Mechanical Properties of Myocardium from Hypertrophied Rat Hearts

A Comparison between Hypertrophy Induced by Senescence and by Aortic Banding

FRANK C.P. YIN, HAROLD A. SPURGEON, MYRON L. WEISFELDT, AND EDWARD G. LAKATTA

SUMMARY Cardiac hypertrophy is a characteristic change that occurs in senescence. Muscles from senescent as compared to mature hearts also demonstrate functional alterations that are similar to the alterations found in muscles from experimentally hypertrophied hearts. Thus, an attractive hypothesis is that functional alterations in senescent muscles are related to the underlying hypertrophy. To test this hypothesis, we used a rat model of aging in which experimental hypertrophy was produced by aortic banding. The time course and extent of cardiac hypertrophy, as well as isometric twitch and viscoelasticity parameters, as a function of age, first were determined in muscles from the rat hearts. Aortic banding then was performed on middle-aged rats to produce the same extent of hypertrophy as seen in the senescent hearts. The resulting functional alterations in muscles from the banded (B) hearts were compared to the senescent (S) and middle-aged (M) muscles. Using tibia length as a reference, we found 14% LV hypertrophy in senescent compared to both young and middle-aged rats, indicating that the hypertrophy occurred during the last quarter of life. The S muscles demonstrated a 25% prolongation in contraction duration (CD) and a 30% increase in slope of the active stiffness-tension line (αA) compared to both young adult and middle-aged muscles. Compared to middle-aged muscles, the B muscles demonstrated a similar spectrum of change in mechanical properties as the S muscles (8% increase in CD and 12% increase in αA), but the quantitative differences between the B and S muscles were significant. Over the functional range of developed tensions, the B muscles demonstrated the lowest and the S muscles the highest values of stiffness. The findings suggest that a portion of the mechanical property alterations seen in the senescent heart are due to the underlying hypertrophy. However, the hypertrophy produced by mechanical loading of the LV cannot explain all of the senescent changes.


WITH advancing age, several characteristic alterations occur in the cardiovascular system. One of the most commonly described changes in senescence is a moderate degree of myocardial hypertrophy in both animals and man (see review by Lakatta, 1979). Other alterations seen in the senescent hearts are a prolonged time course of relaxation (Weisfeldt et al, 1971; Lakatta et al., 1975; Rumberger and Timmerman, 1976; Templeton et al. 1978), a diminished rate of sarcoplasmic reticulum calcium accumulation (Froehlich et al., 1978), a decreased rate of protein and RNA synthesis (Meerson et al. 1978), and an increased active dynamic stiffness in senescent compared with mature heart muscle (Spurgeon et al., 1977) and intact hearts (Templeton et al. 1979). Although the extent to which these functional alterations seen with senescence are related to the concurrent hypertrophy is unknown, a relationship is suggested, since prolonged contraction duration (CD), diminished sarcoplasmic reticulum function, a triphasic increase, plateau, then decrease in RNA and protein synthesis, and increased stiffness have been observed in experimental models of hypertrophy (Bing et al., 1971; Meerson and Kapelko, 1972; Sordahl et al., 1973; Gunning and Coleman, 1973; Alpert et al., 1974; Ito et al., 1974; Jouanatt and Hatt, 1975; Meerson et al., 1978). These alterations, however, are not uniformly observed but depend critically on the duration and type of load imposed on the heart, the species studied, the extent of hypertrophy induced, and whether or not heart failure is produced. There have been no previous studies which have examined the changes in mechanical properties of muscles from hearts in which experimental hypertrophy of the same extent as that existing during the normal aging process has been induced.

The goals of this study were (1) to determine the extent of hypertrophy and its relationship to alterations in mechanical properties as a function of age in the rat model, (2) to create hypertrophy caused
Hypertrophy of Senescence and Aortic Banding

Yin et al.

Methods

Cardiac Hypertrophy of Senescence

In experimental models of hypertrophy, many different indices, including absolute heart weight, heart weight: body weight ratio, and predicted heart weight based on covariance analysis, have been employed to quantify the extent of hypertrophy achieved. However, aging, as well as many experimental interventions, is associated with drastic alterations in body weight which could make an index based on body weight quantitatively inaccurate. The ideal reference would be one that is easily quantifiable, is not altered by the intervention producing the hypertrophy, and does not change on completion of growth. Tibial length in rats fulfills these criteria (Berg and Harrison, 1958) and was chosen as the reference with which to index hypertrophy in the present study.

To delineate the anatomic changes from the completion of growth to senescence, defined as the age at which 50% natural mortality occurs (Schlettwein-Gsell, 1970), 178 male Wistar rats from 6 to 28 months of age from the Gerontology Research Center animal colony were used. All animals were housed four or five to an 18 X 30 X 44-cm wire bottom cage under conditions of controlled light and temperature and were allowed ad libitum access to National Institutes of Health open formula rat pellets and water.

The rats were killed either by spinal transection or by pentobarbital or ether anesthesia. Each rat was weighed and the heart then removed, stripped of fat and appendages, and divided into a right ventricular free wall portion and a left ventricular-septal portion (combined right and left septa) which were blotted dry, weighed, and then reweighed after drying to constant weight at 100°C (24-48 hours). One leg was severed above the knee joint, and the muscle and skin of the tibia were removed by mechanical stripping or by brief immersion in boiling sodium hydroxide solution. The length of the tibia from the condyles to the tip of the medial malleolus was measured by micrometer calipers. The tibia then was dried and the length remeasured. Body weight (BW), dry tibial length (TL), dry right ventricle weight (RVW), and dry left ventricle-septum weights (LVW) were recorded. The extent of hypertrophy as a function of age was ascertained by comparing the ratios LVW/TL and RVW/TL in rats grouped into 2-month age brackets. All data reported are mean ± SEM. Statistical analysis was performed by using analysis of variance, and differences between groups were tested by the range test of Newman and Keuls (Armitage, 1971).

Cardiac Hypertrophy of Aortic Banding

The results of the anatomic studies demonstrated that left but not right ventricular hypertrophy occurred with senescence. This hypertrophy, as indexed by TL, was 14% in senescent compared with middle-aged rats. We sought to duplicate this degree of left ventricular hypertrophy by banding the ascending aorta in a group of 46 mature rats. The aortas were banded for 3-16 weeks so that all rats were about 15 months of age at the time of study. Since the hypertrophy began only in late maturity, the 15-month age was chosen to avoid the transition period around 18 months of age and to still enable the heart to undergo its natural aging process for as much of maturity as possible.

Under ether anesthesia, a left thoracotomy in the 3rd interspace was performed to expose the aortic arch. After the ascending aorta had been carefully dissected free, a tantalum hemoclip (Week Laboratories, Inc.) was placed around the proximal ascending aorta just distal to the coronary ostia. The hemoclip then was pinched with a specially designed applicator to occlude the aorta partially. Pilot studies were performed to determine the degree of occlusion necessary to produce stable hypertrophy of the same extent as that occurring naturally from maturity to senescence without inducing heart failure for banding durations up to 16 weeks. Once it was ascertained that this degree of occlusion produced hypertrophy without heart failure in the majority of hearts, all subsequent aortas were banded to this same degree. By the nature of the protocol, which sought to produce a mild degree of hypertrophy, some hearts were not hypertrophied by the banding. To minimize the number of false positive hypertrophied hearts, only those banded hearts whose LVW/TL ratio exceeded the upper 95% confidence limit for 15-month unoperated animals were considered to have achieved LV hypertrophy.

An estimate of the acute load placed on the left ventricle by this banding technique was made by measuring the aortic input impedance proximal to the clip in seven rats anesthetized with pentobarbital (30 mg/kg). In these rats, the clip was placed between the right innominate and left subclavian arteries. Aortic flow was measured with a Biotronex electromagnetic cuff probe selected to fit snugly around the ascending aorta. Lateral aortic pressure was measured with a Statham P23Db transducer connected to an 8-cm long PE-60 polyethylene cannula whose tip was at the junction of the right innominate artery and aorta. The flowmeter and transducer system had an amplitude response that was flat to 20 Hz and down 10% at 50 Hz. The phase lag was linear (1.3°/Hz) with a damping ratio of 0.75. The frequency response of the pressure trans-
Mechanical Properties

Mechanical studies on strips of excised left ventricular trabeculae carnea from 6- to 8-, 14- to 17-, 23- to 28-month old, and banded rats were performed as described previously (Spurgeon et al., 1977). Briefly, after spinal transection, the hearts were removed and placed in Krebs-Ringer solution at 30°C oxygenated with 95% O2-5% CO2. The trabecular muscle was excised, mounted horizontally, and stimulated through platinum field electrodes at a rate of 24/min. After equilibration for 60 minutes, muscles were stretched to Lmax (defined as the length at which maximum developed force occurred) and allowed to equilibrate for another 60 minutes. Isometric twitch parameters consisting of passive force, peak active force, maximum rate of force development, and CD (defined as the time between each length change). In addition to the isometric twitch parameters, viscoelastic properties consisting of passive and active dynamic stiffness of the muscle strip were measured by means of a penmotor and servocontrol system as previously described (Spurgeon et al., 1977). Sixteen cycles of sinusoidal length oscillation of approximately 0.5% of Lmax at a frequency of 30 Hz were imposed on alternate successive isometric beats beginning 1.5 cycles before the stimulus. The unperturbed beat was subtracted from the perturbed beat and digital filtering was used to remove any remaining low frequency contaminant signals. The remaining high frequency signal represents the dynamic force change during the twitch due only to the length oscillation. All forces were normalized to tensions by dividing by the muscle cross-sectional area obtained by assuming a tissue density of 1.063 so that the data that are reported are passive tension (PT), peak active tension (AT), and peak rate of tension development (dT/dt). Stiffness was calculated from the force and length data as dT/dL, where dT is the dynamic tension change due to the normalized length oscillation dL (oscillation length divided by muscle length). Prior studies (Templeton et al., 1973; Loeffler and Sagawa, 1975; Spurgeon et al., 1977) have shown that dT/dL is a linear function of T, both in the passive and active states, and can be expressed as dT/dL = aT + b, so that the viscoelastic properties are reported in terms of the coefficients a and b. During the twitch, activation data were analyzed from 20 msec after the stimulus to the time of peak force development, and these coefficients are identified by the subscript A. Relaxation data were analyzed from 20 msec after peak stiffness until developed force returned to 10% of its peak value, and these coefficients are identified by the subscript R. The stiffness measured in the resting muscle comprises the passive portion of the data, and these coefficients are identified by the subscript P. All active and passive measurements were made at the four lengths.

Both the isometric twitch and viscoelastic data were recorded at the four muscle lengths, and comparisons between and within groups were performed by analysis of variance. If any significant interaction between the variables tested was found, linear transformation was performed, and a regression analysis of variance was performed to ascertain the presence of significant group differences (Arrmitage, 1971).

Results

Cardiac Hypertrophy of Senescence

The changes with age in BW, LVW, RVW, and TL are illustrated in Figure 1. From the completion of growth at 5–6 months through middle age (18 months), the BW, RVW, and LVW remain relatively constant, whereas TL increases slightly up to 14 months and is constant thereafter. From middle age until senescence, there is a marked decrease in BW, and increase in LVW, but no change in either RVW or TL. Analysis of variance across ages demonstrated significant variance ratios (P < 0.001) for LVW, BW, and TL, but not for RVW. If BW is used as the reference for heart size, the data indicate a 33% LV and 28% RV hypertrophy. However, in view of the marked weight reduction with senescence, TL probably provides a more accurate reference for assessment of the extent of hypertrophy.

The quantitative assessment of LV hypertrophy as a function of age as indexed by the ratio LVW/TL is illustrated in Figure 2. As with absolute LVW, LV hypertrophy of about 14% as indexed by this ratio becomes apparent in senescence with a significant variance ratio (P < 0.001). On the other hand, RVW/TL indicated no RV hypertrophy with senescence. These data establish the age-adjusted normal values for LVW/TL and the degree of hypertrophy based on this ratio which was desired from the banding in the 15-month old hearts to make them comparable to the senescent hearts.

Mechanical properties of trabeculae from the three representative age groups were compared at
the four muscle lengths. For brevity, only the data at Lmax are tabulated in Table 1. There were no differences among the groups in PT, AT, dT/dt, \( \alpha_p \), \( \beta_p \), or \( \beta_m \); however, significant increases occurred in CD, \( \alpha_A \), and \( \alpha_R \), and a decrease occurred in \( \beta_A \) in the senescent (S) compared to middle-aged (M) muscles. The age-associated changes in CD and \( \alpha_A \) are shown in Figure 2 to emphasize their temporal relationship to the age-associated extent of hypertrophy. It is noteworthy that LVW/TL, CD, and \( \alpha_A \) are not altered through middle age (15 months), but all increase with further aging.

Cardiac Hypertrophy of Aortic Banding

The data in Figure 3 demonstrate the effect of aortic banding on the LVW/TL ratio in the 15-month-old rats. Of the 46 banded rats, 27 had LVW/TL outside the 95% confidence interval and were considered to be hypertrophied. As the data demonstrate, the extent of hypertrophy did not vary with duration of banding.

Anatomic features of the 15-month middle-age (M) and banded (B) rats are presented in Table 2. The M and B animals were comparable in size as assessed by both initial BW and TL. The banding produced a 17% increase in LVW/TL ratio and no change in the RVW/TL ratio. Banding did not increase the water content of either LV or lung tissue. Thus, the extent of selective LV hypertrophy induced was quite comparable (17% vs. 14%) to that seen in the normal senescent heart, and there was no apparent heart failure induced by the banding.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Y (6-8 mo.)</th>
<th>M (14-16 mo.)</th>
<th>S (23-28 mo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>PT (g/mm²)</td>
<td>1.68 ± 0.22</td>
<td>1.77 ± 0.17</td>
<td>1.61 ± 0.25</td>
</tr>
<tr>
<td>AT (g/mm²)</td>
<td>3.43 ± 0.51</td>
<td>3.02 ± 0.28</td>
<td>2.93 ± 0.38</td>
</tr>
<tr>
<td>dT/dt (g/mm² per sec)</td>
<td>58.3 ± 8.6</td>
<td>52.0 ± 6.5</td>
<td>46.0 ± 7.2</td>
</tr>
<tr>
<td>CD (msec)</td>
<td>183 ± 9</td>
<td>179 ± 6</td>
<td>226 ± 15†</td>
</tr>
</tbody>
</table>

**Viscoelasticity coefficients**

\( \alpha_p \) (g/mm²): 2.71 ± 0.50, 2.77 ± 0.31, 2.92 ± 0.38

\( \beta_p \) (g/mm²): -0.67 ± 0.48, -0.67 ± 0.28, -0.80 ± 0.33

\( \alpha_A \): 1.46 ± 0.10, 1.43 ± 0.08, 1.86 ± 0.14†

\( \beta_A \) (g/mm²): 2.44 ± 1.0, 1.58 ± 0.84, 0.74 ± 0.80†

\( \alpha_R \): 2.02 ± 0.13, 1.97 ± 0.09, 2.17 ± 0.13†

\( \beta_R \) (g/mm²): -0.80 ± 0.44, -0.62 ± 0.72, -0.23 ± 0.59

All data in this and subsequent tables and figures are shown as mean ± SEM. The cross-sectional areas of the muscles are as follows: Y: 0.48 ± 0.10, M: 0.47 ± 0.07, and S: 0.56 ± 0.03 mm², NS among groups by unpaired t-test.

† P < 0.05, in S compared to Y or M muscles by analysis of variance across lengths.
Data relating to the acute load placed on the LV by the banding procedure are listed in Table 3. The postbanding aortic systolic and mean pressures proximal to the clip were not altered, but flow was reduced. There was an increase in the calculated peripheral resistance and an increase in characteristic impedance, but no change in the total hydraulic power output of the ventricle due to the band. Thus, although the acute load induced by the band was not measurable in terms of hydraulic power, there was a small increase in characteristic impedance, and this small increase was sufficient to induce the desired degree of hypertrophy.

The mechanical properties of trabeculae carneae of B, M, and S hearts at the four lengths are listed in Table 4. Isometric twitch parameters are listed in the upper portion and active viscoelasticity coefficients are listed in the lower portion of this table. There was no significant difference in PT, AT, and dT/dt, although AT and dT/dt were increased at all lengths in B compared to M muscles. There was a significant increase in CD, αp, and αR and a decrease in βA in B compared to M muscles. In addition, there was a decrease in βR in the B muscles. The passive viscoelasticity coefficients for the B muscles are αp = 2.35 ± 0.15, βp = −1.43 ± 0.40; these values are not significantly different from the corresponding values of the M group which are listed in Table 1. Thus, the mechanical properties of muscles from hearts hypertrophied by banding have a striking resemblance to those seen in senescence. To emphasize this, CD and αR as a function of length are plotted for the M, S, and B muscles in Figure 4.

To test quantitatively the similarity between the B and S muscles, a separate analysis of these two groups was performed. There was no difference between groups in AT, PT, αn, αp, or βp. However, the S muscles had significantly higher values for CD, αS, βS, βR and a lower value of dT/dt than the B muscles. Thus, although the changes in mechanical properties resulting from banding are altered in a similar direction as in the S muscles when compared to M muscles, there are still statistically significant differences between the B and S muscles.

One obtains a different perspective on stiffness if the active stiffness vs. total tension lines during contraction at Lmax are considered for the B, M, and S muscles (Fig. 5). Over the range of total tensions for these muscles (about 3–7 g/mm²), the S muscles appear to function at the highest and the B muscles at the lowest level of active stiffness. Thus, in spite of the B muscles being intrinsically stiffer than the M muscles, as judged by their higher values of αS, the B muscles have lower values of dynamic stiffness over this range of tensions.

### Table 2 Anatomic Comparison of the B and M Groups*

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>B</th>
<th>P (M vs. B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Initial BW</td>
<td>649 ± 16</td>
<td>612 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>Final BW</td>
<td>649 ± 16</td>
<td>578 ± 16</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>TL (cm)</td>
<td>4.55 ± 0.02</td>
<td>4.60 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>LVW/TL (g/cm)</td>
<td>0.056 ± 0.002</td>
<td>0.066 ± 0.001</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>RVW/TL (10⁻³ g/cm)</td>
<td>12.5 ± 1.7</td>
<td>12.5 ± 0.64</td>
<td>NS</td>
</tr>
<tr>
<td>Dry:wet LV</td>
<td>0.228 ± 0.006</td>
<td>0.228 ± 0.003</td>
<td>NS</td>
</tr>
<tr>
<td>Dry:wet lung</td>
<td>0.209 ± 0.012</td>
<td>0.199 ± 0.005</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Data are for the same rats in which mechanical performances of trabeculae carneae are compared (Table 4). NS = not significant.

**Discussion**

In the initial phase of this study, the concept of employing a body weight-independent indicator of body size as a reference to quantify cardiac hypertrophy was explored. Since the body weight of the rat decreases dramatically in old age (Fig. 1), we felt that a reference based on body weight might lead to inaccurate interpretation of the extent or even presence or absence of cardiac enlargement. Instead, we chose TL as the reference since it fulfills many of the requirements of an ideal reference parameter. That is, it is an indicator of the maximum body size attained by the rat during its lifetime and is unaffected by changes in body weight (Fig. 1), food consumption, or pathological lesions in other organ systems (Berg and Harmsen, 1958). With this reference, the data demonstrated that there is 14% LV hypertrophy and no RV hypertrophy in the senescent rat. This LV hypertrophy becomes manifest late in life, encompassing only
HYPERTROPHY OF SENESCENCE AND AORTIC BANDING/Yin et al. 297

TABLE 3  Effects of the Mild Aortic Banding on Hemodynamics and Impedance In Seven Middle-Aged Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prebanding</th>
<th>Postbanding</th>
<th>Δ</th>
<th>Δ as % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>110 ± 11</td>
<td>109 ± 13</td>
<td>-0.7 ± 7.9</td>
<td>-4.3 ± 8.3</td>
</tr>
<tr>
<td>Mean pressure (mm Hg)</td>
<td>81 ± 9</td>
<td>85 ± 10</td>
<td>4.1 ± 5.0</td>
<td>5.2 ± 6.5</td>
</tr>
<tr>
<td>Mean flow (ml/sec)</td>
<td>0.87 ± 0.2</td>
<td>0.71 ± 0.2</td>
<td>-0.16 ± 0.06*</td>
<td>-22 ± 7*</td>
</tr>
<tr>
<td>Resistance (10^6 dyn sec/cm^2)</td>
<td>1.71 ± 0.58</td>
<td>2.82 ± 1.37</td>
<td>1.11 ± 0.80</td>
<td>41.6 ± 2.4*</td>
</tr>
<tr>
<td>Characteristic impedance (10^4 dyn sec/cm^5)</td>
<td>2.28 ± 0.50</td>
<td>2.56 ± 0.52</td>
<td>0.28 ± 0.06f</td>
<td>15.3 ± 3.9f</td>
</tr>
<tr>
<td>Hydraulic power (mW)</td>
<td>15.13 ± 4.5</td>
<td>14.20 ± 4.2</td>
<td>-0.93 ± 1.4</td>
<td>-6 ± 11.6</td>
</tr>
</tbody>
</table>

* P < 0.05; † P < 0.005, ‡ P < 0.001, by paired t-test.

Impedance modulus Z was calculated according to the method of Randall (1958) from the power spectra of flow (Sf) and pressure (Sp) as Z = (Sp/Sf), and coherency (C) was calculated using the cospectrum (Sc) and quadrature spectrum (Sq) as C = [(Sc^2 + Sq^2)/(Sf Sp)]. The characteristic impedance was the average of impedance moduli above 3 Hz with coherencies ≥ 0.85. Power was calculated from harmonic analysis of the pressure and flow signals as described by Milnor et al. (1966).

\[ P = P_0 Q_0 + \sum P_i Q_i \cos \phi_i \]

where \( P_i, Q_i \) are the ith harmonic of pressure, flow, and phase angle (pressure minus flow), respectively.

the last quarter of the rats’ normal lifespan. This selective LV but not RV hypertrophy has not been recognized in previous studies of the hypertrophy of senescence. In contrast, use of body weight as the reference estimated more than twice as much hypertrophy as using TL and, in fact, indicated the presence of RV hypertrophy, whereas none was indicated by using TL.

TABLE 4  Mechanical Properties of M, B-Hypertrophy, and S Muscles At Four Lengths

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>90</th>
<th>95</th>
<th>98</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (g/mm²)</td>
<td>M</td>
<td>0.39 ± 0.12</td>
<td>0.84 ± 0.16</td>
<td>1.33 ± 0.17</td>
<td>1.77 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.31 ± 0.07</td>
<td>0.66 ± 0.09</td>
<td>1.15 ± 0.14</td>
<td>1.57 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.26 ± 0.09</td>
<td>0.63 ± 0.11</td>
<td>1.10 ± 0.15</td>
<td>1.61 ± 0.25</td>
</tr>
<tr>
<td>AT (g/mm²)</td>
<td>M</td>
<td>1.49 ± 0.89</td>
<td>2.40 ± 0.25</td>
<td>2.88 ± 0.28</td>
<td>3.02 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.75 ± 0.22</td>
<td>2.71 ± 0.32</td>
<td>3.24 ± 0.37</td>
<td>3.36 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>1.37 ± 0.24</td>
<td>2.23 ± 0.35</td>
<td>2.66 ± 0.38</td>
<td>2.93 ± 0.38</td>
</tr>
<tr>
<td>dT/dt (g/mm² per sec)</td>
<td>M</td>
<td>22.1 ± 3.3</td>
<td>40.1 ± 7.9</td>
<td>50.0 ± 6.5</td>
<td>52.0 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>33.0 ± 4.2</td>
<td>46.9 ± 5.8</td>
<td>54.9 ± 6.6</td>
<td>56.9 ± 5.7</td>
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<tr>
<td></td>
<td>S*</td>
<td>23.9 ± 4.3</td>
<td>36.1 ± 6.2</td>
<td>42.5 ± 7.0</td>
<td>46.0 ± 7.2</td>
</tr>
<tr>
<td>CD (msec)</td>
<td>M*</td>
<td>153 ± 5</td>
<td>165 ± 3</td>
<td>174 ± 6</td>
<td>179 ± 6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>167 ± 4</td>
<td>182 ± 4</td>
<td>189 ± 4</td>
<td>193 ± 4</td>
</tr>
<tr>
<td></td>
<td>S*</td>
<td>192 ± 13</td>
<td>209 ± 14</td>
<td>218 ± 15</td>
<td>226 ± 15</td>
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</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>90</th>
<th>95</th>
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<th>100</th>
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</thead>
<tbody>
<tr>
<td>Viscelasticity coefficients</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>αA (g/mm²)</td>
<td>M*</td>
<td>1.51 ± 0.08</td>
<td>1.54 ± 0.08</td>
<td>1.51 ± 0.07</td>
<td>1.43 ± 0.08</td>
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<tr>
<td></td>
<td>B</td>
<td>1.71 ± 0.07</td>
<td>1.78 ± 0.07</td>
<td>1.75 ± 0.06</td>
<td>1.67 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>St</td>
<td>2.10 ± 0.14</td>
<td>2.01 ± 0.13</td>
<td>1.92 ± 0.11</td>
<td>1.86 ± 0.14</td>
</tr>
<tr>
<td>βA (g/mm²)</td>
<td>M*</td>
<td>-0.83 ± 0.28</td>
<td>-1.42 ± 0.51</td>
<td>-1.06 ± 0.47</td>
<td>-0.93 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>St</td>
<td>-0.66 ± 0.38</td>
<td>-0.64 ± 0.43</td>
<td>-0.16 ± 0.66</td>
<td>0.74 ± 0.80</td>
</tr>
<tr>
<td>βR (g/mm²)</td>
<td>M†</td>
<td>2.08 ± 0.10</td>
<td>2.09 ± 0.07</td>
<td>2.06 ± 0.10</td>
<td>1.97 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.18 ± 0.09</td>
<td>2.27 ± 0.09</td>
<td>2.23 ± 0.09</td>
<td>2.17 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>2.30 ± 0.07</td>
<td>2.26 ± 0.09</td>
<td>2.27 ± 0.11</td>
<td>2.17 ± 0.13</td>
</tr>
</tbody>
</table>

Cross-sectional areas of the B muscles are 0.60 ± 0.06 mm² (not significant compared with the values for the M or S muscles listed in Table 1).

* P < 0.06; † P < 0.005, ‡ P < 0.001, compared to B across lengths by regression analysis of variance.
Together with the LV hypertrophy, we found certain alterations in the mechanical properties of myocardial tissue with senescence, namely, a 25% increase in CD, a 30% increase in the slope, and a decrease in the intercept of the linear stiffness-tension relationship during contraction. These findings confirm the functional alterations demonstrated in senescent compared to adult rat myocardium in this colony in several separate earlier publications (Weisfeldt et al., 1971; Lakatta et al., 1975; Spurgeon et al., 1977). However, the remarkable fact that the temporal course of alterations in mechanical performance closely parallels that of the LV hypertrophy (Fig. 2) had not heretofore been demonstrated. This last finding led us to postulate that the functional alterations seen in senescence are due to the underlying hypertrophy which is caused by an overload on the LV. Other studies provide further indirect evidence in support of this hypothesis. In senescence, prolongation in CD was found to be accompanied by a diminished rate of calcium uptake by the sarcoplasmic reticulum (Froehlich et al., 1978), and similar functional changes have been demonstrated in experimentally overloaded hypertrophied hearts (Sordahl et al., 1973; and Ito et al., 1974). Furthermore, increased dynamic stiffness coefficients with either senescence or overload hypertrophy have been demonstrated previously (Alpert et al., 1974; Spurgeon et al., 1977). Although the precise factors that determine dynamic stiffness in cardiac muscles have not been identified, an increased stiffness during contraction would enable a muscle to maintain or enhance its inherent contractile ability which could be diminished with senescence or prolonged cardiac overloading.

To test the hypothesis, we needed a model that would create LV hypertrophy of the same extent as that found in senescence. We chose aortic banding as our model based on the following considerations: Hypertrophy is the result of an increased load on the heart, but the stimulus for cardiac hypertrophy in the senescent rat is not known, and data pertaining to this question are scanty. A previous study by Rothbaum et al. (1973) on rats from this colony demonstrated a decrease in cardiac and stroke index and a small decrease in systolic and mean blood pressure in awake senescent compared to young rats. Thus, at first glance, it does not seem that either a volume or pressure overload exists on the senescent rat heart, and yet hypertrophy ensues. However, the load faced by the ejecting ventricle consists of more than the mean aortic pressure term. The higher harmonics of the ratio of pressure and flow together with the mean term (resistance) comprise the net aortic impedance which is one means of quantification of the load faced by the heart (Milnor, 1975). An increase in the higher frequency terms of impedance without a change in the mean term could be all the load that is necessary to induce mild hypertrophy. An increase in aortic stiffness could increase the higher frequency terms of aortic impedance by altering the characteristics of the vessel wall (MacDonald, 1974). Whether the senescent rat aorta is stiffer than the young is not clear. Band et al. (1972) demonstrated no age change in the dynamic stiffness of rat aortas. Studies in other laboratories, however, indicate an increase in rat large vessel stiffness with age (Cox,
HYPERTROPHY OF SENESCENCE AND AORTIC BANDING/Yin et al. 299

1977). In other species, there also appears to be increased aortic stiffness with age (LeaRoyd and Taylor, 1966). However, an increased stiffness could be counterbalanced by aortic dilation with age. Without direct measurement of the impedance, it is difficult to predict a priori what the effect of a specific age-induced alteration in size or wall properties would be. However, it is difficult technically to measure the aortic impedance in unanesthetized rats so that definitive studies of the effect of age on impedance in rats have not been performed. Thus, if the stimulus for cardiac hypertrophy in senescence is indeed a mechanical loading, which does not induce a measurable increase in aortic pressure but is manifested only by an increase in the higher frequency terms of the aortic impedance, then mild aortic banding would be a reasonable model to use.

Indeed the increased load on the heart due to our banding method was manifested only by an increase in the characteristic impedance with no increase, at least as measured acutely, in either the systolic or mean blood pressure and no increase in total hydraulic power. This lack of acute increase in aortic pressure is different from previous models of aortic banding in the rat in which acute or chronic increases in pressures proximal to the banding ranged from 40 to 80 mm Hg (Beznak, 1956; Jouannatt and Hatt, 1975; Cutilletta et al., 1977; Kissling et al., 1977). Thus, the load we induced not only produced the desired degree of hypertrophy but may also be quite analogous to the situation existing in the senescent rat. It was not feasible to measure the impedance during or at the end of the banding procedure due to the position of the band. Therefore, the extent to which the load measured acutely after banding reflects the chronic load is uncertain.

We cannot exclude the possibility that compensatory changes in the awake animal occurred with time to change the pattern of the load induced by the banding.

The 17% hypertrophy induced by the banding was about one-half of the 30–40% attained in previous rat models of hypertrophy (Beznak, 1956; Bing et al., 1971; Jouannatt and Hatt, 1975; Cutilletta et al., 1977; Kissling et al., 1977). At the outset of the study, it was not known whether such mild experimental hypertrophy would be associated with any alterations in mechanical properties. The findings demonstrated that despite the mild loading and the small amount of resulting hypertrophy, the B muscles had a definite spectrum of changes compared to their age-matched controls. However, the most impressive aspect of the data was the qualitatively similar nature of the changes in mechanical properties in the B-hypertrophied and the S-hypertrophied compared to M muscles (Table 4, Fig. 4). On the other hand, when the B and S muscles were compared directly, statistically significant differences remained between the two groups. It is unlikely that the strikingly similar spectrum of alterations in mechanical properties in the B and S compared to M muscles is purely fortuitous. However, the fact that the alterations in the B and S muscles were not entirely analogous suggests that (1) the banding model did not entirely duplicate the load on the heart that occurs from maturity to senescence or (2) that there are other factors besides mechanical loading which are responsible for the cardiac hypertrophy of senescence. Thus, although the findings of this study do not prove a causal relationship between hypertrophy and the functional alterations in the senescent myocardium, they are suggestive that at least a portion of the functional alterations with senescence is due to an underlying mechanical overload-induced hypertrophy.

Functional implications of the differences between the banding and senescent hypertrophy can be obtained by examination of Figure 5. Both B and S muscles are intrinsically stiffer than the M muscles as evidenced by an increase in the coefficient $a_\alpha$. However, in the range of tensions over which the muscles function (about 3–7 g/mm²), the B muscles function with the lowest and the S muscles the highest absolute value of stiffness. This finding is analogous to the situation in the intact heart (Mirsky and Parmley, 1973; Fester and Samet, 1974; Peterson et al., 1978) in which patients with hypertrophy were found to have a higher than normal value of the derived stiffness constant in the intact passive left ventricle. The hypertrophied hearts had lower operating wall stress levels enabling the end-diastolic wall stiffness to be in the same range as normals. This normal stiffness level was presumed to be a result of compensatory alterations in the wall thickness of the hypertrophied heart which tend to reduce the level of wall stress. As pointed out by Fester and Samet (1974), the higher value of the stiffness coefficient in the hypertrophied ventricles implies an alteration in the intrinsic properties of the wall independent of changes in thickness.

If the hypertrophied heart were unable to compensate by increasing its thickness, the stiffness level would then be higher than normal. The findings for the B and S muscles, which have the same cross-sectional areas as the control muscles, support the concept that the compensatory changes in hypertrophied muscles involve a fundamental change in muscle structure which is independent of a change in muscle thickness alone. The higher level of stiffness at which S muscles operate may be a manifestation of late stage failure of compensatory hypertrophy such that, even though the muscle is hypertrophied, it no longer is able to function at normal stiffness levels. Although the findings in the intact heart pertain to the passive state and those herein are during activation, the similarity is striking and deserves further and careful investigation.

In summary, selective LV hypertrophy of about 14% in the senescent compared to adult rat heart was found when TL, which is independent of changes in body weight, was used as the reference.
Trabecular muscles from senescent hearts demonstrated a prolongation of CD, an increase in the slope (both in contraction and relaxation), and a decrease of the intercept of the dynamic stiffness-tension line. These temporal changes in the isotropic twitch and viscoelastic properties appeared to parallel the development of hypertrophy, since they were observed in senescent but not middle-aged or young adult rats. LV hypertrophy of a similar extent as that occurring in senescence was induced in middle-aged rats by aortic banding. Measurement of aortic input impedance demonstrated that the acute load induced by the banding was small and was manifest only by a 15% increase in characteristic impedance. The same spectrum of changes in both isotropic twitch and viscoelasticity coefficients as those seen with senescence were demonstrated in these muscles from hearts hypertrophied by banding. However, the magnitude of the changes in the mechanical properties were still significantly less than those observed in senescence. Although the findings of this study do not prove a causal relationship between hypertrophy and the functional alterations with senescence, they are suggestive that at least a portion of the functional alterations are due to the underlying hypertrophy. The reasons for the quantitative differences between the banding and senescent hypertrophy are open to speculation.

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