text{smax}}$</td>
<td>9.4±2.0</td>
<td>4.7±1.8*</td>
<td>8.1±3.1</td>
<td>7.8±2.0</td>
</tr>
</tbody>
</table>

$p<0.05$, Group 1 vs. 2, 3, or 4.

$E_{\text{max}}$, maximum systolic elastance (mm Hg/ml); $E_{\text{smax}}$, slope of stress-strain relation (g/cm² x 10²).

**Table 6. Diastolic Mechanical Properties of the Hypertrophied Long-Tailed Macaque Left Ventricle Studied In Vitro**

<table>
<thead>
<tr>
<th>Weeks of Hypertension</th>
<th>Control (Group 1)</th>
<th>4 (Group 2)</th>
<th>35 (Group 3)</th>
<th>88 (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{\text{ad}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDP</td>
<td>6.1±1.6</td>
<td>20.1±14.3*</td>
<td>8.2±3.8</td>
<td>6.9±4.1</td>
</tr>
<tr>
<td>10</td>
<td>8.2±2.6</td>
<td>21.1±13.3*</td>
<td>9.7±3.5</td>
<td>8.4±4.2</td>
</tr>
<tr>
<td>20</td>
<td>12.5±5.1</td>
<td>23.1±11.6*</td>
<td>13.0±3.6</td>
<td>11.4±4.4</td>
</tr>
<tr>
<td>$E_{\text{ad}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain</td>
<td>0.4±0.2</td>
<td>0.8±0.2</td>
<td>0.7±0.2</td>
<td>0.7±0.5</td>
</tr>
<tr>
<td>5</td>
<td>1.1±0.7</td>
<td>1.5±1.9</td>
<td>1.7±0.7</td>
<td>1.8±1.1</td>
</tr>
<tr>
<td>10</td>
<td>8.8±8.5</td>
<td>5.4±1.9</td>
<td>10.8±8.3</td>
<td>10.8±6.3</td>
</tr>
</tbody>
</table>

*p<0.01, Group 1 vs. 2, 3, or 4.

†p<0.001, Group 2 vs. 3, or 4.

$E_{\text{ad}}$, slope of pressure-volume relation (mm Hg/ml) at end-diastolic pressures (EDP) of 5, 10, and 20 mm Hg; $E_{\text{ad}}$, slope of stress-strain relation (g/cm² x 10²) at Lagrangian strains of 0%, 5%, and 10%.
compensatory or decompensatory phases of LVH in the human myocardium. Finally, the results of this study provide only correlative evidence regarding a cause and effect relation between collagen matrix remodeling and alterations in the systolic and diastolic behavior of the myocardium.

The results of our study do provide hitherto unreported information concerning the biochemical nature of collagen remodeling during various phases of LVH together with the corresponding stress-strain relation of the myocardium. The increase in collagen concentration was demonstrable at 4 weeks when systolic arterial pressure was moderately elevated above control. Collagen volume fraction was also increased at 4 and 35 weeks when there was no light microscopic evidence of cell necrosis. Thus, we would infer that during the evolutionary phase of LVH, enhanced collagen formation is initiated de novo by a signal that may or may not be linked to the muscular compartment. The mechanism involved in mediating collagen accumulation may be secondary to fibroblast proliferation, an augmentation in local fibroblast activity, or both, which accompanies an increase in myocardial wall force. Enhanced collagen synthesis is also observed in workload hypertrophy of skeletal muscle. Hence, it may be more appropriate to consider the increase in collagen formation seen in pressure overload LVH as "collagenosis" or reactive fibrosis and to distinguish it from the reparative fibrosis that accompanies cell necrosis later in LVH. The observation that excess collagen is not seen in all forms of hypertrophy, in pressure overload hypertrophy with prior digoxin treatment, or with cardiac denervation suggests that fibroblast and myocyte growth are under separate controls.

In addition to increased collagen accumulation, we found indirect evidence of collagen reabsorption. At 4 weeks of LVH, thin collagen fibrils traversing muscle fibers across their long axis were no longer observed. The disappearance of these fibrils, which may be related to enhanced collagen degradation mediated by the activation of collagenases, would permit the positive slippage of muscle fibers. Such slippage may in fact have been present given the architectural remodeling of the left ventricle we found at 4 weeks, where chamber volume to mass ratio was decreased.

During this evolutionary phase of LVH, collagen matrix remodeling was associated with an increase in "embryonic" type III collagen. This conversion in collagen types appears to be a characteristic feature of early collagen formation. For example, an increase in type III collagen is seen in response to dermal injury and is thought to provide the initial wound structure and substrate for subsequent healing. Type III collagen, however, does not contribute to the tensile strength of the wound, which is provided by type I collagen fibrils. Type III collagen is also increased in the neonatal heart and in the hypertrophy that accompanies copper deficiency. In copper-deficient hearts, myocardial systolic stiffness is reduced. This reduction in the systolic stress-strain relation of the myocardium is similar to our findings at 4 weeks of pressure overload LVH. In addition to the reduction in stiffness, excess type III collagen is associated with the appearance of myocardial aneurysms and rupture. With the appearance of hypertrophy at 4 weeks, it would therefore appear necessary that the structural proteins have a greater tensile strength in order to minimize the dissipation of generated contractile force by hypertrophied myocytes. This would require an increase in the physical dimension of collagen strands, a restoration of stiffer type I collagen and/or enhanced collagen crosslinking. Accordingly, we would interpret the reduction in the slope of the systolic stress-strain relation of the hypertrophied myocardium noted at 4 weeks, in both the in vivo and in vitro heart, to be secondary to a lag in collagen remodeling, the greater proportion of type III collagen, and the slippage of muscle fibers. The augmented maximal systolic elastance (millimeters mercury per milliliter) of the left ventricular chamber noted during this evolutionary phase of LVH, on the other hand, appears to be a reflection of the reduced unstressed volume and the reduced volume to mass ratio of the chamber. A restoration of this ratio, presumably mediated in part by a rise in intravascular volume secondary to perinephritis and increased dietary sodium, returned chamber elastance to control. In the early phase of established LVH, types I and III collagen had again assumed their normal proportions, collagenous septae were denser, and the slope of the systolic stress-strain relation had returned to control. These findings suggest that the tensile strength of the myocardium had been restored by the structural and biochemical remodeling of collagen. Hence, the rate of collagen remodeling relative to myocyte growth does not appear to be proportional. During the evolutionary phase of LVH there was a marked increase in left ventricular mass and a decrease in the volume-to-mass ratio. Once established, the degree of LVH did not then progress further throughout the 88-week period of observation. These findings would suggest that myocyte hypertrophy occurred early and was unidimensional, whereas collagen remodeling was a slower, more continuous process. In addition, our findings underscore the shortcomings of the hydroxyproline assay in monitoring the nature of collagen remodeling. Thiedemann et al have also emphasized that the physical arrangement of collagen fibrils to myocytes is more relevant to myocardial mechanics than the quantity of collagen or the preponderance of collagen located in the perivascular space.

The average concentration of collagen was again unchanged during the late phase of established LVH. Nevertheless, additional structural remodeling had taken place within the matrix. The collagen network was similar to that found at 35 weeks, but now septa between muscle bundles were quite thick. In some regions, there was even the suggestion that muscle bundles were compressed by collagen. The histological features of the myocardium at 88 weeks suggest that
myocardial necrosis and scar formation had occurred although the interrelation between myocytolytic necrosis and collagen deposition remains obscure. Both reactive and reparative fibrosis would appear to account for collagen remodeling in the late phase of established LVH.

Pfeffer and Pfeffer\(^5\) have noted that developed isovolumetric pressure in the left ventricle of rats with genetic hypertrophy was reduced as a function of advancing age and temporarily coincident with progressively greater amounts of myocardial fibrosis.\(^6\) In hearts examined at 88 weeks in our study, where fibrosis appeared advanced, the slope of the isovolumetric stress-strain relation was reduced below control values. The slope of the systolic stress-strain relation determines the limit to muscle fiber shortening during ejection.\(^7\)\(^,\)\(^8\) and consequently, we would expect that as active stiffness declines, so too would stroke volume for any given filling volume. Pfeffer et al\(^9\) and Averill et al\(^9\) have each reported that the cardiac reserve, or increment in stroke volume for increases in left ventricular filling, was reduced in pressure overload LVH, and they attributed this reduction to an increment in myocardial collagen. Thus, we would infer that the heavy collagenous septae, which we observed at 88 weeks of LVH, could be responsible for the appearance of pathologic hypertrophy and left ventricular systolic dysfunction. The exact manner in which this collagen remodeling adversely influences mechanics, however, remains to be elucidated.

The remodeling of the collagen matrix observed in the present study was not associated with alterations in the diastolic mechanical properties of the ventricle. However, Bing et al\(^8\) found that resting tension increased in hypertrophied papillary muscles obtained from an acute pressure-overloaded model in the rat, which they could relate to an increased collagen concentration of the myocardium. Moreover, this abnormality in diastolic stiffness could be prevented by treating rats after aortic constriction with the lathyrogen, \(\beta\)-amino propionitrile.\(^9\) Thiedemann et al\(^1\) have reported that with LVH secondary to renovascular or genetic hypertrophy in the rat, the passive elastic properties of papillary muscle are abnormal and in keeping with the observed structural remodeling of the collagen matrix. Differences between these studies and our own may be related to a greater degree of subendocardial fibrosis that occurred in these other models of pressure overload and would therefore involve the passive elasticity of papillary muscle. We cannot, however, exclude differences in collagen structure between species. In this regard, Borg et al\(^\circ\) observed a denser collagen weave in rat than in hamster myocardium and suggested that this accounts for the greater passive stiffness of the rat myocardium. Notwithstanding this attractive circumstantial evidence, additional studies will need to be conducted to definitively establish whether the altered collagen composition of the hypertrophied myocardium is causally related to alterations in its mechanical behavior during either systole or diastole.

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K T Weber, J S Janicki, S G Shroff, R Pick, R M Chen and R I Bashey

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