The Contractile State as the Major Determinant in the Evolution of Left Ventricular Dysfunction in the Spontaneously Hypertensive Rat

Israel Mirsky, Janice M. Pfeffer, Marc A. Pfeffer, and Eugene Braunwald

From the Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston Massachusetts

SUMMARY. Female spontaneously hypertensive and normotensive rats were studied at 6, 12, 18, and 24 months of age to determine which characteristics of myocardial performance herald the onset of left ventricular dysfunction. Peak ejection fraction index was derived from measurements of peak stroke volume (in vivo volume loading) and passive pressure-volume relations. The myocardial stiffness constant (\( k_m \)), chamber stiffness constant (\( k_c \)), and left ventricular cavitary volume-to-wall volume ratio at 10 mm Hg were calculated from the pressure-volume data and the contractile state was assessed from the ejection fraction index-afterload relations. In the normotensive rats, the myocardial stiffness constant was not affected by age, whereas, in the spontaneously hypertensive rats, the myocardial stiffness constant remained within normal limits until 18 months, at which time a significant increase in this index of spontaneously hypertensive rats was normal from 6 to 18 months, but were markedly reduced at 24 months. This reduction in cardiac performance was associated with a decrease in the left ventricular chamber stiffness constant, \( k_c \). This decreased chamber stiffness, which occurred at a time when myocardial stiffness was increased, was due to a greater increase in cavity size than in myocardial stiffness. The left ventricular cavity-to-wall volume ratio of normotensive rats was not affected by age, whereas, in the spontaneously hypertensive rats, this ratio markedly declined by 18 months. The ejection fraction index-afterload relations i.e., a measure of the contractile state of the 6- and 12-month-old spontaneously hypertensive rats were similar to those of the normotensive rats of all ages. However, a depression in the contractile state of the spontaneously hypertensive rats occurred at 18 months and was further depressed at 24 months. This abnormality of the contractile state was evident before the deterioration of cardiac performance, as reflected in a decrease in baseline and maximal cardiac indices, and dilation of the left ventricle occurred. The contractile state (ejection fraction index-afterload relation) is thus the most sensitive indicator of left ventricular dysfunction in spontaneously hypertensive rats. (Circ Res 53: 767–778, 1983)

THE ventricle hypertrophies in response to a sustained mechanical stress which may be produced by either a pressure overload, as occurs in systemic hypertension, or a volume overload, as in valvular regurgitation. The development of hypertrophy initially permits the heart to adapt to an increased mechanical stress, but if the overload persists, a depression of the intrinsic contractile state of the myocardium may ensue which ultimately results in heart failure, despite further progression of cardiac hypertrophy.

In experimental models of pressure-overload hypertrophy induced by banding of the pulmonary artery or ascending aorta, the myocardial contractile state has been shown to be normal (Grimm et al., 1963; Pannier, 1971; Sasayama et al., 1976; Williams and Potter, 1974; and Serizawa et al., 1982), enhanced (Kerr et al., 1961; Sasayama et al., 1977), or depressed (Alpert et al., 1974; Bing et al., 1971; Cooper et al., 1973; Gunning and Coleman, 1973; Hamrell and Alpert, 1977; Kaufman et al., 1971; Natarajan et al., 1979; Spann et al., 1967). These conflicting results may well be due to the particular animal model employed, the duration of the pressure overload, or the way in which the contractile state was measured. The spontaneously hypertensive rat (SHR) is an animal model in which the natural progression of left ventricular hypertrophy induced by a pressure overload may be studied. Pfeffer et al. (1979b; 1983) demonstrated that peak pumping ability was normal in mature female SHR with moderate left ventricular hypertrophy, but was impaired in older (24 months) SHR with more advanced cardiac hypertrophy.

The present study was conducted to determine the time at which the transition from compensatory hypertrophy to ventricular dysfunction occurs in the SHR and which characteristics of myocardial behavior might herald the onset of left ventricular dysfunction.
Methods

Hemodynamic experiments were performed on female spontaneously hypertensive rats (SHR) and on two strains of female normotensive rats, the Wistar-Kyoto (WKY) and an American Wistar (NWR), at 6 (12 SHR, 6 WKY, 11 NWR), 12 (8 SHR, 4 WKY, 6 NWR), 18 (9 SHR, 5 WKY, 7 NWR), and 24 (9 SHR, 6 WKY, 11 NWR) months of age. Female rats were studied because of the uniformity of their body weight with age.

Surgical Preparation

The basic surgical preparation has been described elsewhere in detail and is presented here in brief (Pfeffer et al., 1979a, 1979b). After induction of anesthesia with ether, a tracheostomy was performed and ventilation was maintained by a positive pressure respirator connected in series to an ether drip apparatus. The right carotid artery and jugular vein were cannulated and their catheters connected to high fidelity Millar catheter-tip transducers for measurements of systemic arterial and right atrial pressures and heart rate. After a midsternal thoracotomy, the ascending aorta was exposed and fitted with an electromagnetic flow probe for measurements of aortic blood flow.

Volume Loading

Tyrode’s solution was infused into a femoral vein at a rate of 40 ml/min per kg for 45 seconds to produce an increase to a plateau in cardiac output, even though right atrial pressure continued to rise. The maximal cardiac output and stroke volume attained during this volume loading served as indices of the peak pumping ability of the left ventricle (Pfeffer et al., 1976).

Upon return of all hemodynamic parameters to baseline levels, the flow probe was removed and the carotid arterial catheter advanced to the left ventricle for continuous monitoring of end-diastolic pressure. The infusion of Tyrode’s solution was repeated to determine the end-diastolic pressure at which stroke volume occurred. Previous experiments have shown peak stroke volume to occur at left ventricular diastolic pressures between 17 and 22 mm Hg, a pressure range on the steep (volume changes are minimal) portion of the pressure-volume curve (Pfeffer et al., 1976b).

Ejection Fraction Index

An index of ejection fraction (EFI) was derived by dividing the peak stroke volume, attained during the in vivo volume loading, by the ventricular diastolic volume, which occurred at peak stroke volume but which was obtained from the in vitro pressure-volume relation following arrest of the heart. This ventricular diastolic volume was determined as that volume on the in vitro pressure-volume relation which corresponded to the previously determined end-diastolic pressure at which peak stroke volume occurred.

Left Ventricular Afterload

Left ventricular afterload, calculated at the time of peak cardiac output during volume loading, is defined as peak systolic stress (σa) given in the form:

\[ σ_a = P_{AS} \left[ \frac{(2/3)a^3}{(b+a)^3} + \frac{(1/3)a^3}{(b-a)^3} \right] \]

where \( P_{AS} \) is the peak aortic pressure; subscripts d and s denote the end-diastolic and end-systolic states, respectively; and a, b are the inner and outer radii, respectively, of a sphere which is the assumed geometry for the left ventricle. This formula for \( σ_a \) is a modification of one developed earlier by Gaasch et al. (1972) who employed peak left ventricular systolic pressure and assumed an ellipsoidal geometry in their clinical studies. The effects of the spherical geometry assumption are presented in the discussion.

Pressure-Volume Relations and Chamber Stiffness Constants, \( k_c \)

The heart then was arrested in diastole with potassium chloride. A double lumen catheter (PE50 inside PE200) was inserted via the aorta into the left ventricular cavity. A ligature was placed around the atrioventricular groove to secure the catheter and isolate the left ventricular cavity. The right ventricle was incised to eliminate any compressive effects on the left ventricle. Saline was infused at a rate of 0.68 ml/min over a pressure range of 0 to 30 mm Hg, and three repeatable pressure-volume curves were thus obtained within 10 minutes after arrest, before the onset of rigor mortis. The heart was excised and the right and left ventricles were separated and weighed.

The passive pressure-volume relation was curve-fitted in the form, \( P = A e^{k_c} \), over the pressure ranges of 5–20 mm Hg and 15–30 mm Hg. The curve-fitting parameters, \( A \) and \( k_c \), vary with the pressure range employed, as well as from animal to animal (Glantz, 1976). This form of the curve-fit was chosen because it permitted the between-and-within strains comparison of an index of ventricular chamber stiffness. Although polynomial or bi-exponential curve-fits may be more accurate over the entire pressure range, no standard statistical method is currently available for making slope (dP/dV) comparisons at common levels of pressure. The pressure range of 0 to 5 mm Hg was excluded, since the exponential curve does not pass through the point, \( P = 0 \). In this study, therefore, the index of ventricular chamber stiffness is identified with the curve-fitting parameter, \( k_c \), which represents the slope of the chamber stiffness-pressure relation over a given pressure range (i.e., dP/dV = k_cP).

Incremental Modulus, \( E_{INC} \), and Myocardial Stiffness Constant, \( k_m \)

By means of a spherical model for the left ventricle, the geometric parameters may be evaluated from the formulas:

\[ V = \frac{4}{3}a^3, \quad V + V_w = \frac{4}{3}b^3, \quad \text{where } V = \text{cavity volume}, \quad V_w = \text{wall volume} \]

respectively; of a sphere which is the assumed geometry for respectively, and a, b are the inner and outer radii, respectively. The effects of the spherical geometry assumption are presented in the discussion.

\[ \sigma = \frac{\Delta P}{V/V_w} \left( \frac{b}{R} \right)^3 \]

where

\[ \Delta P = P - \sigma R \]

is the difference in midwall circumferential and radial stress components; \( \Delta P = \Delta \sigma / R = \text{incremental midwall circumferential strain} \); \( P \) is the left ventricular cavity pressure; and \( R = (a + b)/2 \) is the midwall radius. If the stress-radius (σ-R) relations are curve-fitted in the form, \( \sigma = bR^a \) (R can be segment, the incremental modu-
lus-stress ($E_{INC} - \sigma$) relation in the form $E_{INC} = (1/2)\alpha(\sigma/R^*)$

$L = (1/2)\alpha = k_n \sigma$ is obtained, where $k_n = \alpha/2$ is the myocardial stiffness constant.

Statistical Analysis

Results are expressed as mean ± SEM. A two-way analysis of variance was conducted [General Linear model (GLM) of SAS, 1979] to determine whether there was a significant interaction effect between strains and with age for each variable. The significance of the differences within strains and between strains (eight) were made between SHR and age-matched NWR and WKY.

An analysis of covariance was conducted to determine whether the various linear EFI-afterload relations within each strain could be pooled (Snedecor and Cochran, 1967). The analysis then was repeated to test for significant differences in slopes and elevations between each combined normotensive group and the SHR. The EFI-afterload relation was determined by a standard linear regression analysis. Confidence intervals were also evaluated for each group.

Results

The prethoracotomy mean arterial pressure of the WKY and NWR remained constant from 6 to 24 months of age (Table 1). In the SHR, from 6 to 18 months of age, these pressures were markedly elevated, compared with WKY and NWR, but were not different from both normotensive groups at 24 months. When left ventricular weight was expressed relative to body weight, the ratios increased progressively with age in the SHR from 44 (at 6 months) to 71% (at 24 months) above those of combined, age-matched WKY and NWR. Despite the presence of moderate hypertrophy and elevated systemic arterial pressures, the 6- to 18-month-old SHR maintained normal baseline and maximal (volume loading) cardiac indices. However, by 24 months of age, both the baseline and maximal aortic blood flows of SHR were significantly reduced compared with WKY and NWR, even though the mean arterial pressure of the SHR was now normal.

Left Ventricular Chamber and Myocardial Stiffnesses

Over the diastolic pressure range of 5–20 mm Hg, the chamber stiffness constant ($k_c$) of the left ventricle of NWR was not affected by age, whereas the values at 12 and 18 months for WKY were significantly lower ($P < 0.005$) than that at 6 months of age (Fig. 1A). In the SHR, $k_c$ was unchanged from 6 to 18 months, but was significantly ($P < 0.025$) decreased by 24 months, when compared with the 6-month-old group. In contrast, over the diastolic pressure range of 15–30 mm Hg, $k_c$ was unaffected

| Table 1 |

| Body and Ventricular Weights, Mean Arterial Pressure, and Cardiac Output of Normotensive (WKY and NWR) and Spontaneously Hypertensive Rats (SHR)* |
|------------------------|--------|--------|--------|--------|
| Age (mos.) 6 12 18 24 |
| W (g)       | WKY    | NWR   | SHR    |
| LV (mg)     | WKY    | NWR   | SHR    |
| LV/W (mg/g) | WKY    | NWR   | SHR    |
| MAP (mm Hg) | WKY    | NWR   | SHR    |
| CI (ml/min per kg) | WKY    | NWR   | SHR    |
| pCI (ml/min per kg) | WKY    | NWR   | SHR    |

* In each strain the rats were 6-, 12-, 18-, and 24-month-old females.
† $P < .05$, ‡ $P < .025$, § $P < .005$, WKY or NWR compared to age-matched SHR. W = body weight; LV = left ventricular weight; MAP = prethoracotomy mean arterial pressure; CI = cardiac index; pCI = peak cardiac index; number of animal studies shown in parentheses.
by age for each strain (Fig. 1B). At each age, the $k_c$ of SHR was equal to or less than that of WKY or NWR. Thus, despite a progressive increase in cardiac mass, the left ventricular chamber of the SHR was not stiffer than that of age-matched normotensive rats and, indeed, was strikingly more compliant ($P < 0.005$) at 24 months of age, when hypertrophy was most severe.

The myocardial stiffness constant, $k_m$, of WKY and NWR was not affected by age over the physiological stress range of 5–35 g/cm² (Fig. 1C). However, the $k_m$ of SHR was significantly higher at 18 months of age compared with both age-matched controls ($CP < 0.05$) and the WKY (Fig. 3A) at 18 and 24 months of age, alterations in the chamber stiffness constant did not parallel those in the myocardial stiffness constant. In particular, the myocardial stiffness constant of the 24-month-old SHR tended to be increased at a time when the chamber stiffness constant was decreased.

The changes that occurred in the chamber, and myocardial properties of the left ventricles of the normotensive and hypertensive rats over the physiological ranges of pressure and stress, are presented in Figures 2–4. The diastolic volumes over the pressure range of 5 to 20 mm Hg of the NWR (Fig. 2A) and the WKY (Fig. 3A) did not change from 6 to 24 months of age. However, a significant increase in diastolic volumes occurred at all levels of pressure in the 24-month-old SHR when compared to the 6-, 12-, and 18-month-old SHR.

The chamber stiffness ($dP/dV = k_cP$) of NWR was not affected by age at common levels of pressure over the range of 5–20 mm Hg (Fig. 2B). However, the chamber stiffness of the 12- and 18-month-old WKY ($P < 0.005$) (Fig. 3B) and 24-month-old SHR ($P < 0.025$) (Fig. 4B) was decreased, compared with that of the respective 6-month-old group.

Myocardial stiffness (or incremental modulus, $E_{INC} = k_mP$) was not affected by age in NWR (Fig. 2C) and in WKY (Fig. 3C) at common levels of stress over the range of 5–35 g/cm². In the SHR, myocardial stiffness was significantly greater at 18 ($P < 0.005$) and 24 ($P < 0.025$) months than at 6 months of age (Fig. 4C). Of particular note, the left ventricular chamber of the 24-month-old SHR was more compliant (i.e., $dP/dV$ and $k_c$ were decreased, $P < 0.005$) than that of age-matched WKY and NWR, whereas the myocardium was stiffer (i.e., $k_m$ was elevated, $P < 0.05$) than that of NWR, but not of WKY.

**Left Ventricular Cavity Volume-to-Wall Volume Ratio**

The ratio of left ventricular cavity volume-to-wall volume ($V/V_w$) was determined at a common diastolic pressure of 10 mm Hg and plotted as a function of age (Fig. 5). The parameter, $V/V_w$, is closely related to diastolic wall stress at common levels of pressure. There was no change in the ratio of left ventricular cavity volume to wall volume with age in either WKY or NWR. However, the $V/V_w$ ratio in the SHR was significantly lower at 18 ($P < 0.005$) and 24 ($P < 0.01$) months, compared with the 6-month-old group; i.e., the left ventricular mass of the SHR increased more than did volume from 6 to 18 months of age but not from 18 to 24 months of age.
Peak Systolic Stress and Ejection Fraction Index Relationships with Age

The peak systolic stresses, \( a_s \), of the WKY and NWR were unaffected by age in contrast with the SHR in which the peak systolic stress at 18 and 24 months was significantly (\( P < 0.005 \)) lower than that at 6 months (Fig. 6A).

Ejection fraction index was unaffected by age in NWR, WKY, and SHR (Fig. 6B). There was, however, a tendency for the ejection fraction index of the SHR to decline after 12 months of age, so that, by 24 months, it was significantly less than that of WKY and NWR (\( P < 0.005 \)).

Ejection Fraction Index-Afterload Relation

An analysis of covariance for each strain indicated that it was possible to combine the data from all age groups for NWR and WKY, but only from the 6- and 12-month-old groups for SHR, as a significant (\( P < 0.005 \)) difference in the elevation of the EFI-\( a_s \) relation was observed for the 18-month-old SHR. The 24-month-old SHR was excluded from this analysis, since the range of afterload was outside that for the 6- and 12-month-old groups. A repeated analysis of covariance for the combined NWR, combined WKY, and 18-month-old SHR demonstrated a significant difference in the elevation of the EFI-\( a_s \) relation between the 18-month-old SHR and both pooled normotensive groups. The ejection fraction index-afterload relations of the combined NWR, combined WKY, combined 6- and 12-month-old SHR, and the 18-month-old SHR are presented in Figure 7. (No significant linear relation was obtained for the 24-month-old SHR and, thus, only mean values are given.)
The ejection fraction-afterload relation has been used to assess the contractile state in pressure overload hypertrophy (Ross, 1976; Sasayama et al., 1976). The relation of ejection fraction index and peak systolic stress demonstrated the alteration in the contractile state of the hypertrophying myocardium of the SHR. Although the ejection fraction index of the 6-month-old SHR ($S_1 = 71 \pm 4\%$) was less than that of NWR ($N_1 = 90 \pm 2\%$), the left ventricles of these SHR were pumping against a much higher afterload ($S_1 = 228 \pm 12$ vs. $N_1 = 158 \pm 6$ g/cm$^2$). In fact, the EFI-$\alpha_s$ relation for the pooled 6- and 12-month-old SHR was not statistically different from that of the pooled WKY or NWR. In 18-month-old SHR, there was a shift to the left and downward in this relationship which was statistically different ($P < 0.005$) from the relations in normotensive rats at all ages and 6- and 12-month-old SHR, indicating a depression of the contractile state. A further decline in the level of the contractile state of the SHR myocardium occurred between 18

---

**FIGURE 4.** Pressure-volume, chamber stiffness-pressure, and myocardial stiffness-stress relations in female SHR for the low ranges of pressure and stress. Part A: at each level of pressure, significant differences in ventricular volume occurred between the 24-month- and the 6- to 18-month-old SHR. Part B: there was a significant decrease in the slope of the chamber stiffness-pressure relation at 24 months in the SHR, compared with those at 6 months ($P < 0.025$). Part C: myocardial stiffness-stress relations in the SHR were similar to those of NWR and WKY from 6 to 12 months of age. The stiffness constants, $k_*$, at 18 and 24 months of age are significantly greater than at 6 months ($P < 0.005, P < 0.025$). Note that the $k_*$ of SHR at 24 months is significantly greater than that of age-matched NWR ($P < 0.05$) in the presence of a compliant chamber.

---

**FIGURE 5.** Left ventricular cavity volume-to-wall volume ratio $(V/V_w)$ at 10 mm Hg vs. age relations in the SHR (●), WKY (△), and NWR (○). This ratio in NWR and WKY was unaffected by age. In the SHR, $V/V_w$ at 18 ($P < 0.005$) and 24 ($P < 0.01$) months was significantly lower than at 6 months. When compared with age-matched WKY, the $V/V_w$ of SHR was significantly lower at 12 months ($P < 0.05$) and at 18 and 24 months ($P < 0.005$).

---

**FIGURE 6.** Part A: peak systolic stress ($\alpha_s$) vs. age. Peak stress in NWR (○) and WKY (△) was unaffected by age in contrast to the SHR (●) in which peak stress at 18 and 24 months was markedly lower than that at 6 months ($P < 0.005$). Compared with age-matched WKY, peak stress in the SHR was significantly higher at 6 months ($P < 0.01$) and significantly lower at 24 months ($P < 0.01$). Part B: ejection fraction index (EFI) vs. age. Age had no effect on EFI for NWR (○). WKY (△) and SHR (●). Compared with age-matched controls, EFI in the SHR was significantly lower at 6 months ($P < 0.005$ vs. NWR) and at 24 months ($P < 0.005$).
and 24 months. The linear relations for the various groups are given by

$$EFI = 1.21 - 0.00220 \sigma_0 \quad (n = 32, r = 0.48, P < 0.010)$$
(combined NWR)

$$EFI = 1.27 - 0.00271 \sigma_0 \quad (n = 21, r = 0.59, P < 0.010)$$
(combined WKY)

$$EFI = 1.18 - 0.00220 \sigma_0 \quad (n = 19, r = 0.65, P < 0.005)$$
(6-month and 12-month SHR)

$$EFI = 1.00 - 0.00221 \sigma_0 \quad (n = 9, r = 0.73, P < 0.025)$$
(18-month SHR)

An analysis of covariance indicated no significant difference in slopes, but a significant ($P < 0.005$) difference in the position of the relation between 18-month-old SHR and both normotensive strains.

To confirm the conclusions drawn from the analyses of the $EFI-\sigma_0$ relation (Fig. 7), we conducted a confidence interval analysis, and the results are shown in Figure 8A and 8B. Although the range of overlap of afterload was relatively small between the combined NWR and combined 6- and 12-month-old SHR ($171 < \sigma_0 < 205 \text{ g/cm}^2$), this was not the case with WKY ($171 < \sigma_0 < 234 \text{ g/cm}^2$) (Fig. 8B). Thus, on the basis of the overlapping confidence intervals, it can be concluded that contractile state is normal in the combined 6- and 12-month SHR.

Employing the ejection fraction index-afterload relations displayed in Figure 7, the ejection fraction index may be evaluated at common levels of stress. The relationship between ejection fraction index (at 190 g/cm$^2$) and the myocardial stiffness constant, $k_m$, (over the range of 5–35 g/cm$^2$) for the 6- and 12-month-old SHR was similar to that for the pooled (combined age groups) WKY and NWR. However, the lower ejection fraction index of the 18-month-old SHR was associated with increased myocardial stiffness. The ejection fraction index could not be evaluated at 190 g/cm$^2$ for the 24-month-old SHR.

### Discussion

Although systemic hypertension has emerged as the dominant etiological factor in the development of congestive heart failure, as reported in the Framingham study by Kannel (1974), the manner in which hypertension without concurrent ischemic heart disease leads to heart failure is unclear. The...
by guest on April 28, 2017

FIGURE 9. Myocardial stiffness constant ($k_m$) for the low stress range vs. ejection fraction index (EFI) at a common level of stress for NWR (○), WKY (△), and SHR (●). Employing the linear regression relations described in Figure 7, we evaluated the ejection fraction index at a common level of stress ($\sigma_s = 190$ g/cm$^2$) for each strain of rats. The combined age groups of NWR and WKY are denoted by N and W, respectively, and S1 + S2 represents the combined 6- and 12-month-old SHR. From 6 to 18 months in SHR, an inverse relationship existed between $k_m$ and EFI. Although the ejection fraction index was further depressed after 18 months of age in SHR (S3) in the presence of an elevated stiffness constant, no relation between these two parameters was apparent.

present study sought to determine which parameters of left ventricular systolic and diastolic function might signal the transition from compensated hypertrophy to ventricular dysfunction in the SHR.

Various studies in experimental animals and patients have shown myocardial stiffness in diastole to be either normal or increased in pressure overload hypertrophy. In pulmonary artery banded cats, Spann et al. (1967) demonstrated a depression in the contractile state of papillary muscles from right ventricles that had doubled in mass. When the data from this study were analyzed by Mirsky (1976), the stiffness of the papillary muscle from the hypertrophied right ventricles was found to be increased compared with normal. Similar results were also reported by Alpert et al. (1974) and Natarajan et al. (1979), who found an increased stiffness of right ventricular papillary muscles taken from rabbits and cats, respectively, which had been subjected to pulmonary artery banding. On the other hand, Serizawa et al. (1982) found no alteration in myocardial stiffness in the intact hearts of dogs that had undergone subcoronary aortic constriction as puppies.

However, only a modest (banded, 5.2 vs. control, 4.1 g/kg) increase in left ventricular weight occurred during 34 weeks of aortic constriction. In two models of pressure overload with moderate left ventricular hypertrophy, O’Connor et al. (1980) demonstrated both no alteration (perinephric hypertension) and an increase (aortic banding) in myocardial stiffness in dogs. In the present study, the myocardial stiffness constant over the physiological range of stress in the 6- and 12-month-old SHR was similar to that of both NWR and WKY, even in the presence of moderate (40–50%) hypertrophy (Fig. 1C). From 6 to 24 months of age, there was no statistically significant change in the myocardial stiffness constant in either WKY or NWR. On the other hand, there was a significant age-related increase in $k_m$ in SHR (18 and 24 months, Fig. 1C). It is true, however, that, at various age levels, differences between groups occurred.

The lack of correlation between myocardial and chamber stiffness in the SHR is also of note. At no age examined was the left ventricle of the SHR stiffer, i.e., less compliant, than that of WKY or NWR (Fig. 1, A and B). Indeed, there was an age-related decrease in the chamber stiffness constant of the SHR (Fig. 1A). The tendency for chamber stiffness ($k_c$) to decline while myocardial stiffness ($k_m$) tended to be elevated requires a more detailed explanation.

The passive diastolic left ventricular pressure-volume relation depends on a number of factors, the most important of which are myocardial elasticity; ventricular size, wall mass, and geometry; external pressures; and coronary perfusion pressure (Glantz and Parmley, 1978; Mirsky and Rankin, 1979). In the present study, ventricular geometry, external pressures, and perfusion pressure were of secondary importance since (1) there were no shape changes in the SHR with age, (2) the pericardium was excised, and (3) pressure-volume relations were obtained following arrest of the heart. Thus, the dominant factors affecting the pressure-volume relations in this study were myocardial elasticity and ventricular size and wall mass. In Appendix I, we have derived an expression in which chamber stiffness is directly proportional to myocardial stiffness ($E_{inc}$) and inversely proportional to ventricular size (or volume, V) and the cavitory volume to wall-tovolume ratio (V/Vw), i.e., $\Delta P/\Delta V \sim (4/9) (E_{inc}/V)/(1 + V/Vw)$. It is the interplay between these three parameters that enables a chamber with an elevated myocardial stiffness constant to manifest a lower chamber stiffness constant under certain chronic conditions. In the particular case of the chronically dilated ventricle of the 24-month-old SHR, ventricular size (or volume) is the dominating factor which results in a more compliant chamber when compared to that at 6 to 18 months of age (Fig. 4A). Furthermore, the marked degree of hypertrophy of these SHR effectively maintains operating myocardial stiffness ($E_{inc}$) at normal levels in the presence...
of an elevated $k_m$ and volume. Clinical studies by Peterson et al. (1978), Pasipoularides et al. (1980), and Mirsky and Krayenbuehl (1981) have also shown that left ventricular myocardial stiffness may be either normal or increased in patients with aortic stenosis. The conflicting results obtained from both clinical and animal studies may be due to the type of cardiovascular disorder or the animal model employed, i.e., the manner, magnitude, duration, and rapidity with which the pressure overload was applied.

A relationship between systolic and diastolic myocardial function has been suggested in several studies. Spann et al. (1967) and Natarajan et al. (1979) demonstrated a depression in the contractile element shortening velocity in the presence of an increase in stiffness (Mirsky, 1976; Natarajan et al., 1979) of papillary muscles from the hypotrophied right ventricles of pulmonary artery-banded cats. On the other hand, contractile function was normal (Taylor and Hopkins, 1972), as was myocardial stiffness (Mirsky, 1976), in dogs with aortic regurgitation. However, Williams and Potter (1981) reported that contractile function in papillary muscles was depressed in cats 2–3 weeks after pulmonary artery banding, and was normal 24 weeks after banding, whereas muscle stiffness was modestly increased at both times.

The ejection fraction-afterload relation has been used to assess the contractile state in pressure overload hypertrophy (Ross, 1976). The dependence of ejection fraction on preload and afterload has been demonstrated by Mahler et al. (1975) and Spiro and Sonnenblick (1964). To eliminate the possible effect that a variation in preload may have on ejection fraction, we determined the ejection fraction index at its optimal value, i.e., during maximal loading when the peak value for stroke volume had been attained and the end-diastolic pressure ranged from 17 to 22 mm Hg. Spiro and Sonnenblick (1964) demonstrated that the relation between ejection fraction and end-diastolic pressure in both normal and depressed ventricles from rats reached a maximum and remained constant over this pressure range.

The ejection fraction-afterload relations displayed in Figures 7 and 8 (A and B) clearly demonstrate the normality of the contractile state for the 6- and 12-month-old SHR. However, by 18 months of age in the SHR, the occurrence of a significant shift to the left and downward of the relationship indicated a depression of the contractile state, as evidenced by the non-overlapping confidence intervals for $S_3$, NWR, and WKY [Figs. 7 and 8 (A and B)]. This shift does indeed represent a depression in the inotropic state of the myocardium, since the significant decline in stress was not accompanied by an increase in the ejection fraction index. Furthermore, this fall in stress was associated with a significant ($P < 0.05$) increase in the myocardial stiffness constant, compared with 18-month-old NWR and WKY. A further decline in the level of contractility occurred between 18 and 24 months in the SHR as both the ejection fraction index and afterload were reduced. These findings are consistent with those of Burger and Strauer (1981), who showed that an elevation of myocardial stiffness in 15-month-old male SHR was associated with a depression in ejection fraction.

**Limitations of the Analyses**

There are several limitations to the analyses employed in this study. The range of values over which the data from the pressure-volume and stress-strain relations should be curve-fitted is controversial (Glantz, 1976; Mirsky, 1977). As Glantz (1976) has noted, the indices of chamber and myocardial stiffness change with the range of pressure or stress that is employed. In the present study, we evaluated these indices over two pressure ranges (5–20 and 15–30 mm Hg) and two stress ranges (5–35 and 30–60 g/cm²). The low ranges of pressure and stress correspond to the physiological ranges for these rat strains, and the overlapping high ranges were chosen so as to include equal numbers of measured data in both ranges. The use of single exponential curve-fits and power-law fits for the data from each range of pressure and stress, respectively, permitted the expression of the chamber and myocardial stiffness as indices that could be compared statistically. The values for $k_m$ and $k_c$ in the high ranges of pressure and stress were always significantly higher than those in the low ranges. These findings could be attributed to the fact that the chamber, as a structure, and the myocardium become more resistant to stretch in the high ranges due to the recruitment of more collagen fibers which, in general are unstretched in the low pressure and stress ranges, as reported previously by Roach and Burton (1957) in studies on the distensibility of arteries. An alternative explanation is that the splitting up of data into two ranges is not entirely satisfactory, and that it may be more appropriate, in future studies, to employ polynomial or biexponential curve-fits over the entire range; i.e., we should give priority to mathematical accuracy over standard statistics.

The second limitation to the analysis relates to the assumption of a spherical geometry for the left ventricle of each strain over the entire age range of 6–24 months. In Appendix II, we have developed a model based on an ellipsoidal geometry of the left ventricle for the calculations of global average stress and myocardial stiffness constants. The values of $k_m$ and $k_c$ based on both geometric models are presented in Table 2 for individual rats of all strains and ages. Although the table does not include 24-month-old SHR, earlier studies by Pfeffer et al. (1979a) on male rats showed no significant changes in the geometric shape factors with age or strain. The results demonstrated that (1) percent difference in $k_m$ ranged from −8.6 to 10.1%, (2) percent difference in $k_c$ ranged from 4.6 to 8.9%, (3) shape factor (epicardial long-axis-to-short axis ratio) is independ-
ent of strain and age, and (4) based on 24 geometric
studies, no significant difference occurred between
the values for k_m or σ derived from either the
spherical or ellipsoidal models. Thus, the assump-
tions of a spherical geometry for the left ventricle did
not markedly alter the conclusion of this study.

In conclusion, alterations in the myocardial stiff-
ness constant, peak systolic stress, ejection fraction
index, and the left ventricular cavity volume-to-
wall volume ratio accompany the onset of left ven-
tricular dysfunction in female SHR from 18 to 24
months old. However, the ejection fraction index-
afterload relation appears to be the most sensitive
parameter for the early detection of left ventricular
dysfunction, since a depressed contractile state was
already evident in SHR by 18 months of age (Fig.
7), whereas baseline and peak aortic blood flows
were normal. This finding has also been shown to
be a useful predictor of postoperative performance
in patients who have undergone valve replacement
(Mirsky et al., 1981). Thus, in the SHR, a phase of
compensated hypertrophy exists in which the con-
tractile state and stiffness of the myocardium remain
within normal limits. However, with further hyper-
trophic growth, a phase eventuates in which con-
tractility is depressed as the myocardial stiffness
constant is increased.

**Appendix I**

**Functional Relation between Left Ventricular
Chamber Stiffness, Ventricular Size, Myocardial
Stiffness, and Cavitary Volume-to-Wall Volume
Ratio**

For a sphere of internal and external radii (a and
b, respectively) subjected to an internal pressure (P),
the radial displacement (u) at the endocardial surface
(assuming an incompressible material) is given by
the expression (Love, 1944):

\[
    u = \frac{(3/4)Pb^3a}{b^3 - a^3}E,
\]

where \( E \) is Young's modulus, which is identified
with the incremental elastic modulus, \( E_{inc} \), in
the present study. Thus, an incremental change (Δa)
in the radius due to an increment in pressure (ΔP) is
approximated by:

\[
    \Delta a = \Delta u = \frac{(3/4)\Delta PB^3a}{(b^3 - a^3)E_{inc}}.
\]
If $V$ = cavitary volume and $V_w$ = wall volume, we have $V = (4/3)\pi a^2$, $V_w = (4/3)\pi(h^3 - a^3)$, and $\Delta V = 4\pi a^2 \Delta a$. Substituting these relations into Equation (I.1) and rearranging terms, we obtain:

$$\Delta P/\Delta V \sim (4/9)(E_{INC} / V)/(1 + V/V_w).$$  \hspace{1cm} (I.2)

Note that if a similar analysis is conducted on the basis of a radial displacement at the midwall, the relationship is more complex, but still contains the three important parameters: $E_{INC}$, $V$, and $V/V_w$.

### Appendix II

#### An Ellipsoidal Geometric Model for the Evaluation of Global Stresses and Myocardial Stiffness Constants

As an alternative model for the calculation of global stresses and myocardial stiffness constants, we assume an ellipsoidal geometry for a left ventricle of uniform wall thickness and constant major axis-to-minor axis ratio at the epicardium. Thus, the equations for the determination of the various geometric parameters are given by:

$$V = (4/3)\pi a h^2$$  \hspace{1cm} (II.1)

$$V + V_w = (4/3)\pi(a + h)(b + h)^2$$  \hspace{1cm} (II.2)

$$A_t/B_t = c = \text{constant} = A_e/B_e$$  \hspace{1cm} (II.3)

where $V$ = cavitary volume; $V_w$ = wall volume; $h$ = wall thickness; and $a$, $b$, $A_r$, and $B_r$ are the semi-major and semi-minor axes at the epicardium and midwall, respectively. The shape factor ($c$) is evaluated from measurements of the long axis ($2A_r$) and short axis ($2B_r$) midway at the epicardium and are obtained by fixation at 5 mm Hg after potassium arrest of the heart.

Solving Equations (II.1), (II.2), and (II.3), we obtain:

$$B_r = [(3/4 \pi)(V + V_w)^{1/3}; \ A_r = cB_r$$  \hspace{1cm} (II.4)

and $h$ is determined from the cubic equation:

$$h^3 - h^2(A_r + 2B_r) + h(B_r^2 + 2A_rB_r) - 3V_w/4 \pi = 0.$$  \hspace{1cm} (II.5)

Thus, it is possible to evaluate the geometric parameters $A_r$, $B_r$, and $h$ at each level of pressure from known measurements of $V$, $V_w$, and $c$.

#### Expression for Global Peak Systolic Stress

The global average circumferential stress ($\sigma_r$) may be obtained directly from the force equilibrium equation:

$$P \pi a b_r = \sigma_r \pi (A_r b_r - a_rb_r),$$  \hspace{1cm} (II.6)

where $P$ is the left ventricular pressure. This may be written as:

$$\sigma_r = P(A_r - h)(B_r - h)/h(A_e + B_e - h).$$  \hspace{1cm} (II.7)

Employing a modification of a formula developed by Gaasch et al. (1972), one may approximate peak systolic stress ($\sigma_s$) by the expression

$$\sigma_s = P_{AP} \left\{ (2/3)(A_r - h)(B_r - h) \right\} + P_{AP} \left\{ (1/3)(A_r - h)(B_r - h) \right\},$$  \hspace{1cm} (II.8)

where $P_{AP}$ is the peak systolic aortic pressure and subscripts $d$ and $s$ denote the end-diastolic and systolic states, respectively. The % differences reported in Table 2 were evaluated from the expression, ($\sigma_s - \sigma_d$)/$\sigma_d$, where $\sigma_s$ is the peak systolic stress based on the spherical geometry.

#### Evaluation of the Myocardial Stiffness Constants

Based on an ellipsoidal geometry, the stress difference ($\sigma$) may be written as (Mirsky and Rankin, 1979):

$$\sigma = \sigma_r - \sigma_s,$$  \hspace{1cm} (II.9)

$$\sigma = P(B_m/h)(1 - B_m^2/2A_m^2 - 3h^2/8B_m^2).$$  \hspace{1cm} (II.10)

The incremental elastic modulus ($E_{INC}$) is evaluated from the expression:

$$E_{INC} = K \Delta \sigma/(\Delta B_m/B_m),$$  \hspace{1cm} (II.11)

where $K$ = average value of $(3/2)/(2 + B_m^2/A_m^2)$, and $A_m$ and $B_m$ are the midwall semi-major and semi-minor axes, respectively.

The stress-radius ($\sigma - B_m$) relations are curve-fitted in the form, $\sigma = BB_m^\gamma$ ($\beta$, $\gamma$ being curve-fitting parameters), and thus the incremental modulus is expressed in terms of the myocardial stiffness constant ($k_r$) in the form, $E_{INC} = K(BB_m^\gamma) = k_r \sigma$, where $k_r = K\gamma$. The stiffness constants ($k_r$) are reported in Table 2 for the physiological range of stress, $5 < \sigma < 35$ g/cm$^2$.

We gratefully acknowledge the fine technical and secretarial assistance of Cindy Steinberg, Sally Cassells, Margaret Milne, Dale Taber, Eleanor Brennan, and Angela Moscaritolo.

This work was presented at the 54th Scientific Sessions of the American Heart Association, Dallas, Texas, November 18, 1981.

Supported by U.S. Public Health Services Grants HL 20552, HL 28238, and HL 12711 from the National Heart, Lung, and Blood Institute.

Dr. J. Pfeffer is the recipient of Research Career Development Award HL 01186. Dr. M. Pfeffer is the recipient of an Established Investigatorship of the American Heart Association.

Address for reprints: Dr. I. Mirsky, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

Received July 26, 1982; accepted for publication September 22, 1983.

### References

The contractile state as the major determinant in the evolution of left ventricular dysfunction in the spontaneously hypertensive rat.
I Mirsky, J M Pfeffer, M A Pfeffer and E Braunwald

_Circ Res._ 1983;53:767-778
doi: 10.1161/01.RES.53.6.767

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4371

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/53/6/767

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/