Pressure-Induced Cardiac Enlargement in Neonatal and Adult Rats

Left Ventricular Functional Characteristics and Evidence of Cardiac Muscle Cell Proliferation in the Neonate

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SUMMARY Pressure-induced cardiac enlargement was created in neonatal (21 days of age) and adult (250-275 g) rats by abdominal aortic constriction. Sham-operated and aorta-constricted neonates were studied 2, 3, 4, and 5 weeks after surgery. Left ventricular weight was elevated by approximately 50% at all times studied. Radioautography demonstrated a marked elevation in \(^{3}H\)-thymidine-labeled nuclei in cardiac muscle and nonmuscle cells in aorta-constricted neonates. Other experiments examined the functional characteristics of hearts subjected to pressure overload while in either the neonatal or the adult state. Five weeks after surgery, left ventricular pressure and left ventricular weight were elevated to nearly identical degrees in both groups of experimental rats. Under control conditions, heart rate and the maximum rate of left ventricular pressure development were not altered significantly in either neonatal or adult rats with aortic constriction. Aortic peak flow velocity, cardiac index, and stroke index also were within normal limits in neonates with aortic constriction; however, these measurements were reduced significantly in adults with aortic constriction. Stroke volume augmentation in response to saline infusion and inotropic responsiveness to isoproterenol were unaltered in aorta-constricted neonates but were markedly attenuated in aorta-constricted adults. The maintenance of normal heart functional characteristics represents a major point of distinction between pressure-induced cardiac enlargement in neonatal and adult rats which correlates with the presence or absence of cardiac muscle cell proliferation during adaptive heart growth.

WHEN THE WORK requirements of the heart are increased by a variety of experimental methods, including pressure or volume overload, a relatively rapid increase in heart mass occurs.\(^{1,2}\) Although cardiac enlargement is an end result common to each experimental method, there has been controversy as to whether cardiac enlargement results from growth of pre-existing cells (hypertrophy), increased cell numbers due to mitotic division (hyperplasia), or a combination of both processes. It has been suggested\(^{3,4}\) that the animal's age at the time an elevated workload is imposed on the heart may be one major factor that determines the heart's cellular response to overload, i.e., hypertrophy or hyperplasia.

In the rat heart, spontaneous mitotic activity rapidly declines during the first few weeks of neonatal life, and, presumably, muscle cells become fully differentiated approximately 16-30 days after birth.\(^{6,7}\) Thus, an elevated workload imposed on the hearts of rats well beyond 30 days of age would be expected to elicit an increased myocardial mass due primarily to larger muscle cells without an increase in muscle cell number. The expected results have been clearly demonstrated in mature rats following aortic constriction\(^{8-12}\) and nutritional anemia.\(^{3,4}\) Cellular proliferation does occur in the enlarged adult rat heart; however, radioautographic analysis of the labeled nuclei indicates interstitial cell proliferation with essentially no DNA synthesis within cardiac muscle cells.\(^{5,11-12}\) As yet no stimulus has been identified that will produce cardiac muscle cell proliferation in the adult heart. Conversely, when nutritional anemia is created in neonatal rats (21 days of age) to induce a volume overload, cardiac muscle cells, as well as interstitial cells, are stimulated to undergo active mitotic division.\(^{4}\)

It was not clear from the above experiments whether any stimulus which increases the work requirements of the neonatal heart will elicit muscle cell proliferation or whether this response is limited to nutritional anemia (volume overload). To answer this question, our first series of experiments characterized pressure-induced cardiac enlargement in neonatal rats. Biochemical and \(^{3}H\)-thymidine radioautographic results indicated that cardiac muscle cells as well as nonmuscle cells were stimulated to undergo mitotic division.

The significance of a mechanism that produces cardiac enlargement in the adult heart without benefit of additional cardiac muscle cells may be reflected ultimately in the functional characteristics of the enlarged hearts. A depressed contractile state has been reported for papillary muscles from pressure-overloaded cat\(^{13}\) and rat\(^{14}\) hearts. Clinical observations\(^{15-17}\) on patients with hypertrophied but nonfailing left ventricles have associated depressed ventricular function with the hypertrophic condition. The functional characteristics of the enlarged heart may be
related to the heart's cellular responses to overload, i.e., hypertrophy or hyperplasia. Therefore, additional experiments were conducted to examine the functional characteristics of hearts subjected to pressure overload while in either the neonatal or the adult state.

Methods

Experiments on Cardiac Enlargement in Neonatal Rats

Surgical Procedures

Pressure-induced cardiac enlargement was effected in neonatal rats by abdominal aortic constriction. Male Sprague-Dawley rats, 21 days of age, were selected at random from groups of rats born on the same day. No attempt was made to match littersmates in the control and experimental groups. Under ether anesthesia, the abdominal aorta was isolated surgically above the renal vessels, and the ligature-needle technique described by Morkin and Ashford was used for aortic constriction. A blunt 22-gauge needle produced comparable degrees of abdominal aortic constriction, pressure overload, and left ventricular enlargement in adult rats. Control neonatal and adult rats were subjected to sham operations. No attempt was made to match littermates in the control and experimental groups. In chronic overload experiments, all sham-operated and aorta-constricted rats were studied 5 weeks after surgery.

Time Course Experiments

Aorta-constricted and sham-operated rats were studied 2, 3, 4, and 5 weeks following surgery. Rats were weighed, anesthetized with pentobarbital (50 mg/kg, ip), and placed on positive-pressure ventilation with room air, using a small-animal respirator. A midline thoracotomy was performed to expose the heart. Left ventricular peak systolic pressure was measured by puncturing the ventricle with a 20-gauge needle attached directly to a Statham P37 miniature pressure transducer. After pressure measurements were completed, the heart was excised and placed in crushed ice. Right and left atria were dissected, pooled into a single sample, and weighed. Extraneous tissue and great vessels then were dissected from the base of the heart. The remaining portion of the heart was separated into right ventricular free wall and left ventricular (including septum) samples and weighed. For chemical analyses, the entire left ventricle was homogenized in ice-cold distilled water by using a ground-glass homogenizer. Samples were taken for protein, DNA, and hydroxyproline assay.

Tritiated Thymidine Radioautography

The time course experiments described above established that heart weight was increasing rapidly 1–2 weeks after aortic constriction. The cell population(s) proliferating during this period of cardiac enlargement were identified by injecting [methyl-3H]thymidine (1 μCi/g body weight; 44 Ci/mM), intraperitoneally, at this time. The injection schedule described by Neffgen and Korecky was followed, and standard histological radioautographic techniques were used to determine the relative numbers of labeled cardiac muscle and nonmuscle cell nuclei present in the left ventricular sections. Sections were examined at 10×-100× magnification, and at least 2500 nuclei were counted in each left ventricle. Fields showing major blood vessels or portions of blood vessels were disregarded. An arcsin transformation was performed prior to statistical evaluation of the radioautographic results.

Experiments on Left Ventricular Function

Experimental Models of Pressure-Induced Cardiac Enlargement

Experiments on neonatal animals were initiated with male Sprague-Dawley rats, 21 days of age. Male rats initially weighing 250–275 g (6–10 weeks of age) were used for experiments on adult animals. The abdominal aorta was constricted in neonatal rats with a blunt 22-gauge needle, as described above. A blunt 20-gauge needle produced comparable degrees of abdominal aortic constriction, pressure overload, and left ventricular enlargement in adult rats. Control neonatal and adult rats were subjected to sham operations. No attempt was made to match littersmates in the control and experimental groups. In chronic overload experiments, all sham-operated and aorta-constricted rats were studied 5 weeks after surgery.

Acute Aortic Constriction Experiments

On the basis of preliminary experiments, we anticipated that decrements in cardiac functional characteristics would be detected in the enlarged adult heart. To establish that this effect is a function of cardiac enlargement in the adult heart rather than aortic constriction per se, acute aortic constriction experiments were conducted. Rats having body weights equivalent to those of adult rats 5 weeks after surgery were anesthetized, and acute aortic constriction was created, as described above. Subsequently, myocardial function was evaluated within 15–20 minutes after the creation of pressure overload.

Evaluation of Myocardial Function

Functional measurements were made in situ, using methods previously described in detail. Rats were weighed, anesthetized with pentobarbital, and placed on positive-pressure ventilation with room air via a tracheotomy. A cannula was placed in the jugular vein for volume infusion or drug administration. The heart and ascending aorta were exposed by midline sternotomy, and an electromagnetic flow probe (2-mm internal diameter) was placed around the aorta. Intraventricular pressures were measured by puncturing the left ventricle with a 1-inch, 20-gauge needle attached directly to a Statham P37 miniature pressure transducer. Left ventricular end-diastolic pressure was measured by high amplification of the left ventricular pressure signal. Heart rate was determined continuously with a cardiograph triggered by the left ventricular pressure pulse. The maximum rate of left ventricular pressure development (dP/dt max) was derived by using an analog differentiator. This measurement was used as an index of myocardial contractile function. All hemodynamic measurements were recorded simultaneously on a Beckman type R dynograph.

After initial (control) hemodynamic measurements were completed, cardiac output was augmented by infusing isotonic saline via the jugular vein cannula at 6 ml/min. When the mean aortic flow reached its highest steady state level (approximately 1 minute), infusion was
discontinued. A period of 10 minutes was allowed for the animal to recover, and new control hemodynamic measurements were made. Rats in which hemodynamic values had returned to within 10% of initial values then were infused with isoproterenol (0.05 μg/min) at 0.3 ml/min for approximately 3 minutes. Peak heart rate and dP/dt max responses were determined subsequently.

To evaluate left ventricular pump performance during rapid volume infusion, ventricular function curves were drawn. Stroke index (stroke volume/kg) was calculated from the continuous record of cardiac output and heart rate. Stroke index then was related to left ventricular end-diastolic pressure to obtain function curves.

**Left Ventricular Weight**

After functional measurements had been completed, the heart was excised. Atria, extraneous tissue, and great vessels were dissected from the base of the heart. The remaining portion of the heart was separated into right ventricular and left ventricular (including septum) samples. Left ventricles then were weighed to verify the establishment of cardiac enlargement, but chemical analyses were not performed.

**Statistical Methods**

Experimental results were compared to appropriate control values by Student's t-test. P < 0.05 was considered statistically significant.

**Results**

**Experiments on Cardiac Enlargement in Neonatal Rats**

**Left Ventricular Pressure**

Measurements of left ventricular peak systolic pressure in sham-operated and aorta-constricted rats are shown in Table 1. Two weeks after surgery, left ventricular pressure was significantly elevated in aorta-constricted rats. These results demonstrate that a substantial pressure overload of approximately 40 mm Hg was in effect at that time.

<table>
<thead>
<tr>
<th>Weeks post surgery</th>
<th>n</th>
<th>Left ventricular pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>10</td>
<td>78 ± 4</td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
<td>114 ± 5*</td>
</tr>
<tr>
<td>3 Weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>9</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
<td>138 ± 5*</td>
</tr>
<tr>
<td>4 Weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>13</td>
<td>111 ± 3</td>
</tr>
<tr>
<td>AC</td>
<td>12</td>
<td>135 ± 7*</td>
</tr>
<tr>
<td>5 Weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>22</td>
<td>124 ± 3</td>
</tr>
<tr>
<td>AC</td>
<td>21</td>
<td>165 ± 5*</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = number of rats.

Pressure measurements made 3, 4, and 5 weeks after surgery indicate that a similar degree of pressure overload was maintained throughout the experimental period. It should be noted that marked differences are apparent when left ventricular pressure values obtained from sham-operated rats 2 weeks after surgery are compared with those obtained 5 weeks after surgery. A similar left ventricular pressure response was observed in aorta-constricted rats. The lower left ventricular peak systolic pressures in the younger rats may be attributed to barbiturate anesthesia and/or may represent a normal developmental response. Nevertheless, a substantial left ventricular pressure overload was present in all aorta-constricted rats used for these experiments.

**Body Weight-Heart Weight**

The experimental procedures used in the present studies did not disrupt the growth rate of neonatal rats, as shown in Figure 1. Therefore, the efficacy of abdominal aortic constriction in producing cardiac enlargement could be assessed without resorting to procedures to normalize body weight. As shown in Figure 2, left ventricular weight was significantly elevated after 2 weeks of pressure overload. Left ventricular weight continued to increase in a fashion that appeared to be linear throughout the experimental period in both sham-operated and aorta-constricted rats. An elevation of approximately 50% in left ventricular mass resulted from aortic constriction at all times studied. Abdominal aortic constriction had no significant effect on right ventricular weight; however, pooled atrial weight was significantly elevated at all times studied (data not shown).

**Protein, DNA, and Hydroxyproline Concentrations**

The results of biochemical analysis of left ventricles of sham-operated and aorta-constricted rats are shown in Table 2. A uniform protein concentration was observed in sham-operated rats, and there were no significant differences in left ventricles of aorta-constricted rats. In sham-operated rats, a time-dependent DNA concentration was observed, with a peak at 5 weeks post-surgery. In aorta-constricted rats, a similar pattern was observed, but the peak was slightly lower.

**TABLE 1 Left Ventricular Peak Systolic Pressure Measurements in Sham-Operated and Aorta-Constricted (AC) Neonatal Rats**

![Graph showing body weights of sham-operated and aorta-constricted neonatal rats. See Table 1 for number of rats at each point; AC = aorta-constricted. Body weights of sham-operated and aorta-constricted rats are not significantly different.](http://circres.ahajournals.org/content/40/2/305)
tion response was apparent. DNA concentration was significantly lower at the 5-week point, compared with other values, however, no significant deviation from this pattern was noted in aorta-constricted rats. Although hydroxyproline concentration was slightly elevated in aorta-constricted rats at all times studied, these increases achieved statistically significant levels only at 4 weeks after surgery.

In a separate group of sham-operated and aorta-constricted rats, left ventricular tissue samples were weighed and dried to constant weight in an oven at 100°C to determine tissue water content. Two weeks after surgery, the water content in the left ventricles of sham-operated rats \((n = 5)\) was 75 ± 1% (mean ± se) and was not significantly different in any of the other groups.

**Left Ventricular Total DNA**

Left ventricular DNA content was calculated as the product of left ventricular wet weight and left ventricular DNA concentration. In sham-operated rats, a significant increase in total DNA content was observed between 2 and 3 weeks after surgery (Fig. 3). At that point, total DNA content in the left ventricle of sham-operated rats remained constant. If it is assumed that the DNA content per cell nucleus remains constant in the rat heart, then the significant increase in total DNA content observed in sham-operated rats indicates proliferation of some cellular population(s) until approximately 6 weeks after birth. The apparent cellular proliferation was not stimulated by sham operation, because similar total DNA results were obtained from control rats (data not shown). Aortic constriction resulted in a significant elevation in total DNA content 2 and 3 weeks after surgery (Fig. 3). Therefore, pressure overload stimulated additional mitotic activity over and above that observed in sham-operated rats. In addition, the continued increase in total DNA content in aorta-constricted rats throughout the experimental period indicates that mitotic cell division was sustained for the duration of the pressure overload stimulus.

**Incorporation of Tritiated Thymidine**

The incorporation of \(^{3}H\)-thymidine into cardiac muscle and nonmuscle cell nuclei was studied in six sham-operated and six aorta-constricted rats. In sham-operated rats, the labeled nuclei index (labeled nuclei/1000) was 23 ± 4 and 9 ± 2 in nonmuscle and muscle cells, respectively. The labeled nuclei indices in aorta-constricted rats were increased significantly in both nonmuscle (104 ± 23) and muscle (73 ± 15) cells, compared with those of sham-operated rats. Therefore, it appears that cardiac enlargement due to pressure overload in neonatal rats is accompanied by mitotic division of muscle as well as nonmuscle cell populations.

**Left Ventricular Function Experiments**

**Body Weight-Left Ventricular Weight**

Abdominal aortic constriction did not disrupt the growth rate of either neonatal or adult rats, as shown by

![Figure 2](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.42.3.306/-/DC1/figure2.png)

**Figure 2** Left ventricular weights of sham-operated and aorta-constricted neonatal rats. See Table 1 for number of rats at each point. LV = Left ventricle; AC = aorta-constricted. *P < 0.05 compared to sham.

![Figure 3](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.42.3.306/-/DC1/figure3.png)

**Figure 3** Total left ventricular DNA content of sham-operated and aorta-constricted neonatal rats. See Table 1 for number of rats at each point. LV = Left ventricle; AC = aorta-constricted. *P < 0.05 compared to sham.

**Table 2 Biochemical Results from Left Ventricles of Sham-Operated and Aorta-Constricted (AC) Neonatal Rats**

<table>
<thead>
<tr>
<th>Weeks postsurgery</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>165 ± 4</td>
<td>3.52 ± 0.16</td>
<td>0.74 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
<td>155 ± 4</td>
<td>3.17 ± 0.12</td>
<td>0.78 ± 0.03</td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>168 ± 7</td>
<td>3.60 ± 0.14</td>
<td>0.86 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
<td>163 ± 4</td>
<td>3.63 ± 0.20</td>
<td>0.92 ± 0.03</td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>153 ± 5</td>
<td>3.33 ± 0.13</td>
<td>0.83 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>12</td>
<td>162 ± 9</td>
<td>3.16 ± 0.10</td>
<td>0.93 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are mean ± SE; \(n\) = number of rats; OH-P = hydroxyproline. *P < 0.05 compared to sham.
the data on body weight presented in Table 3. In neonatal rats, left ventricular weight was elevated significantly by approximately 45% as the result of aortic constriction (Table 3). Nearly identical left ventricular weight increases were observed in aorta-constricted adult rats.

Control Functional Measurements

The initial hemodynamic measurements obtained in sham-operated and aorta-constricted rats are shown in Table 4. In neonatal rats, an increase of approximately 40 mm Hg in left ventricular peak systolic pressure was established by abdominal aortic constriction, and a nearly identical degree of pressure overload was achieved in aorta-constricted adult rats. In contrast to left ventricular pressure, heart rate and dP/dt max were not altered significantly in either neonatal or adult aorta-constricted rats. Aortic peak flow velocity, cardiac index, and stroke index also were within normal limits in aorta-constricted neonatal rats; however, these flow-related measurements all were reduced significantly in aorta-constricted adults compared with those of sham-operated adults. Marked differences in cardiac index and stroke index were apparent between sham-operated neonatal and adult rats (Table 4). As the result of reduced stroke volume, cardiac output was approximately 15% lower in adult rats prior to normalization for individual differences in body weight. Disproportionate body weight increases in older rats contributed to an equal degree toward the 30-40% reductions in cardiac index and stroke index that were observed. Nevertheless, it can be concluded that left ventricular pump function is compromised under control conditions due to pressure-induced cardiac enlargement in adult rats.

Heart Pump and Contractile Functional Reserve Capacity

Although initial hemodynamic measurements were unaltered in aorta-constricted neonatal rats, the possibility existed that this normal functional state had been achieved at the expense of a reduced myocardial pump and/or contractile functional reserve capacity. The responses to rapid volume infusion and catecholamine challenge were used to evaluate this possibility.

As shown in Figure 4, both sham-operated and aorta-constricted neonatal rats demonstrated rapid and significant augmentation of stroke volume in response to saline infusion. No marked alterations were apparent in either the initial or steady state portions of the ventricular function curves. In contrast, augmentation of stroke volume was markedly attenuated in hearts of aorta-constricted adult rats (Fig. 5). This effect is a function of cardiac enlargement in adult rats rather than aortic constriction per se, because normal ventricular function curves were observed in adult rats that had undergone acute aortic constriction without sufficient time to develop cardiac enlargement (Fig. 5).

The chronotropic and inotropic responses to isoproterenol infusion are shown in Table 5. When challenged by catecholamine, significant and nearly identical increases in dP/dt max were achieved in both sham-operated and aorta-constricted neonatal rats. In a similar manner, heart rate was increased to nearly identical levels by isoproterenol infusion in both groups of rats. Adult sham-operated rats demonstrated a greater responsiveness of dP/dt max to an isoproterenol challenge which was identical to that of neonatal rats. However, aorta-constricted adult rats did not demonstrate a similar inotropic responsiveness, as evidenced by a significantly reduced index of contractility during catecholamine challenge. Directionally similar heart rate effects were noted, but these responses did not achieve statistical significance.

Discussion

Pressure- and volume-induced cardiac enlargement has been investigated extensively on a biochemical basis. Studies dealing with responses of the protein, nuclei acid, and connective tissue have been of particular interest, because this information provides some insight into the cellular mechanisms responsible for adaptive heart growth. It generally is accepted that compensatory cardiac enlargement in the adult heart is achieved by growth of pre-existing muscle cells (hypertrophy) and nonmuscle cell mitotic division (hyperplasia). This conclusion has been reached, in part, on the basis of quantitative determination of DNA in enlarged hearts. The total DNA content in enlarged left ventricles has been shown to increase significantly; however, the amount of DNA synthesized is not proportional to the overall increase in tissue mass when a sustained enlarging stimulus is encountered. Therefore, a "dilution" of nuclear material occurs, and this dilution is reflected in a reduced DNA concentration (mg/g) within the enlarged ventricle. A similar but spontaneous dilution effect was observed in the present studies at the time the left ventricular total DNA content assumed a constant value in sham-operated rats. In the enlarged adult heart, substantial amounts of connective tissue are synthesized, and this results in marked elevations in both total hydroxyproline content and concentration.

A direct comparison of mechanisms for cardiac enlargement in neonatal and adult hearts has been made by using nutritional anemia to create a heart volume overload. These studies demonstrated that a volume overload in neonatal rats results in significant cardiac enlargement accompanied by mitotic division of both muscle and nonmuscle cell populations. Cell division was limited to nonmuscle cells in adult rats. A major conclusion from these studies was that "the response of the cardiac cell is dependent upon the age at which cardiomegaly is produced." The present studies provide evidence that sup-

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Body Weight and Left Ventricular Weight in Sham-Operated and Aorta-Constricted (AC) Rats Used in Functional Experiments</th>
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</thead>
<tbody>
<tr>
<td>Group</td>
<td>n</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>Neonates</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>10</td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>11</td>
</tr>
<tr>
<td>AC</td>
<td>10</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = number of rats; LV = left ventricular.

* P < 0.05 compared to sham.
ports this hypothesis. A substantial pressure overload was created by abdominal aortic constriction in neonatal rats. Sequential left ventricular pressure measurements indicated that the stress of pressure overload was in effect within the time period during which cardiac muscle cell proliferation has been shown to occur with dietary anemia. Significant left ventricular enlargement was established by pressure overload, and marked elevations in total DNA content indicated mitotic cell division within the enlarged ventricle. In contrast to chronic pressure-induced cardiac enlargement in adult hearts, DNA accumulated in proportion to the increased left ventricular mass in pressure-overloaded neonatal rat hearts, and no consistent, significant elevation in hydroxyproline concentration could be demonstrated. These biochemical results are similar to those of previous studies on volume overload in which muscle cell division has been documented by radioautography. The biochemical responses in aorta-constricted neonatal rats are qualitatively different from those of previous studies on volume overload, and suggest that muscle proliferation has been stimulated in the neonatal rat heart.

From the biochemical results obtained in aorta-constricted neonatal rats, it is clear that proliferation of some cellular population(s) accompanies cardiac enlargement. Qualitatively, the biochemical results are consistent with the possibility that muscle cell proliferation has been stimulated; however, the proliferating cell population(s) required confirmation by other methods. Radioautographic results indicate that a greater percentage of non-muscle cell nuclei incorporated 3H-thymidine than did muscle cell nuclei in the sham-operated rat left ventricle. Therefore, the significant increase in left ventricular total DNA content observed in sham-operated rats between 2 and 3 weeks after surgery appears to be largely attributable to nonmuscle cell proliferation. It also appears that all heart cellular populations ceased DNA synthesis approximately 6 weeks after birth, because total DNA content of the left ventricle of sham-operated rats remained constant at 3, 4, and 5 weeks after surgery. Radioautographic data of Neffgen and Korecky and Klinge and Stocker showed that 3H-thymidine incorporation reaches a minimum at 32–34 days and 4 months of age, respectively. A more recent study indicates that 3H-thymidine incorporation into DNA extracted from rat heart essentially ceases 17 days after birth, and the enzymatic activity of DNA

![Figure 4](image-url)  
**Figure 4.** Composite left ventricular function curves from sham-operated and aorta-constricted neonatal rats. AC = Aorta-constricted; LVEDP = left ventricular end-diastolic pressure. Composite curves represent seven sham-operated and six aorta-constricted rats.

![Figure 5](image-url)  
**Figure 5.** Composite left ventricular function curves from sham-operated and aorta-constricted adult rats. AC = Aorta-constricted; LVEDP = left ventricular end-diastolic pressure. Composite curves represent seven sham-operated and six aorta-constricted rats. Three acute aorta-constricted rats were studied, as described in Methods.
polymerase declines dramatically over the same time period. Despite the lack of consensus regarding the stage of postnatal development in the rat at which heart cellular proliferation is arrested, the present studies clearly demonstrate increased 3H-thymidine labeling in both muscle and nonmuscle cell nuclei during the early phases of the left ventricular enlargement that results from abdominal aortic constriction in 21-day-old rats. Enhanced synthesis of "metabolic" DNA28 and/or the development of polyplid nuclei could contribute to the increases in total DNA and labeled cell nuclei. However, previous studies4-10 have considered these factors to be of little significance in experimentally induced cardiac enlargement. We believe the increased 3H-thymidine incorporation indicates proliferation of both muscle and nonmuscle cell populations.

Despite numerous investigations, the functional characteristics of the heart which has enlarged due to overload stress remain controversial. Inherent in this controversy is the possibility that divergent functional responses accompany cardiac enlargement consequent to volume or pressure overload. Nevertheless, no consensus is currently available when considering only pressure-induced cardiac enlargement. Normal21-23 depressed,13-14 and enhanced cardiac heart function has been reported, but many different experimental animal species, forms, and durations of pressure overload and methods of functional evaluation are represented. Failure to recognize that several discrete biochemical phases occur during the myocardial response to pressure overload25 and/or that time-dependent hemodynamic fluctuations24 take place following aortic constriction undoubtedly contributes to the lack of consensus. In addition, an age-related factor determines the proliferative capacity of the cardiac muscle cell. Volume overload4 and pressure overload (this paper) stimulate cardiac muscle cell mitotic activity in neonatal rats, whereas muscle cell proliferation does not occur in the enlarged adult rat heart.4,10-12,26 Because the present studies sought to examine the functional characteristics of hearts that had enlarged either with or without cardiac muscle cell proliferation, care was exercised to ensure comparable experimental conditions. Abdominal aortic constriction was used as a common stimulus for cardiac enlargement in neonatal and adult rats. Nearly identical degrees of pressure overload and cardiac enlargement were achieved after 5 weeks of aortic constriction. This time point should correspond to the "stable" biochemical phase of cardiac enlargement described by Meerson.29 Identical procedures were used to compare heart function in the neonatal and adult groups. Therefore, the observed alterations in heart function probably reflect differences in mechanisms for cardiac enlargement rather than extraneous experimental conditions.

In the present studies, left ventricular function was evaluated in the intact heart in situ. Flow-related measurements (cardiac index and stroke index) and measurements related to left ventricular ejection (dP/dt max and peak aortic flow velocity) served as estimates of heart pump and muscle contractile function, respectively. In addition, the pump and contractile functional reserve capacities of the left ventricle were determined.

From the data on dP/dt max it was concluded that cardiac muscle contractile function was not significantly altered under control conditions in either neonatal or adult aorta-constricted rats. However, we recognize that dP/dt max may be influenced by myocardial loading conditions,36-37 and the use of this measurement alone as an index of contractile function in pressure-overloaded hearts must be viewed with caution. Peak aortic flow velocity also has been proposed as an index of heart contractile function.36-38 This contractile function index and dP/dt max were elevated slightly in aorta-constricted neonatal rats. Because both stroke index and cardiac index were maintained within normal limits in aorta-constricted neonatal rats, it appears that myocardial contractile function was adequate to sustain normal pump performance under control conditions, despite a substantial elevation in afterload. Furthermore, the unaltered ability to augment both myocardial pump and contractile functions indicates that normal control heart function was not achieved at the expense of a reduction in functional reserve capacity. In contrast to neonatal rats, peak aortic flow velocity was significantly reduced in aorta-constricted adult rats. Concomitant reductions in cardiac index and stroke index demonstrate a decrement in heart pump performance which may be mediated by inadequate left ventricular contractile responses even though dP/dt max was essentially unchanged. Reduced responsiveness of dP/dt max to isoproterenol in aorta-constricted adult rats provides additional evidence for such a conclusion. Possible differences in sensitivity to adrenergic agonist, as previously reported for enlarged hearts of spontaneously hypertensive rats,40-41 cannot be ruled out. Nevertheless, compromised inotropic responsiveness to any given level of catecholamine challenge would represent a decrement in contractile functional reserve capacity. The observed reductions in pump functional reserve capacity also suggest inadequate left ventricular contractile responsiveness in the enlarged adult heart.

The contractile properties of the enlarged heart have been investigated by using isolated papillary muscle preparations for which loading conditions can be carefully controlled. Depressed contractile function has been reported for papillary muscles from pressure overloaded, adult cat13 and rat14 hearts. These results lend support to our present conclusions, which are based upon functional evaluation of the intact heart.

The presence or absence of cardiac muscle cell prolifer-
vation represents a significant qualitative difference between cardiac enlargement mechanisms in the neonatal and adult heart. Maintenance of normal heart functional characteristics also represents a major point of distinction between pressure-induced cardiac enlargement in neonatal and adult rats. Therefore, the presence or absence of cardiac muscle cell proliferation during adaptive heart growth seems to exert profound influences on myocardial function. The divergent left ventricular functional characteristics observed following pressure-induced cardiac enlargement in neonatal and adult rats illustrate this influence; however, the associated cellular and subcellular mechanisms and the relationship of these responses to the functional characteristics of the heart remain to be determined.

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