Pulmonary Arterial Tree following Prolonged Experimental Reduction of Pulmonary Blood Flow

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With the technical assistance of Burton S. Tabakin, M.D., and John S. Hanson, M.D.

Widespread pulmonary arterial thrombosis is a well-known complication of severe pulmonary valvular stenosis or atresia.1-6 With time such thrombi may recanalize or organize to form arterial intimal proliferative lesions. In the current study, such pulmonary arterial lesions were produced experimentally in dogs by a prolonged reduction of pulmonary blood flow. In human beings the pathogenesis of these lesions has been attributed to increased blood viscosity, i.e., polycythemia1,3,5 or to various combinations of polycythemia, reduced blood flow and hypoxemia.2,4 The absence of polycythemia in our animals made it possible to dissociate the effects of increased viscosity from the influence of reduced flow on the genesis of the thrombotic lesions.

Methods

Six-week-old dogs weighing approximately 2.3 kg each were selected for study. On six dogs a Blalock anastomosis7 was performed between the end of the left subclavian artery and the side of the left pulmonary artery using a continuous suture of 5-0 or 7-0 silk posteriorly and an interrupted suture anteriorly under microscopic control. These operations were performed to increase pulmonary blood flow. As will be shown, the operations were successful in four of the dogs and pulmonary blood flow was increased. The current study was made possible by an unexpected complication of surgery which reduced blood flow to the left lung in two of the dogs. In these two animals the left pulmonary arteries became occluded by fibrous diaphragms just proximal to the shunt anastomotic sites. In addition, in each instance a fibrous diaphragm formed at the point where the subclavian artery was anastomosed to the left pulmonary artery. In each of these two dogs only a pinpoint aperture in the diaphragms at the anastomotic site permitted blood flow into the left lung.

For each experimental animal, a paired control from the same litter was prepared by ligation and division of the subclavian artery. No anastomoses were performed on these control animals. Two of the shunt animals were maintained for 15 months and the remaining four were sacrificed 22 months after operation along with their controls. During this period, all dogs with anastomoses grew and developed normally as did their litter mate controls. Right heart catheterizations and retrograde aortic angiograms were performed under anesthesia eight months after the anastomoses.

At postmortem examination multiple measurements were made of the hearts, major blood vessels and subclavian shunts. Multiple tissue blocks were taken from all lobes of the lungs, sectioned at seven microns and stained with Verhoeff’s and Van Gieson’s stains. Occasional sections were also stained with Mallory’s trichrome stain and phosphotungstic acid hematoxylin stains.

Results

Findings are summarized in table 1. In dogs #1 and #2 right heart catheterization eight months after operation and some 14 months before death failed to demonstrate a left to right shunt when the catheter was advanced into the pulmonary artery. These findings were clarified by retrograde aortic angiograms which revealed that the patent subclavian shunts filled only the left pulmonary arteries. This was due to the aforementioned diaphragms which occluded the left pulmonary arteries proximal to the shunt site. These findings were clarified by retrograde aortic angiograms which revealed that the patent subclavian shunts filled only the left pulmonary arteries. This was due to the aforementioned diaphragms which occluded the left pulmonary arteries proximal to the shunt site. Although the proximal portions of the shunts were large and filled promptly in these two animals, there was a marked delay in passage of dye from the shunts into the left pulmo-
### Table 1

**Summary of Clinical and Autopsy Data on Five Dogs with Blalock Anastomoses**

<table>
<thead>
<tr>
<th></th>
<th>Dog 21</th>
<th>Dog 22</th>
<th>Dog 23</th>
<th>Dog 24</th>
<th>Dog 25</th>
<th>Dog 26</th>
<th>Control dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Duration of experiment (months)</td>
<td>22</td>
<td>22</td>
<td>15</td>
<td>22</td>
<td>15</td>
<td>22</td>
<td>15-22</td>
</tr>
<tr>
<td>2. Dog’s weight (kg)</td>
<td>21</td>
<td>20</td>
<td>19</td>
<td>19</td>
<td>16</td>
<td>21</td>
<td>18-22</td>
</tr>
<tr>
<td>3. Shunt by cardiac catheterization utilizing oxygen measurements (liters/min)</td>
<td>0</td>
<td>0</td>
<td>2.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>4. Delay of shunt emptying on angiogram</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>5. Blood flow through left lung by angiogram</td>
<td>decreased</td>
<td>decreased</td>
<td>increased</td>
<td>increased</td>
<td>increased</td>
<td>increased</td>
<td>—</td>
</tr>
<tr>
<td>6. Mean pulmonary arterial blood pressure (mm Hg)</td>
<td>22</td>
<td>16</td>
<td>16</td>
<td>18</td>
<td>—</td>
<td>—</td>
<td>14-22</td>
</tr>
<tr>
<td>7. Hemoglobin (gm %)</td>
<td>15.7</td>
<td>15.7</td>
<td>16.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8. Femoral arterial oxygen saturation %</td>
<td>90</td>
<td>94</td>
<td>91</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>77-95</td>
</tr>
<tr>
<td>9. Minute ventilation in liters/kg dog’s weight</td>
<td>0.30</td>
<td>0.38</td>
<td>0.34</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.17-0.48</td>
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</table>

**NECROPSY OBSERVATIONS**

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<tbody>
<tr>
<td>1. Diameter of subclavian shunt before opening (mm)</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td>16</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>less</td>
<td>less</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2. Diameter of shunt at anastomotic site (mm)</td>
<td>than 1</td>
<td>than 1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3. Proximal occlusion of left pulmonary artery</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>4. Enlarged collateral blood supply to left lung</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>5. Weight of heart (g)</td>
<td>151</td>
<td>141</td>
<td>168</td>
<td>187</td>
<td>142</td>
<td>209</td>
<td>137-202</td>
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<tr>
<td>6. Thickness: right ventricle (mm)</td>
<td>5.5</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>5.5</td>
<td>4-6</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Thickness: left ventricle (mm)</td>
<td>16.0</td>
<td>14</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>17</td>
<td>14-18</td>
</tr>
<tr>
<td>8. Microscopic changes in pulmonary arteries of left lung:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Thrombi</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>b) Intimal proliferative lesions</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>c) Atrophy of media</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>
Reduction in Pulmonary Blood Flow

Figure 1
The media is very thin in this muscular pulmonary artery from the left lung of dog #1. Blood flow and pressure in this lung were presumably greatly reduced. Verhoeff's and Van Gieson's stains. X 400.

Figure 2
Muscular artery showing media of normal thickness. This is from the right lung of dog #1. Blood flow through this lung was probably increased. Verhoeff's and Van Gieson's stains. X 400.

Pulmonary arteries in each instance. This was related to the pinpoint apertures at the anastomotic sites through which blood had to pass to reach the left lungs. The pulmonary vessels in the left lungs of these two dogs did not completely opacify on angiograms suggesting low blood flows. Pulmonary arterial and systemic arterial blood pressures were normal as were hemoglobin values. Femoral arterial oxygen saturation was somewhat decreased in dog #1 (90%). The dogs were anesthetized and this mild unsaturation was attributed to hypoventilation. Minute ventilation in liters/kg of dog's weight could be directly correlated with arterial oxygen saturation in both experimental and control animals. Two of the sham operated control animals had arterial oxygen saturations of 77% and 83% with minute ventilations of 0.17 and 0.23 liter/kg respectively. Animals with a normal arterial oxygen saturation had a minute ventilation of 0.36 to 0.48 liter/kg.

In both dogs #1 and #2, blood also reached the left lungs through collateral channels. Evaluated by the method of Liebow et al., the total cross sectional areas of bronchial arteries in the left lungs of these animals were increased four-fold over values for the right lungs. These same cross sectional areas for bronchial arteries of the left lung were about 50% of the cross sectional areas recorded for accompanying pulmonary arteries. Collateral supply to the left lungs of these two dogs was also increased through arterial channels formed through postoperative pleural adhesions. These latter channels were much smaller than the bronchial arteries. Apparently neither the subclavian shunts nor the collateral channels were sufficient to maintain normal pulmonary arterial pressures in the left lungs of these dogs. This can be inferred from the observation that muscle about pulmonary arteries in the left lungs of these two animals was atrophic when compared with that found about arteries in the right lungs (figs. 1 and 2). Such muscular atrophy was found proximal as well as distal to occlusive thrombotic arterial lesions. A correlation has previously been reported between pulmonary arterial muscle mass and pulmonary arterial pressure.

In dogs #1 and #2, intimal proliferative lesions were widespread in the pulmonary arteries of the left lungs but absent in comparable vessels of the right lungs. Such lesions were interpreted as thrombotic in origin. They were randomly distributed throughout elastic and muscular arteries of all sizes. The lumina of some of the involved arteries were narrowed or even occluded (figs. 3 to 5). The lesions were comprised of proliferated endo-
Intimal proliferative lesions in a muscular pulmonary artery from the left lung of dog #2. Reduplicated elastic tissue is admixed with endothelial cells and smooth muscle in the lesion. Blood flow through this lung was presumably reduced. Verhoeff's and Van Gieson's stains. X 400.

Muscular pulmonary artery from the left lung of dog #1. The vessel contains a large eccentric organized thrombus. Phosphotungstic acid hematoxylin stain. X 500.

Bronchial arteries but were often prominent in the enlarged collateral arteries which had formed in the enlarged pulmonary arteries. In a few bronchial arteries involved, the lesions were comprised of small, eccentric foci of subendothelial fibrosis which never encroached upon the lumina. In the pleural arteries, these proliferative lesions sometimes considerably narrowed the vessel lumina. The presence of thrombus in a few of these vessels suggested a thrombotic origin for the lesions (fig. 4).

The pulmonary arteries and subclavian shunts were everywhere widely patent in dogs #3, #4, #5, and #6, permitting increased blood flows through the lungs. Although a diaphragm had formed at the subclavian-left pulmonary artery anastomotic site in dogs #4 and #6, the aperture which permitted flow into the left lung was adequate to permit a large blood flow (table 1). These postmortem findings supported the earlier angiographic and cardiac catheterization data in dogs #3, #4, #5, and #6. In dog #3, pulmonary blood flow 14 months before death was 2.2 times the systemic flow indicating a large left to right subclavian shunt. Although precise data is lacking in dogs #4, #5, and #6, a left to right shunt was demonstrated indirectly just before death in all three cases by comparing the color of blood removed from the right ventricle with that removed from the pulmonary artery.
REDUCTION IN PULMONARY BLOOD FLOW

Several observers were easily able to distinguish blood from the pulmonary artery in each instance by its brighter red color. Pulmonary arterial muscle mass was normal in the lungs of dogs #3, #4, #5, and #6. This observation correlated with the normal pulmonary arterial pressures recorded during right heart catheterization (table 1). None of the six animals had myocardial hypertrophy of the right or left ventricular walls when compared with their matched controls (table 1). Thrombotic or intimal proliferative lesions were entirely absent from the pulmonary arteries of dogs #3, #4, #5, and #6. Neither bronchial nor pleural arteries were enlarged. No pulmonary capillary or pulmonary venous abnormalities were detected in any of the anastomotic or control animals.

Discussion

Widespread pulmonary arterial thrombotic lesions developed in the left lungs of two dogs after a prolonged experimental reduction of blood flow to these organs. The duration of the reduced flow was at least 14 months and may have been as long as 22 months. The reduction in flow was caused by narrowing and occlusion of arterial channels leading into the involved lungs. In both dogs, medial muscle about the pulmonary arteries of the left lung was atrophic. Such atrophy was presumably a reflection of low pulmonary arterial pressure and flow and has been noted previously in humans with severe stenosis or atresia of the pulmonic valve. Another consequence of reduced left pulmonary blood flow in these two dogs was the development of an enlarged collateral arterial supply to the involved organs.

Although fresh thrombi were rare in the involved arteries, the intimal proliferative lesions had many characteristics suggestive of a thrombotic origin. They were randomly distributed in arteries of all sizes. They often formed eccentric plaques about the luminal margin of arteries, a common form for thrombi or emboli to take after they have been organized. It is not surprising that such organized lesions were more common than fresh thrombi in our animals since such thrombi can presumably organize in less than two weeks, and the factors which predisposed to their development in our cases had persisted for at least 14 months. Thrombi, rather than emboli, seem a more probable origin for the lesions since no obvious source for emboli was found. Such widespread pulmonary arterial thrombosis is a well-known complication of severe pulmonic stenosis or atresia in human beings.

In human beings, development of the thrombotic lesions has most often been attributed to secondary polycythemia and its associated increase in blood viscosity. However, reduced pulmonary blood flow and hypoxemia have also been implicated in their pathogenesis. In our dogs, hypoxemia, polycythemia and presumably an increase in blood viscosity were absent. This was due to the fact that blood flow through one lung in each dog was at least normal and probably increased. Therefore, the arterial lesions in the left lungs of our dogs can most easily be attributed to reduced flow. In this regard, a second group of animals with some increase in blood flow through their left lungs failed to develop arterial lesions. This correlates with the observation in human beings that such lesions in patients with pulmonic stenosis can be prevented by shunting procedures designed to increase pulmonary blood flow. In such cases, the lesions may be prevented while some degree of hypoxemia and mild polycythemia persist, whereas, other individuals having prolonged hypoxemia and polycythemia without reduced pulmonary blood flow do not commonly develop pulmonary arterial thrombotic lesions.

The proliferative lesions noted in pleural and bronchial arteries of dogs #1 and #2 have previously been noted in the enlarged bronchial arteries which develop after pulmonary artery occlusion. Their cause is presumably other than low blood flow. Except at sites where thrombi had organized, the media of these arteries was as thick as that found about other systemic arteries, suggesting a high intraluminal pressure. Blood flow through
these channels is presumably large\textsuperscript{14} and the lesions which develop in them might be compared with those found in pulmonary arteries in other disorders where flow is increased. It is well known that large increases in blood flow through arteries can independently induce intimal proliferative lesions.\textsuperscript{13} The evidence in our animals suggests that such lesions might have a thrombotic origin (fig. 4).

**Summary**

A Blalock anastomosis between the left subclavian artery and the left pulmonary artery was performed on six dogs soon after birth. As an unexpected complication of surgery, blood flow through the left lungs of two of the dogs was greatly reduced. Over a period of 22 months, widespread arterial thromboses developed in these two lungs. The thrombotic lesions were comparable to those which develop in human beings with severe pulmonary valvular stenosis or atresia. Hypoxemia and polycythemia have often been implicated in the development of these lesions. Our study demonstrates that such lesions can be induced in the dog by reduction in pulmonary blood flow alone since hypoxemia and polycythemia were absent in our experimental animals.

**References**

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