Morphometric Analysis of Cardiac Hypertrophy During Development, Maturation, and Senescence in Spontaneously Hypertensive Rats

Gary L. Engelmann, John C. Vitullo, and Ross G. Gerrity

Hypertrophy of the mammalian heart, regardless of the initiating event, results in architectural remodeling of ventricular components that maintain structural and functional characteristics of this organ. Ventricular components that vary their morphology and morphometry in a hypertrophic state are the muscle cells, connective tissue elements, vasculature, or a combination of some or all of the above. Morphologic quantification of the progressive tissue changes occurring throughout the natural life span of the spontaneously hypertensive and normotensive Wistar-Kyoto rats has not been thoroughly documented. Using perfused-fixed tissue from both strains at 1, 6, 12, 18, and 24 months of age, we have determined the morphometric changes that occurred in the subepicardial and midwall regions of the left ventricle. Myocyte cell size, wall thickness, and arterial blood pressure were elevated in 1-month-old spontaneously hypertensive rats, reached significance by 6 months, and remained significantly greater throughout the 24 months examined. Tissue morphometry demonstrated significant tissue component volumetric differences at 6 months in the spontaneously hypertensive rat. Age-related morphometric tissue changes occurred in both strains yet were exacerbated (percent volume of myocytes) or diminished (percent volume interstitial space) in the mature and aging spontaneously hypertensive rat. Capillary density of SHR left ventricle showed a drastic decline so that 6-month-old SHR had the same density as a senescent Wistar-Kyoto. Tissue morphometry and capillary density data strongly support the hypothesis that tissue oxygenation is diminished in the spontaneously hypertensive rat, and as a result, tissue necrosis and myocyte cell death occur.

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Materials and Methods

Age-matched, 4-week-old male SHR and WKY were obtained from Taconic Farms (Germantown, N.Y.), maintained in our AAALAC-approved facility, and sampled at 1, 6, 12, 18, and 24 months of age. Animals were housed 3/cage and fed ad libitum normal Purina rat chow pellets (0.4% Na content). Body weight and tail cuff blood pressure measurements were obtained weekly for the first 8 weeks and monthly thereafter. Immediately before sampling, animals were anesthetized with sodium amytal, and blood pressure was recorded on a Brush recorder via a pressure transducer attached to a PE-50 catheter inserted into the carotid artery.

Fixation Techniques

For all studies, the carotid cannula was pushed into the left ventricle (LV) and LV diastolic pressure recorded. Rats were killed by injection of 1 ml of 3.3 M KCl via the jugular vein for diastolic arrest, and hearts were rapidly removed and cannulated via the thoracic aorta at the arch for retrograde perfusion fixation via the ventricle and coronary arteries. After flushing residual blood from the LV and coronary vasculature with oxygenated Krebs solution, fixative was perfused at intraventricular diastolic pressure to fill the heart. The heart was then immersed and perfused with 2% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4) at room temperature for 30 minutes. Perfusion was stopped, heart bisected at right angles to the long axis, and 1-mm slices of the total thickness of the LV were removed from the cut surface with a razor blade. Similar sites were sampled from each rat. The slices were diced in fixative into rectangular full-wall thickness (1 × 2 mm) blocks, which provided cross-sectional muscle bundles and capillary profiles of subepicardial and midwall regions of the LV. After 1 hour in fixative, blocks were washed for 1 hour in two changes of 0.1 M Na-cacodylate buffer (pH 7.4) containing 7.5% sucrose and post-fixed for 1½ hours at 4 °C in 1% OsO₄ in the same buffer. The samples were then dehydrated from 50% ethanol and embedded in Spurr’s resin. For light microscopy, sections 1 μm thick were cut using an LKB-III ultramicrotome. All sections were mounted on glass slides and stained with 1% toluidine blue in 1% borax.

Quantitative Morphological Techniques

Wall thickness. For each heart, 10 equidistant measurements of LV-free-wall thickness were taken at a fixed magnification. The overall mean thickness was calculated for each age and strain and plotted to determine the developmental course of LV hypertrophy.

Capillary density. For capillary density studies, 1-μm thick sections containing cross-sections from the subepicardial and midwall regions of the LV from 4–6 rats/strain/age group were examined. Capillaries were counted using an ocular grid at a final magnification of 300 X. A minimum of 5 sites from each ventricle were analyzed. Capillary counts were performed on 5–8 fields from each site so that 25 or more fields from each ventricle were quantified. Fields were selected at random, with the sole criterion being cross-sectional orientation of myocytes and capillaries. At younger ages (1, 6, and 12 months), these fields were characterized by tightly packed myocytes and capillaries. However, areas of focal and subsequently widespread necrosis were evident at the later (18 and 24 months) stages. These areas were not excluded from the morphometric analysis because they represented the pathological alterations that occurred in hypertension-mediated hypertrophy and necrosis.

Densities were expressed as capillary profiles/mm² cross-sectional area of myocardium. From this data, diffusion distance was calculated using the following equation: R = 10²/VN²r, where N is the number of capillaries/mm².¹⁹ This value is equal to the mean half-distance between two capillaries in cross-section and represents an average index of the capillary supply. However, the capillary bed is not isotropically arranged in the myocardium. As an index of the variability of capillary spacing, the range of capillary densities was reported for each age and strain.

Tissue morphometry. The quantitative stereologic methods used are largely derived from those of Weibel²³ and Underwood²⁵ and have been recently reviewed by Loud and Anversa.²⁷ The present study utilized a systematic two-dimensional point lattice to estimate volume fractions for different tissue components.²⁶ From a total of 15 blocks/animal, 5 blocks were sectioned and 3–5 fields/block were photographed. Negatives were enlarged onto the surface of a digitizer pad connected to a Wang 2200 computer programmed to accept point counts on the various tissue components under study. A grid system, on transparent overlay, was placed on each micrograph, and point counts overlaying the various tissue components were made by touching the intercepts with a magnetic stylus that fed the counts directly to the computer. Volume fraction of muscle cells, extracellular space, and vasculature were quantitated within a defined area. Means were determined for each ventricle and statistical comparisons made between age groups and strains. In addition, individual myocyte cross-sectional areas (MCSA) were determined for 200 myocytes/animal/age. All volume fraction estimations were performed only in tissue areas wherein myocytes were arranged in cross section.

Statistical analysis. All data are presented as the mean ± SEM and were analyzed by two-way analysis of variance (ANOVA), and follow-up analysis of pairwise comparisons between age-matched strains was performed using the Bonferroni modified t test.²⁷ Age-related changes within each strain were analyzed by one-way ANOVA, and all possible comparisons performed using the Newman-Keul multiple range test.

Results

Systolic blood pressure (BP) in SHR approached 200 mm Hg by 12 months of age, compared to the normotensive WKY, which stabilized at approximately 120 mm Hg by 6 months (Table 1). Although SHR...
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had an elevated BP at 1 month of age, the difference between WKY and SHR, at this time, failed to achieve statistical significance. Significant differences \((p<0.05)\) in BP levels between the strains persisted from 6 months onward. There was an age-related increase in left ventricular wall thickness up to 18 months in both SHR and WKY. However, while the age-related increase in wall thickness in WKY showed a gradual progression, SHR wall thickness rapidly reached a temporary plateau by 6 months of age. SHR LV wall thickness did not increase significantly between 6 and 12 months. Between 12 and 18 months, LV wall thickness of both strains reached a maximum (Table 1). In contrast to WKY, senescent SHR \((24 \text{ months})\) demonstrated a significant \((p<0.05)\) thinning of the LV free wall.

The increase in LV wall thickness during the first 6 months in both SHR and WKY was due primarily to an increase in myocyte cell size, i.e., cellular hypertrophy. To assess cellular growth, myocyte cross-sectional area (MCSA) was determined (Table 1). As early as 1 month, SHR myocytes were already larger than WKY, at an age prior to sustained, elevated BP. Between 1 and 6 months, myocyte cell size increased in both strains, presumably representing normal cell growth. However, in association with the continued increase in BP between 6 and 12 months, SHR myocyte MCSA increased by 65\% \((p<0.01)\) while the MCSA of WKY did not increase significantly in this period (Table 1). The accelerated growth of the SHR myocyte is documented by the fact that the MCSA plateaued at 12 months with no significant increase or decrease during senescence. In contrast, the WKY MCSA increased significantly \((p<0.01)\) between 12 and 18 months and decreased significantly \((p<0.01)\) between 18 and 24 months (Table 1).

As both strains matured (1–6 months), LV volumetric measurements revealed an age-related decrease in the fractional volume of interstitial space and corresponding increase in myocyte fractional volume (Figure 1A and B). However, whereas these measurements normalized in adult WKY (6–12 months), the percent volume of left ventricle occupied by myocytes in SHR remained significantly \((p<0.01)\) elevated until 12–18 months. During this period, ventricular volume of interstitial space in SHR was significantly \((p<0.01)\) reduced (Figure 1A and B). Between 12–18 months in the SHR, there was a significant \((p<0.01)\) decrease in the percentage volume of LV occupied by myocytes and a corresponding significant \((p<0.01)\) increase in percentage volume of LV containing interstitial material (Figure 1A and B).

Myocyte hypertrophy in both SHR and WKY during the growth period (1–6 months) was associated with an

### Table 1. Age-Related Changes in Blood Pressure, Ventricular Wall Thickness, and Myocyte Cross-Sectional Area of WKY and SHR

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Arterial blood pressure (mm Hg)</th>
<th>Left ventricular wall thickness (mm)</th>
<th>Myocyte cross-sectional area (MCSA) (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WKY: 90 ± 11 (4)</td>
<td>WKY: 0.92 ± 0.04 (5)</td>
<td>WKY: 208 ± 21 (5)</td>
</tr>
<tr>
<td></td>
<td>SHR: 110 ± 6 (3)</td>
<td>SHR: 1.07 ± 0.01 (6)</td>
<td>SHR: 244 ± 10 (6)</td>
</tr>
<tr>
<td>6</td>
<td>WKY: 123 ± 3 (12)</td>
<td>WKY: 1.65 ± 0.06 (7)*</td>
<td>WKY: 494 ± 27 (6)*</td>
</tr>
<tr>
<td></td>
<td>SHR: 175 ± 4 (12)*↑</td>
<td>SHR: 2.13 ± 0.14 (5)*↑</td>
<td>SHR: 640 ± 50 (6)*↑</td>
</tr>
<tr>
<td>12</td>
<td>WKY: 104 ± 5 (5)</td>
<td>WKY: 1.91 ± 0.06 (5)*</td>
<td>WKY: 564 ± 38 (5)*NS</td>
</tr>
<tr>
<td></td>
<td>SHR: 208 ± 5 (5)*↑</td>
<td>SHR: 2.38 ± 0.08 (5)*↑</td>
<td>SHR: 990 ± 25 (5)*↑</td>
</tr>
<tr>
<td>18</td>
<td>WKY: 117 ± 3 (5)</td>
<td>WKY: 2.16 ± 0.05 (4)*</td>
<td>WKY: 778 ± 29 (4)*</td>
</tr>
<tr>
<td></td>
<td>SHR: 205 ± 7 (5)*↑</td>
<td>SHR: 2.79 ± 0.10 (4)*↑</td>
<td>SHR: 1002 ± 52 (4)*NS</td>
</tr>
<tr>
<td>24</td>
<td>WKY: 120 ± 3 (5)</td>
<td>WKY: 2.13 ± 0.03 (6)*NS</td>
<td>WKY: 610 ± 46 (6)*</td>
</tr>
<tr>
<td></td>
<td>SHR: 206 ± 15 (5)*↑</td>
<td>SHR: 2.30 ± 0.04 (5)*NS</td>
<td>SHR: 904 ± 23 (5)*NS</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM (number of animals/group).

*Significantly different from preceding strain-matched value \((p<0.01)\); †significantly different from age-matched WKY \((p<0.05)\); NS, not statistically significant from preceding strain-matched value \((p>0.05)\).
The growth (1-6 month) and maturation (6-12 month) capillaries, which subsequently stabilized at 6 months. During the periods in both strains, the capillary density decreased significantly (p<0.01). However, SHR capillary density was significantly (p<0.05) less than WKY values. In addition to reduced vascular profiles, SHR LV also had the widest range of capillary density values, which substantiates the nonhomogeneous capillary spacing theory of cardiac hypertrophy, since areas of both normal and severely diminished vasculature exist within the same ventricular regions studied.

Directly associated with decreased capillary density is a corresponding increase in computed average intercapillary diffusion distance (AIDD) (Table 2). This value rapidly equilibrates in the WKY by 6 months with a significant (p<0.05) increase observed only between 12 and 18 months. In contrast, the SHR AIDD rapidly and significantly increases at 6 and 12 months (p<0.01 and 0.05, respectively) before reaching a plateau. The AIDD progressively increases throughout the SHR life span and eventually exceeds 17 μm by 24 months. From 6 months onward, SHR AIDD significantly (p<0.05) exceeds WKY values.

The volumetric tissue changes can be observed quantitatively from light micrographs (Figure 3). At 6 months (Figure 3A and B), established myocyte hypertrophy is evident in SHR vs. WKY with otherwise normal ventricular architecture. Additionally, 6-month SHR showed distinct, small foci of necrotic cells, which were most pronounced in subepicardial and midwall regions compared to subendocardial.

Aged and senescent SHR had near-complete necrosis of the subendocardial ventricular region. However, at 24 months, WKY hearts showed age-related myocyte hypertrophy and areas of focal necrosis characterized by slightly elevated connective tissue components.

Table 2. Age-Related Vascular and Myocyte Size Changes in WKY and SHR

<table>
<thead>
<tr>
<th></th>
<th>MCSA (μm²)</th>
<th>Capillary density</th>
<th>Average diffusion distance (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean profiles/mm²</td>
<td>Range</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month (5)</td>
<td>208 ± 21</td>
<td>4,081 ± 110</td>
<td>3,273-5,134</td>
</tr>
<tr>
<td>6 months (5)</td>
<td>494 ± 27</td>
<td>3,119 ± 217*</td>
<td>2,232-3,162</td>
</tr>
<tr>
<td>12 months (5)</td>
<td>564 ± 38</td>
<td>2,434 ± 219†</td>
<td>1,302-3,162</td>
</tr>
<tr>
<td>18 months (4)</td>
<td>778 ± 29</td>
<td>1,815 ± 151NS</td>
<td>670-2,604</td>
</tr>
<tr>
<td>24 months (6)</td>
<td>610 ± 46</td>
<td>2,043 ± 196NS</td>
<td>707-3,385</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month (5)</td>
<td>244 ± 10</td>
<td>3,972 ± 102</td>
<td>3,088-4,724</td>
</tr>
<tr>
<td>6 months (6)</td>
<td>640 ± 50‡</td>
<td>1,973 ± 123*‡</td>
<td>1,339-3,162</td>
</tr>
<tr>
<td>12 months (5)</td>
<td>990 ± 25‡</td>
<td>1,539 ± 170‡</td>
<td>409-2,716</td>
</tr>
<tr>
<td>18 months (4)</td>
<td>1,002 ± 52‡</td>
<td>1,370 ± 122NS</td>
<td>260-2,158</td>
</tr>
<tr>
<td>24 months (5)</td>
<td>904 ± 23‡</td>
<td>1,113 ± 87NS‡</td>
<td>298-2,418</td>
</tr>
</tbody>
</table>

Values represent means ± SEM (number of animals/group).
*Significantly different from preceding strain-matched value (p<0.01); †Significantly different from preceding strain-matched value (p<0.05); ‡Significantly different from age-matched WKY (p<0.05); NS, not statistically significant from preceding strain-matched value (p>0.05).
In contrast, aged SHR ventricles (Figure 3D) demonstrated myocyte hypertrophy, widespread infarction, and tissue necrosis characterized by a large fibrous component and the influx of inflammatory cells throughout the ventricle.

**Discussion**

Hypertrophy of the mammalian ventricular myocyte is a time-dependent process that continuously evolves during the life span of the animal. Because of the extensive use of the SHR as an animal model of human essential hypertension, a complete life-span analysis of the myocardial response to long-term hypertension is warranted but has not been completely described. Since the life span of most rodents reaches or exceeds 2 years, analysis of only selected younger age groups may obscure potentially significant aging changes that may occur in combination with hypertrophy. By assessing morphometric changes in the subepicardial and midwall regions of the LV at the times indicated, cardiac hypertrophy was analyzed during development, maturation, aging, and subsequent ventricular deterioration. The results indicate that subepicardial and midwall regions are the first to demonstrate focal necrosis, which can be seen as early as 6 months in SHR. Although the subendocardium of the LV appears to be more susceptible to damage in transient ischemic attacks, it may be protected from chronic ischemic necrosis by oxygen diffusion from the ventricular chamber in a manner analogous to that seen in large elastic arteries, the inner two-thirds of which are avascular. In contrast, the subepicardial and midwall areas are totally dependent on the capillary network for oxygenation. Our results would suggest that the increased diffusion distance resulting from myocyte hypertrophy in these regions may lead to early focal ischemia and necrosis, which, with age, becomes widespread.

Cardiac hypertrophy represents a complex biological process that involves the remodeling of the architecture of the heart, probably in response to an increased work load. The stimuli for this process appear to be both mechanical and humoral in nature. Because fetal and neonatal SHR are exposed to elevated blood pressures, development of cardiac hypertrophy may be temporally and quantitatively accelerated. Accelerated myocyte growth was seen in both LV wall thickness and MCSA of the 6-month-old SHR (Table 3.

**Figure 3.** Age-related changes in ventricular tissue morphology in SHR and WKY. Light micrographs of 1-μm thick plastic section of ventricular tissue from 6-month-old WKY (3A), 6-month-old SHR (3B), 24-month-old WKY (3C) and 24-month-old SHR (3D). (Toluidine Blue stain.) All micrographs are 125×.
1). During development of cardiac hypertrophy in SHR and WKY (1–6 months), capillary density significantly declines due to the increase in MCSA. Differences in MCSA, rather than cell length, represent the most accurate index of interventricular and hypertrophic variations in myocyte size. The fractional tissue volume occupied by capillaries also declines, yet only reaches significance (p < 0.05) in SHR. This finding, together with the reduction in capillary density, would indicate that compensatory neovascularization does not occur during this period or at least is inadequate to maintain a normal vascular profile in the SHR. Anversa et al have recently described several structural compensatory mechanisms in SHR less than 2 months old. During this period, capillary proliferation in SHR exceeded that of WKY, yet capillary density remained unchanged. This suggests that the capillary deficiency detected in 6-month-old SHR occurs after 2 months since Tomanek and Hovanek and Tomanek et al have reported that 2.5, 4, and 7-month SHR have reduced capillary densities. Inadequate neovascularization, relative to muscle cell growth, occurs during the developmental phase of cardiac hypertrophy in both strains (Table 2). A vascular deficiency is particularly acute in the SHR since capillary profiles at 6 months old are one-half of the 1-month-old animals. In association with deficient neovascularization in SHR, an expanding myocyte cell volume results in a dramatic increase in the AIDD. In agreement with Tomanek et al, the similar AIDD of the WKY and SHR at 1 month rapidly expands such that 6-month-old SHR AIDD had a value not significantly different from 24-month-old WKY (Table 2). In contrast, Rakusan et al have reported that 5-month-old SHR and WKY have similar Krogh cylinder radii, which suggests that the oxygen diffusion distance is not different between strains at this age. The failure of revascularization during the remodeling of the SHR ventricle during the developmental phase of cardiac hypertrophy may lead to the eventual decline in pumping ability of the aged myocardium and to the ventricular necrosis observed in old age in the present study. During the maturation phase (6–12 months) of cardiac hypertrophy, LV wall thickness of the SHR remains stable, yet the MCSA continues to increase (Table 1). In WKY, LV wall thickness increased with no significant increase in MCSA. The morphometric consequences of these changes are seen in Figure 1. The percent volume of LV occupied by myocytes in the SHR remained significantly (p < 0.01) elevated and the amount of interstitial material significantly (p < 0.01) decreased. During this same period, WKY myocytes occupied significantly (p < 0.01) less of the LV wall while the percentage of LV wall occupied by interstitial material increased significantly (p < 0.01). As previously seen during the developmental phase, the increased MCSA in SHR exceeds any neovascularization that may have occurred and exacerbated the already established vascular deficiencies. Although there are no other literature data on LV-free-wall capillary density in 12-month-old WKY or SHR, functional criteria of SHR hearts begin to deteriorate from this age onward. Pfeffer and Pfeffer report that after 12 months increased SHR ventricular weight is not associated with an augmentation in pressure generating capacity. Wikman-Coffelt et al reported that when compared to a 6-month-old SHR, a 12-month-old SHR consumes 32% less oxygen/g tissue/beat, and this decrease was associated with isomyosin shifts toward V₁ and V₂. Hochachka has recently described the cellular strategies available to hypoxic tissues such as the ischemic myocardium. Additionally, Ingwall and Fossel have reported that the myocardial creatine kinase system shows a decline after 12 months. During the aging phase of cardiac hypertrophy (12–18 months), the BP of the SHR reaches a plateau, yet LV wall thickness and MCSA continue to increase (Table 1). This period of cardiac hypertrophy may represent a secondary period of ventricular growth that is associated with both muscle and nonmuscle cell types and their associated extracellular matrices. This hypothesis is substantiated by morphometric analysis of the SHR (Figure 1) since fractional volume of the LV occupied by myocytes decreases significantly while the corresponding volume of interstitial material increases. Because MCSA of the SHR does not decrease during this period, morphometric analysis suggests that the decreased percent volume occupied by myocytes may represent a dilution of remaining cells in an increasing tissue mass occupied by interstitial material. This is substantiated by the fact that the LV wall thickness demonstrated a secondary increase (Table 1) in concert with a significant increase in interstitial material (Figure 1). Biochemical analysis of SHR has documented an age-related increase in ventricular collagen content. Secondary growth of SHR LV after 12 months has been reported by others, yet a stabilization of ventricular growth has been reported by Tomanek and Hovanek and Tomanek et al between 7 and 15 months. Because Tomanek and Hovanek and Tomanek et al have utilized a SHR colony maintained exclusively at their institution, population differences may be associated with the variation in results when comparing their aged (15 months old) animals to those used by Rakusan et al and in this report. The reported "normalization" of the subepicardial vascular profiles of 15-month-old SHR may reflect the stable growth characteristics of the animal colony utilized by Tomanek et al as well as methodological differences in tissue areas analyzed. Tomanek et al reported capillary density data only on "fields containing closely packed myocytes and capillaries with virtually no visible separation of cells." In the current study, areas of both focal and widespread necrosis were included on the rationale that these tissue changes accurately reflect the actual pathological consequences of hypertension-mediated cardiac hypertrophy. In agreement with our results, Rakusan et al have described a similar decrease in capillary density and concomitant increase in AIDD such that 15-month and 18-month SHR (Table 2) have significant vascular abnormali-
ties. The increased interstitial space and collagen content, possible myocyte attrition, deficient anisotropic vasculature, and expanded AIDD may all contribute to the ventricular failure that occurs during the senescent phase of cardiac hypertrophy.

During senescence (18–24 months), the ventricular myocardium demonstrated morphological (Figure 3) and morphometric (Figures 1 and 2) characteristics of degeneration. Although the WKY myocardium showed some cellular characteristics of senescent degeneration, the SHR myocardium demonstrated marked architectural alterations. During this period in SHR, LV wall thickness decreases significantly (thinning) while MCSA remained relatively constant (Table 1). These results would suggest that this decrease in LV wall thickness may result from both a loss of myocytes, since percent volume occupied by myocytes declines significantly (Figure 2B), and fibrotic scarring of infarcted areas shown qualitatively (Figure 3) and quantitatively (Figure 2A). Olivetti et al have also reported an age-related loss of myocytes in the LV of normotensize rats. Pfeffer et al reported a dramatic increase in LV fibrosis with age in SHR such that approximately 50% of the free wall was fibrotic at 90 weeks of age. Tomanek et al have also documented increased lysosomal-associated cellular degeneration in aged and senescent SHR. In agreement with both Tomanek and Hovane and Rakusan et al, our results also indicate a significant decrease in LV capillary density and expanded AIDD. Biochemical and physiological deterioration of senescent SHR heart manifest themselves such that the transition of the compensated, hypertrophied heart toward failure often occurs during this period. Biochemical abnormalities in SHR creatine kinase (CK) system become particularly acute during senescence. The senescent loss of CK is primarily associated with the mitochondrial isozyme form, which correlates with the mitochondrial structural abnormalities seen by Tomanek and Hovane in senescent SHR and Frenzel and Feimann in senescent Wistar rats. In concert with loss of mitochondrial volume, Beyer and Starnes and Beyer et al have reported that mitochondrial coenzyme Q content declines significantly after 18 months in many rodents. The loss of oxidative pathways via decreased tissue perfusion and organellar damage in the SHR myocardium may be related to the age-related increase in glycolytic energy production, decreased contractile protein ATPase activity, and corresponding decline in cardiac performance.

When analyzed through its entire life span, the ventricular consequences of long-term hypertension in the SHR can be better appreciated. The heart is an organ that is in a steady state regarding its energy producing and consuming cells and organelles. Therefore, any impairment in the flow of energy producing substrates and/or oxygen is compounded by a corresponding increase in metabolic byproducts that may profoundly influence contractility and function. Although these functions of cardiac physiology are well appreciated, structural correlates of these proposed physiological deficiencies of the SHR have not been documented. SHR vascular deficiencies are evident at 6 months, which suggests that the aging process may be accelerated since vascular profiles of the 6-month-old SHR are not significantly different from the 24-month-old WKY. A similar form of "accelerated aging" in the pressure-overload myocardium has been proposed for protein synthesis and degradation. Therefore, age-related changes in LV myocardial architecture of the SHR may represent accelerated aging of the myocyte and vascular components that contribute to a progressively deteriorating hypertrophied ventricular pump.

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References
2. Frohlich ED, Tarazi RC: Is arterial pressure the sole factor responsible for hypertensive cardiac hypertrophy? Am J Cardiol 1979;44:959–963
17. Lund DD, Tomanek RJ: Myocardial morphometry in spontaneous


39. Pfeffer JM, Pfeffer MA: Transition to cardiac failure in sponta-


42. Hochachka PW: Defense strategies against hypoxia and hypo-


46. Olivetti G, Hiler B, Ricci R, Guideri G, Anversa P: Myocyte cell loss and myocyte hypertrophy in the aging rat heart (ab-
stract). *Circulation* 1986;74 (suppl II):II-175


none) and protein concentrations over the life span of the labo-


52. Bishop SP: Structural alterations in the hypertrophied and fail-


54. Liedtke AJ, Orie JE, Toggart EJ: Carbohydrate and lipid me-

**Key Words**: cardiac hypertrophy • tissue morphometry • capillary density • spontaneously hypertensive rats
Morphometric analysis of cardiac hypertrophy during development, maturation, and senescence in spontaneously hypertensive rats.

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