SUMMARY Cardiac pumping ability was assessed during the natural development of left ventricular hypertrophy by elevating venous pressure by infusing Tyrode's solution intravenously to produce peak cardiac output. This experiment was performed on spontaneously hypertensive rats (SHR) of three age groups (11, 24, and 83 weeks). From 11 to 24 weeks, peak cardiac output of SHR increased in direct proportion to the abnormally increased ventricular mass; thus peak cardiac output per gram of left ventricle (LV) remained stable. Similar results were obtained for two strains of normotensive rats at each of the same three age groups. Thus, in the normotensive animal peak cardiac output per gram of LV remained stable over a wide range of ages and varying left ventricular weights. However, with progressive elevation of arterial pressure in aging SHR (83 weeks), we observed severe ventricular hypertrophy (100% increases in left ventricular to body weight ratio). In this oldest SHR group, unlike age-matched normotensive rats, there was a marked reduction in the pumping ability per gram of LV. Thus, during the natural development of left ventricular hypertrophy SHR demonstrated both a stable stage of hypertrophy in which the increased left ventricular mass maintained its pumping ability, and a later stage of deterioration in which there was a loss of the normal relationship between ventricular mass and pumping ability.

STUDIES ON the mechanical performance of models of pressure-induced ventricular hypertrophy have produced conflicting results. Thus, contractile function in experimental models of both right and left ventricular isometric hyperfunction has been reported as supranormal,1-2 normal,3 and depressed.4-6 These experiments have used the rat,5-6 dog,2,4 and cat.4-6 The most common means for producing pressure-induced ventricular hypertrophy has been by the surgical production of outflow tract obstruction or banding, a method which rapidly increases ventricular mass while often depressing contractile performance. Meerson7 described three stages in the development of ventricular hypertrophy. In the first stage, before myocardial mass increases, contractile function is impaired. Subsequently, during the second stage myocardial mass increases to maintain normal myocardial function; however, in Meerson's third stage there is a progressive deterioration of myocardial function, and eventually cardiac failure is believed to supervene. In contrast, the development of ventricular hypertrophy has been characterized by others as a progressive downhill course leading to overt failure.4 In support of this latter observation, many experimental models of pressure-induced ventricular hypertrophy failed to demonstrate Meerson's second stage of stable ventricular hyperfunction.

Williams and Potter1 recently have reported that the contractile function of the hypertrophied right ventricle (from cats in which the pulmonary artery was banded) was reduced at six weeks but returned to normal levels after 24 weeks of banding. They suggested that the often reported progressive deterioration in performance of the hypertrophying myocardium may be related to the method used to produce hypertrophy and not be a function of the increased muscle mass per se.

The spontaneously hypertensive rat (SHR) provides a model for longitudinal studies during the spontaneous development of isometric hyperfunction and subsequent left ventricular hypertrophy. In these rats left ventricular mass increases gradually in response to the progressive hypertensive disease process and not abruptly as a consequence of a surgical procedure. Thus, the occurrence and nature of left ventricular hypertrophy in SHR may be more analogous to the most common form of left ventricular hypertrophy in man, that associated with systemic hypertension.

Therefore, we assessed cardiac pumping ability in three age groups of SHR and two strains of age-matched normotensive rats by rapidly increasing venous pressure until cardiac output reached a plateau despite further elevations in venous pressure. The peak cardiac output produced thereby is a highly reproducible index of myocardial pumping ability8 and thus provides valuable information to aid in determining whether the chronic and progressive development of left ventricular hypertrophy in the SHR is characterized by a progressive deterioration of cardiac pump function or whether the increased mass provides an adaptation to the sustained hyperfunctional state and thereby permits a period of stable function.

Methods

Experiments were performed on three strains of Wistar rats. The SHR was that originally developed by Okamoto and Aoki10,11 and has been maintained at the University of Oklahoma Health Sciences Center since 1969 by brother-to-sister inbreeding. The Wistar-Kyoto (WKY) strain, the
immediate progenitor strain from which the SHR was derived, was obtained from the National Institutes of Health in 1973; they also have been maintained by brother-to-sister inbreeding. A second control group of commercially obtained normotensive Wistar rats (NR) (West Jersey Biological Supply, Wenonah, N.J.) also was used for comparison with the Japanese strains. All rats were provided standard rat chow (Purina) and water ad libitum, were housed in polyethylene cages (14 1/2 x 12 1/2 by 6 1/2 inches; a maximum of four rats per cage), and were maintained on a 12-hour light-dark cycle.

We studied three age groups (11 ± 0.5, 24 ± 1.0, and 83 ± 3.7 weeks) of each of the three strains. These age groups were selected to provide a wide age range which includes a period of rapid body and ventricular growth (11-24 weeks) and a period of more gradual growth (24-83 weeks). In each of the age groups studies were performed on 24 male rats (eight from each strain).

Each rat was anesthetized with ether and hemodynamic measurements were made using a preparation previously described. In brief, the left carotid artery and right jugular vein were cannulated and the catheters were advanced centrally (the venous catheter being positioned in the right atrium) and connected to Statham pressure transducers (P23Db and P23Bd, respectively). A femoral vein was cannulated for intravenous infusions. The trachea was directly intubated with PE 240 tubing and, after stabilization, control prethoractomy pressures and heart rates were recorded on a multichannel polygraph (Hewlett-Packard, model 7784A). The rat then was ventilated artificially and maintained under anesthesia by an ether drip apparatus (Phipps and Bird) arranged in series with the respirator (Harvard Apparatus, model 680). The chest was entered by cauterizing the sternoclavicular junction and the first four to five sternocostal articulations. The ascending aorta was isolated by passing the blunt end of a previously threaded curved needle under the vessel; this facilitated the placement of an electromagnetic flow probe [with inside diameter (i.d.) of either 1.5, 2.0, or 25.0 mm] around the vessel. Two sets of probes and electromagnetic flow meters were used in this study (Micron Instruments, model RC1000, and Statham Instruments, model SP2202). No difference in total aortic blood flow was observed with either instrument. Flow probes were calibrated by several previously described methods using whole blood of comparable hematocrit.

With this preparation, cardiac output was defined as total ascending aortic blood flow and, as such, excluded coronary blood flow. Cardiac index was obtained by dividing cardiac output by body weight, and is expressed as flow (milliliters per minute per kilogram). Stroke work was calculated from the product of the difference between the mean arterial and right atrial pressures, the stroke volume, and 0.0136 (the factor to convert from mm Hg to meters of water). Left ventricular minute work was obtained by multiplying stroke work by heart rate. Body temperature was maintained within ±1°C of the original value by an electric heating pad.

After a stabilization period of 10 minutes Tyrode’s solution (composition, in g/liter: 0.10 MgCl2·6 H2O; 8.00 NaCl; 0.20 KCl; 1.00 NaHCO3; 1.00 glucose; 0.05 NaH2PO4·H2O; 0.20 CaCl2) was infused for 1 minute into a femoral vein at the rate of 40 ml/min per kg. This infusion rate produced a rapid increase and then a plateau in cardiac output within 60 seconds (usually between 30 and 45 seconds).

During the infusion the point at which cardiac output failed to increase further despite continued elevation in right atrial pressure was defined as the peak cardiac output (Figure 1). Under these experimental conditions this peak cardiac output for each rat served as the prime index of the pumping ability of the left ventricle. The peak stroke and minute work usually did not occur at the same time as peak cardiac output because the increases in venous pressure reduced the difference between mean arterial and venous pressures. To obtain these values, measurements of cardiac output, heart rate, and arterial and venous pressures were made for each increment of 0.5 mm Hg in venous pressure. Peak values of external work were then selected from these calculations whenever they occurred during the infusion period.

At the conclusion of the hemodynamic study the heart was excised, the atria and great vessels were removed, and the right ventricular free wall was carefully dissected from the left. Measurements of left ventricular weight thus include intraventricular septum.

The “unpaired” Student’s t-test was used for statistical comparisons of data for the three strains of rats. Analysis of variance was used to determine the statistical significance of differences within a given strain over the three age groups examined.

Results

All three strains of rats demonstrated increased body and left ventricular weights with age (Table 1). The hemodynamic characteristics of these nine groups of rats are similar to those reported previously (Table 2). Because of the wide range in age, and thus in weight (138-635 g), of the rats, left ventricular mass was expressed with respect to body weight. In mature (24-83 weeks) normotensive rats this ratio of left ventricular to body weight remained constant. In contrast, the ratio progressively increased in the SHR as left ventricular mass increased out of proportion to body growth. The changes in weights from 24 to 83 weeks serve to underscore the intensive hypertrophic process in the left ventricle of SHR. During this time the increase in body weight of all strains was comparable (NR = 55, WKY = 52, and SHR = 46 g), although there was a marked dissimilarity in left ventricular growth (NR = 136, WKY = 79, and SHR = 473 mg).

For the 11-week-old normotensive group the left ventricular to body weight ratios were slightly greater than those for the more mature groups of normotensive rats. This follows the normal pattern of development of rats and most mammals, a pattern in which the immature animal has a greater heart weight with respect to body size. However, this normal pattern of growth was not observed in the SHR. In these rats both the left ventricular to body weight ratio and the arterial pressure demonstrated severe and progressive increases with age. In the normotensive groups arterial pressure remained stable (Table 2). The central venous pressures of all nine groups of rats were comparable and
there was no evidence of overt cardiac failure in any of the animals studied (Table 2).

The intravenous infusion of Tyrode's solution produced a prompt rise in cardiac output which rapidly obtained plateau (peak) values (Fig. 1). Associated with the increase in central venous pressure there was a reduction in arterial pressure and heart rate (Table 2). These variables started to return toward control levels with the immediate postinfusion reduction in venous pressure. Within 15 minutes after stopping the infusion the venous pressure and other hemodynamic variables returned to their respective preinfusion levels. Determinations of hematocrit in seven animals demonstrated a reduction of less than 4 vol% within 5 minutes after infusion.

Two strains of normotensive Wistar rats were used for comparison with SHR to provide more evidence as to whether interstrain differences are related to the hypertrophic process. Although the two normotensive strains had different physical and hemodynamic characteristics (Table 1), we observed there were no differences between these strains in peak performance (cardiac output, stroke volume, stroke work, and minute work) per gram of left ventricle. Thus, over a wide range of age and weight two strains of normotensive Wistar rats demonstrated very similar values of peak cardiac performance per gram of left ventricle. Both strains are progenitors of the SHR but neither, by itself, provides an adequate control for the SHR.

Although comparisons between SHR, WKY, and NR can be made for each of the three age groups, the present study was designed primarily to permit evaluations of cardiac function longitudinally during the natural aging and hypertrophic growth processes. Thus, the emphasis of this report is placed on intrastrain comparisons of performance from 11 to 24 weeks, and 24 to 83 weeks.

In each of the three strains of rats the rapid body and ventricular growth which occurred between 11 and 24 weeks was accompanied by an increased ability of the heart to pump blood. This increased pumping performance was directly related to the increase in ventricular mass, since the peak flow per gram of left ventricle remained unaltered (Figs. 2 and 3). Similarly, external work increased in proportion to total ventricular growth; therefore, peak minute work per gram of left ventricle also remained constant (Fig. 4). Thus, with normal ventricular growth in NR and WKY rats [ratio of left ventricular to body weight (LV/B wt) at 24 weeks = 1.8 ± 0.06 and 1.9 ± 0.04,

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**Table 1** Weight and Prethoracotomy Pressure Values of Normotensive and Spontaneously Hypertensive Wistar Rats

<table>
<thead>
<tr>
<th>Age group</th>
<th>NR</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 weeks</td>
<td>213 ± 5°</td>
<td>265 ± 20</td>
<td>242 ± 21</td>
</tr>
<tr>
<td></td>
<td>0.465 ± 0.013</td>
<td>0.558 ± 0.050</td>
<td>0.655 ± 0.57°</td>
</tr>
<tr>
<td></td>
<td>2.18 ± 0.04</td>
<td>2.09 ± 0.06</td>
<td>2.70 ± 0.06°</td>
</tr>
<tr>
<td></td>
<td>122 ± 7</td>
<td>106 ± 6</td>
<td>147 ± 5°</td>
</tr>
<tr>
<td>24 weeks</td>
<td>490 ± 24</td>
<td>375 ± 5°</td>
<td>352 ± 12°</td>
</tr>
<tr>
<td></td>
<td>0.877 ± 0.56°</td>
<td>0.708 ± 0.29</td>
<td>0.987 ± 0.41°</td>
</tr>
<tr>
<td></td>
<td>1.78 ± 0.06</td>
<td>1.88 ± 0.04</td>
<td>2.80 ± 0.05°</td>
</tr>
<tr>
<td></td>
<td>132 ± 6°</td>
<td>111 ± 2</td>
<td>159 ± 6°</td>
</tr>
<tr>
<td>83 weeks</td>
<td>545 ± 35</td>
<td>427 ± 16°</td>
<td>398 ± 9°</td>
</tr>
<tr>
<td></td>
<td>1.013 ± 0.062°</td>
<td>0.787 ± 0.024</td>
<td>1.400 ± 0.073°</td>
</tr>
<tr>
<td></td>
<td>1.89 ± 0.06</td>
<td>1.85 ± 0.05</td>
<td>3.67 ± 0.17°</td>
</tr>
<tr>
<td></td>
<td>130 ± 11</td>
<td>113 ± 7</td>
<td>176 ± 8°</td>
</tr>
</tbody>
</table>

NR = commercially obtained normotensive rats; WKY = normotensive Wistar-Kyoto strain; SHR = spontaneously hypertensive rats.

Results are expressed as mean ± 1 SEM.

° Differs from age-matched NR, *P < 0.05.

°° Differs from age-matched WKY, *P < 0.05.

°°° Differs from both age-matched NR and WKY, *P < 0.01.
In their study, the slopes of the curve relating ventricular performance. In addition, thoracotomy and positive pressure ventilation are known to shift the curve of ventricular performance. In these studies on conscious dogs, these investigators demonstrated that the plateau of ventricular output (peak cardiac output) is extremely reproducible, varying by only 2% from one study to another. Other aspects of the ventricular function curves were not as reproducible. Thus, although the plateau levels of flow were the same, the peak flow did not always occur at the same level of right or left atrial pressure. Therefore, in their study, the slopes of the curve relating filling pressure to output were variable within the same dog. Because of this variability it is essential to obtain peak levels of ventricular performance. In addition, thoracotomy and positive pressure ventilation are known to shift the curve relating cardiac output to right atrial pressure; however, once again, the plateau levels of flow remain unaltered. 

One apparent problem in comparing peak cardiac output of intact normotensive and hypertensive animals is the difference in aortic pressure between these groups. It often is assumed that aortic pressure is an important determinant of cardiac output. However, Herndon and Sagawa have quantified the effects of alterations in mean aortic pressure on aortic flow and found that peak aortic flow remained constant as mean arterial pressure was raised from 50 to 180 mm Hg. To this end, in a previous study we reported that peak aortic flow remained constant as mean arterial pressure was raised from 50 to 180 mm Hg. To this end, in a previous study we reported that peak aortic flow remained constant as mean arterial pressure was raised from 50 to 180 mm Hg. To this end, in a previous study we reported that peak aortic flow remained constant as mean arterial pressure was raised from 50 to 180 mm Hg.
arterial pressure was acutely raised from 110 to 190 mm Hg. In our present study all rats examined had arterial pressures well within the range in which differences in pressure alone should have negligible effects on aortic blood flow. In addition, the most important comparisons in the present study were within strains of rats at different ages (i.e., hypertensives of three different ages).

In the normotensive rats, associated with body and ventricular growth there was a proportionate increase in pumping ability when peak cardiac output was measured during rapid volume loading and used as a prime index of cardiac pumping ability. Most important, even the oldest group of normotensive rats maintained a stable relationship between ventricular mass and cardiac pumping ability. Lee et al.21 have demonstrated that even 24-month-old Wistar rats respond to preload stress as well as do their respective 6- and 12-month-old counterparts.

Normal ventricular growth is, in essence, a hypertrophic process, since the number of myocardial cells seems to remain stable throughout life.22 The hypertrophic process in the left ventricle of SHR is pathological, since the ventricular mass increases out of proportion to body size11 and is associated with a reduction in myocardial deoxyribonucleic acid concentration23 and an increased muscle fiber diameter.44 Although ventricular hypertrophy is, in actuality, a physiological adaptation to permit sustained increases in cardiac performance, the functional ability of this pathologically induced hypertrophied myocardium is unresolved at present. Frequently, the models of experimentally induced hypertrophy caused by pressure-load fail to demonstrate a stable stage of ventricular function in which the increased myocardial mass compensates for the imposed hyperfunctional state. However, in the naturally developing left ventricular hypertrophy of the SHR, a stage of stable function was demonstrated; this is so, presumably, because of the gradual but progressive nature of the disease. Thus, from 11 to 24 weeks, proportionate increments in maximum pumping performance were associated with large increases in ventricular weight.

Biochemical determinations of cardiac energy generation and utilization potential for this age group of SHR support the physiological observations of the increased mass sustaining more external work. Thus, Aoki et al.26 have shown that myofibrillar ATPase activity per weight of protein was normal in 15- to 25-week-old SHR. In addition, Farmer et al.24 reported normal myocardial cytochrome oxidase activity and mitochondrial protein concentration in SHR of comparable age. Thus, total myofibrillar ATPase activity and mitochondrial protein levels are greater in the SHR by virtue of the increased myocardial mass and not as a result of alterations in tissue concentrations. Therefore, we were able to demonstrate for the middle-aged SHR (6 months) Meerson’s second stage of adaptive hyperfunction in which

![Graph](image-url)
the increased myocardial mass was able to maintain normal ventricular function despite the increased workload imposed upon the heart by the progressively increasing afterload.

With unaltered progression of the hypertension, the oldest group of SHR demonstrated an increase of approximately 100% in the left ventricular to body weight ratio; this was not observed in either strain of normotensive rats. Hypertrophy of this magnitude, though reported in clinical forms of ventricular hypertrophy, in general has not been observed in experimental models of hypertrophy caused by increased afterload. Hence, in our oldest group of SHR with massive hypertrophy there was a gross reduction in pumping ability per gram of left ventricle which was not observed in age-matched normotensive rats. Therefore, our SHR group demonstrated Meerson's third, and final, stage of ventricular hypertrophy in which there is a progressive deterioration of myocardial function leading to eventual deterioration of cardiac function. By virtue of this natural progression of increasing afterload, we were able to demonstrate in our oldest group of SHR that the stable phase of adaptive hyperfunction was overcome by a further increase in left ventricular afterload.

It is possible, therefore, that many of the surgically induced models of cardiac hypertrophy caused by increased afterload did not demonstrate the stable stage of ventricular function because the initial stress was so severe that it provided too rapid a response to overcome the adaptive process of ventricular hypertrophy. Ventricular hypertrophy in SHR, in contrast, was a much slower cardiac response to a naturally occurring stimulus for ventricular hypertrophy. Thus, although surgical banding of the outflow tract produces an abrupt hyperfunctional state and triggers an adaptive increase in ventricular mass, this situation does not seem to be analogous to the increased workload associated with nonmalignant forms of systemic hypertension. The SHR, therefore, provides a model for study of the pathogenesis of hypertensive heart disease; and these rats, in our present study, demonstrated both the stable (Meerson, stage II) and deteriorating (Meerson, stage III) stages of ventricular performance during the natural development of left ventricular hypertrophy.

Acknowledgments

We gratefully acknowledge the excellent assistance rendered by Bruce Ferrell and Grace Lawson in the performance of these experiments and the analysis of the data. The excellent secretarial assistance of Elizabeth Murray is also greatly appreciated.

References

10. Okamoto K, Aoki K: Development of a strain of spontaneously
Regional Myocardial Blood Flow during Acute Myocardial Infarction in the Conscious Dog

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SUMMARY Regional myocardial blood flow was studied in the conscious dog at periods of 5 minutes to 4 days after occlusion of a major branch of the left coronary artery. Dogs were instrumented with aortic electromagnetic flow probes, occlusive cuffs on either the anterior descending or circumflex branch of the left coronary artery, and a left atrial Silastic catheter for injection of microspheres (15 ± 5 μm) labeled with either 85Sr, 141Ce, or 51Cr. Microspheres were injected into 25 fully conscious dogs during three of the following time periods: control preocclusion and 0.1, 2, 6, 24, or 96 hours postocclusion. In the conscious dog, before occlusion, the endocardial half of the left ventricular myocardium averaged 63 ml/100 g per min during the control period, was reduced to 12–18 ml/100 g per min at 0.1–6 hours in the ischemic region, and least reduced in the marginal region of the infarct. Blood flow was most severely reduced in the subendocardial center of the ischemic region, less so in the epicardial ischemic region, and least reduced in the marginal region of the infarct. Blood flow was increased to the nonischemic tissue. There was no change in this pattern of reduced blood flow by 6 hours postocclusion, but by 24 hours, flow was modestly increased to all areas except the central subendocardial core, and was further increased at 96 hours. Blood flow to the endocardial half of the left ventricular myocardium averaged 63 ml/100 g per min during the control period, was reduced to 12–18 ml/100 g per min at 0.1–6 hours in the ischemic region, increased to 29 ml/100 g per min at 24 hours, and to 48 ml/100 g per min by 96 hours. These findings indicate that there is a reversal of the flow ratio within ischemic myocardium with relative underperfusion of the endocardial half of the wall, which is not corrected by 4 days. There is a modest increase of collateral blood flow to ischemic tissue by 24 hours and this increase is considerably augmented by 96 hours, apparently as a result of the growth and enlargement of collateral vessels.

THE ABILITY of existing coronary collateral vessels to deliver blood to myocardial tissue acutely deprived of its primary source is a key factor in determining survival of the tissue. It has long been recognized that hearts with a well developed collateral circulation are better protected from the effects of major coronary artery occlusion than those with poor collateral circulation. Numerous studies of naturally occurring ischemic heart disease in man and acute experiments on anesthetized dogs have demonstrated a subendocardial localization of the most severe ischemic necrosis. Few studies have attempted to quantify the development of changes in regional distribution of collateral blood flow to the myocardium after sudden occlusion of a coronary artery in the conscious animal. Previous studies have used methods which directly measured blood flow in the unoccluded artery or a collateral branch, or depended upon indirect means such as measurement of peripheral coronary pressure, isotope washout, retrograde flow, or anatomical evidence to estimate collateral blood flow.

Previous studies from our laboratory demonstrated anatomical enlargement of coronary collateral circulation by 4 days postocclusion along with an increase in peripheral coronary pressure and retrograde flow which suggested increased collateral flow to the ischemic tissue. The develop-