Morphologic Development of the Pulmonary Vascular Bed in Experimental Coarctation of the Aorta

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SUMMARY Although electrocardiographic evidence of right ventricular hypertrophy is considered common in newborn infants with coarctation of the aorta, the reason for this finding is not well established. Investigations of the pulmonary vascular bed of these infants have resulted in variable findings, probably due to the differences in morphometric techniques, coexisting cardiac defects, and variable postnatal age at time of death. To study more carefully the pulmonary vascular bed, we produced coarctation of the aorta in fetal lambs at 103–126 days gestation. Twelve to 32 days later the fetuses were reoperated on and systemic and pulmonary arterial blood pressures, and arterial blood gas tensions were determined to be normal. At autopsy, juxta ductal coarctations extended a mean of 2.8 mm into the aortic lumen and occupied 9.5 mm of the aortic circumference. The fifth-generation pulmonary resistance vessels had increased medial width (p < 0.01), decreased external diameter (p < 0.001), and increased medial width/external diameter ratios (p < 0.001) compared with vessels from control fetuses. The number of small muscular pulmonary vessels/cm² lung tissue was significantly reduced (p < 0.01) in the study animals compared with the control animals. These alterations of the pulmonary vascular bed were not due to fetal pulmonary arterial hypertension or fetal hypoxemia. These pulmonary vascular changes may explain the occurrence of pulmonary hypertension and right ventricular hypertrophy in newborn infants with coarctation of the aorta.

ELECTROCARDIOGRAPHIC EVIDENCE of right ventricular hypertrophy is considered a typical finding in newborn infants with coarctation of the aorta; but the reasons for this finding have not been well established. Since many of these infants also have pulmonary arterial hypertension in the newborn period, it has been suggested that intrauterine pulmonary hypertension might exist secondary to left-sided obstruction caused by the coarctation. Intrauterine pulmonary hypertension could result in increased pulmonary arterial smooth muscle and right ventricular work resulting in the newborn infant in pulmonary hypertension and electrocardiographic evidence of right ventricular hypertrophy. Morphologic analysis of the pulmonary vasculature in infants with coarctation of the aorta has been performed by several investigators, with various results. Naeye found an increase in pulmonary arterial smooth muscle, although Haworth and Reid found the normal amount of muscle and normal number of pulmonary vessels. The differing results of these investigators may be the result of differences in techniques, coexisting cardiac lesions, and variable postnatal age at the time of death.

We have examined the morphologic development of the pulmonary vasculature in fetal lambs with isolated, experimentally created coarctation of the aorta.

Material and Methods

The Surgical Preparation

Six pregnant ewes, 103–126 days gestation, were given spinal anesthesia with 2–3 ml of 1% tetracaine HCl. Intraoperative anesthesia was supplemented with 1 mg/kg ketamine and 0.01 mg/kg atropine i.v. as needed. The uterus was exposed through a midline abdominal incision and a hysterotomy was performed. The left fore limb of the fetus was exteriorized and, with 1% xylocaine local anesthesia, a left posterior-lateral thoracotomy performed through the fourth intercostal space. The junction of the ductus arteriosus and aortic isthmus as they form the descending aorta was identified (fig. 1A). Previously described techniques for creating coarctation of the aorta were modified as follows. A length of 0.054 inch o.d. polyvinyl chloride tubing was placed around the posterior-lateral aspect of the aorta in the juxta ductal position and retracted in a medial-anterior direction (fig. 1B). Using two to four 6–0 prolene horizontal mattress sutures the posterior-lateral wall of the aorta was sutured together incorporating the polyvinyl chloride tubing into an invagination in the wall (fig. 1C). Care was taken to place the sutures in the aortic wall and avoid penetrating the lumen. The anterior-medial ends of the tubing were loosely opposed and a single suture was placed through the ends to prevent the tubing from slipping out of place. The last suture was carefully placed to prevent the tubing from becoming constrictive throughout the entire circumference of the aorta. The fetal thorax and hysterotomy were closed and the ewe was allowed to recover.

Twelve to 32 days postoperatively, the ewes were again given spinal anesthesia and the uterus was exposed. Through small hysterotomies and using local anesthesia, 0.054 inch o.d. by 0.034 inch i.d. polyvinyl chloride catheters were inserted into the fetal femoral...
and carotid arteries. Through a left lateral fetal thoracotomy, a catheter was also placed through a pursestring suture directly into the fetal pulmonary trunk just distal to the pulmonary valve. The incisions were closed, a catheter was placed into the amniotic cavity, and all catheters were brought out through the left flank of the ewe and filled with heparin.

Each day postoperatively, blood pressure recordings were made using a Beckman Dynograph recorder and Statham P23Db pressure transducers. Intra-amniotic pressure was used as zero reference. Carotid or femoral arterial blood gas tensions and pH determinations were performed using standard Radiometer electrodes.

**Preparation of Tissues**

**The Lungs**

Shortly after the fetuses died, the heart and lungs were dissected free and prepared by methods previously described. Briefly, the pulmonary artery was perfused with 3% glutaraldehyde at the pulmonary arterial mean blood pressure measured in vivo. A pressure of 10 cm H₂O was used in the airways. An India ink-Micropaque-gelatin mixture was then infused into the pulmonary arterial tree, and using a dissecting microscope, small tissue blocks were prepared. Sets of 150 7-µ serial sections were cut and stained with hematoxylin and Van Geison's solution. Using the light microscope, fifth-generation resistance vessels were identified, photographed and measured. Mean medial width, mean external diameter, and mean medial width/external diameter ratio were determined for 288 vessels from the six fetuses. These were compared by t test for independent means to 529 fifth-generation resistance vessels from six normal lamb fetuses previously studied. All the small muscular arteries in 25 randomly selected sections from four of the fetal lungs of the study animals were counted. The sections were traced and planimetered and the number of vessels per area (cm²) of lung tissue was determined. Because one of these animals was 125 days and three were 137 days gestation at the time of death, the number of vessels/cm² lung tissue was compared by t test for independent means to vessels from the lungs of four normal fetuses which were 120, 122, 135 and 140 days gestation.

**The Hearts**

The hearts of the six study animals were fixed in 3% glutaraldehyde and dissected in the following manner:

![Diagram of fetal sheep's heart and arteries](image)

**Figure 1.** A) Diagramatic representation of normal anatomy of the great arteries in the fetal sheep. The ductus arteriosus is a direct extension of the pulmonary trunk. The left and right pulmonary arteries arise from a short main pulmonary artery which comes off the pulmonary trunk. There is one brachiocephalic vessel in the sheep. B) The 0.054 inch o.d. polyvinyl chloride (PVC) tubing was placed around the posterior-lateral aspect of the aorta at the junction of the ductus arteriosus and the aortic isthmus. The tubing was retracted in the medial-anterior direction, causing the posterior-lateral wall of the aorta to invaginate. C) The tubing was sutured into the aortic wall invagination and the medial ends were loosely approximated and sutured.
The right ventricular free wall, the interventricular septum, and the left ventricular free wall were dissected free from the atria and weighed and the values compared to those from nine normal fetuses by unpaired $t$ test.

## Results

### The Coarctation

There was a mild-to-moderate postcoarctation dilatation of the aorta present in all cases. Upon opening
the aorta a juxtaductal coarctation was apparent in the fixed specimens (fig. 2). In the six fetuses, the mean width of the ridge was 2.6 mm, the mean height 2.8 mm, and the mean length 9.5 mm (fig. 3).

**Physiology**

Four of the animals survived the reoperation long enough to obtain in utero steady-state blood pressure recordings, and the carotid, femoral and pulmonary arterial blood pressures were within normal range. The pulmonary arterial blood pressure was slightly greater than the femoral arterial blood pressure which was normal\(^7\) (fig. 4). The carotid arterial blood pressure was also slightly greater than the femoral arterial blood pressure. The mean systemic arterial blood oxygen tensions were 22.4 ± 5.6 mm Hg, carbon dioxide tensions 34.1 ± 5.7 mm Hg and pH was 7.42 ± 0.01 (n = 28) — all within the normal range.\(^18\)

**Pulmonary Vessels**

The mean medial width was increased and the mean external diameter decreased in the vessels from study animals compared with those from the control animals (table 1). This resulted in a highly significant increase in the mean medial width/external diameter ratio in the study group compared with the control group (table 2). The number of smaller muscular vessels per unit of tissue was decreased in the lungs from the study animals compared with those from the control animals (\(p < 0.01\)) (table 1).

**The Heart**

The right ventricular free wall weight significantly increased (table 3). However, the left ventricular free wall weight was also increased, so the right ventricular/left ventricular free wall ratio was unchanged.

**Discussion**

The surgical technique we used results in a pathological specimen which appears similar to isolated juxtaductal coarctation of the aorta in the human infant.\(^1, 2, 19, 20\) It does, therefore, not only provide a good model for the study of the anatomical effects of coarctation of the aorta but also allows us to derive reasonable conclusions about the in utero hemodynamics of infants with this lesion. Although blood flow studies were not performed, the blood pressure determinations indicated that there was only a mild pressure difference across the area of the coarctation from both the pulmonary trunk and the ascending aorta compared to the descending aorta. The pulmonary arterial blood pressure was within the normal range.

In spite of these findings, morphologic alterations of the pulmonary vascular bed exist. There is both an increase in pulmonary arterial medial wall smooth muscle and a decrease in the external diameter of the vessels which have been found in other experimental circumstances with pulmonary hypertensions.\(^6, 21\) In addition, there was a decrease in the number of small muscular vessels/cm\(^2\) lung tissue. An alteration in the quantity of pulmonary arterial smooth muscle has been described previously in both experimental animals\(^6, 21, 22\) and human infants\(^6, 8, 12, 23-20\) with arterial hypertension or left-sided obstruction. Although fetal pulmonary arterial hypertension is one explanation for an increase in smooth muscle development, alterations have occurred with normal pulmonary arterial blood pressure.\(^22\) If the ductus arteriosus is widely patent in the fetus,\(^26\) blood pressure can be equal in both the pulmonary and systemic circuits. Both an altered kinetic energy of the pulmonary arterial blood flow and reversal in the direction of flow through the ductus arteriosus resulting in a change in pulmonary arterial blood oxygen tension have been postulated as possible reasons for the alteration of the development of fetal pulmonary arterial smooth muscle.\(^1, 14, 22\) It is possible that the normal ejection characteristics of the right ventricle
TABLE 1. *Fifth-Generation Resistance Vessels in Experimental Coarctation of the Aorta*

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Medial width (µ)</th>
<th>External diameter (µ)</th>
<th>Medial width/external diameter</th>
<th>Number of vessels/cm² lung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85 days</td>
<td>70</td>
<td>6.09 ± 2.25</td>
<td>40.58 ± 0.97</td>
<td>0.16 ± 0.06</td>
<td>25</td>
</tr>
<tr>
<td>105 days</td>
<td>100</td>
<td>6.99 ± 4.24</td>
<td>40.80 ± 10.20</td>
<td>0.17 ± 0.04</td>
<td>25</td>
</tr>
<tr>
<td>120 days</td>
<td>124</td>
<td>5.69 ± 1.07</td>
<td>36.39 ± 7.90</td>
<td>0.16 ± 0.04</td>
<td>25</td>
</tr>
<tr>
<td>122 days</td>
<td>93</td>
<td>6.42 ± 1.71</td>
<td>44.02 ± 10.45</td>
<td>0.15 ± 0.04</td>
<td>25</td>
</tr>
<tr>
<td>135 days</td>
<td>32</td>
<td>7.12 ± 2.07</td>
<td>42.74 ± 14.37</td>
<td>0.17 ± 0.06</td>
<td>25</td>
</tr>
<tr>
<td>140 days</td>
<td>101</td>
<td>5.92 ± 1.29</td>
<td>34.19 ± 7.28</td>
<td>0.18 ± 0.04</td>
<td>25</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 days</td>
<td>22</td>
<td>10.58 ± 2.13</td>
<td>32.45 ± 5.63</td>
<td>0.33 ± 0.05</td>
<td>25</td>
</tr>
<tr>
<td>137 days</td>
<td>23</td>
<td>6.78 ± 1.57</td>
<td>30.15 ± 5.21</td>
<td>0.23 ± 0.05</td>
<td>25</td>
</tr>
<tr>
<td>137 days</td>
<td>25</td>
<td>8.79 ± 3.20</td>
<td>33.25 ± 8.71</td>
<td>0.26 ± 0.06</td>
<td>25</td>
</tr>
<tr>
<td>137 days</td>
<td>32</td>
<td>9.42 ± 2.88</td>
<td>32.33 ± 7.18</td>
<td>0.29 ± 0.07</td>
<td>25</td>
</tr>
<tr>
<td>138 days</td>
<td>12</td>
<td>8.23 ± 2.18</td>
<td>32.32 ± 6.38</td>
<td>0.26 ± 0.07</td>
<td>25</td>
</tr>
<tr>
<td>144 days</td>
<td>23</td>
<td>7.02 ± 1.87</td>
<td>31.58 ± 5.48</td>
<td>0.23 ± 0.07</td>
<td>25</td>
</tr>
</tbody>
</table>

Values are mean ± sd.

Abbreviation: COA = coarctation of the aorta.

TABLE 2. *Fifth-Generation Pulmonary Resistance Vessels in Experimental Coarctation of the Aorta: Comparison of Groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Medial width (µ)</th>
<th>External diameter (µ)</th>
<th>Medial width/external diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>6.19 ± 1.70</td>
<td>39.10 ± 10.15</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>Experimental</td>
<td>6</td>
<td>8.47 ± 2.18</td>
<td>32.10 ± 6.38</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± sd.

May be altered by the juxtaductal coarctation and there is an increased distribution of kinetic force in the pulmonary vessels due to more direct force of ejection into the pulmonary artery. These mechanisms may be operative in this experimental circumstance. In utero pulmonary hypertension is not the cause of increased muscle found in these experiments.

The cause for the decrease in the number of small muscular pulmonary vessels is not well understood. Alteration of the number of vessels has been found in experimental pulmonic stenosis in fetal lambs, hypoxia in newborn rats, and several human conditions, including congenital left-sided diaphragmatic hernia, exposure to prostaglandin synthetase inhibitors in utero, and aortic stenosis and atresia.

Exposure to various stimuli in utero may alter the normal proliferation of small muscular pulmonary arteries, although what these stimuli are remain to be delineated.

The alterations in the fetal pulmonary vasculature in these animals explain the pulmonary arterial hypertension and right ventricular hypertrophy commonly seen in newborn infants with coarctation of the aorta.

TABLE 3. *Fetal Heart Weights: Experimental Coarctation of the Aorta*

<table>
<thead>
<tr>
<th></th>
<th>Right ventricular free wall (g)</th>
<th>Interventricular septum (g)</th>
<th>Left ventricular free wall (g)</th>
<th>RV/LV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>4.54 ± 1.22</td>
<td>5.06 ± 1.02</td>
<td>4.63 ± 1.10</td>
</tr>
<tr>
<td>Coarctation of the aorta</td>
<td>6</td>
<td>6.38 ± 0.84</td>
<td>6.14 ± 1.10</td>
<td>6.27 ± 1.65</td>
</tr>
</tbody>
</table>

Values are mean ± sd.

Abbreviations: RV/LV = ratio of right ventricular to left ventricular free wall.
Acknowledgment

The authors appreciate the assistance of Drs. Ronald M. Perkin and David E. Fixler, and Mrs. Jean Pitzer in the preparation of this material.

References

Morphologic development of the pulmonary vascular bed in experimental coarctation of the aorta.
D L Levin, L J Mills and M Parkey

Circulation. 1979;60:349-354
doi: 10.1161/01.CIR.60.2.349
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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