Contribution of ventricular remodeling to pathogenesis of heart failure in rats

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Received 27 January 2000; accepted in final form 22 September 2000

Brower, Gregory L., and Joseph S. Janicki. Contribution of ventricular remodeling to pathogenesis of heart failure in rats. Am J Physiol Heart Circ Physiol 280: H674–H683, 2001.—We previously reported an approximately 50% incidence of rats with symptoms of congestive heart failure (CHF) at 8 wk postinfrarenal aorto-caval fistula. However, it was not clear whether compensatory ventricular remodeling could continue beyond 8 wk or whether the remaining animals would have developed CHF or died. Therefore, the intent of this study was to complete the characterization of this model of sustained volume overload by determining the morbidity and mortality and the temporal response of left ventricular (LV) remodeling and function beyond 8 wk. The findings demonstrate an upper limit to LV hypertrophy and substantial increases in LV volume and compliance, matrix metalloproteinase activity, and collagen volume fraction associated with the development of CHF. There was an 80% incidence of morbidity and mortality following 21 wk of chronic volume overload. These findings indicate that the development of CHF is triggered by marked ventricular dilatation and increased compliance occurring once the myocardial hypertrophic response is exhausted.

METHODS

All experiments were performed using adult male Sprague-Dawley (Hsd:SD) rats initially weighing between 380 and 440 g. The rats were housed under standard environmental conditions and maintained on commercial rat chow and tap water ad libitum. All studies conformed with the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the protocol was approved by our Institution’s Animal Care and Use Committee. Anesthesia for surgical procedures and subsequent euthanasia at the experimental end point was effected by pentobarbital sodium (50 mg/kg) injected into the intraperitoneal cavity. Postoperative analgesia was provided by buprenorphine HCl (0.05 mg/kg sc) administered to the rats at the time of surgery.

Surgical preparation. Infrahepatic abdominal AV fistula was created in rats as previously described (5). Briefly, a ventral abdominal laparotomy was performed to expose the aorta and caudal vena cava ~1.5 cm below the renal arteries. An 18-gauge needle was inserted into the exposed abdominal aorta and advanced through the medial wall into the vena cava to create the fistula. The needle was withdrawn, and the ventral aortic puncture was sealed with cyanoacrylate. Creation of a successful AV fistula was visually evident by the pulsatile flow of oxygenated blood into the vena cava. The animals would have subsequently developed overt CHF. Therefore, the purpose of this study was to determine the temporal response of ventricular remodeling and function beyond 8 wk postfistula and the morbidity and mortality due to CHF in this model of sustained volume overload. To this end, LV mass, dilatation, compliance, function, matrix metalloproteinase (MMP) activity, and collagen concentration of hearts from rats with chronic AV fistula for 15 and 21 wk and controls were compared. The findings of this study provide experimental verification of the hypothesis first put forward by Linzbach (21) that the transition to heart failure is triggered by marked ventricular dilatation occurring once the myocardial hypertrophic response is exhausted. Together these studies characterize the temporal progression of ventricular hypertrophy and size and morbidity and mortality, and they clearly establish the AV fistula in rats as an appropriate model to study the pathogenesis of CHF.

AN INCREASED WORKLOAD imposed on the heart by a sustained volume overload induces compensatory cardiomyocyte hypertrophy and ventricular dilatation (12, 31). In a previous study, we described an early and progressive decrease in myocardial contractility, marked hypertrophy, and ventricular dilatation in response to a biventricular volume overload induced by infrarenal aorto-caval (AV) fistula (5). There was also an initial increase in in vivo left ventricular (LV) end-diastolic pressure (EDP), which peaked at 3 wk. A subsequent decline in in vivo LVEDP was associated with significant ventricular dilatation and increased LV compliance. Approximately 50% of the rats at the 8-wk postfistula time point developed symptoms consistent with congestive heart failure (CHF). However, it was not clear from that study whether the compensatory ventricular remodeling induced by an AV fistula would continue beyond 8 wk or whether the remaining
abdominal musculature and skin incisions were closed by standard techniques with absorbable suture and autoclips, respectively.

**Experimental protocol.** Rats were randomly selected from the colony and studied at 15 (n = 7 rats) and 21 wk (n = 9 rats) following creation of the AV fistula. Age-matched control groups for each study period consisted of sham-operated rats (n = 7 each time period). At the end of the study period, the rats were weighed and anesthetized, the fistula patency was visually confirmed, and the heart was removed and attached to a perfusion apparatus for evaluation of ventricular function (see Assessment of ventricular size and function). After the functional studies were completed, the atria and great vessels were removed and the LV (plus septum) and RV were separated and weighed. Lung and function were evaluated in vitro using a blood-perfused isolated heart preparation as previously described (5). Briefly, the apparatus consisted of a pressurized perfusion reservoir and a collection reservoir connected in circuit with a support rat. Arterial blood from the carotid artery of the support rat was pumped to a pressurized reservoir for retrograde perfusion of the heart. The coronary venous effluent was collected and returned to the support rat through a jugular vein catheter to filter and oxygenate the blood supply to the isolated heart. The temperature of the blood in the perfusion reservoir was maintained at 37 ± 1°C, and the environment around the isolated heart was kept constant at 35 ± 2°C. Coronary perfusion pressure was maintained between 100 and 110 mmHg.

Before the heart was removed from an anesthetized rat, the coronary arteries were ligated, and a cannula was inserted into the thoracic aorta at a level just proximal to the first pair of intercostal arteries and secured with a silk ligature. Retrograde perfusion of the coronary arteries with blood from the perfusion reservoir was begun as soon as the support rat had developed stable isovolumetric contractions. Once the heart developed stable isovolumetric contractions, blood from the perfusion reservoir was begun as soon as the support rat had developed stable isovolumetric contractions. The temperature of the blood in the perfusion reservoir was maintained at 37 ± 1°C, and the environment around the isolated heart was kept constant at 35 ± 2°C. Coronary perfusion pressure was maintained between 100 and 110 mmHg.

Morbidity and mortality assessment. To determine the time course of morbidity and mortality in this model, 116 rats with AV fistula were evaluated weekly for signs of CHF. For the purpose of this study, overt CHF (i.e., morbidity) was defined as a significant increase in body weight (i.e., >50 g in a 7-day period) together with labored respiration and/or pitting edema. Once symptoms of overt CHF were identified, a 7-day period (i.e., <1 mmHg) for the volume range 0–1,240 μl. Statistical analyses were performed using SYSTAT 7.0 software (SPSS: Chicago, IL). All group data were expressed as means ± SD. Grouped data comparisons were made by one-way analysis of variance. When a significant F ratio (P < 0.05) was obtained, intergroup comparisons were made using a modified t-test and Bonferroni bounds. Statistical significance was taken to be P < 0.05/k, where k equaled the number of comparisons. Before the peak isovolumetric pressure-volume relationships for each study group were compared, the pressure-volume data for individual hearts were adjusted so that all pressure-volume curves began at the origin of the graph. The adjustment was made by subtracting V₀ and peak isovolumetric pressure at V₀ (P₀), respectively, from all subsequent volume and pressure data.
RESULTS

Control groups. No significant trends were observed in LV, RV, and lung weights in the individual control groups (Table 1). Body weight increased with age, but these differences were not statistically significant. Statistical comparisons of the EDP-volume and peak isovolumetric pressure-volume relationships from the individual age-matched control groups (data not shown) revealed no significant differences. Therefore, these data were combined and considered as a single group (control) for comparison purposes.

AV fistula groups. Average LV, RV, lung, and body weights for the control and volume overload groups are presented in Table 1. There was a significant increase in both LV and RV weights ($P < 0.001$) relative to the age-matched controls. Most of the myocardial hypertrophy induced by the AV fistula occurred during the first 8 wk postfistula, but there was a trend for ventricular weights to increase further with time, although this was only significant for the RV weights of the 15- and 21-wk groups relative to the 8-wk AV fistula group. The average lung weight in all of the AV fistula groups was significantly increased over control, but this decrease did not attain statistical significance. There was no further shift to the right in the LV pressure-volume relationships beyond 8 wk postfistula (when compared strictly on a time postfistula basis).

LV systolic function. Measures of LV systolic function, namely $P_0$ and peak isovolumetric pressure at an LVEDP of 25 mmHg ($P_{25}$); the peak isovolumetric pressure-EDV ($P_{\text{max}}$-$V$) relationship slope, which is an index of contractility; and the range of regression coefficients for the $P_{\text{max}}$-$V$ relationship are given in Table 3 for each group. There was a tendency for the $P_0$ and $P_{25}$ values to be slightly lower in the AV fistula groups relative to control, but this decrease did not attain statistical significance. The relationship between peak isovolumetric pressure and EDV was highly linear, as evidenced by correlation coefficients, which were all $>0.88$ with the exception of one curve in the 8-wk group. The slopes of the normalized $P_{\text{max}}$-$V$ relationships for all of the fistula groups were significantly less.

dilatation was made by comparing the values for $V_0$, whereas an indication of ventricular compliance was ascertained from the volume required to increase EDP from 0 to 25 mmHg ($\Delta V_{0–25}$). Thus it can be seen from the data in Table 2 and Fig. 1 that, although the LV in the AV fistula groups are significantly dilated and much more compliant than the normal hearts, there was no further shift to the right in the LV pressure-volume relationships beyond 8 wk postfistula (when compared strictly on a time postfistula basis).

<table>
<thead>
<tr>
<th>Group</th>
<th>LV Weight, mg</th>
<th>RV Weight, mg</th>
<th>Lung Weight, mg</th>
<th>BW, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 wk Control</td>
<td>935±98</td>
<td>241±15</td>
<td>1,945±228</td>
<td>484±33</td>
</tr>
<tr>
<td>15 wk Control</td>
<td>1,037±128</td>
<td>270±46</td>
<td>1,833±242</td>
<td>475±27</td>
</tr>
<tr>
<td>21 wk Control</td>
<td>1,049±123</td>
<td>276±55</td>
<td>1,856±59</td>
<td>564±31</td>
</tr>
</tbody>
</table>

| 8 wk Fistula         | 1,667±266†    | 537±95†       | 2,593±620‡     | 547±107‡ |
| 15 wk Fistula        | 1,731±237†    | 652±127‡*     | 3,258±1,073‡§  | 529±42‡§ |
| 21 wk Fistula        | 1,801±184†    | 656±126‡*     | 3,437±1,453‡   | 587±76  |

| 8 wk Compensated     | 1,671±314†    | 528±94†       | 2,167±217      | 525±102 |
| 15 wk Compensated    | 1,577±31†     | 480±11†       | 2,013±95       | 488±18  |
| 21 wk Compensated    | 1,705±237‡†   | 556±84†       | 2,317±168†     | 568±28  |
| Avg. compensated     | 1,690±275†    | 529±85†       | 2,191±202      | 526±78  |
| Fistula failure      | 1,757±192†    | 663±119‡$§$   | 3,674±1,157‡$§$ | 570±76  |

Values are means ± SD. LV, left ventricle; RV, right ventricle; BW, body weight. *$P < 0.05$ vs. 8 wk fistula group; †$P < 0.001$ vs. age-matched control; ‡$P < 0.05$ vs. age-matched control; §$P < 0.01$ vs. compensated fistula group.

### Table 2. Isolated heart: diastolic function

<table>
<thead>
<tr>
<th>Group</th>
<th>$V_0$, µl</th>
<th>$\Delta V_{0–25}$, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>211±65</td>
<td>185±36</td>
</tr>
<tr>
<td>8 wk Fistula</td>
<td>370±105*</td>
<td>476±264*</td>
</tr>
<tr>
<td>15 wk Fistula</td>
<td>367±160*</td>
<td>402±187*</td>
</tr>
<tr>
<td>21 wk Fistula</td>
<td>381±94*</td>
<td>425±197*</td>
</tr>
<tr>
<td>8 wk Compensated</td>
<td>316±32*</td>
<td>364±93*</td>
</tr>
<tr>
<td>15 wk Compensated</td>
<td>268±56</td>
<td>338±171*</td>
</tr>
<tr>
<td>21 wk Compensated</td>
<td>355±29*</td>
<td>277±31*</td>
</tr>
<tr>
<td>Avg. compensated</td>
<td>317±52*</td>
<td>323±106*</td>
</tr>
<tr>
<td>Fistula failure</td>
<td>475±127†</td>
<td>632±187†</td>
</tr>
</tbody>
</table>

Values are means ± SD. $V_0$, left ventricular volume at an end-diastolic pressure (EDP) of 0 mmHg; $\Delta V_{0–25}$, the change in LV volume between EDP 0 and 25 mmHg. *$P < 0.01$ vs. control; †$P < 0.01$ vs. compensated fistula group. For statistical consideration age-matched controls were combined into a single group.
than control ($P < 0.05$), indicating a decrease in contractility.

**Morbidity and mortality.** The incidence of symptoms or death attributable to CHF can be derived from the plot of survival postfistula depicted in Fig. 2. The initial occurrence of morbidity and mortality due to CHF was noted at 4 wk after inducing chronic volume overload, with the incidence of overt CHF subsequently exceeding 50% and 80% at 14 and 21 wk postfistula, respectively. Thirty-three of the rats (28%) died acutely without noticeable increases in body weight; however, almost all of these rats had lungs that did not collapse when the thorax was opened, together with marked increases in lung weight and lungs with a wet, heavy appearance; typically, there was also excess fluid in the thoracic cavity. No adverse events occurred in the control group during the same period.

**Compensated volume overload versus overt heart failure.** These experiments were initially designed to progress to a specific temporal end point; however, comparisons based solely on time postfistula revealed no identifiable trends with regard to the development of overt CHF. Nevertheless, it was clear that individual rats were developing CHF after differing periods of compensated LV remodeling. Therefore, each rat from the 8-, 15-, and 21-wk fistula groups was separated into compensated or failure groups based on the presence or absence of symptomatic CHF. The average values obtained for the morphological and functional parameters based on this grouping are presented in Fig. 3 and Tables 1–3. In comparing the compensated and failure groups, there was, as expected, a marked increase in lung weight observed in rats with overt CHF. The hypertrophic response in the RV was also correlated with lung weight ($r = 0.76$). The initial RV hypertrophy after opening the fistula stabilized after an ~100% increase in mass. This initial rate of RV hypertrophy roughly paralleled that of the LV and was typically associated with relatively normal lung weights. However, coincident with the development of LV dysfunction, as evidenced by a marked increase in lung weight, the extent of RV hypertrophy became disproportionately greater than that of the LV, as reflected by a significant decrease in the LV-to-RV ratio ($3.20 \pm 0.20$

### Table 3. Isolated heart: systolic function and contractility

<table>
<thead>
<tr>
<th>Group</th>
<th>$P_{25}$ mmHg</th>
<th>$P_{25}$ mmHg</th>
<th>Slope $P_{\max-V}$, mmHg/mmHg/µl</th>
<th>$r_{P_{\max-V}}$ Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$121 \pm 76$</td>
<td>$198 \pm 67$</td>
<td>$0.412 \pm 0.160$</td>
<td>0.95–1.00</td>
</tr>
<tr>
<td>8 wk Fistula</td>
<td>$99 \pm 55$</td>
<td>$167 \pm 27$</td>
<td>$0.207 \pm 0.136^*$</td>
<td>0.76–1.00</td>
</tr>
<tr>
<td>15 wk Fistula</td>
<td>$108 \pm 53$</td>
<td>$174 \pm 48$</td>
<td>$0.119 \pm 0.045^*$</td>
<td>0.88–0.98</td>
</tr>
<tr>
<td>21 wk Fistula</td>
<td>$79 \pm 36$</td>
<td>$163 \pm 34$</td>
<td>$0.208 \pm 0.105^*$</td>
<td>0.95–0.99</td>
</tr>
<tr>
<td>8 wk Compensated</td>
<td>$86 \pm 59$</td>
<td>$173 \pm 51$</td>
<td>$0.280 \pm 0.039$</td>
<td>0.98–1.00</td>
</tr>
<tr>
<td>15 wk Compensated</td>
<td>$66 \pm 6$</td>
<td>$141 \pm 28$</td>
<td>$0.143 \pm 0.039^*$</td>
<td>0.98–0.98</td>
</tr>
<tr>
<td>Avg. compensated</td>
<td>$86 \pm 39$</td>
<td>$167 \pm 41$</td>
<td>$0.244 \pm 0.088^*$</td>
<td>0.97–1.00</td>
</tr>
<tr>
<td>Fistula failure</td>
<td>$98 \pm 53$</td>
<td>$170 \pm 35$</td>
<td>$0.118 \pm 0.068^*$</td>
<td>0.88–0.99</td>
</tr>
</tbody>
</table>

Values are means ± SD. *$P < 0.05$ vs. control; †$P < 0.01$ vs. compensated fistula group. For statistical consideration, age-matched controls were combined into a single group. $P_{25}$, peak isovolumetric pressure at an end-diastolic pressure of 0 and 25 mmHg, respectively; $P_{\max-V}$, peak isovolumetric pressure-volume relationship; Slope $P_{\max-V}$, slope of $P_{\max-V}$; $r_{P_{\max-V}}$, the upper and lower range of the $P_{\max-V}$ regression coefficients.
The duration of time post-fistula before rats developed symptoms of overt CHF was correlated with LV weight ($r = 0.75$). However, surprisingly there was very little difference between the compensated and failing hearts with regard to LV weight ($P = 0.92$), with the LV weights being comparable in both the compensated and overt CHF groups, ranging from 1,252 to 2,279 mg and 1,425 to 2,009 mg, respectively.

The most striking difference between the compensated and failure groups was the marked increase in LV volume and compliance seen in the failing hearts. Although the LV dilatation and increased compliance in the compensated fistula group were significant (50% larger $V_0$ and 75% increase in $\Delta V_{0-25}$ relative to control, $P < 0.01$), this remodeling was relatively modest compared with the marked LV dilatation and increased compliance in hearts from the fistula failure group (125% larger $V_0$ and 242% increase in $\Delta V_{0-25}$ relative to control, $P < 0.01$). There were corresponding changes in LV systolic function, with moderate depression of the $P_{\text{max}}$-$V$ slope index (i.e., assessment of LV contractility) in the compensated groups (an average 41% decrease relative to control). However, it was only in the 15-wk compensated fistula group that this contractility index became significantly less than control ($P < 0.05$). In fact, the significant decrease in the slope of $P_{\text{max}}$-$V$ in the 15-wk compensated group was similar to the marked depression in contractility (i.e., 71% less than control) observed in the rats with symptomatic CHF.

**Histological evaluation.** As can clearly be seen from Fig. 4, the fistula hearts were grossly larger, but other than an obvious cardiomyocyte hypertrophy, differences from normal hearts at the light microscopic level were subtle. Muscle fiber thinning and widened interstitial spaces were seen in failing hearts, with the latter being attributable to the diffuse systemic edema characteristic of CHF. There was no evidence of myocyte necrosis, inflammatory infiltrates, or scar tissue deposition, and the occasional foci of mild to moderate interstitial fibrosis localized to the subendocardium and papillary muscles present in a few of the hearts was consistent with the pattern of cardiac fibrosis described as an incidental aging lesion in rats (2). However, there was a generalized increase in the myocardial interstitial collagen concentration in the rats with overt CHF (CVF of $1.42 \pm 0.26\%$ compared with a control value of $1.02 \pm 0.39\%$, $P < 0.01$), whereas the compensated rats had CVF values comparable to control ($1.08 \pm 0.35\%$). There were no other consistent differences in the histological appearance of the myocardium among the normal, compensated, and overt heart failure groups.

**MMP activity.** As can be seen in Fig. 5, LV MMP activity is significantly increased in rats with symptomatic CHF (27% greater than control, $P < 0.01$) compared with normal levels in rats from the compensated group.

**DISCUSSION**

The cardiovascular remodeling secondary to chronic volume overload is characterized by progressive ventricular dilatation, hypertrophy, and ultimately failure. Although ventricular function in the clinically manifested stage of CHF has been well studied in both human subjects and experimental animal models (20, 31, 35, 37), very little is known about ventricular remodeling and function during the compensated period of chronic volume overload preceding the development of clinical heart failure. Such information would provide valuable insight into the pathogenesis of CHF. We previously established that the ventricular remodeling during the first 8 wk in a rat model of AV fistula
Values are means ± SD.

This is the first comprehensive study to document the temporal changes in ventricular mass, size, and function; myocardial MMP activity; and the interstitial collagen content associated with the compensated and decompensated stages of chronic volume overload induced by AV fistula. Furthermore, no other studies have previously documented the consistent development of overt CHF in the rat AV fistula model and characterized the temporal morbidity and mortality associated with CHF in this model. Accordingly, these results together with our previous article (5) provide guidance for investigators to design studies to examine specific molecular, biochemical, or cellular events relevant to different stages in the progression from compensated to decompensated heart failure. These findings also represent experimental confirmation of the hypothesis that the transition to heart failure is triggered by marked ventricular dilatation first put forward by Linzbach (21).

Hypertrophic response. Together with our earlier findings (5), this study establishes that myocardial hypertrophy induced by an AV fistula is progressive during the compensatory phase of chronic volume overload but appears to reach an upper limit before the development of CHF. This hypothesis is supported by no difference in the LV weights of both the compensated and failure groups. However, there does not appear to be a straightforward correlation between the degree of LV hypertrophy and development of overt CHF or death, because several rats that developed overt failure had relatively small LV weights (as little as 1,425 mg compared with a high value of 2,009 mg). A proportionally greater hypertrophic response may partially explain why particular hearts did not fail earlier, as suggested by the observation that the compensated phase of ventricular remodeling tends to have a longer duration in hearts with larger LV weights.

During the course of the cardiac remodeling secondary to an AV fistula, the temporal pattern of RV hypertrophy appears to parallel the increase in LV mass. However, unlike the LV hypertrophic response that plateaus once LV function begins to deteriorate, the increase in RV mass continues, as evidenced by the significant decrease in the LV-to-RV ratio in the overt CHF group. The fact that there is a correlation between RV and lung weights suggests that the development of pulmonary edema secondary to LV systolic dysfunction is responsible for the subsequent RV hypertrophy and development of symptomatic CHF.

LV diastolic properties. Ventricular dilatation is the initial compensatory response to maintain an appropriate stroke volume in the volume-overloaded heart (16, 24). This time-dependent process of LV dilatation and associated changes in LV shape has been termed “remodeling” (31). We previously demonstrated that ventricular dilatation during the first 8 wk of postfistula is progressive. In addition to permissive dilatation, a significant increase in ventricular compliance developed between the third and fifth week of volume overload. However, as can be seen in Fig. 1, there was essentially no difference in ventricular size or compliance in the AV fistula groups beyond 8 wk when grouped on the basis of time postfistula. However, when these same animals were divided into groups on the basis of whether they were compensated or in heart failure, as depicted in Fig. 3, there was an appreciable increase in LV dilatation and compliance in the failing hearts with no additional increase in LV weight. These findings suggest that progressive ventricular hypertrophy associated with a compensated ventricle is limited, and additional size increases are detrimental. Linzbach
bach (21) asserted that as the ventricle enlarges, a substantially greater force is necessary to eject an equivalent volume of blood. This adverse effect of dilatation on ventricular function is compounded by the fact that the increased ventricular size produces LV wall thinning, effectively requiring the amount of work per unit of contracting muscle to be still greater. Linzbach’s hypothesis is supported by the finding by Gulch and Jacob (13) that stroke volume increases with growing anatomic heart size but ultimately reaches a maximum beyond which it rapidly declines. Thus this marked ventricular dilatation is potentially the primary impetus for the development of overt CHF.

Histological evaluation. With the exception of Z band misalignment, the histological appearance of the myocardium has been reported to be relatively normal in the AV fistula model (14, 30, 33). A report by Hatt et al. (14) found that degenerative changes identified at earlier stages of remodeling had largely resolved after 6 mo of chronic volume overload. Thus our findings are overall in agreement with the existing literature, even as to the normal appearance of the myocardium at the light microscopic level in rats with overt CHF.

Conversely, this is the first study to report significant increases in interstitial collagen associated with CHF secondary to an AV fistula. However, note again that in the majority of these studies examining myocardial collagen, none of the animals were documented to be in overt CHF. Instead, the animals were studied at points postfistula where it would be expected that the hearts were well compensated. Thus our findings are in contrast to the majority of studies examining chronic volume overload secondary to AV fistula, which have either shown no change in myocardial collagen concentration or in the ratio of collagen types (15, 26, 40), or the findings of Ruzicka et al. (34), that the LV collagen concentration was significantly decreased at 4 and 10 wk post-AV fistula. Clearly, the rats with CHF have a significant increase in the amount of myocardial collagen. This suggests the possibility that the marked increase in ventricular compliance is not simply due to collagen degradation.

MMP activity. The rats with symptomatic CHF have a significant increase in myocardial MMP activity, which has not previously been observed in hearts with marked remodeling that have not yet decompensated (26). This in itself is not a dramatic finding, given the several studies that have previously demonstrated that experimentally induced myocardial collagen degradation leads to ventricular enlargement and an increase in ventricular compliance (7, 19, 28). This increase in MMP activity seen in the failing hearts corresponds to a period of time when marked changes in LV dilatation and compliance were seen. Although this does not definitively prove that collagen degradation is responsible for the increases in chamber volume and compliance, it does support that hypothesis. However, these results also offer the first clear experimental evidence that significant ventricular dilatation can occur even when collagen synthesis exceeds degradation. This observation lends credence to the hypothesis that factors other than total collagen concentration, such as decreased collagen interactions with integrin receptors, altered collagen cross-linking, or internal architectural changes in the cardiomyocyte, are responsible for changes in ventricular compliance. Given the increases in myocardial MMP activity, it is likely that changes in the composition and organization of the collagen matrix occur even if the total percentage of collagen is maintained or increased (i.e., decreases in collagen cross-linking and/or alterations in the ratio of collagen type I to type III). New collagen fiber deposition in the heart tends to consist predominantly of the more compliant type III collagen. The greater compliance of this new collagen, together with less extensive collagen cross-linking, could account for much of the progressive increase in ventricular compliance over the course of chronic volume overload in this model. Similarly, a decrease in integrin-mediated cardiomyocyte adhesion to the extracellular matrix could also account for increased ventricular compliance that would not be dependent on changes in interstitial collagen in the myocardium. Finally, the fact that marked LV dilatation occurred without a concomitant increase in LV weight suggests other mechanisms, such as alterations in the arrangement of muscle layers in the ventricular wall or intracellular remodeling in cardiomyocytes, contribute to the pathogenesis of heart failure. This might consist of the sliding displacement (i.e., slippage) of heart muscle cells leading to a decrease in the number of muscle layers in the ventricular wall proposed by Linzbach (22). However, even more likely is the contribution of myocyte lengthening to chamber dilatation. Intracellular reorganization consisting of in series sarcomeric addition has been shown to be a major component of ventricular remodeling secondary to chronic volume overload (23), although in these compensated hearts, Liu et al. found that increased cross-sectional area accounted for more of the increase in myocyte volume (i.e., hypertrophy) than cell lengthening. From our observations, however, we hypothesize that in the decompensated heart there is a progressive reduction in myocyte diameter (i.e., loss of myofilaments) together with further cellular elongation, which could account for the marked ventricular dilatation and wall thinning without a concurrent increase in ventricular weight. This hypothesis is consistent with the histological changes reported herein for the failing hearts. In addition, this intracellular remodeling is also likely to be a major factor contributing to myocardial dysfunction and decreased contractility associated with the development of CHF.

LV systolic function. The slope of the linear $P_{\text{max}}$ vs. $V$ relationship in the isolated canine heart has been shown to be sensitive to pharmacological changes in contractility, independent of preload and afterload, and therefore is considered to be an index of contractility (36). That is, positive and negative inotropic agents result in larger and smaller slope values, respectively. The results herein indicate the slope of the $P_{\text{max}}$ vs. $V$ was decreased for all of the AV fistula groups, suggesting that the intrinsic myocardial contractility...
was significantly depressed relative to control. The relatively greater decrease observed in the $P_{\text{max}}$-V relationship of the compensated 15-wk fistula group seemingly indicates that these rats were closer to developing CHF than the 8- and 21-wk compensated groups, which had $P_{\text{max}}$-V values that were not significantly different from the control group. This interpretation is also supported by the normal body weight of the 21-wk compensated group, indicating an absence of ascites. Accordingly, the 8- and 21-wk compensated rats had not progressed to the point of developing CHF and more than likely could have maintained compensated ventricular function beyond the study end point. The marked decrease in contractility observed in the fistula failure group was significantly greater ($P < 0.01$) than that seen in the compensated group; however, despite decreases in contractility, systolic function (i.e., peak isovolumetric pressure) was maintained, albeit at higher EDVs.

Given that the $P_{\text{max}}$-V relationship was linear over the range of volumes studied and contained regions of overlap between the control and AV fistula groups, it is reasonable to conclude that the reduction in the slope of $P_{\text{max}}$-V in the AV fistula groups was due to impaired contractility and not related to differences in the range of LV volume between groups in our study. Additionally, hearts from the overt failure group did not exhibit a typical Frank-Starling response; rather, these hearts reached a near maximum peak isovolumetric pressure at ventricular volumes near $V_0$, and systolic pressure generated during isovolumetric contraction increased only marginally with additional volume. The absence of a Frank-Starling response in the overt failure group is consistent with the findings of Ross et al. (33) and suggests that these hearts were already functioning at or near the upper range of sarcomeric length.

Several investigators have suggested that sympathetic stimulation may contribute to the increase in cardiac output, at least in part, by increasing myocardial contractility via its direct effect on cardiac muscle. However, Alyono et al. (1) found that while cardiac hemodynamic performance was normal or increased at 1 wk postfistula, intrinsic myocardial contractility was significantly depressed. Nakano et al. (25) also found contractile dysfunction in chronic volume overload associated with an inadequate hypertrophic response in dogs with mitral regurgitation. They postulated that the muscle dysfunction might be the result of an excessive load placed on the existing, insufficient myocardial mass. Our finding that progressively depressed myocardial contractility was associated with the development of heart failure postfistula is consistent with these studies. Furthermore, we have recently made the observation that hearts subjected to chronic volume overload (e.g., 8 wk postfistula and beyond) are not responsive to $\beta$-adrenergic stimulation with dobutamine, suggesting that contractility might be decreased because of $\beta$-adrenergic receptor downregulation or uncoupling (6).

Morbidity and mortality. To date we have accumulated data on 116 rats postfistula, which illustrates the consistent development of overt CHF after an extended period of compensated volume overload (between 4 and 21 wk postfistula) in this model of chronic volume overload. The cumulative CHF-related morbidity and mortality in untreated fistulas at 21 wk postfistula is $\geq 80\%$. Furthermore, the morbidity and mortality study demonstrates that the development of overt CHF is not limited to a specific time interval following the imposition of a sustained volume overload. Although the extent of hypertrophy was highly variable in hearts that failed, the extent of LV dilatation was remarkably similar. Taken as a whole, the current findings suggest that the development of overt heart failure is triggered when the compensatory responses (i.e., hypertrophy, contractility, and Frank-Starling) are exhausted and marked ventricular dilatation is induced.

Rapid development of CHF is relatively common when an AV fistula is created in dogs (1, 30, 33). In contrast, the majority of studies using the rat AV fistula model have not reported the development of CHF symptoms (8, 26, 34), and in the few studies that have reported symptoms or mortality due to heart failure, the incidence of CHF has been relatively low ($\sim 33\%$) (5, 14, 23, 29). This raises the question of why chronic volume overload does not consistently lead to the development of CHF in rats with an AV fistula. There are several factors that are likely to account for this variability. One obvious factor is experimental design that is not targeted to identify symptoms (i.e., lung weights not obtained) or which is of insufficient duration for the transition from compensated ventricular hypertrophy to CHF to occur. A difference in the size of the AV fistula relative to body size produced by the various methods that have been used to create fistulas is another variable. That is, a method that creates a smaller AV fistula (i.e., an incision or 20-gauge needle) would not produce as great a stimulus to remodel as a larger fistula. Therefore, the time required to induce CHF should be longer and conceivably the heart might reach a stable compensated state that does not continue to progressively remodel. This would be analogous to the previous report by Pfeifer et al. (31), in which hearts with a relatively small myocardial infarction did not significantly remodel and failed to develop CHF, whereas larger myocardial infarcts produced progressive ventricular dilatation and the subsequent development of CHF. Conversely, a substantially larger fistula tends to overwhelm the compensatory reserves, accelerating the development of pulmonary edema and producing death at a much earlier time point without expressing the full range of compensatory mechanisms (29).

Additional factors to consider are the potential influence of age and gender on myocardial remodeling. In a study by Chen et al. (8), there is no indication that any of the AV fistula rats developed CHF (i.e., as evidenced by RV weight), although these rats were subjected to $\sim 21$ wk of chronic volume overload and developed LV hypertrophy comparable to that reported herein. However, in this study, the fistula was created in male rats at 4 wk of age. It is possible that because the heart is
still in a rapid growth phase at 4 wk of age, there might be an enhanced hypertrophic response, greater neovascularization, or even the potential for additional cardiomyocyte hyperplasia. Another study by Liu et al. (23) is of interest because it highlights the potential gender difference in the development of CHF. In that study using a similar AV fistula model in female rats, there were no deaths, and most indexes of cardiac function were normal or elevated even though the duration and extent of hypertrophy was similar to our present study in males. This raises the question of whether gender-related differences in myocardial remodeling are responsible for the favorable outcome in females subjected to chronic volume overload. This appears to be the case, because the incidence of CHF in preliminary studies utilizing female rats in our laboratory was only 16% at 20 wk, and the female heart developed a relatively larger hypertrophic response (38% greater increase above control values) and markedly less pulmonary edema (53% less increase in lung weight) than in comparable male rats (3). A similar study on the effects of gender by Tamura et al. (37) using spontaneously hypertensive heart failure (SHHF) rats also demonstrated that females have a greater hypertrophic reserve than males.

These interesting differences in the literature underscore the importance of further characterizing the mechanisms responsible for the development of heart failure in the rat AV fistula model. In addition, the AV fistula has some distinct advantages over the other currently described rat models known to progress to overt heart failure. Foremost among these is that the studies can be completed at a lower cost. For example, the acute postoperative mortality is negligible, with <3% of the rats dying in the first week compared with an initial mortality as high as 50% in the myocardial infarct model. The length of time required for CHF to develop is also significantly shorter than in the SHHF rat strain, and interpretation is not complicated by the superimposed hypertension. Furthermore, this is by no means a new animal model, and there is already an established body of literature describing various aspects of the cardiovascular remodeling, even though there are important questions remaining to be addressed with regard to the pathogenesis of CHF.

Possible mechanisms. Chronic volume overload consistently produces marked hypertrophy and ventricular dilatation regardless of the animal model; however, the specific mechanisms responsible for progressive ventricular dilatation, wall thinning, and spheroidization are still unknown. The traditional hypothesis is that ventricular systolic dysfunction leads to chamber dilatation; however, a more contemporary hypothesis is that development of overt systolic dysfunction is preceded by an adverse increase in chamber volume (9). Several recent investigations have emphasized that cardiac dilatation is a precursor of LV dysfunction and clinical heart failure (11, 32, 39). Our results are in agreement with these studies, clearly demonstrating that extensive myocardial remodeling producing marked ventricular dilatation and increased compliance precedes the development of overt CHF. However, stroke volume is generally increased by ventricular dilatation even if contractility is decreased (13). Therefore, cardiac output would be maintained until compensatory hypertrophic responses and contractility reserves were exhausted, leading to significant increases in EDP and additional ventricular dilatation. A possible contributing factor to the development of CHF could be compromised mitral valve integrity secondary to marked ventricular dilatation, with the resulting regurgitant flow further impairing cardiac output and increasing the degree of volume overload, inducing sudden decompensation and overt failure.

Conventional understanding of ventricular remodeling suggests that for progressive dilatation and hypertrophy to occur, attachments between cardiomyocytes and interstitial collagen must be disrupted. The increase in MMP activity seen in the CHF hearts is consistent with previous findings demonstrating that myocardial collagen degradation is correlated with activation of MMPs, inducing ventricular dilatation in the cardiomyopathic Syrian hamster (10, 17) and the rapid pacing model of heart failure (35). Global degradation of myocardial collagen, characterized by loss of fibrillar collagen at the perimysial and endomysial levels, is followed by ventricular dilatation and an increase in ventricular compliance (18). Interestingly, it is possible that tumor necrosis factor-α, which is known to be elevated in patients with CHF, may induce increased cardiac MMP expression (27). Although these findings suggest collagen degradation as the likely instigator of the ventricular dilatation and increased compliance in this model, another possible mechanism that could produce dilatation is cytokine-induced alterations in the myocardial extracellular matrix. A recent study by Bozkurt et al. (4) demonstrated that tumor necrosis factor-α infusion can produce reversible ventricular dilatation. However, at this time, the mechanisms responsible for the marked ventricular dilatation and wall thinning characteristic of heart failure remain unknown.

In summary, chronic volume overload secondary to AV fistula induces a progressive LV dilatation and myocardial hypertrophy and ultimately results in the development of CHF. Whereas the literature frequently makes reference to heart failure inducing remodeling of the myocardium, it is more accurate to attribute the initial tissue remodeling to compensatory mechanisms attempting to normalize physiological parameters. CHF develops when the remodeling becomes maladaptive, having reached a point where hypertrophy is maximal, contractile reserves are depleted, and additional ventricular dilatation can no longer sustain cardiac output. Thus we would conclude that the inability to further hypertrophy, together with LV dilatation and increased compliance, are critical factors responsible for the development of overt CHF in this model.

We thank James Stewart and Phil Svor for technical assistance and Lisa Henegar for histological preparation.
REFERENCES


