Cellular and Ventricular Contractile Dysfunction in Experimental Canine Mitral Regurgitation

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This study was designed to answer two questions. First, does the left ventricular contractile dysfunction resulting from mitral regurgitation (MR) reflect a primary defect in the cardiac muscle cell? Second, what is the basis for any change in cellular contractile function that might be observed? Left ventricular volume overload was produced in 10 dogs by catheter transection of mitral chordae tendineae. Three months later in these and in seven control dogs, left ventricular contractile function was characterized by the end-ejection stress–volume relation (EESVR). Investigators who were blinded to these results then characterized the contractile performance of cardiac muscle cells, or cardiocytes, from these same left ventricles in terms of the viscosity (graded external load)–velocity relation. Finally, the tissue and cellular components of these same left ventricles were analyzed morphometrically. Both the left ventricles from the MR group and their constituent cardiocytes showed marked contractile abnormalities. By matching ventricles with cells from the same MR dogs, ventricular EESVR was correlated with cardiocyte peak sarcomere shortening velocity (SSV). The correlation coefficient between EESVR and SSV was 0.63, but between a size-independent measure of active ventricular stiffness and SSV, it was 0.88. No change in left ventricular interstitial volume fraction was found in MR dogs, but both ventricular and cellular contractile dysfunction strongly correlated with a decreased volume fraction of cardiocyte myofibrils. Last, in an attempt to relate the degree of contractile dysfunction to the hypertrophic response, left ventricular mass in the MR dogs was correlated with both cellular and ventricular contractile indexes; no significant correlation was found. Three conclusions are warranted by these studies. First, chronic left ventricular volume overload from mitral regurgitation leads to contractile defects at both the ventricular and cellular levels, the extent of which correlates well in individual animals. Second, no quantitative interstitial change resulted from MR. Taken together, these two findings strongly suggest that the contractile defect is intrinsic to the cardiocyte. Third, while the contractile abnormality in MR remains undefined, the most basic defects appear to be a combination of myofibrillar loss with the failure of compensatory hypertrophy to occur in response to progressive decrements in cellular and ventricular function. (Circulation Research 1992;70:131–147)

For ventricular hypertrophy in response to hemodynamic overloads in the adult mammal, whether in humans or in nonrodent animal models, the right and left ventricles exhibit a dichotomous response in terms of pump performance: The right ventricle tends to tolerate volume overload well and pressure overload poorly, whereas the left ventricle tends to tolerate volume overload poorly and pressure overload well. This dichotomy raises two questions: First, since there is no a priori reason to assume a major basic difference in the intrinsic...
contractile properties of right versus left ventricular myocardium, does the poor pump performance of the volume-overloaded left ventricle, particularly in terms of systemic perfusion when that volume overload is caused by mitral regurgitation (MR), reflect abnormal loading conditions or, instead, abnormal contractile properties? Second, does any potential contractile deficit in the left ventricle volume overloaded by mitral regurgitation result from a primary defect in the cardiac muscle cell or, instead, from changes in the interstitial environment of this cell?

In an attempt to answer these questions, we have combined two recent developments from these laboratories: a closed-chest canine model of mitral chordal rupture2,3 and a technique that allows the contractile function of isolated cardiac muscle cells, or cardiocytes, to be characterized in terms of sarcomere shortening in response to graded external loads.4 The chordal rupture model exhibits severe mitral regurgitation that, within several months, is associated with both hypertrophy progressing to congestive heart failure and marked dysfunction of the left ventricle as measured in vivo by time-varying elastance indexes.5 The technique for measuring cellular contractile function allows the intrinsic contractile properties of externally loaded cardiocytes to be characterized in the absence of the ordinary in situ contributions from myocardial interstitial, neural, and vascular elements.4 The application of graded external loads to the contracting cardiocyte, which contrasts with the usual characterization of externally unloaded cardiocyte contractions, is important in view of potential changes in intracellular compliance, or cytoskeletal load, during cardiocyte hypertrophy.5

The removal of the native interstitial environment of the cardiocyte before defining contractile behavior is important in view of potential contributions from interstitial changes to the abnormal contractile behavior seen in some forms of cardiac hypertrophy.1 Given that, because of both theoretical and practical concerns about the time-varying elastance model, there is no universally accepted measure of left ventricular contractile function in vivo,6 especially when longitudinal measurements of contractile function are coincident with the marked geometric changes of ongoing volume overload hypertrophy, the combination of these two recent developments provided an opportunity to rigorously test the proposition that in vivo ventricular contractile dysfunction detected by time-varying elastance indexes reflects primarily cellular contractile dysfunction in this pathological entity.

Finally, since contractile function was to be characterized on both the ventricular and the cellular levels, a morphometric analysis of structure was performed on both of these levels to provide potential structural correlates for any functional changes observed.

Materials and Methods

Preparation and Evaluation of the Experimental Model

Seventeen previously unreported adult mongrel dogs of random sex weighing 18–35 kg were studied. After anesthesia was induced by 0.15 ml/kg i.m. droperidol plus fentanyl (Innovar-Vet), the dogs were intubated and allowed to breathe spontaneously through a rebreathing apparatus, which, along with supplemental doses of Innovar-Vet, provided ongoing anesthesia by inhalation of N2O and O2 in a 3:1 ratio. This combination of anesthetic agents has very little effect on cardiac contractile function.7

Ten dogs were instrumented as described before3 for hemodynamic and ventriculographic studies with a pulmonary artery thermodilution Swan-Ganz catheter, a left ventricular angiographic catheter, and an externally and then internally calibrated left ventricular catheter (Millar Instruments, Inc., Houston, Tex.). After baseline pressure recordings, triplicate thermodilution cardiac outputs were obtained, and the values were averaged. β-Blockade with 300 μg/kg/min i.v. esmolol hydrochloride was next instituted to prevent adrenergic effects on left ventricular function and thus render conditions as comparable as possible to those later used for studying contractile function of cardiocytes isolated from these same left ventricles. Left ventricular contractile function was studied as follows. A first left ventriculogram was performed in the 30° right anterior oblique position at 60 frames per second after injection of nonionic radiographic contrast at a rate of 10 ml/sec for 1.5 seconds. The Swan-Ganz catheter was then withdrawn, and a 20-mm balloon catheter was placed in the inferior vena cava. Balloon inflation caused a decrease in venous return, followed by a decrease in aortic pressure; however, to preserve coronary perfusion pressure, aortic diastolic pressure was not allowed to fall below 50 mm Hg. Subsequent balloon deflation then caused an incremental, beat-to-beat rise in venous return and, thus, in systolic and diastolic left ventricular load. Simultaneously, a second left ventriculogram was performed at 60 frames per second after injection of nonionic radiographic contrast at a rate of 6 ml/sec for 5 seconds. This incrementally loaded series of contractions allowed the end-ejection stress–volume relation (EESVR) to be determined from a single contrast injection.

After this baseline study, but as part of the same procedure, MR was created in these 10 dogs via a transvascular approach. As described in detail elsewhere,2 a grasping forceps was introduced through a sheath that had been inserted into the left ventricle from the left carotid artery. Mitral chordae were grasped and transected in successive steps until stroke volume decreased by ≥50%, systemic systolic pressure had fallen to ≤85 mm Hg, and/or pulmonary artery wedge pressure was ≥20 mm Hg. The adequacy of the lesion was then assessed by measuring thermodilution cardiac output and contrast ventriculographic regurgitant and ejection fractions. The adequacy of ongoing β-blockade was then validated by a rise in heart rate of ≤5 beats per minute in response to 1 μg/kg/min i.v. isoproterenol in each dog. Three months after vascular repair and recovery from these procedures, a second analysis of left
ventricular function was done in the same way in these dogs, and a single such analysis was done in a group of seven control dogs.

The effects of MR on the left ventricle in terms of size, mass, and function were assessed by the use of cineangiography. Volume was calculated by the arealength method.8 Left ventricular mass was calculated angiographically; we have shown earlier that this measure of left ventricular mass correlates linearly in both control and MR dogs over a wide range of values with gravimetric mass, with a correlation coefficient of 0.96.3 Regurgitant fraction (RF) was calculated as follows:

\[
RF = \frac{SV_A - SV_v}{SV_A}
\]

where \(SV_A\) is angiographic stroke volume (end-diastolic volume minus end-systolic volume) and \(SV_v\) is stroke volume calculated from thermodilution cardiac output divided by heart rate. End-diastolic volume was taken as the largest angiographic volume. End-systolic volume was taken to be the smallest, or end-ejection, volume, recognizing that, although end-systolic volume and end-ejection volume may not be identical in MR, end-ejection volume at least has the distinct advantage of being subject to exact definition. Wall stress was calculated at end ejection using the formula of Mirsky:10

\[
\text{Stress} = \frac{(p \cdot b)h[1-(h/2b)-(b^2/2a^2)]}{L+h/2} \cdot 1,332 \text{ dyne/cm}^2/\text{mm Hg}
\]

where \(p\) is end-ejection pressure, \(b\) is semiminor axis at end ejection \([D+h/2]\), where \(D\) is diameter, \(a\) is semimajor axis at end ejection \([L+h/2]\), where \(L\) is length, and \(h\) is end-ejection thickness, which was calculated by measuring end-diastolic thickness at the mid anterior wall and then calculating the amount of thickening that occurred from the measured amount of shortening.11 To evaluate left ventricular function, we examined the slope of EESVR; this slope was multiplied by the simultaneously determined angiographic left ventricular mass to obtain a mass-corrected relation (EESVR\textsubscript{mc}), and this slope was multiplied by the simultaneously determined angiographic end-diastolic volume to obtain a volume-corrected relation (EESVR\textsubscript{vc}). The slope of the EESVR was also determined by linear regression using the least-squares method. Finally, we used a size-independent index\textsuperscript{12} of active ventricular stiffness, relating a change in end-ejection stress (\(\sigma\)) to the corresponding change in a measure of strain, that is, the natural logarithm of the reciprocal of wall thickness (1/H):

\[
\sigma = Ce^{k_\text{ss} \ln(1/H)}
\]

This size-independent index (\(k_\text{ss}\)) is the end-systolic elastic stiffness constant of the myocardium; it was obtained by curve-fitting of the end-systolic \(\sigma-\ln(1/H)\) relation to the above equation.

Preparation and Evaluation of the Isolated Cardiocytes

The method used to isolate cardiocytes from the canine left ventricle was modified from a previously reported procedure.\textsuperscript{13} Two days after in vivo evaluation of left ventricular function by one subset of investigators, another subset of investigators, who were not informed of the results of this evaluation, isolated and evaluated cardiocytes from these same left ventricles. The dogs were anesthetized in the same manner as that described for the in vivo studies, but without \(\beta\)-blockade. The hearts were removed quickly via a left lateral thoracotomy and placed in cold, nominally calcium-free buffer of the following composition (mM): NaCl 130.0, KCl 4.8, MgSO\(_4\) 1.2, NaH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 2.5, HEPES buffer 12.0, and glucose 12.5; 6 mg/l insulin was also added. The atria and great vessels were next removed, leaving the left circumflex coronary artery intact. A wedge of left ventricular free wall perfused by either the first or second branch of this artery was then dissected free of the heart, the arterial branch was cannulated, and the specimen was cleared of blood by brief perfusion with buffer warmed to 37°C and gassed with 100% O\(_2\). To dissociate the cardiocytes from the tissue, perfusion was continued for 15 minutes at 37°C by recirculating the buffer, now supplemented with 155 units/ml type II collagenase and 10 \(\mu\)M Ca\(^{2+}\); mean pressure (\(-80\) mm Hg) and pH (\(-7.40\)) were kept within the physiological range. Any undissociated portions of the specimen, primarily the borders and epicardium, were then discarded. The remaining tissue was placed in fresh enzyme-containing buffer, to which was added 3% salt-free bovine serum albumin and 300 \(\mu\)M Ca\(^{2+}\), and minced into \(-2\)-mm cubes. The minced tissue was then gently agitated for 5 minutes at 37°C while being gassed with 100% O\(_2\). The cardiocytes were harvested by drawing off the supernatant in which they were suspended for filtration through 210-\(\mu\)m nylon mesh. They were kept for 1 hour at 37°C and pH 7.4 in collagenase-free buffer supplemented with 2.5 mM Ca\(^{2+}\) before defining contractile function.

The use of laser diffraction techniques for measuring sarcomere motion in isolated cardiocytes is well established.\textsuperscript{14,15} A brief outline of our method\textsuperscript{4} is given below. An aliquot of isolated cardiocytes was added to 4 ml of the 2.5 mM Ca\(^{2+}\) buffer in a well affixed to a glass slide. The cardiocytes came to rest on the bottom of this chamber, which was placed on the stage of an inverted microscope. The buffer was kept at 37±0.1°C by a thermostated heating stage. The cardiocytes were stimulated to contract between platinum wire electrodes by 0.25-Hz, 100-\(\mu\)A DC pulses of alternating polarity. Changes in sarcomere length were measured from movement of the first-order diffraction pattern cast by a substage laser light passing through the sarcomeres of a given cardiocyte onto diametrically opposed optical sensors situated above the microscope stage, as shown in Figure 1.
Inclusion of methylcellulose in the cardiocyte superfusate was used to impose graded external loads on the cells during contraction, as we have described in detail before. Briefly, 2% solutions of methylcellulose of increasing polymer length were prepared in the standard 2.5 mM Ca\(^{2+}\) buffer. The viscosity of each methylcellulose solution was measured at 37°C by both a Brookfield viscometer and by falling ball viscometry; the two techniques produced identical results. To be sure that contractile function and morphology were not affected by potential osmotic changes in the viscous solutions, the osmolarity of each methylcellulose solution was measured by both equilibrium vapor pressure and freezing-point depression. The osmolarity of the standard buffer was 290±5 mosm/kg, and as would be expected for 2% solutions of very large molecules, this value did not increase significantly in any of the methylcellulose solutions.

Sarcomere motion was characterized as described before. The cardiocytes (five left ventricular cells from each of seven control dogs and five left ventricular cells from each of 10 MR dogs) were stimulated to contract at 0.25 Hz either in the standard 1-cp buffer or in buffer-methylcellulose solutions of known viscosities ranging from 12 to 500 cp. When the extent of shortening was stable after 10–15 contractions, 10 contractions were sampled and averaged to yield a final profile of sarcomere length and velocity versus time during contraction. Only cardiocytes with the following characteristics were analyzed: single, rod-shaped cells unattached to adjacent cells, which contracted with each stimulus and were quiescent between stimuli. To apply a viscous load to contracting cardiocytes, the cells were immersed in superfusates of differing viscosities. Superfusate viscosities (at 37°C) of 1, 12, 160, and 500 cp were used in studying the cardiocytes from each left ventricle; they were applied in random sequence to prevent any systematic sampling bias, and each cell was studied at all four viscosities.

**Cardiac and Cardiocyte Morphology**

The morphology of each cardiocyte used for the study of contractile function was examined after the cell was photographed during the course of the study. Cardiocyte surface area was determined by digitizing the lateral edges of the cell images obtained from a \(×7\) magnification of the resulting 35-mm negatives (final magnification, \(×1,400\)). Cell length and width were determined directly from these cell images when digitized by using a computer program that automatically found the maximum value for each of these two parameters after the cellular long-axis orientation was selected by the operator.

Tissue and cardiocytes were also fixed during each study, both to provide a more detailed characterization of cardiocyte dimensions and to provide a light and electron microscopic analysis of left ventricular composition. Tissue fixation was accomplished as before by perfusion fixation of the left ventricular free wall remaining after the wedge of left ventricle used for cardiocyte isolation was removed. For this purpose, the proximal left anterior descending coronary artery was cannulated and perfused with buffered 300 mosm of 1.5% glutaraldehyde fixative at 120 mm Hg pressure. A transmural specimen from the center of the fixed myocardium was then dissected free and postfixed in glutaraldehyde solution for a further 12 hours. For cardiocyte fixation, a portion of the cells to be used for studies of sarcomere dynamics was immersion-fixed in two changes of the same glutaraldehyde fixative for a total of 1 hour.

After tissue fixation, midmyocardial and endomyocardial specimens were dissected with reference to cardiocyte polarity, so that accurate cross sections could then be obtained. Specimens for light micro-
copy were processed and embedded in methacrylate; 2-µm sections were cut and affixed to glass slides. The sections were stained with methylene silver to demarcate cardiocyte borders. Cross-sectional areas were found by using a computerized image analysis system that identified the boundaries and calculated the areas of operator-selected cardiocytes. Thirty cell profiles per specimen were measured at the level of the nucleus at a magnification of ×1,600; this sample size had an interobserver variability of <7% for this measurement. Volume fractions of cardiocyte and interstitial components in each of 12 regions of each slide were estimated on the basis of densitometric differences using the computerized video imaging system. Morphometric analysis of the cardiocytes was done on the same tissue specimens, in this case postfixed in 1% OsO₄, embedded in Epon, cut as 60–90-nm cross sections, and stained with uranyl acetate and lead citrate. Electron micrographs of five regions of each micrograph were analyzed at a magnification of ×25,000; the imaging system allowed operator-determined myofibrillar and mitochondrial outlines to be expressed as percentages of total image area.

Cell length was measured at ×575 on 20 fixed, isolated cardiocytes per left ventricle by using an inverted microscope equipped with a computerized video imaging system. The sample size was selected on the basis of a progressive means test, wherein the mean based on this sample size was consistently found to be within 10% of that obtained from a larger sample.

All of the image analysis for the above measurements was done using a microcomputer-based image processing system and software that we have developed. This system provides an integrated hardware and software work environment for image capture, processing, and analysis.

Data Analysis

The mean value and the standard error of the mean are shown for each group of data. Differences in selected measures were evaluated via either a paired or unpaired Student’s t test, as appropriate, with a significant difference said to exist at p<0.05 both for these and for the following further statistical comparisons, except where Bonferroni’s inequality (p<0.05 per number of comparisons) was applied to multiple comparisons between groups. Where applicable, group means were first compared by a one-way or two-way analysis of variance, and if a difference was found, then each experimental mean was compared with that of the control group by a Newman-Keuls t test.

Table 1. Experimental Model Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Time 0</th>
<th>Time 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (kg)</td>
<td>18.5±1.1</td>
<td>26.3±2.2*</td>
<td>26.5±1.5</td>
</tr>
<tr>
<td>LVₐ wt (g)</td>
<td>72.9±5.2</td>
<td>101.2±6.3*</td>
<td>120.8±6.3†</td>
</tr>
<tr>
<td>LVₐ wt/body wt (g/kg)</td>
<td>3.94±0.16</td>
<td>3.85±0.11</td>
<td>4.56±0.15†</td>
</tr>
<tr>
<td>RV wt/body wt (g/kg)</td>
<td>1.56±0.09</td>
<td>...</td>
<td>1.49±0.07</td>
</tr>
<tr>
<td>LVₐ wt/RV wt (g/g)</td>
<td>2.53±0.13</td>
<td>...</td>
<td>3.06±0.13*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MR, mitral regurgitation; LVₐ, angiographically determined left ventricular weight; RV, gravimetrically determined right ventricular weight. There were seven control dogs and 10 MR dogs, each of which was studied both at time 0 (immediately before MR) and at time 1 (3 months after MR). For this MR model, mortality (~20%) has been confined to the first postoperative month; all dogs surviving at 3 months were studied without exception. Statistical comparisons for the first three rows are of time 0 vs. control (unpaired) and of time 1 MR vs. time 0 MR (paired); Bonferroni’s inequality was applied in assessing significance. Statistical comparisons for the last two rows are of time 1 vs. control (unpaired).

*Significant difference from the control value by Student’s unpaired t test.
†Significant difference from the time 0 value by Student’s paired t test.

Table 2. Left Ventricular Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Time 0</th>
<th>Time 1</th>
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</thead>
<tbody>
<tr>
<td>ESV/body wt (ml/kg)</td>
<td>1.65±0.13</td>
<td>1.46±0.12</td>
<td>2.18±0.17*</td>
</tr>
<tr>
<td>EDV/body wt (ml/kg)</td>
<td>3.34±0.14</td>
<td>2.89±0.15</td>
<td>4.77±0.35*</td>
</tr>
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</table>

Values are mean±SEM. MR, mitral regurgitation; ESV, end-systolic volume; EDV, end-diastolic volume. These data, from the same 17 dogs characterized in Table 1, were obtained during β-blockade with esmolol hydrochloride. The MR dogs were studied both at time 0 (immediately before MR) and at time 1 (3 months after MR). Statistical comparisons are of time 0 MR vs. control (unpaired) and of time 1 MR vs. time 0 MR (paired); Bonferroni’s inequality was applied in assessing significance.

*Significant difference from the time 0 value by Student’s paired t test.
test. For linear regression data, the significance of the correlation coefficient was assessed by a t test; 95% confidence bands and t tests for zero slope and parallelism were also used where specified. For morphometric data, the samples were first assessed for normality of distribution by the Kolmogorov-Smirnov test; a nested analysis of variance followed by Scheffe’s test to identify differing variables was then used.

**Results**

**Characterization of the Experimental Model**

A total of seven control and 10 MR dogs were studied. Several features of the animal model used in this study are shown in Table 1. Although the initial body and left ventricular weights of the two groups of dogs differed, the initial ratio of left ventricular to body weight did not differ significantly. For the MR group, body weight did not change between time 0, the time when MR was produced, and time 1, when the dogs were killed 3 months later. The extent of left ventricular hypertrophy produced by this volume overload was shown by significant increases in left ventricular weight (time 0 versus time 1 MR), in the ratio of left ventricular to body weight (time 0 versus time 1 MR), and in the ratio of left to right ventricular weight (control versus time 1 MR); the right ventricular to body weight ratio did not change (control versus time 1 MR). The left ventricular regurgitant fraction for the time 1 MR dogs was 0.61±0.04. Among the 10 MR dogs, two dogs exhibited pleural effusion and pulmonary edema after death, but the remaining dogs did not exhibit these signs of overt heart failure. For the 10 time 0 versus time 1 MR dogs as a group, left ventricular end-diastolic pressure (6.3±1.1 mm Hg at time 0 before chordal transection versus 20.0±2.0 at time 0 just after chordal transection and 18.4±2.1 at time 1) and pulmonary capillary wedge pressure (6.0±0.6 mm Hg at time 0 before chordal transection versus 18.8±1.8 at time 0 just after chordal transection and 17.1±2.0 at time 1) both increased significantly with chordal transection but did not change further at time 1. Table 2 shows the angiographically calculated left ventricular end-systolic volumes and end-diastolic volumes

<table>
<thead>
<tr>
<th>Table 3. Left Ventricular Contractile Function</th>
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<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>EES (kdyne/cm²)</td>
</tr>
<tr>
<td>EDS (kdyne/cm²)</td>
</tr>
<tr>
<td>EESVR [{(kdyne/cm²)/ml}</td>
</tr>
<tr>
<td>EESVR_{mc} [{(kdyne/cm²)/ml} · g]</td>
</tr>
<tr>
<td>EESVR_{e} [{(kdyne/cm²)/ml} · ml]</td>
</tr>
<tr>
<td>k_{mc}</td>
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</tbody>
</table>

Values are mean±SEM. MR, mitral regurgitation; EES, end-ejection stress; EDS, end-diastolic stress; EESVR, end-ejection stress-volume relation; EESVR_{mc}, EESVR corrected for left ventricular mass (g); EESVR_{e}, EESVR corrected for left ventricular volume (ml); k_{mc}, size-independent active ventricular stiffness (see text). These data, from the same 17 dogs characterized in Table 1, were obtained during ß-blockade with esmolol hydrochloride. The MR dogs were studied both at time 0 (immediately before MR) and at time 1 (3 months after MR). Statistical comparisons are of time 0 MR vs. control (unpaired) and of time 1 MR vs. time 0 MR (paired); Bonferroni’s inequality was applied in assessing significance.

*Significant difference from the time 0 value by Student’s paired t test.
†Significant difference from the control value by Student’s unpaired t test.

![Figure 3. Modulation contrast photomicrographs of left ventricular cardiocytes from control dogs (panel A) and left ventricular cardiocytes from dogs with induced mitral regurgitation (panel B). These immersion-fixed cardiocytes are from the same groups used to generate the data shown in Table 4. Calibration bar, 100 μm.](image-url)
corrected for body weight. Neither end-systolic volume nor end-diastolic volume differed significantly in control versus time 0 MR dogs before chordal transection. However, 3 months after MR was produced, both end-systolic volume and end-diastolic volume had increased significantly (time 0 versus time 1 MR).

Characterization of Left Ventricular Function

As shown in Table 3, the first left ventriculogram, obtained during normal venous return, was used to calculate afterload as active left ventricular stress at end ejection and preload as passive left ventricular stress at end diastole; for the MR group, the former was decreased, whereas the latter was substantially increased (time 0 versus time 1 MR). We then used the second left ventriculogram, obtained after deflation of the vena caval balloon, to assess left ventricular contractile function as the slope of EESVR, where incremental values of left ventricular end-ejection stress were plotted against accompanying increments of left ventricular volume. An example of the decrease in the slope of the EESVR relation after MR in a single dog is shown in Figure 2; summary data for all 10 MR dogs are shown in Table 3 (time 0 versus time 1 MR). It is known, however, that the EESVR slope is affected not only by contractile function but also by cardiac size and shape. In an effort to correct for this problem, we first multiplied the EESVR slope by the simultaneously determined end-diastolic volume to obtain a volume-corrected relation, EESVRVC. In a second, separate correction we multiplied the EESVR slope by the simultaneously determined angiographic left ventricular mass to obtain a mass-corrected relation, EESVRM. Finally, the values of a measure of size-independent active ventricular stiffness derived from end-ejection stress and In 1/H were used to generate the geometric constant, ksm, of this relation. As shown in Table 3, there was a significant decrease in the value of each of these relations after MR (time 0 versus time 1 MR). Thus, by each of these indexes, there was a decrement in left ventricular contractile function after MR.

Characterization of Left Ventricular Cardiocyte Function

The cardiocytes used in this study were isolated from same seven control and 10 MR dogs in which ventricular function had been characterized 2 days before. The average yield of cardiocytes from these left ventricles, which was the same for control and MR dogs, was 71 ± 2% rod-shaped cells, which were quiescent in 2.5 mM CaCl2. The morphological features of a random sampling of left ventricular cardiocytes are shown in Table 4. For cardiocytes used for studies of contractile function, the length (175.3 ± 3.2 versus 199.2 ± 4.0 μm), width (32.5 ± 0.7 versus 36.9 ± 0.8 μm), and surface area (4,465 ± 98 versus 5,676 ± 142 μm2) of left ventricular cardiocytes from the MR dogs were each increased significantly (control versus time 1 MR), but resting sarcomere length was the same (1.87 ± 0.01 versus 1.86 ± 0.01 μm) in the two groups of cells. Of note, these latter values are the same as those both in explanted superfused myocardium at slack length and in perfusion-fixed unloaded diastolic myocardium when the fixative is isosmotic and contracture is avoided. The most striking difference between these two groups of cells, however, was produced, both end-systolic volume and end-diastolic volume had increased significantly (time 0 versus time 1 MR).

<table>
<thead>
<tr>
<th>Width (μm)</th>
<th>Control (n=7)</th>
<th>MR at time 1 (n=10)</th>
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<tbody>
<tr>
<td>32.5 ± 0.7</td>
<td>36.9 ± 0.8</td>
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</table>

Values are mean ± SEM. MR, mitral regurgitation; time 1, 3 months after MR. These data were obtained from the same 17 dogs characterized in Table 1. For cell length, a random sample of 20 fixed, isolated cardiocytes from each dog was studied; these are not the cells that were used to define contractile function. Statistical comparison is of the means for the cells from each dog in the time 1 MR group vs. the means for the cells from each dog in the control group via a Student's unpaired t test. For cell cross-sectional area, the outline of 30 cardiocytes at the level of the nucleus was measured both in a midmyocardial block and in an endocardial block from each left ventricle. Statistical comparisons are of time 1 MR vs. control via a nested analysis of variance followed by Scheffe's test. For both length and cross-sectional area, a Kolmogorov-Smirnov test showed that the samples were normally distributed.

*Significant difference from control.

![Figure 4](http://circres.ahajournals.org/DownloadedFrom.jpg)

**FIGURE 4.** A digital display of sarcomere dynamics during the contraction of single cardiocytes in 1-cp superfusate. Panel A: Control cardiocyte. Panel B: Cardiocyte obtained 3 months after mitral regurgitation was produced. The sarcomere length and velocity tracings shown are the average for 10 contractions of each cardiocyte at 37°C. The real-time resolution of the sensor array was 1 msec.
as illustrated by Figure 3, was the increased length of the MR cardiocytes. Figure 4 is an example of the sarcomere shortening patterns for a control and an MR cardiocyte during externally unloaded contractions in the standard 1-cp superfusate. As shown by this figure, sarcomere length decreased with stimulation and then returned to the initial length during cardiocyte relaxation. The velocity of sarcomere shortening reached a maximum value at ~40 msec after the initiation of shortening and became zero when the extent of shortening reached a maximum. Thereafter, sarcomere length returned toward the rest length in an approximately exponential decay pattern. Resting sarcomere length and sarcomere shortening behavior were observed as before to be uniform when obtained at any given point within a cell, excluding nuclear regions. The mechanical characteristics of the cardiocytes in 1-cp superfusate are summarized in Table 5. Sarcomere shortening velocity, extent of shortening, and relaxation velocity each decreased significantly in the MR group, whereas time to peak shortening, time to 50% relaxation, and time to peak relaxation velocity each increased significantly in the MR group (control versus time 1 MR).

The contractile function of cardiocytes from the control and MR left ventricles as a function of varying viscous afterloads is shown in Figure 5. As seen in Figure 5A, there was a significant reduction in sarcomere shortening velocity for the MR cardiocytes at each viscous afterload used. To determine whether the form of the viscosity–velocity relation is similar to that of the classical force–velocity relation of the isolated papillary muscle preparation of cardiac muscle, we plotted the dog cardiocyte viscosity–velocity data in a semilogarithmic form in Figure 5B. This both linearized the relation in the same manner as that observed for the isolated papillary muscle and clearly demonstrated the symmetrical depression of this entire relation for the MR cardiocytes. Significant differences in sarcomere mechanics seen in the 1-cp superfusate between cardiocytes from control and time 1 MR dogs in terms of extent of shortening, relaxation velocity, time to peak shortening, time to 50% relaxation, and time to peak relaxation velocity were maintained at each higher viscosity superfusate used.

Of note, there were no differences in cardiocyte morphology or in resting sarcomere length at any viscosity used. It is also important to emphasize that, when examined as part of each study, cardiocyte mechanics in the 1-cp superfusate did not differ when

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**TABLE 5. Cardiocyte Contractile Function**

<table>
<thead>
<tr>
<th>Description</th>
<th>Control (n=7)</th>
<th>MR at time 1 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortening velocity (μm/sec)</td>
<td>3.11±0.09</td>
<td>2.18±0.09*</td>
</tr>
<tr>
<td>Shortening (μm)</td>
<td>0.191±0.004</td>
<td>0.158±0.004*</td>
</tr>
<tr>
<td>Time to peak shortening (msec)</td>
<td>151±3</td>
<td>170±3*</td>
</tr>
<tr>
<td>Relaxation velocity (μm/sec)</td>
<td>3.93±0.15</td>
<td>2.68±0.15*</td>
</tr>
<tr>
<td>Time to 50% relaxation (msec)</td>
<td>57±2</td>
<td>69±2*</td>
</tr>
<tr>
<td>Time to peak relaxation rate (msec)</td>
<td>53±2</td>
<td>62±2*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MR, mitral regurgitation; time 1, 3 months after MR. These data, from the same 17 dogs characterized in Table 1, were obtained in the 1-cp superfusate for the cardiocytes from each dog that were used to characterize cellular contractile function. Statistical comparisons are of time 1 MR vs. control (unpaired).

*Significant difference from the control value by Student’s unpaired t test.
the contractile indexes were compared at the beginning and at the end of the full study protocol.

**Comparison Between Ventricular and Cardiocyte Function**

The relation for each of the 17 dogs studied between ventricular function defined as either EESVR, corrected EESVR, or k\textsubscript{em} and cardiocyte function defined as peak sarcomere shortening velocity in the 1-cp superfusate is shown in Figure 6; again, the left ventricle and left ventricular cardiocyte data were gathered independently in a blinded manner. For each panel of Figure 6, the linear regression line, equation, statistics, and 95% confidence bands pertain only to the 10 MR dogs; the two MR dogs in overt heart failure at the time of death are indicated by arrows. The correlation shown in Figure 6A between EESVR and sarcomere shortening velocity was significant, but because of the increase in left ventricular size in the MR group, there was an expected downward shift in the EESVR values for all of the MR dogs, including those MR dogs with the best left ventricular and cardiocyte function. Nonetheless, the correspondence between decrements in ventricular and cardiocyte contractile function in individual MR dogs is clearly apparent, and the two MR dogs with congestive heart failure are in their expected position in terms of this relation. Two attempts were made to compensate for the contractility-independent effects of changing left ventricular size on left ventricular function as measured by the EESVR index. As shown in Figure 6B, correction of EESVR for left ventricular mass has two effects: first, left ventricular function as measured by EESVR\textsubscript{mc} in those MR dogs wherein left ventricular and cardiocyte function are best preserved now corresponds to that of the control dogs; second, the correlation between ventricular and cardiocyte contractile function is improved by the use of EESVR\textsubscript{mc}. However, the fact that Table 3 demonstrates a significant difference in terms of EESVR\textsubscript{mc} between two groups of normal dogs (control versus time 0 MR) that differ significantly from each other in terms of body and left ventricular weight (Table 1) raises serious concerns about the validity of EESVR\textsubscript{mc} as a size-independent measure of left ventricular function. As shown in

**Figure 6.** The relation between ventricular and cellular mechanics. Time 1 MR, dogs in which cardiocytes were obtained 3 months after mitral regurgitation (MR) was produced; CHF, congestive heart failure; EESVR, end-ejection stress volume relation; EESVR\textsubscript{mc}, mass-corrected EESVR; EESVR\textsubscript{vc}, volume-corrected EESVR; k\textsubscript{em}, size-independent ventricular stiffness. In each panel, the abscissa is the same index of cardiocyte contractile function, peak sarcomere shortening velocity in the 1-cp superfusate, and the ordinate is one of four indexes of ventricular contractile function. Arrows indicate the two MR dogs in overt heart failure at the time of death. The linear regression lines, equations, statistics, and 95% confidence bands pertain only to the 10 MR dogs. Panel A: The slope of EESVR vs. sarcomere shortening velocity. Panel B: The slope of EESVR\textsubscript{mc} vs. sarcomere shortening velocity. Panel C: The slope of EESVR\textsubscript{vc} vs. sarcomere shortening velocity. Panel D: The slope of k\textsubscript{em} vs. sarcomere shortening velocity.
Figure 6C, correction of EESVR for left ventricular volume has the desirable effect of producing a correspondence between control and MR dogs in those MR dogs wherein left ventricular and cardiocyte function are best preserved, but the correlation between decrements in ventricular (EESVR) and cardiocyte (sarcomere shortening velocity) function is now insignificant.

In contrast to these three comparisons of ventricular and cellular function, the comparison in Figure 6D of k_m with sarcomere shortening velocity demonstrates three points quite well: First, ventricular and cellular function in the control and in the optimal MR dogs is coincident. Second, the correlation between ventricular and cellular contractile function in the MR group is very highly significant, and the correlation coefficient is significantly ($p<0.0001$) better than that in the other three panels of this figure. Third, the two MR dogs in overt failure at the time of death segregate clearly from the remaining MR dogs. Further, $k_m$ is an index of left ventricular function that is truly size-independent,12 as is seen here in the comparison of the $k_m$ of the control dogs with that of the larger time 0 MR dogs in Table 3.

**Comparison Between Cardiac Structure and Function**

Potential structural bases for the ventricular and cellular contractile dysfunction described above were sought through a morphometric analysis of the left ventricular myocardium on both the light and electron micrographic levels. As is shown in Table 6, no difference was found at the light micrographic level between control and experimental groups in terms of tissue composition when divided into the cardiocyte and interstitial components. However, on the electron micrographic level, as is illustrated in Figure 7 and defined numerically in Table 6, major differences were found. In comparison with Figure 7A, which is an electron micrographic cross section from control left ventricular myocardium, Figure 7B, which is from an MR dog with relatively well-preserved ventricular and cellular contractile function, shows a modest loss of myofibrils, whereas Figure 7C, which is from one of the two MR dogs exhibiting frank congestive heart failure at the time of death, shows a striking loss of myofibrils. Indeed, the relatively greater degree of cardiocyte as opposed to ventricular hypertrophy in MR (Table 1 versus Table 4) suggests that this process might have eventuated in actual cardiocyte

<table>
<thead>
<tr>
<th>TABLE 6. Left Ventricular Morphometrics</th>
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<tbody>
<tr>
<td>Control (n=5)</td>
</tr>
<tr>
<td>MR at time 1 (n=10)</td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td><strong>Myocardial volume density</strong></td>
</tr>
<tr>
<td>Cardioocyte (% of myocardium)</td>
</tr>
<tr>
<td>Midmyocardial</td>
</tr>
<tr>
<td>Endomyocardial</td>
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<tr>
<td>Interstitium (% of myocardium)</td>
</tr>
<tr>
<td>Midmyocardial</td>
</tr>
<tr>
<td>Endomyocardial</td>
</tr>
<tr>
<td><strong>Cardiocyte volume density</strong></td>
</tr>
<tr>
<td>Myofibrils (% of cardiocytes)</td>
</tr>
<tr>
<td>Midmyocardial</td>
</tr>
<tr>
<td>Endomyocardial</td>
</tr>
<tr>
<td>Mitochondria (% of cardiocytes)</td>
</tr>
<tr>
<td>Midmyocardial</td>
</tr>
<tr>
<td>Endomyocardial</td>
</tr>
<tr>
<td><strong>MR-NL (n=5)</strong></td>
</tr>
<tr>
<td>MR-ABNL (n=5)</td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
</tbody>
</table>
| Values are mean±SEM. MR, mitral regurgitation; time 1, 3 months after MR; MR-NL, MR with relatively well-preserved ventricular and cellular contractile function; MR-ABNL, MR with poorly preserved ventricular and cellular contractile function. These data were obtained from the same 17 dogs characterized in Table 1, but tissue was available for analysis in only five of the seven control dogs. The 10 MR dogs were divided into two groups (MR-NL and MR-ABNL) on the basis of combined ventricular and cellular contractile function. For myocardial volume density measurements, the percentages for each dog of cardiocytes and interstitium were digitized in 12 distinct regions of each of two slides from separate blocks, first of the left ventricular midmyocardium and then of the left ventricular endomyocardium. Statistical comparisons are of time 1 MR vs. control, by layers and by groups, via a nested analysis of variance followed by Scheffe’s test. No significant difference was found at any level, and the MR-NL and MR-ABNL data are therefore presented as MR group means. For cardiocyte volume density measurements, the percentages for each dog of myofibrils and mitochondria were digitized in five distinct regions of each of three electron micrographs from separate blocks, first of the left ventricular midmyocardium and then of the left ventricular endomyocardium. Statistical comparisons are of time 1 MR vs. control, by layers and by groups, via a nested analysis of variance followed by Scheffe’s test; only the noted differences were found. For both myocardial and cardiocyte volume density, a Kolmogorov-Smirnov test showed that the samples were normally distributed.

*Significant difference from control within that layer.
†Significant difference from all other samples, both within and between layers.

Myofibrils

Midmyocardial 56.81±0.50 52.06±0.68* 45.69±0.75**
Endomyocardial 55.59±0.49 53.17±0.84 40.74±0.98**

Mitochondria

Midmyocardial 23.18±0.36 24.18±0.52 26.20±0.55
Endomyocardial 24.06±0.42 24.43±0.47 24.70±0.58
loss from these left ventricles. When the group of 10 MR dogs was divided into those five dogs with the worst combined ventricular and cellular contractile function (NR-ABNL) as opposed to those five dogs with either a lesser or no degree of contractile dysfunction (NR-NL), as is shown in Figure 8A, it is evident from Table 6 that there was a substantial decrement in myofibrillar volume density in those MR dogs with abnormal function, with the endomyocardium being more severely affected than the mid-myocardium. Mitochondrial volume density was unaffected. Of interest, when the two myocardial layers are considered as a whole, and ventricular and cellular contractile function are compared separately with myofibrillar volume density, Figures 8B and 8C demonstrate a striking correlation between decrements in contractile function and in myofibrillar volume density.

**Hypertrophic Response to Contractile Dysfunction**

A reasonable working assumption is that hypertrophy is the basic cardiac adaptive response to hemodynamic overloads, such that any decrements in
intrinsic chamber or cardiocyte contractile function are counterbalanced by increments in mass until the biological limits of hypertrophy are reached and heart failure ensues. But as shown in Figure 9, such an assumption is invalid for this experimental model of MR. As was the case for Figure 6, for each panel of Figure 9 the linear regression line and equation pertain only to the 10 MR dogs. Not only is there no significant correlation between left ventricular mass and either cardiocyte (Figure 9A) or ventricular (Figure 9B) contractile function, but the slope of neither relation differs significantly from zero. Thus, despite the fact that modest but significant left ventricular hypertrophy occurred in the MR dogs as a group (Table 1), there is no tendency in the MR dogs for increasing left ventricular hypertrophy to occur in response to decreasing contractile function, even in those two dogs exhibiting overt congestive heart failure at the time of death.

Discussion

The data generated during this study did provide answers, at varying levels of completeness, to the three questions initially posed. First, the left ventricular volume overload imposed by MR results not only in compromised ventricular pump performance but also in compromised intrinsic ventricular contractile performance. Second, while a cause and effect relation is not established unequivocally, the extent of ventricular and cellular contractile abnormalities corresponded rather closely in individual dogs with MR, suggesting that the contractile dysfunction observed on the ventricular level can be attributed to cellular contractile dysfunction. Further, no change in the interstitial volume fraction, which might contribute to the observed abnormalities of ventricular contractile function, is apparent in our data. Third, the pathophysiological basis for both the ventricular and the cellular dysfunction observed in this entity appears to be a combination of deficient hypertrophy resulting in inadequate muscle mass on the ventricular level and myofibrillar loss resulting in inadequate contractile performance on the cellular level.

The Effect of Mitral Regurgitation on Left Ventricular Contractile Performance

We have suggested elsewhere that the left ventricle tolerates a volume overload less well than does the right ventricle. However, a series of studies over the past quarter century using either arteriovenous fistulas or heart block to produce chronic left ventricular volume overload has shown that left ventricular pump performance is relatively well maintained despite hemodynamic concomitants of congestive heart failure unless the circulatory overload is particularly severe or prolonged. Similarly, clinical experience demonstrates that right ventricular volume overload is well tolerated in the absence of a superimposed pressure overload, as validated experimentally in terms of myocardial contractile function.

Given that contractile performance in the two most common clinical forms of left ventricular volume overload may differ substantially, with aortic regurgitation and its attendant high afterload being accommodated better than MR and its attendant low afterload, the suggestion that left ventricular volume overload is relatively poorly tolerated would thus seem by exclusion to be based on the experience with mitral regurgitation. Indeed, both clinical...
and experimental studies suggested to us the hypothesis that depressed contractile performance may be an intrinsic property of the left ventricle volume overloaded by MR.

Our experimental MR model has shown that after 3 months of severe MR, left ventricular contractile function, when assessed via a number of standard indexes, is distinctly abnormal, and this conclusion is unaltered by isochronal analysis of maximum fiber elastance or an analysis of stress-shortening behavior. But given our concerns about the validity of such indexes, especially when based on the time-varying elastance concept in chambers of changing size and shape, this study provided valuable new information in two areas. First, as shown in Figure 6D, left ventricular contractile function is depressed after chronic MR when measured by a size-independent measure of active stiffness. Second, as shown in Figure 5, variably afterloaded cardiocytes isolated from these same ventricles showed a similar decrement in intrinsic contractile function. Thus, although left ventricular volume overloads in the setting of a normal or even increased afterload may be fairly well tolerated, left ventricular volume overloading by MR, which is associated with a distinctly abnormal reduced afterload, results in unequivocally abnormal intrinsic myocardial contractile function on both the ventricular and cellular levels if the hemodynamic load is of substantial degree and duration.

**Ventricular and Cellular Contractile Defects in Mitral Regurgitation**

Although the practical utility of measures of left ventricular function based on the time-varying elastance concept is well established, and it has even been suggested recently that it may not be necessary to correct elastance measurements for ventricular geometry, as intimated above there are theoretical concerns about the physical meaning of such measurements. More germane to the present study, there are practical concerns about how to deal with changes in left ventricular size and shape when using such indexes to measure longitudinal changes in ventricular function after imposing lesions that cause progressive alterations in ventricular geometry. For instance, it may be seen in Figure 6A that the uncorrected EESVR would suggest that ventricular function is depressed in all dogs with MR without regard to whether contractile function of cardiocytes from the same ventricles is well preserved. The use of such geometric corrections as the mass correction shown in Figure 6B or the volume correction shown in Figure 6C produces results that are intuitively more sensible but that are not necessarily based on a sound theoretical rationale in either case. This was one of the major reasons that a size-independent measure of active ventricular stiffness was developed and validated in these laboratories. As seen in Figure 6D, the usage of this index in the present study revealed both a close correlation between ventricular and cellular contractile function when measured independently and placed the dogs with overt congestive heart failure at the time of death in their expected position within the combined relation.

We developed the technique of measuring cellular contractile function in terms of sarcomere shortening velocity in variably afterloaded cardiocytes both as a means of characterizing intrinsic cellular contractile function and as a means of characterizing such contractile function in response to graded, reproducible cellular afterloads. To date, this technique has shown a close overall correspondence between contractile function measured on the tissue and cellular levels in feline right ventricular pressure-overload hypertrophy and, in the present study, a close correspondence even in individual dogs between ventricular and cellular function in left ventricular volume-overload hypertrophy. It is reasonable to
conclude from this correspondence that, at least in these two models of pressure- and volume-overload hypertrophy, the contractile defect observed at the tissue level may largely be attributed to contractile abnormalities on the cellular level.

Considerable attention has been given recently to the potential role of interstitial fibrosis, with accompanying decreased compliance, in the pathophysiology of the contractile dysfunction observed in some forms of cardiac hypertrophy. This has especially been the case with left ventricular pressure-overload hypertrophy. In left ventricular volume overload induced by an aortocaval fistula, compliance has also been found to be decreased, in this case in association with a greater degree of cross-linking of some forms of interstitial collagen. However, in clinical left ventricular volume overload from either aortic or mitral insufficiency, the interstitial volume fraction is not increased; in the model of mitral regurgitation used in the present study, left ventricular compliance is actually increased, and as shown in Table 6, the interstitial volume fraction is unchanged. Given this information, and the close correspondence of ventricular and cellular dysfunction found in this study, it seems reasonable to conclude that interstitial changes are not likely to play a major role in the contractile dysfunction that we observed in this model of mitral regurgitation.

Basis for Contractile Dysfunction in Mitral Regurgitation

We have begun our search for the etiology of the contractile dysfunction found herein for MR with the most obvious functional and structural underpinnings of sustained cardiac contractile function in the face of increased hemodynamic demands. Functionally, because hemodynamic overloads increase the energy needs of the affected chamber, attention has been given to the possibility that coronary flow reserve may be inadequate in hypertrophied myocardium. Indeed, almost uniformly in substantial pressure-overload hypertrophy and in some instances of volume-overload hypertrophy, such has been found to be the case. However, for the canine model of MR used here, no abnormality of regional left ventricular perfusion has been found either at rest or during pacing stress.

Structurally, because the most basic cardiac adaptation to hemodynamic overloads is myocardial hypertrophy, the adequacy of the hypertrophic response to left ventricular volume overloading in terms of normalizing an initially elevated wall stress is also of interest. In aortic regurgitation, which is a combined volume and pressure overload of the left ventricle, it has been found that the initially elevated left ventricular end-diastolic wall stress that follows the induction of aortic regurgitation is renormalized by the hypertrophic response, although this finding may not obtain when the growth capacity of the left ventricle is compromised by age. However, as seen in Table 3 of the present study, left ventricular end-diastolic stress, which was markedly elevated immediately after MR was induced, remained markedly elevated 3 months later. Further, as is seen in Figure 9, the degree of left ventricular hypertrophy was modest for the MR group as a whole, and there was no tendency for those dogs with the worst ventricular and cellular contractile function to exhibit a more pronounced hypertrophic response. Indeed, the regurgitant fraction initially produced in these dogs was in excess of 60% for the group as a whole, and there was no tendency for those with the greatest initial regurgitant fraction to exhibit a greater degree of left ventricular hypertrophy at study 3 months later. Certainly, none of these dogs with mitral regurgitation developed a degree of left ventricular hypertrophy that even approaches that seen with other hemodynamic stimuli, most notably pressure overload. Thus, this canine model, where mitral regurgitation is abruptly imposed on a formerly normal left ventricle, closely mimics only a minor fraction of MR in humans, and the modest left ventricular mass response is less than that seen clinically with chronic MR of gradual onset. However, the left ventricular mass response is qualitatively analogous to that seen in human disease: the mean increase in human left ventricular mass is 53% with MR and 155% with combined aortic stenosis and regurgitation; the maximum observed increase in left ventricular mass with mitral regurgitation is only half of that observed for combined aortic stenosis and regurgitation.

Therefore, it would appear that the most basic reason for the development of congestive heart failure after the induction of MR is the inadequacy of the left ventricular hypertrophic response. Although no conclusive explanation is available for this phenomenon, two points are of interest. First, it has been a major theme of our research for some time that the single factor most important in providing dynamic control of cardiac mass in the adult mammal is the cardiac loading environment and that the load signal is sensed and transduced at the level of the cardiocyte. Although diastolic left ventricular stress and strain are clearly increased after MR, the incompetent mitral valve results in a systolic load that is actually lower than normal at end ejection, with this disparity being even more marked in early systole. Yet active rather than passive stress is the more effective stimulus for cardiac protein synthesis. Thus, the most basic problem resulting from MR may well be the simultaneous imposition of the demand for a greater stroke volume at the same time that this lesion reduces rather than increases the signal that would allow the hypertrophic response to this demand to be generated.

Although an inadequate hypertrophic response might well explain decreased ventricular pump performance, it does not explain the decreased contractile function that we observed in this study on both the ventricular and cellular levels. Further, it does not explain the failure of left ventricular function in patients with MR to respond as well as might be expected to surgical correction of this hemodynamic lesion. These characteristics of MR predict instead
a mass-independent myocardial defect intrinsic to the cardiocyte. Thus, the ultrastructural abnormalities that we found in the dogs with MR are of considerable interest. The myofibrillar loss described in both Figure 7 and Table 6 appears to be characteristic not simply of MR but rather of MR associated with poor ventricular and cellular contractile function, as is shown in Figure 8. Although a cause and effect relation cannot be ascribed with certainty, it would certainly be reasonable to expect that cells exhibiting myofibrillar loss such as that shown in Figure 7C would exhibit abnormal contractile function. Such myofibrillar loss would not, however, appear to be specific to MR, because it has been shown to be characteristic of failing myocardium in a variety of settings, including MR. It is certainly not a morphological feature characteristic of volume-overload hypertrophy itself. Rather, given that this ultrastructural feature has no relation to the degree of hypertrophy, as is seen in Figure 9, but rather relates well to the degree of ventricular and cellular contractile dysfunction, as is seen in Figure 8, it would appear that the myofibrillar loss seen here is a marker for ventricular failure rather than an element of volume-overload ventricular hypertrophy.

Conclusion
This canine model of closed-chest MR closely mimics the features of this disease as encountered in the clinical setting. The decreased ventricular contractile function would appear to have its basis in the decreased contractile function of the cardiocytes that compose that ventricle, with no contribution from an altered interstitial volume fraction. The two most noteworthy pathophysiological hallmarks of the failing left ventricle in this MR model are the lack of a hypertrophic growth response appropriate to the hemodynamic demand and a loss of myofibrils from the cardiocytes of the ventricles exhibiting the poorest contractile function. Since there is no evidence that these most severely affected cells and ventricles experienced either a greater hemodynamic challenge or a greater hypertrophic response than their fellows with better preserved function and morphology, it is reasonable to suspect that a superimposed pathophysiological process might be responsible for both the functional and structural changes seen in the most abnormal dogs in this study, as well as in the most severely affected patients with MR. Given both the suggestion that increased adrenergic drive may be important to maintaining ventricular function in MR and the documentation of increased circulating catecholamines in a dog model of left ventricular volume overload, one may speculate that perhaps the inadequate hypertrophic response after MR leads to a sustained adrenergic drive to maintain forward cardiac output and systemic perfusion. In light of the direct cardiocyte toxicity of catecholamines that we have recently found, it might well be that such an initially compensatory adrenergic response might eventually be responsible for the irreversible deterioration of left ventricular myocardium in the setting of severe MR.

Acknowledgments
The technical assistance of Stephen Vinciguerra, Robert Biederman, and Shelby Yoakum, the computer programming by John Lindroth, and the secretarial assistance of Betty Owens are all very gratefully acknowledged. The laser diffraction system used for measuring sarcomere dynamics was fabricated by Michael Loughnane at Instech Laboratories, Horsham, Pa.

References


**KEY WORDS**
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- cardiocytes
- left ventricle
- mechanics
Cellular and ventricular contractile dysfunction in experimental canine mitral regurgitation.
Y Urabe, D L Mann, R L Kent, K Nakano, R J Tomanek, B A Carabello and G Cooper, 4th

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