Morphological Development of the Pulmonary Vascular Bed in Experimental Pulmonic Stenosis

Daniel L. Levin, M.D., Michael A. Heymann, M.D., and Abraham M. Rudolph, M.D.

SUMMARY The main pulmonary trunk was banded in four fetal sheep at 63–69 days of gestation. The fetuses were killed after they had developed progressive pulmonary stenosis at 98, 123, 134 and 135 days of gestation. The right lung of each animal was perfused with glutaraldehyde and serial sections followed microscopically. The medial width/external diameter ratios for fifth generation resistance vessels were significantly less (0.13) than those from six normal control lungs (0.16, p < 0.001). In addition, the number of resistance vessels per cm² lung tissue in the lungs of the animals with experimental pulmonic stenosis was less than in normal controls.

The altered in utero hemodynamics with severe pulmonic stenosis results in thin-walled pulmonary arterial vessels. This may be caused by an increased blood oxygen tension of the blood perfusing the pulmonary circulation via reversed flow through the ductus arteriosus, or altered pulmonary arterial pressure characteristics in the pulmonary vessels distal to the obstructed pulmonary trunk.

THE EFFECTS OF CONGENITAL cardiac lesions on pulmonary vascular development in utero are unknown. Histologic studies of lungs of infants with severe pulmonary stenosis or atresia are conflicting; some show the pulmonary arterioles to be normal,1,2 and others show them to be thin-walled.3 Also, it is not known whether the pulmonary vascular alterations in patients with severe pulmonic stenosis or pulmonary atresia are secondary to the valvar lesion or a separate, coincident anomaly.4

In this study we reexamined these issues in a controlled experimental situation rather than using routine human autopsy material. This method of histologic analysis avoids a variety of previous problems with fixation, vessel selection and quantitation.5

Materials and Methods

Animal Preparation

Four pregnant ewes at 63–69 days of gestation (term 145–150 days) were given spinal anesthesia with 1% tetracaine hydrochloride and sedated with intravenous sodium pentobarbital intraoperatively. Through an anterior midline incision, the uterus was exposed, a hysterotomy performed and the left forelimb of the fetus exteriorized, exposing the left axilla and hemithorax. A fetal thoracotomy was performed in the third or fourth left intercostal space and the pulmonary trunk was identified. A slightly constricting 2-0 braided silk suture was tied around the pulmonary trunk just above the pulmonic valve, the thoracotomy was closed, the fetus returned to the uterus and the ewe allowed to recover.

At 98, 123, 134 and 135 days of gestation the ewes were again given spinal anesthesia, the fetuses exteriorized and prevented from ventilating by a fluid-filled bag placed over their heads. As part of a study on the hemodynamic effects of right ventricular outflow obstruction, catheters were inserted into a femoral artery and vein and carotid artery. A catheter inserted into the jugular vein was advanced until its tip was in the right ventricle. When possible, this catheter was also advanced briefly across the area of constriction. The fetus was killed and the thorax rapidly opened to expose the right lung.

Preparation of the Lungs

The right lung of each animal was prepared as previously described.6 Briefly, the right pulmonary artery and right main stem bronchus were cannulated and perfused with 0.9% sodium chloride and glutaraldehyde fixative. The pressure used in the arterial system was that measured in vivo. A pressure of 10 cm H₂O was used in the airway. After fixation the arterial system was gently filled with an India ink-gelatin-Micropaque mixture and the lungs cut in serial blocks perpendicular to the open right main stem bronchus. These were dehydrated with alcohol and cleared with xylene and placed under the dissecting microscope.

We defined the main pulmonary artery as the first generation, the right pulmonary artery as the second generation, and successively smaller generations consecutively until six, after which nonmuscular vessels were found. Under the dissecting microscope, smaller blocks were prepared and embedded in paraffin. From these, 500 7-µ serial sections were cut and stained with iron hematoxylin and Van Geison solution.

Measurements

The first large vessel on the first section of each serially prepared set of slides was third generation.

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The vessels were serially followed and fifth generation vessels (designated as resistance vessels from our previous studies) were photographed and measured using a calibrated reticle. Only vessels which could be clearly followed in serial sections were included. Photographic slides were projected and the external diameter measured between the outer margins of the media, and the media measured between the outer margin of the intima and the outer margin of the media. All measurements were repeated at 90° from the first measurements and the separate figures added and averaged.

Under 100 X magnification, all the fifth and sixth generation vessels of any shape in 25 randomly selected sections from each set of slides from each of four fetuses and the six normal controls were counted. The area of the sections was planimetered and the number of vessels per cm² lung tissue calculated.

The medial width, external diameter, and the medial width/external diameter ratio of the vessels from the four experimental fetal animals were compared by t test for independent means to those of six normal fetuses. Linear regression equations for the number of vessels per cm² lung tissue were determined for the six normal control fetuses and the four experimental fetuses and compared using the t test for the difference between the slopes.

Results

All four animals demonstrated a significant degree of pulmonary stenosis. This was indicated by a higher pressure in the right ventricle than in the aorta or pulmonary artery distal to the band, hypertrophy of the right ventricle, and marked anatomic constriction of the main pulmonary trunk (fig. 1).

A total of 161 fifth generation resistance vessels were analyzed from the four experimental animals (table 1). The medial width/external diameter ratios were significantly less than the controls (p < 0.001, table 2). This was primarily due to thin-walled medial muscular coats, since the external diameters were not significantly different (p = 0.167). The mean medial wall thickness in each animal was significantly less than in the controls (fig. 2).

The total number of resistance vessels (fifth and sixth generation vessels) per cm² of lung tissue was less in the experimental animals compared with the normal controls. This difference tended toward significance (p < 0.1).

Discussion

The results of this study indicate that experimental supravalvar pulmonic stenosis affects the in utero development of the pulmonary vascular bed. In these fetuses pulmonary arterial resistance vessels have thinner walls than those of normal animals. The decreased medial muscular layer could be due to a change in the composition of the blood perfusing the pulmonary vascular bed in the fetus. If right ventricular output was reduced as a result of increased afterload, the flow of blood through the ductus arteriosus could be reversed, providing the pulmonary circulation with blood which has a higher oxygen tension than normal. Since the fetal pulmonary resistance vessels are sensitive to even small changes in oxygen tension, particularly close to term, pulmonary vascular tone could be reduced; this could result in a decreased stimulus to the development of pulmonary vascular smooth muscle.

Another factor which might lead to decreased pulmonary vascular tone, and thus a decreased stimulus to development of pulmonary vascular smooth muscle, might be an altered perfusion pres-
sure of the pulmonary circulation. This is unlikely, however, since the pulmonary vascular bed in the fetus could still be perfused by reverse flow through the ductus arteriosus.

Altered velocity characteristics of the pulmonary blood flow could alter the normal kinetic effects of right ventricular ejection on the developing pulmonary vascular bed. This altered velocity profile and flow pattern may be sufficient to effect the normal stimulus to growth of the pulmonary vascular smooth muscle.

Although it has been suggested that decreased pulmonary blood flow in utero may result in decreased stimulus to pulmonary arterial muscle development, \textsuperscript{6} hemodynamic studies in experimental animals have shown normal pulmonary blood flow. \textsuperscript{12}

The decreased number of pulmonary arterial resistance vessels in animals with experimental pulmonic stenosis is not understood. The possible hemodynamic arrangement of this experimental situation would lead to an increase in blood oxygen tension in the pulmonary circulation, which may decrease the stimulation to new vessel development.

Compared with normal lungs, the presence of thin-walled pulmonary arterial resistance vessels in fetuses with experimental pulmonic stenosis could cause a greatly reduced pulmonary vascular resistance at birth. This could explain the deleterious effects of systemic-pulmonary shunts in newborn infants with previously marked diminished pulmonary blood flow. \textsuperscript{3, 12} The pulmonary resistance vessels could have a limited ability to regulate flow and distal pressure. Exposure of the vessels distal to the resistance vessels to high pressure and flow may explain severe congestion and hemorrhage and the high incidence of cardiac failure in newborns after systemic-pulmonic shunts. The use of formalin infiltration of the ductus arteriosus \textsuperscript{13} as an initial procedure to maintain a moderate pulmonary blood flow early, while preserving all the vascular structures as the pulmonary vascular bed develops \textsuperscript{4} might be beneficial in this situation.

### References

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9. Levin DL, Heymann MA, Rudolph AM: Morphologic develop-

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**Table 1. Resistance Vessels in Experimental Pulmonic Stenosis**

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>N</th>
<th>Medial width (μ)</th>
<th>External diameter (μ)</th>
<th>m/d</th>
<th>Number of vessels/cm²</th>
<th>Lung resistance (mmHg × λ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></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 (10.97)</td>
<td>0.16 (0.06)</td>
<td>25</td>
<td>45.4 ± 19.0</td>
</tr>
<tr>
<td>100 days</td>
<td>100</td>
<td>6.90 (4.24)</td>
<td>40.80 (10.20)</td>
<td>0.17 (0.04)</td>
<td>25</td>
<td>73.5 ± 19.8</td>
</tr>
<tr>
<td>120 days</td>
<td>124</td>
<td>5.60 (4.01)</td>
<td>36.39 (7.90)</td>
<td>0.16 (0.04)</td>
<td>25</td>
<td>92.6 ± 30.8</td>
</tr>
<tr>
<td>122 days</td>
<td>93</td>
<td>5.62 (1.71)</td>
<td>44.02 (10.45)</td>
<td>0.15 (0.04)</td>
<td>25</td>
<td>119.8 ± 36.9</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>
<td>104.5 ± 37.6</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>
<td>170.0 ± 70.9</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>98 days</td>
<td>48</td>
<td>3.70 (0.97)</td>
<td>33.66 (7.08)</td>
<td>0.11 (0.04)</td>
<td>25</td>
<td>18.6 ± 32.8</td>
</tr>
<tr>
<td>123 days</td>
<td>40</td>
<td>4.52 (1.28)</td>
<td>34.33 (5.94)</td>
<td>0.13 (0.03)</td>
<td>25</td>
<td>51.7 ± 20.5</td>
</tr>
<tr>
<td>137 days</td>
<td>38</td>
<td>4.88 (1.48)</td>
<td>38.97 (11.88)</td>
<td>0.14 (0.07)</td>
<td>25</td>
<td>25.4 ± 9.3</td>
</tr>
<tr>
<td>138 days</td>
<td>35</td>
<td>4.94 (0.93)</td>
<td>38.68 (8.95)</td>
<td>0.13 (0.04)</td>
<td>25</td>
<td>245.8 ± 41.0</td>
</tr>
</tbody>
</table>

Parentheses = ± SD.

**Table 2. Fifth Generation Resistance Vessels in Experimental Pulmonic Stenosis: 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</td>
<td>39.05</td>
<td>0.16</td>
</tr>
<tr>
<td>Experimental</td>
<td>4</td>
<td>4.51*</td>
<td>36.41</td>
<td>0.13*</td>
</tr>
</tbody>
</table>

*Significant difference compared to Control.*

\*p <0.001, *t* test for independent means.
Diagnostic Value of Visualization of the Right Ventricle Using Thallium-201 Myocardial Imaging

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SUMMARY The diagnostic significance of visualizing the right ventricle on thallium-201 myocardial perfusion scans (T-scan) at rest was studied in 53 patients. In 33 patients the right ventricle was visualized clearly on the T-scan (group A). Hemodynamic evidence of right ventricular hypertension with systolic pressure \( \geq 30 \text{ mm Hg} \) was present in 28 of 33 (85\%) of these patients. Right ventricular volume overload with left-to-right shunt > 2:1 was present in three patients. Other tests were diagnostic for right ventricular enlargement and or pulmonary hypertension as follows: chest x-ray (58\%), echocardiogram (36\%) and electrocardiogram (15\%). In an unselected group of 20 patients (group B) where resting T-scan did not show visualization of the right ventricle, the right ventricular systolic pressure was < 30 mm Hg in all. The other noninvasive tests did not reveal presence of right ventricular hypertrophy or enlargement. T-scan appears to be a useful and sensitive test in detecting right ventricular pressure or volume overload compared with other noninvasive tests. This may be useful in detection of patients with right ventricular hypertrophy or enlargement secondary to pulmonary hypertension or other causes.

MANY NONINVASIVE TECHNIQUES are being used to evaluate right ventricular hypertrophy. Echocardiography is helpful in identifying some patients with pulmonary hypertension, but the assessment of right ventricular hypertrophy with noninvasive techniques has variable reliability.\(^1\)\(^4\) Although the right ventricle is usually not well-visualized during thallium-201 myocardial perfusion scanning, Cohen et al. observed that this technique was more useful than the electrocardiographic criteria for the determination of right ventricular hypertrophy in patients with chronic pulmonary hypertension.\(^5\) This report emphasizes the value of the thallium scan in the diagnosis of right ventricular pressure or volume overload compared with echocardiographic and electrocardiographic techniques in 53 patients with documented hemodynamic studies.

Material and Methods

Fifty-three patients with known or suspected heart disease were studied. Each had a complete clinical evaluation, including ECG, chest x-ray, echocardiogram, and left heart catheterization, and resting thallium-201 myocardial images. There were 32 males and 21 females. The mean age for the whole group was 50.1 years (range 23–72 years). Informed consent for performing thallium-201 imaging was obtained in all patients. Myocardial images were obtained in the resting state 20 minutes after intravenous administration of 2 mCi of thallium-201. Images were obtained in the anterior, 30° and 60° left anterior oblique (LAO) and left lateral projections. A Picker gamma camera 4/10 with parallel hole collimator and a 20° window was used. A 1-mm lead ring ‘mask’ was placed on the collimator to limit the field of view. Each image contained 300,000 total counts, and computer views were made with 30% background subtraction. The 30° LAO projection provided the best separation of the right and left ventricles and was used for evaluation of the right ventricular images. The scans were interpreted by three observers without knowledge of the hemodynamic, echocardiographic or electrocardiographic findings. Visualization of the right ventricle was considered to be present when at least two of the

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