IT IS generally accepted that resistance to ventricular filling is increased in hearts hypertrophied by pressure loading. However, it has not been possible to determine conclusively which of the several determinants of ventricular diastolic stiffness are altered and in particular whether intrinsic muscle stiffness is changed (for review, see Grossman and Parmley, 1973; Alpert et al., 1974; Bing et al., 1978).

Although in vitro measurements of passive muscle stiffness circumvent many of the problems associated with measurements in intact hearts and a large amount of data is available from such studies, conflicting conclusions have been reported. Thus, some studies have reported unaltered stiffness of hypertrophied muscle (Grimm et al., 1963; Williams et al., 1966; Spann et al., 1967; Pannier et al., 1968; Bassett and Gelband, 1973; Cooper et al., 1973; Williams and Potter, 1974; Jouannot and Hatt, 1975), whereas others have found muscle stiffness to be increased (Mirsky and Parmley, 1973; Alpert et al., 1974; Bing et al., 1978).

Assessment of passive muscle stiffness in vitro has been based almost exclusively on the comparison of resting length-tension relationships in normal and hypertrophied muscles. However, the validity of assessing muscle stiffness solely from resting length-tension relations has been challenged by Mirsky (1976), who has proposed the elastic stiffness-stress relationship as a more accurate measure of passive stiffness. To emphasize this point, Mirsky (1976) determined the elastic stiffness-stress relationship from resting length-tension data published in a previous study (Spann et al., 1967) in which the resting length-tension relation of hypertrophied muscles was similar to that of non-hypertrophied muscles. According to Mirsky's (1976) calculations, the slope of the elastic stiffness-stress relationship and elastic stiffness at any stress level was greater in the hypertrophied muscles.

In addition to the use of different methods for assessing passive stiffness, it seemed possible that variations in contractile state of the muscles might also contribute to the varying conclusions regarding...
stiffness of hypertrophied myocardium. Certainly, contractile function varies after pulmonary or aortic banding in experimental animals in a time-dependent manner (Meerson, 1969; Williams and Potter, 1974; Jouannot and Hatt, 1975), and it has been reported that alterations in contractile force do affect passive stiffness, at least acutely (Sonnenblick et al., 1966; Hoffman et al., 1969). Thus, we compared stress-strain and elastic stiffness-stress relationships of hypertrophied right ventricular papillary muscles from pulmonary artery-banded cats with normal and depressed contractile function to those derived from non-banded cats. Our results indicate that elastic stiffness is increased modestly in hearts hypertrophied by pressure loading independent of changes in contractile function.

Methods

Adult cats weighing between 1.5 and 2.5 kg anesthetized with intraperitoneal sodium pentobarbital (35 mg/kg) and artificially ventilated underwent pulmonary artery constriction with a 4.0-mm i.d. band. The band was composed of Silastic tubing containing a copper wire 4.0 mm in length and a silk suture. After the band had been placed around the main pulmonary artery, the ends were approximated and the suture tied to maintain band size. Two to 24 weeks later, the animals were killed, the hearts removed, and the smallest right ventricular papillary muscle placed in a myograph containing modified Kreb's solution of the following composition (mm): Na⁺, 144; K⁺, 4.0; Ca²⁺, 2.5; Mg²⁺, 0.5; H₃PO₄, 10; HCO₃⁻, 25; Cl⁻, 128; and glucose, 5.6. The solution was maintained at a temperature of 30°C and was gassed vigorously with 95% O₂-5% CO₂ which produced a pH of 7.4 and a PO₂ exceeding 500 mm Hg. The non-tendinous end of the muscle was held rigidly by a plastic clip attached to a short metal rod which passed through the bottom of the myograph and was connected to a Statham force transducer (Statham Instruments, Inc., model G1-250). The tendinous end was secured by a short silk suture to the long arm (10:1 ratio) of a lever attached to a displacement transducer (Schaevitz Engineering, model R4BS) which, in turn, was secured to a rigid stand. The compliance of the system without muscle was 2 µm/g and the equivalent mass of the level system was 150 mg. Micrometers appropriately placed above the lever were used to obtain isometric contractions at various known muscle lengths or isotonic contractions with light preloads. The muscle was stimulated with rectangular pulses 4-5 msec in duration at a frequency of 12/min and 10% above threshold voltage by use of field electrodes parallel to the long axis of the muscle. After the muscle had contracted isotonically with a light preload for 45-60 minutes, maximal velocity of shortening was measured. Isometric contractions then were produced and length-tension relations determined after 0.1-mm increments in length from a point at which resting force first rose above zero force to that where active force first declined from its maximum. Resting tension was determined after a change in length only when stress relaxation appeared complete. Three length-tension curves were obtained for each muscle. Muscle length at the peak of active force development (Lₘₐₓ) was measured with a calibrated reticle. The right ventricle was dissected from the left ventricle plus septum and the weight of each specimen obtained. Dry weight also was obtained after oven drying to a constant weight.

Active muscle performance was assessed from measurements of maximal velocity of isotonic muscle shortening with a light preload and from active force development at Lₘₐₓ. Passive stiffness was assessed from the stress-strain relationship as described by Glantz and Kernoff (1975): stress (σ) = force/instantaneous cross-sectional area whereas strain (Lagrangeian) = (L - L₀)/L₀, where L₀ = muscle length at zero stress. The elastic constants β and α then were determined from the equation σ = α(e^β - 1). The tangent modulus or elastic stiffness (da/dd) = βσ + αβ (Glantz and Kernoff, 1975). Instantaneous cross-sectional area was calculated from the instantaneous length and wet weight of the muscle. Statistical analyses were performed using analysis of variance unless otherwise specified (Snedecor, 1956).

Results

The data obtained in 18 banded and 21 non-banded cats are presented in Table 1. Although right ventricular pressure was not measured in banded animals, in previous studies using a similar model, pulmonary artery cross-sectional area was reduced by an average of 60-70% and right ventricular systolic pressure increased by approximately 100% (Williams and Potter, 1974). The mean right ventricular:left ventricular weight ratio of 0.53 in banded animals, in previous studies using a similar model, was similar to that noted in our previous study and is approximately 70% above that of non-banded animals. Dry weight:wet weight ratios of ventricular specimens were not significantly different in the two groups and averaged 0.24. Papillary muscle cross-sectional area at L₀ and wet weight ratio; /9 and a - elastic constants.

<table>
<thead>
<tr>
<th></th>
<th>Papillary muscle cross-sectional area at L₀ (mm²)</th>
<th>RV/LV</th>
<th>α (g/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-hypertrophied</td>
<td>21 ± 0.2</td>
<td>0.03</td>
<td>±0.01</td>
</tr>
<tr>
<td>Hypertrophied</td>
<td>18 ± 0.1</td>
<td>0.03</td>
<td>±0.02</td>
</tr>
</tbody>
</table>

Values represent mean ± sem. RV/LV = right ventricular:left ventricular weight ratio; L₀ = unstressed muscle length; β and α = elastic constants.
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area at $L_\text{max}$ although slightly larger than optimum, also was similar in the two groups. Papillary muscles weighed an average of $8.1 \pm 0.5$ and $9.2 \pm 0.7$ mg in the control and banded groups, respectively, a difference that is not significant statistically. The difference in initial muscle length also was not significant. Initial length of individual muscles was 75–80% of that at $L_\text{max}$.

Examination of the three resting length-tension curves obtained in each muscle revealed that the resting tension at any given length in the first curve generally was higher than in the second or third curves, whereas no systematic difference was observed between the second and third curves. In our experience, this is a common occurrence when isotropic contractions are first initiated after isotonic equilibration. Thus, the data from the first curve were discarded and those from the latter two curves averaged. The average difference in resting tension at a given length between the second and third curves was 12%.

The elastic constants, $\beta$ and $\alpha$, are presented in Table 1. The $\alpha$ values are not significantly different, whereas the difference between $\beta$ values is of questionable significance ($P = 0.625$). However, elastic stiffness over the entire stress range is greater in hypertrophied muscles (Fig. 1) and by analysis of covariance the difference is significant ($P < 0.05$). There was no significant correlation in hypertrophied muscles between $\beta$ ($r = 0.213$) or $\alpha$ ($r = 0.149$) and the degree of hypertrophy as assessed by RV: LV weight.

Since we had demonstrated previously in this model that contractile function varied in a time-dependent manner after banding (Williams and Potter, 1974), we determined whether passive stiffness might be related to the level of contractile function. Passive stiffness was determined in three subgroups of muscles from animals banded for 2 to 3, 6, or 24 weeks before study. The groups contained 5, 7, and 6 animals, respectively, with right ventricular: left ventricular weight ratios averaging 0.58, 0.51, and 0.50, respectively, values which are not significantly different.

Active force at $L_\text{max}$ and the maximal velocity of muscle shortening at a light preload for these groups and for the non-hypertrophied muscles are given in Figure 2. Both variables were significantly reduced 2–3 weeks after banding and this change was followed by recovery to control values by 24 weeks. Elastic stiffness-stress relations of these groups of hypertrophied and non-hypertrophied muscles are presented in Figure 3. The stiffness-stress relationship was not significantly different among hypertrophied muscles.

**Discussion**

Our conclusion that passive stiffness of pressure-induced hypertrophied myocardium is increased is in accord with that of previous studies (Alpert et al., 1974; Mirsky, 1976; Bing et al., 1978). However, the increase in stiffness we observed was modest and of lesser magnitude than that reported in previous studies. Whether this difference is due to use of a different animal model or method for assessing passive stiffness (Alpert et al., 1974; Bing et al., 1978) or Mirsky's (1976) use of data from cat hearts with greater degrees of pulmonary artery constriction (Spann et al., 1967) is unclear.

Several methods for assessing passive stiffness have been proposed, but it is unclear as to which is the most valid (Glantz, 1979; Mirsky, 1979). A principal argument against the use of stress-strain relations employing either natural or Lagrangian strain has been the necessity for determining un-
stressed muscle length. Certainly, at low levels of stress, small increments in stress are associated with large increments in muscle length and accurate measurements of this relationship are difficult. However, we found that, with high sensitivity tracings, measurements of muscle length at the point where resting stress first rises above zero are reproducible, i.e., ±8%. Furthermore, Glantz and Kernoff (1975) have shown that $\beta$ is relatively insensitive to small changes in initial muscle length in contrast to $\alpha$. Our values for $\beta$ are quite similar to those of Glantz and Kernoff (1975) who used isolated canine papillary muscle.

With one exception (Bing et al., 1978), resting length-tension relations of aortic or pulmonary artery-banded animals have not varied significantly from those of non-banded animals (Grimm et al., 1963; Williams et al., 1966; Pannier, 1971; Cooper et al., 1973; Bassett and Gelband, 1973; Williams et al., 1974; Jouannot and Hatt, 1975). However, Mirsky’s (1976) recalculation of the data of Spann et al. (1967) amply demonstrated that the elastic stiffness-stress relation is a more sensitive measure of change in passive stiffness than is the resting-length tension curve. Our results support Mirsky’s conclusion, since resting length-tension relations in our hypertrophied and non-hypertrophied groups also were not significantly different.

A principal finding of this study was that passive stiffness was not related to the level of contractile function. Elastic stiffness-stress relations of hypertrophied muscles with markedly depressed contractile function (2-3 week banded) and comparably hypertrophied muscles with normal function (24-week banded) were essentially identical. Thus, a chronic change in contractile function does not affect resting stiffness as has been reported with acute changes in contractile force (Sonnenblick et al., 1966).

The weight of our hypertrophied papillary muscles was slightly but not significantly greater than that of the non-hypertrophied ones. Although this would suggest that the papillary muscles did not participate in the hypertrophic process, our results are due to selection rather than lack of hypertrophy. Muscles greater than 2.0 mm$^2$ in cross-sectional area (approximately 30%) were excluded from analysis in an effort to limit problems associated with core hypoxia. Importantly, others have demonstrated that pulmonary artery banding in cats does produce hypertrophy of both the free wall and the papillary muscles of the right ventricle (Cooper et al., 1973).

Although the cross-sectional area of both non-hypertrophied and hypertrophied muscles was greater than optimum, there was no significant difference between the groups and the results should be comparable. Also, we calculated cross-sectional area at initial muscle length rather than at L$_{max}$, as is customary. Had we employed the latter cross-sectional area, it would be approximately 25% smaller than that listed.

The elastic stiffness-stress relationship as a measure of passive stiffness is based on the stress-strain concept and elastic stiffness or tangent modulus defines the slope at any point on the stress-strain curve (Mirsky, 1976). Our calculation of elastic stiffness requires the determination of the elastic constants based on the assumption that the stress-strain relationship is an exponential one. Although Mirsky and Parmley (1973) reported that the stress-strain relationship deviated from a single exponential at both large and small stress, our stress-strain data points fit a single exponential, with coefficients of determination ($r^2$) averaging 0.979 (range 0.960-0.998) and 0.984 (range 0.942-0.998) in the hypertrophied and non-hypertrophied groups, respectively.

Functionally, cardiac muscle has been considered primarily in terms of a three-element model, i.e., a freely extensible contractile element with series and parallel elastic components (Brady, 1967; Hefner and Bowen, 1967; Glantz, 1975). In such a model, the elastic constant $\beta$ would reflect the characteristics of the parallel elastic component as previously suggested (Alpert et al., 1974; Mirsky, 1976). However, there is evidence that neither model is correct (Hoffman et al., 1969; Loeffler and Sagawa, 1975) and that the contractile elements may not be freely extensible (Hoffman et al., 1969). Until the correct...
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model is determined, the elastic constant cannot be equated definitively with any particular element of the model.

Similarly, the anatomic determinants of resting stiffness have not been elucidated completely, although the connective tissue matrix has been considered to play a major role (Sonnenblick and Skelton, 1974). An increase in collagen has been observed in hearts hypertrophied by pressure loading (Buccino et al., 1969; Bing et al., 1978). Although increased collagen may account for or contribute to the increased stiffness of hearts hypertrophied in this manner, a correlation between changes in resting stiffness and connective tissue does not exist under all circumstances. Urthaler et al. (1978) observed a growth-dependent decrease in passive stiffness in canine atrial myocardium, whereas connective tissue increased.

It should be appreciated that studies reporting increased stiffness of pressure-induced hypertrophied myocardium have employed animal models in which the load is applied abruptly. Bishop and Melsen’s (1976) observation that myocardial necrosis and fibrosis occurred in hearts of cats hypertrophied by pulmonary artery banding but not in hearts with congenital pulmonary valve stenosis indicate that caution must be exercised in concluding that all pressure-induced hypertrophied myocardium is stiffer than normal. The abruptness with which the load is applied and the rapidity with which hypertrophy develops may be major determinants of resting stiffness.

Whatever structures in the myocardium determine passive stiffness, the elastic constants should reflect the overall passive elastic behavior of the muscle. However, this alone is an insufficient measure of passive stiffness, since the β constant of two muscles could be identical, yet elastic stiffness at any stress level significantly different. Alternatively, with different β constants, elastic stiffness of one muscle could be greater at certain levels of stress but less at other stress values, as exemplified by the study of Alpert et al. (1974). Elastic stiffness can be considered the “operational” stiffness of the muscle and both elastic constants and elastic stiffness at various levels of stress are necessary to characterize functionally the passive stiffness properties of the muscle as stated previously by Mirsky and Parnley (1973).

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