CONGESTIVE HEART FAILURE (CHF) is caused by a predominant abnormality in systolic function (systolic CHF), diastolic function (diastolic CHF), or both. Fifty percent of the patients who develop CHF over the age of 70 yr have a predominant abnormality in diastolic function causing diastolic CHF (2, 31, 35, 38, 49). Diastolic CHF is associated with a 5-yr mortality rate, which approaches 50% in patients over 70 yr old (35, 38, 49). Treatment for diastolic CHF is difficult and uncertain, at least in part because the underlying mechanisms causing diastolic dysfunction have not been completely defined, particularly in a fashion that provides clear targets for effective treatment strategies. It has been hypothesized that diastolic dysfunction develops when functional and structural changes within the cardiac muscle lead to a fundamental alteration in the material properties of the myocardium. Understanding the mechanisms underlying these alterations in myocardial material properties is the primary focus of this study.

Cardiac muscle is a composite material consisting of cardiac muscle cells (cardiocytes), fibroblasts, blood vessels, and the extracellular matrix that surrounds them. Changes in any one of these component elements within the myocardium may affect its material properties and may alter myocardial diastolic function. We hypothesized that the changes in myocardial material properties that occur during the development of pressure overload hypertrophy (POH) are caused by changes in the constitutive viscoelastic properties of the cardiocytes themselves.

To test this first part of this hypothesis, we examined the effects of POH on the constitutive properties of cardiocytes isolated from hypertrophied myocardium and found that 1) POH caused significant alterations in the cardioytic viscoelastic properties, 2) the POH-induced increase in cardioytic viscous damping was associated with alterations in the intracellular cytoskeleton, and 3) these abnormalities in the cellular cytoskeleton could be acutely corrected and that correcting these cellular abnormalities completely normalized viscous damping in the POH cardiocytes (43, 53). However, whether these POH-induced changes in the constitutive properties of the component cardiocytes contribute causally to changes in the material properties of the composite cardiac muscle as a whole remained uncertain. In a composite structure, each element may contribute, to a greater or lesser degree, to the overall material properties. Either because of its...

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large volume within the composite or because of its exceptional physical properties, one element may have a dominant effect that overwhels the effects of all of the other elements within the composite. Therefore, it is quite plausible that the cellular changes described above are not large enough to alter the material properties of the composite myocardium and that some other component has a larger, more dominant effect that limits or negates the effects that the changes in cellular constitutive properties have on the myocardium. To address this question, and further test the hypothesis stated above, a selective, isolated, acute change in cardiocyte properties must be imposed in an in vitro system capable of measuring the resultant effects on the properties of the composite cardiac muscle as a whole. Therefore, the current study was designed to 1) measure composite myocardial material properties in papillary muscle isolated from normal and POH cats, 2) acutely alter or correct the constitutive viscoelastic properties of the cardiocytes within the papillary muscle, and 3) measure the effect that this change in cardiocyte properties has on each of the material viscoelastic properties of the papillary muscle, thus defining the relative contribution that this cellular property has on the composite myocardium. In the present study, we used these methods to determine whether, and to what relative extent, the POH-induced changes in the material properties of the cardiac muscle were caused by changes in the intrinsic constitutive properties of the cardiocytes themselves.

METHODS

Right ventricular (RV) papillary muscles were isolated from eight normal cats and eight cats with chronic RV POH induced by pulmonary artery banding (PAB) for 4 wk. This model was used because both cardiac tissue and cardiocytes from this preparation have been fully characterized in terms of morphology, function, and energetics. The extent of POH was determined by catheterization just before surgical isolation of the papillary muscles. A computer-controlled servo motor system was used to mechanically test the papillary muscles. Data examining the effect of POH and treatment with colchicine on myocardial contractile function from these same papillary muscles have been previously published (52). The current study examined the effect of POH and treatment with colchicine on myocardial constitutive viscoelastic properties. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, Revised 1985).

Pulmonary Artery Banding

RV POH was induced by partially occluding the pulmonary artery with a 2.9-mm inner diameter band using previously described methods (52). Briefly, adult cats weighing 2.6–3.9 kg were first anesthetized with ketamine hydrochloride (15 mg/kg im), meperidine (2.2 mg/kg im), and acepromazine maleate (0.25 mg/kg im), intubated, and then placed on a respirator. A left thoracotomy was performed, and a band was placed around the proximal pulmonary artery. Eight cats underwent PAB and then recovered for 4 wk. Four normal adult cats and four sham-operated cats served as controls.

Hemodynamic Studies

Four weeks after PAB or sham operation, catheterization was performed using previously described methods (52). Cats were anesthetized with ketamine hydrochloride (25 mg/kg im). Right heart pressures were obtained using a fluid-filled catheter inserted through the right external jugular vein and advanced into the right atrium and RV. Arterial pressure was monitored using a second fluid-filled catheter, which was positioned in the proximal left common carotid artery. Each catheter was attached to a strain gauge, and the midchest position was taken as a zero reference point for pressure measurements. Arteriovenous oxygen content was used as a measure of cardiac output and was determined in duplicate by measuring simultaneous blood samples obtained from the carotid artery and RV.

Papillary Muscle Isolation

After assessment of hemodynamic status by catheterization, RV papillary muscles were isolated using previously described methods (52). A median sternotomy was performed, the pericardium was bluntly dissected away from the heart, and the cat was heparinized (1,000 units iv). A perfusion cannula was placed in the proximal aorta. The inferior and superior vena cava were ligated, the aorta was cross-clamped, and the heart was perfused antegrade with a Krebs-Henseleit cardioplegia solution consisting of (in mM) 98.0 NaCl, 4.7 KCl, 1.2 MgSO4, 1.1 KH2PO4, 24.0 NaHCO3, 20.0 NaAc, 2.5 CaCl2, 11.2 glucose, and 30.0 2,3-butanediol monoxide (BDM) and 10 U/l insulin. The cardioplegia solution, which was continuously bubbled with 95% O2-5% CO2 at room temperature and pH 7.38, was initially infused as a 50-ml bolus over a 1-min period, causing cardiac standstill, and then continuously infused at a slower rate of 5–10 ml/min while the papillary muscles were excised. During this continuous administration of the cardioplegia solution, the heart was removed, the RV free wall was incised close to the interventricular septum, and one to three RV papillary muscles were dissected free. A 6.0 silk suture was tied to the top of each papillary muscle at the junction of the chordae tendineae with the papillary muscle. The base was attached to a spring clip. The papillary muscles were immediately placed in a 250-ml container of the cardioplegia solution and bubbled continuously with 95% O2-5% CO2 (pH 7.38, room temperature). The muscles were kept in oxygenated cardioplegia solution for 30 min and then placed vertically in a 250-ml acrylic isolated muscle chamber containing oxygenated cardioplegia solution without BDM for a 15-min washout period before electrical stimulation was begun. Once the papillary muscle was placed in the isolated muscle chamber, and throughout the subsequent study, the temperature was held at a constant 29°C.

Papillary Muscle Servo Control System

After the washout period, the muscle was electrically stimulated by parallel platinum electrodes delivering 5-ms pulses at a voltage 10% over threshold. The silk suture on the upper end of the papillary muscle was attached to a dual-mode Cambridge 300 B Servo Control system, and the lower clip was attached to a semiconductor strain-gauge transducer (DSC-3, Kistler-Morse). A digital computer with an analog-to-digital interface controlled either tension or length of the preparation. Tension and length data were sampled at a rate of 1 kHz and stored for later analysis. The precision of the force and length settings was 5 mg and 2 μm, respectively. The step response of the system to an imposed length change
was 95% complete in 2 ms. Equipment compliance was <1.0 μm/mN (52).

**Experimental Protocols**

**Baseline.** After isolation, each papillary muscle was allowed to equilibrate in the isolated muscle chamber by contracting isotonically at a light, 0.5 g of preload for a period of 120 min. During this preconditioning period, at 15-min intervals, the muscle was gradually stretched to the peak of the active tension versus length curve ($L_{\text{max}}$). In addition, isotonic contractions at 0.5 g of preload and isometric contractions at $L_{\text{max}}$ preload were performed. This protocol was necessary to precondition the muscle so that its constitutive properties reached a stable, reproducible state (16). The papillary muscle was determined to be at mechanical equilibrium (fully preconditioned) when values for muscle length at $L_{\text{max}}$, shortening extent during isometric contraction, and active tension during isometric contraction reached a steady state during three consecutive measurements separated by 15-min intervals. Once mechanical equilibrium was achieved, baseline values were obtained. At baseline, three determinations of $L_{\text{max}}$ were made. A series of four uniaxial variable rate stretches were then performed in the baseline state.

**Colchicine treatment.** After the baseline stretches, colchicine was added to the buffer to achieve a final concentration of $10^{-5}$ M. This concentration, which was higher than that used in isolated cardiocyte studies, was chosen to ensure thorough diffusion throughout the muscle (43, 52, 53). After treatment with colchicine for 90 min, $L_{\text{max}}$ was determined, and another series of four uniaxial variable rate stretches were performed. Colchicine causes microtubule depolymerization by binding to β-tubulin and preventing α/β-tubulin heterodimer polymerization into microtubules. Because the half-life of microtubules is ~30 min, colchicine causes a reduction in the number of microtubules over 30–90 min. The effects of POH and the effects of colchicine treatment on 1) the relative amounts of free and polymerized β-tubulin in papillary muscles, characterized by immunoblotting; 2) the appearance and density of the cardiocyte microtubule network, visualized by direct immunofluorescence micrographs; and 3) the effects on myocardial contractile state have been presented in detail in a previous study (52). To ensure that time itself did not result in the changes ascribed to 90-min treatment with colchicine, three normal and three RV POH muscles under went 120 min of preconditioning, and baseline measurements were made, followed by an additional 90 min of observation (without treatment with colchicine) and repeated measurements. This additional 90 min of time did not significantly alter the stiffness or viscosity constants.

At the conclusion of each experiment, muscle length was measured while the muscle was held at a force equal to the passive tension at $L_{\text{max}}$. The muscle was removed from the clips, blotted, and weighed. The muscle was then dried at 110°C for 24 h and weighed again. Muscle cross-sectional area was determined, assuming a uniform cross section, from muscle length at $L_{\text{max}}$, the muscle dry weight, a wet weight-to-dry weight ratio of 4:1, and a specific gravity of 1.0. Muscles with cross-sectional areas <0.5 or >1.5 mm² were excluded from further analysis. Previous studies have clearly shown that if muscle cross-sectional area is <1.5 mm², there is no central core hypoxia in the isolated muscle preparation used under the conditions employed in the present study (8, 14, 40). There was no significant difference between control versus experimental groups in either muscle length (6.0 ± 1.0 mm in normal vs. 6.2 ± 1.0 mm in PAB) or muscle cross-sectional area (1.0 ± 0.2 mm² in normal vs. 1.2 ± 0.3 mm² in PAB).

**Measurements of Viscoelastic Properties**

At each study point, myocardial viscoelastic properties were assessed by defining $L_{\text{max}}$ and then performing uniaxial variable rate stretches. $L_{\text{max}}$ was determined by stretching the muscle at a slow rate of 0.1 mm/min to the peak of the active tension versus muscle length relationship. $L_{\text{max}}$ was defined as that resting muscle length resulting in peak active tension generation.

Uniaxial variable rate stretches were performed in quiescent muscles by increasing muscle length at 0.1 mm/min and 0.1, 1.0, and 10 mm/s. Muscles were stretched under length control over a range of passive tension from a minimum length at 0.2 g of passive tension to a maximum length at 20% above the passive tension at $L_{\text{max}}$. An example of passive muscle force, muscle length, and strain rate (\(\epsilon\)) data obtained using these methods are shown in Fig. 1.

Muscle stress (\(\sigma\)) was calculated from force measurements as follows

\[
\sigma = \text{Force}/\text{CSA}
\]

where CSA is the muscle cross-sectional area.
Muscle strain ($\varepsilon$) was calculated from length measurements as follows

$$\varepsilon = (L_N - L_0)/L_0$$  \hspace{1cm} (2)

where $L_0$ is the muscle length at 0.2 g of preload and $L_N$ is the muscle length during the uniaxial stretches.

Muscle $\varepsilon'$ was calculated from lengthening rate measurements as follows

$$\varepsilon' = 1/L_0(dL/dt)$$  \hspace{1cm} (3)

where $dL/dt$ is the muscle lengthening rate.

The myocardial $\sigma$ versus $\varepsilon$ relationship at any $\varepsilon'$ can be affected by a number of factors that may alter the material properties of the myocardium. These factors include passive elastic stiffness and viscous damping. Changes in both of these determinants acting individually or in concert can alter the $\sigma$ versus $\varepsilon$ relationship. The experimental methods described above and the analytic model described below were used to examine the effects of POH and the effects of an acute change in cardiocyte constitutive properties on elastic stiffness and viscous damping separately.

The papillary muscle was modeled using two elements in parallel, a nonlinear spring ($\sigma_1$) where

$$\sigma_1(\varepsilon) = Ae^{P\varepsilon} + B$$  \hspace{1cm} (4)

and a nonlinear viscous damper ($\sigma_2$) where

$$\sigma_2(\varepsilon') = C - De^{-\eta\varepsilon'}$$  \hspace{1cm} (5)

Total stress for this two-element parallel model is

$$\sigma(\varepsilon, \varepsilon') = \sigma_1(\varepsilon) + \sigma_2(\varepsilon')$$  \hspace{1cm} (6)

To calculate elastic stiffness ($\beta$) and viscous damping constants ($\eta$), measurements of muscle stress, strain, and strain rate were fit by a constitutive equation for a nonlinear viscoelastic composite biomaterial where

$$\sigma(\varepsilon, \varepsilon') = (Ae^{P\varepsilon} + B) + (C - De^{-\eta\varepsilon'})$$  \hspace{1cm} (7)

where $A$, $B$, $C$, and $D$ are curve fitting constants.

The elastic stiffness constant ($\beta$) was assessed using the slowest uniaxial stretch at 0.1 mm/min. Under these experimental conditions, strain rate approximated zero, the second portion of $\sigma$ versus $\varepsilon$ became negligible, and stress became a function of strain alone. Therefore, the stress versus strain data obtained from the slow uniaxial stretch at 0.1 mm/min were fit to the first half of $\sigma$ versus $\varepsilon$ ($i.e.$, Eq. 4) and $\beta$ was determined. We hypothesized that if POH increased elastic stiffness, the $\sigma$ versus $\varepsilon$ relationship would shift up and to the left and $\beta$ would increase (Fig. 2).

The viscous damping constant ($\eta$) was assessed using all four uniaxial variable rate stretches (Fig. 2). From these stretches, the relationship between stress and strain rate was defined at a selected, constant value of strain (Fig. 2). At any selected, constant value of strain, the relationship between stress and strain rate was curvilinear. Under these experimental conditions, strain was constant, the first portion of $\sigma$ became constant, and stress became a function of strain rate alone. Therefore, the stress versus strain rate data obtained at a constant strain (0.05) from the variable rate stretches were fit to the second portion of $\sigma$ and $\eta$ was determined. We hypothesized that if POH increased viscous damping, the $\sigma$ versus $\varepsilon'$ relationship would shift up and $\eta$ would increase (Fig. 2).

**Statistics**

Data are presented as means ± SE for each data group. Differences between normal and POH groups at baseline and after colchicine treatment were determined using a two-way repeated-measures ANOVA and were considered significant at $P < 0.05$.
RESULTS

Hemodynamic Studies In Vivo

The effects of PAB on in vivo measurements of pressure, oximetry, and mass are summarized in Table 1. The data for normal and PAB cats were similar to those in our previous studies (52) in that PAB caused significant increases in RV systolic pressure and mass.

Effects of POH on Myocardial Elastic Stiffness

An example of the effects of POH on myocardial elastic stiffness is shown in Fig. 3A. Summary data examining myocardial elastic stiffness for all animals studied are shown in Fig. 3B. POH caused the myocardial stress versus strain relationship to shift upward and to the left so that for any given strain, the stress in the papillary muscle was greater for the POH muscle than for the normal muscle. When these data were fit by Eq. 3, this analysis showed that POH caused a significant increase in the elastic stiffness constant \( \beta \) from 20.5 \( \pm \) 1.3 in the normal papillary muscles to 28.4 \( \pm \) 1.8 in the POH papillary muscles \( (P < 0.05) \). This shift in the stress versus strain relationship and the increase in the elastic stiffness constant indicate that POH increased myocardial elastic stiffness.

Effects of POH on Myocardial Viscosity

An example of the effects of POH on myocardial viscosity is shown in Fig. 4A. Summary data examining myocardial stiffness for all animals studied are shown in Fig. 4B. POH caused the myocardial stress versus strain rate relationship to shift upward so that for any given strain, as the strain rate increased, the stress on the papillary muscle increased more rapidly in the POH muscle than it did in the normal muscle. When these stress versus strain data were fit to Eq. 4, this analysis showed that POH caused a significant increase in the viscous damping constant \( \eta \) from 15.2 \( \pm \) 1.1 s in the normal papillary muscles to 19.8 \( \pm \) 1.5 s in the POH papillary muscles \( (P < 0.05) \).

Table 1. Characteristics of the pulmonary artery banding model

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pulmonary Artery Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV systolic pressure, mmHg</td>
<td>21 ( \pm ) 3</td>
<td>69 ( \pm ) 5*</td>
</tr>
<tr>
<td>( \Delta O_2 ) %saturation (SVC vs. RA)</td>
<td>-0.5 ( \pm ) 0.2</td>
<td>-0.2 ( \pm ) 0.6</td>
</tr>
<tr>
<td>RV weight/body weight, g/kg</td>
<td>0.52 ( \pm ) 0.04</td>
<td>1.10 ( \pm ) 0.05*</td>
</tr>
<tr>
<td>RV weight/tibial length, g/cm</td>
<td>0.11 ( \pm ) 0.01</td>
<td>0.27 ( \pm ) 0.02*</td>
</tr>
<tr>
<td>LV weight/body weight, g/kg</td>
<td>2.2 ( \pm ) 0.1</td>
<td>2.0 ( \pm ) 0.2</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>2.4 ( \pm ) 0.1</td>
<td>2.7 ( \pm ) 0.1</td>
</tr>
<tr>
<td>Arteriovenous ( O_2 ) difference, ml/l</td>
<td>35.4 ( \pm ) 2.1</td>
<td>32.1 ( \pm ) 1.0</td>
</tr>
<tr>
<td>RV diastolic pressure, mmHg</td>
<td>2 ( \pm ) 1</td>
<td>6 ( \pm ) 1</td>
</tr>
<tr>
<td>Liver weight/body weight, g/kg</td>
<td>24.2 ( \pm ) 1.0</td>
<td>26.7 ( \pm ) 1.2</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n \) = no. of cats. RV, right ventricle; SVC, superior vena cava; RA, right atrium; LV, left ventricle. Statistical comparisons were by unpaired Student’s t-test. *\( P < 0.05 \) vs. control.

Effects of Colchicine on Myocardial Stiffness and Viscosity

The effects of colchicine treatment on myocardial elastic stiffness and viscous damping are shown in Figs. 5 and 6. When isolated papillary muscles were treated with 10\(^{-5}\) M colchicine for 90 min, there was no significant change in elastic stiffness in normal or POH muscles. In contrast, colchicine caused a significant decrease in the myocardial viscous damping constant in POH muscles from 19.8 \( \pm \) 1.5 s at baseline to 14.7 \( \pm \) 1.3 s after colchicine treatment \( (P < 0.05) \). In fact, whereas there was a tendency for the viscosity to increase in normal muscles after treatment with colchicine, the viscosity in POH muscles returned toward normal after treatment with colchicine.
Stress vs. Strain at Physiological Lengthening Rates: Effects of POH and Colchicine

The data presented thus far support the conclusions that changes in cellular viscoelastic properties induced by POH and altered by colchicine contribute to the viscoelastic material properties of the myocardium. In particular, changes in POH myocardial viscosity can be normalized by altering cellular cytoskeleton. However, no studies have yet been presented that use methods that mimic in vivo myocardial lengthening. This raises a set of critical questions. Do these findings have clinical relevance? Do the changes in viscosity produced by colchicine alter the stress versus strain relationship when the muscle lengths at a rate that corresponds to in vivo lengthening rate? At rapid in vivo lengthening rates, do alterations in elasticity predominate and obscure effects of changes in viscosity?

When the myocardium is stretched at very slow rate, such as 0.1 mm/min, the contribution made by the viscous damper to the σ versus ε relationship is negligible. In contrast, when the myocardium is stretched at a physiological rate, such as 10.0 mm/s, the viscous damper becomes engaged such that the σ versus ε relationship becomes a function of both the elastic spring and the viscous damper. Therefore, in a POH muscle, it was expected that an upward shift in the σ versus ε relationship at a strain rate of 10.0 mm/s was caused by increases in both β and η. Furthermore, a decrease in η produced by treatment of a POH muscle with colchicine should affect the overall σ versus ε relationship when stretched at 10.0 mm/s. However, because colchicine treatment did not alter β, the overall σ versus ε relationship would not be expected to return completely to normal. To test this hypothesis, the σ versus ε relationship was examined in POH versus normal muscle when stretched at 10.0 mm/s. An example of such an experiment is shown in Fig. 7.

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The purpose of this study was to test the hypothesis that changes in the composite material properties of the myocardium induced by POH are caused, at least in part, by changes in the intrinsic constitutive properties of the component cardiocytes themselves. To test this hypothesis, five steps must be taken. First, determine whether POH alters the constitutive viscoelastic properties of the cardiocyte. Second, identify one or more intracellular structures or processes responsible for causing these changes in cellular properties. Third, establish a method that will acutely and selectively correct the abnormality in this cellular structure or process. Fourth, develop an in vitro system capable of measuring changes in the material properties of the myocardium that result from an acute correction of this cellular structure or process. Finally, determine whether POH-induced changes in the material viscoelastic properties of the myocardium are changed by correcting this cellular structure or process.

The first three steps were accomplished in our previous studies (43, 52, 53). The last two steps were taken in the current study. Current data support the following conclusions: 1) POH caused a significant change in the material properties of the composite myocardium by increasing both myocardial viscosity and passive elastic stiffness and 2) The composite material properties of the myocardium were altered by selectively and acutely correcting the POH induced abnormalities in the intracellular cytoskeleton. Acutely depolymerizing the excessive microtubule network present in POH caused a decrease in cardiocyte viscosity and a decrease in myocardial viscosity. This cellular change led to a partial, but not complete, correction of the viscoelastic properties of the composite POH myocardium. 3) Therefore, changes in the constitutive properties of the cardiocyte itself contributed causally to the abnormalities in myocardial viscoelasticity and myocardial diastolic function that occur during the development of POH. These data may have important clinical application because if and when it becomes possible to correct selective cellular abnormalities, this may provide therapeutic methods to improve or even normalize the myocardial diastolic dysfunction present in specific disease processes.

Bioengineering Model

A number of mechanical models have been proposed to describe the material properties of myocardial tissue (9, 11, 12, 17, 19–22, 30, 37, 42, 50). These models range from simple to complex. Choosing a model requires a balance between one that is simple but ignores important factors relevant to the questions posed versus one that takes every possible factor into account but is so complex it cannot be readily applied to in vitro experimental design. Bearing this fact in mind, and based on the fact that all biological material, including cardiac muscle, behaves as a nonlinear viscoelastic material, we chose a two-element model composed of a nonlinear spring in parallel with a nonlinear viscous damper. We believe that the assumptions that define this model are reasonable and justifiable; however, they impose some limitations that must be acknowledged. For example, the model does not mimic the response of isolated muscle to a quick stretch or quick release, in which there is an immediate change in strain and stress, followed by additional changes over time reflecting the viscous element because the parallel viscous element will not allow it. To overcome this limitation requires the use of a three-element model with one viscous element in series with and a second viscous element in parallel with the spring. However, the additional complexity that this three-element model would add is not likely to change the outcome or conclusions stated in this study. Because the limitation is applicable both to the normal and RV POH muscles at baseline and after cochinacine treatment, the differences observed in each of these experimental conditions should not be significantly effected by the limitation in model design and should not limit the validity of the study conclusions.

The bioengineering model and material testing methods chosen in this study were similar to those used by previous investigators (9, 11–13, 15, 17, 19–22, 30, 36, 37, 42, 50). Throughout the 1970s–1990s, attempts to apply these principles and methods to in vivo studies of the intact myocardium were made in animal models and patients with heart disease (11, 37, 50). The most common model used was a nonlinear spring in parallel with a linear viscous damper. However, two features of in vivo studies limited the value of this model and limited the apparent value of examin-
ing the elastic spring separately from the viscous damper. In vivo, muscle lengthening occurs at very rapid rates, making the differences between normal and abnormal muscle that result from changes in viscosity appear small and insignificant because only one strain rate can generally be examined. Furthermore, from a pragmatic perspective, previous investigators have suggested that characterizing viscosity did not add significantly to an understanding of the physiology of the heart disease being examined. For these and other reasons, viscous damping appeared less important, was not examined closely, and was frequently omitted from consideration. As a result, myocardial material properties have been generally characterized as myocardial stiffness based on the stress vs strain relationship. However, when we and other investigators attempted to examine the underlying cellular and extracellular mechanisms that characterize myocardial material properties, it became evident that assessment of at least two major determinants of the stress vs strain relationship, elastic stiffness and viscous damping, are crucial (3, 4, 12, 24–27, 30, 41–43, 47, 53).

There are a number of important reasons to examine elastic stiffness and viscous damping separately and independently. Among the most important is the fact that this kind of analysis may provide clues as to which specific structures or processes are altered by a particular pathological state in which specific structures or processes lead to abnormalities in diastolic function. For example, given the anatomic structure of extramyofilamentous cytoskeleton, their relation to myofilaments, and their own physical viscoelastic properties, we hypothesized that microtubules would have a significant impact on viscosity. Therefore, when it became clear that POH altered cellular viscosity, the excess microtubules found in POH became a reasonable target (43, 53). The results of current and previous studies make it likely that changes in the passive elastic spring and the viscous damper can be altered by separate and distinct changes in cellular and extracellular structures and processes (3, 4, 12, 24–27, 30, 41–43, 47, 53). This possibility is borne out by the results of the current study. Treatment with colchicine and the resultant depolymerization of the microtubules normalized cellular and myocardial viscosity but did not alter passive elastic stiffness. Because of this, myocardial lengthening at rates comparable to in vivo physiological rates improved but remained abnormal in POH treated with colchicine. Therefore, the current study leaves open the question of what other intracellular and extracellular processes are abnormal in and contribute to the diastolic dysfunction that accompanies POH.

**Cellular and Extracellular Mechanisms That Alter Myocardial Material Properties**

A number of previous studies have shown that changes in intracellular calcium homeostasis, myofilaments, energetics, the cytoskeleton, and neurohumoral activation can result in changes in myocardial diastolic function (1, 5, 10, 18, 28, 29, 32, 33, 39, 45, 51). Disease processes that result in myocardial ischemia, hypertrophy, or aging or disease processes that result from exposure to toxins or infection can alter diastolic myocardial function by changing one or more of these intracellular structures or processes. The difficulty with these studies is assigning specificity to the results. For example, exposing myocardium to ischemia-reperfusion or hypoxia/reoxygenation clearly results in changes in a number of intracellular and extracellular processes that may individually or in combination cause changes in the material properties of the myocardium and result in diastolic dysfunction (7, 34, 44, 46). Therefore, to determine which of these intracellular and extracellular processes are central to the development of diastolic dysfunction, a number of investigators have studied the relationships between cellular and myocardial material viscoelastic properties in normal cardiomyocytes and muscles. ter Keurs and others (12, 30, 42) have clearly shown that changes in intracellular calcium homeostasis (and other intracellular processes) result both in changes in cellular and myocardial viscoelasticity. Granzier and others (4, 24–27, 41, 43, 47, 53) have shown that changes in the intracellular cytoskeleton can contribute to changes in cellular and myocardial viscoelasticity. Changes in cytoskeletal proteins such as titin isotypes have been shown to contribute to differences in viscoelasticity among animal species (48). None of these studies, however, has examined these intracellular processes in myopathic cells or muscles. In addition, none of these studies have been able to acutely correct intracellular processes, correct cellular viscoelastic properties, or define the relative contribution to myocardial properties that a specific intracellular process or structure makes to the presence of diastolic dysfunction. These facts make the results presented in the current study unique and important.

**Specificity of Colchicine Effects**

Recent studies have raised but not definitively answered the question of whether free, nonpolymerized β-tubulin may act as a β-adrenergic agonist increasing calcium, cAMP, and protein kinase A via the β-adrenergic G protein signaling cascade (6, 23). These data have potentially important implications for the current study. There is no question that the diastolic relaxation rate can be altered and improved if calcium is released more rapidly from troponin C (as would occur if troponin I were phosphorylated by cAMP) or if calcium were sequestered more rapidly into the sarcoplasmic reticulum (as would occur if phospholamban were phosphorylated by cAMP). In addition, it is clear that persistent, residual myofilament activation during diastole can impede muscle relaxation and lengthening rate and effect both passive elastic stiffness and viscosity. However, for this mechanism, which is dependent on an increase in colchicine induced β-adrenergic G protein signaling, to have an effect on myocardial material...
properties, it must lower resting, quiescent, diastolic calcium concentration and increase the rate of calcium sequestration. In previous studies, we and other investigators (6, 52) have been unable to demonstrate that colchicine alters resting, quiescent, diastolic calcium concentration or the rate of calcium sequestration. Whether an increase in β-adrenergic G protein signaling itself alters resting, quiescent, diastolic calcium concentration in other experimental conditions is less clear. Nonetheless, it is possible that an increase in β-adrenergic G protein signaling may alter myocardial material properties. Most important to the results of the current study, however, colchicine, regardless of whether the mechanism of action is single or multiple, does alter the intracellular process that results in a change in constitutive cellular viscoelastic properties, and this change in constitutive cellular viscoelastic properties is at least partially and causally responsible for the alterations in myocardial material properties that develop during chronic POH.

In conclusion, POH caused a significant change in the material properties of the composite myocardium by increasing both myocardial viscosity and passive elastic stiffness. The composite material properties of the myocardium were altered by selectively and acutely correcting the POH induced abnormalities in the intracellular cytoskeleton. Acutely correcting alterations in the cellular cytoskeleton present in POH caused a decrease in cardiocyte viscosity and a decrease in myocardial viscosity. This cellular change led to a partial, but not complete, correction of the viscoelastic properties of the composite POH myocardium. Therefore, changes in the constitutive properties of the cardiocyte itself contributed causally to the abnormalities in myocardial viscoelasticity and myocardial diastolic function that occur during the development of POH.

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