The Relation of Blood Pressure and Flow to the Development and Regression of Experimentally Induced Pulmonary Arteriosclerosis

By Donald J. Ferguson, M.D. and Richard L. Varco, M.D.

Systemic artery-pulmonary artery shunts have been made in dogs and the relation between pulmonary artery pressure and blood flow and the development of vascular lesions has been studied. Arteriolar medial hypertrophy and intimal proliferation are not necessarily incited by a large blood flow, but such lesions are more readily induced by an end-to-end than by an end-to-side type of shunt. Intimal proliferation is not associated with stainable alterations in elastic tissue or lipid infiltration. Vascular lesions do not readily regress when the inciting factor is removed.

The development of pulmonary arteriolar lesions in dogs in which a systemic artery is anastomosed to the pulmonary artery has been previously demonstrated. The lesions are similar to those which occur in human beings with various types of pulmonary hypertension; in early stages the arterioles show medial hypertrophy, and later there is intimal and adventitial proliferation with eventual obliteration of the lumen in some of the vessels.

Clinical studies have indicated that pulmonary vascular lesions develop in proportion to the degree of pulmonary hypertension, but not directly in proportion to the pulmonary blood flow, and a similar situation was described in the experimental animal.

It is the purpose of this paper to describe more extensive experiments relating pulmonary blood pressure and flow to development of pulmonary vascular lesions, to present further descriptions of the histology of the lesions, and to indicate the extent to which they may regress when inciting factors are removed.

METHODS

The dogs used were puppies nearly full grown or young adults, of both sexes. Two general types of systemic-pulmonary shunts were prepared as shown in figure 1. In three of the animals in group II, the brachiocephalic artery was used instead of the subclavian. In two other dogs in group II, bilateral carotid-jugular end-to-end shunts were also made.

Blood Pressure.—Pulmonary arterial, pulmonary venous or left auricular, and aortic or femoral pressures were measured before and after making the anastomosis in 4 of the 46 dogs in group I, and in 3 of the 16 dogs in group II. In most of the remaining dogs pulmonary arterial pressure and systemic pressure were measured. Cannulas were filled with heparinized saline and connected to strain gauge manometers and a Sanborn amplifier-recorder. The pulmonary artery cannula entered the vessel approximately one centimeter distal to the anastomosis. Mean pressures were recorded by an attached electronic integrator. Subsequent pressure measurements were made through a thoracotomy at the time of lung biopsy. All pressure and flow measurements were made during anesthesia. The respirator was shut off for a few seconds as the pressures were taken.

Blood Flow measurements were made by applications of the Fick principle, using Van Slyke manometric measurements of blood oxygen content, and a spirometer connected to a tracheal cannula for oxygen consumption.

In dogs of group I the left main bronchus and the trachea were each connected with one lumen of a double-lumen and endo-tracheal tube, and two balloons were inflated to make the connections air tight. A cardiac catheter was placed in the pulmonary artery and a cannula was inserted in a femoral artery. The left lung was connected to the oxygen supply and the right lung to a rebreathing bag. The animals became cyanotic and respirations increased in rate and depth, these signs being hardly perceptible with large shunts and very severe with small ones. There was usually a moderate rise in...
both systemic and right pulmonary artery pressure, which in some animals was transient and in others sustained. A state of equilibrium, as determined with a cuvette oximeter and the spirometer, was reached in about 15 minutes, except where the shunt was very small, in which case equilibrium was not attained. In any event, the flow was probably altered somewhat by the procedure. When equil- 

brium was reached, samples were drawn and oxygen consumption was measured.

In dogs of group II, right ventricular output was obtained by measuring oxygen uptake from both lungs, while blood samples were drawn from the right ventricle and femoral artery for oxygen content. The oxygen content of mixed pulmonary artery blood was obtained from multiple samples drawn at thoracotomy through a needle from 3 or 4 branches of the right pulmonary artery into one syringe. In addition, the mixed pulmonary artery sample was determined in those dogs with parts of both lungs remaining, by connecting one main bronchus to an oxygen supply, while the other was connected to a small tonometer and rebreathing bag, rotating in a water bath at 37 C. A sample of blood in the tonometer came into equilibrium after 20 to 30 minutes with the rebreathed air and at the same time with the mixed pulmonary artery blood, and the sample was analyzed for oxygen content. For each animal, it was required that at least two flow measurements, taken within one month, should agree within 15 per cent of their mean, for

the data to be included here. Values other than the two or more in agreement were not used in obtaining the final mean for the animal; it is believed that such extraneous values probably represent an unobserved error in the technique. There are, of course, other types of error associated with use of the Fick method. In the present experiment only large and consistent differences in blood flow are considered significant.

**Calculations**

**Group I.** Blood flow through left lung, in liters/min. = \( O_2 \) consumption through left lung ml./min. STPD (right lung rebreathing)/(\( O_2 \) content, ml./liter, of aortic blood when 100 per cent oxygen being supplied both lungs) — (\( O_2 \) content, ml./liter, of aortic blood when 100 per cent oxygen supplied to left lung and right lung rebreathing). The latter blood sample is drawn while \( O_2 \) consumption from the left lung is being measured; the saturated sample, drawn before or afterward, is assumed to correspond in oxygen content to left pulmonary venous blood when only the left lung breathes oxygen.

**Group II.** Blood flow through the lungs in l./min. = output of right ventricle (\( Q_{rv} \)), plus flow through shunt (\( Q_{sh} \)) = output of left ventricle. \( Q_{rv} = O_2 \) consumption/\( O_2 \) content, ml./liter, of aortic blood) — (\( O_2 \) content, ml./liter, of right ventricle blood).

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**Fig. 1.** Diagrams of the systemic artery—pulmonary artery shunts in the two groups of dogs. Percentages represent average of total lung weight (see text), obtained from 10 normal dogs.
(O₂ content mixed pul. a. blood)
\[ Q_{sh} = \frac{Q_T - (O_2 \text{ content r. vent. blood})}{(O_2 \text{ content aortic blood}) - (O_2 \text{ content mixed pul. a. blood})} \]

**Index of pulmonary blood flow**, to left lung in group I and to total remaining lung in group II, = liters of blood flow per minute per square meter of body surface per total lung (intact, bilateral). Surface area was calculated from body weight by Meeh’s formula: \[ S \text{ sq.m.} = 11.2 \sqrt[3]{\text{wt. in Gm.}} \]
The amount of lung perfused was calculated from the per cent of total lung weight contributed by each of the lobes. These percentages, shown in figure 1, were obtained as the averages from 10 normal young adult dogs which died while in supine position in the course of other experiments and were autopsied immediately.

**Resistance**, in arbitrary units, = mean pulmonary artery pressure (mm. Hg.)/index of pulmonary blood flow. Pulmonary venous pressure was usually not measured; when it was measured, it was not significantly elevated by the shunt (table 3).

**HISTOLOGIC EVALUATION**

Lung biopsies were fixed in formalin. Pieces 1 x 2 x 2 cm. were taken from the periphery of the lobe. Three or more biopsies were taken at most of the operations, and specimens were taken repeatedly from the same animals at various intervals. Paraffin sections were stained with Verhoeff’s and Van Gieson’s stain. The pulmonary arterioles, identified by structure and location, included vessels from 20 to 200 microns in diameter of the external elastic lamina. Since it is rare to find any visible intima in the pulmonary vessels of the normal young adult dog, any intimal thickening was considered pathologic. When only medial hypertrophy was found, or only one or two arterioles with intimal change were present in a square centimeter of lung area, the specimen was graded 1; if 3 to 5 vessels with intimal proliferation were present, 2; and if more than that, 3. Adventitial changes were not separately evaluated. Larger arteries up to and including the main branch of the pulmonary artery were seen in autopsy specimens and were affected with lesions similar to those in the arterioles.

**RESULTS**

**Vascular Lesions.**—Histologic changes in the pulmonary arterioles were found in 25 of the 62 dogs studied 2 weeks or more after operation. Dogs considered to be without changes had

**Fig. 2.** Photomicrograph of pulmonary arteriole, from group Ic, observed 6 months after operation; 200 μ, X 180. Verhoeff’s and Van Gieson’s stain. Each of the three coats is greatly thickened, and the lumen is nearly obliterated.

**Fig. 3.** Photomicrograph from group Ib, taken 15 months after shunt was made, and 8 months after it was removed and the pulmonary artery reanastomosed; 80 μ, X 360. Medial hypertrophy, intimal proliferation, and some increase in adventitia are present.

**Fig. 4.** Photomicrographs from group Ic, observed 8 months after shunt was made, and 2 months after it was removed. Two areas at top appear to be recanalized; section is oblique; 180 μ, X 180.
negative biopsies on two or more occasions. Medial muscular hypertrophy of arterioles with significant reduction of the lumen/medial thickness ratio can be shown after two weeks in some animals. After two or three months, intimal proliferation of fibroblasts and adventitial collagenous thickening appear (fig. 2 to 4), and obliteration of the lumen may be seen in many vessels. Hyaline changes were not observed. Capillaries, alveoli, and veins showed little change. Seventy-two control sections were made from parts of lung removed at the first operation, from the right lungs in group I dogs, and from intact animals of all ages. The medial hypertrophy of pulmonary arterioles, with occasional intimal thickening, that is present at birth, disappears completely within two months.

Sections of lung from the nine animals with grade two and three lesions were fixed in lead acetate-formalin, stained with toluidine blue, and compared with similarly treated sections of normal lung. The thickened arterioles showed no increase of metachromasia, (supposed to indicate the presence of mucopolysaccharides) such as has been described in arteries in which the elastic tissue has fragmented.

Pieces of lung from the same nine animals were frozen after fixation in formalin and sections were stained with Sudan IV. Sudanophile material could not be seen in the affected vessels, or in the normal controls. The proliferation of intima apparently was not induced or accompanied by an infiltration of lipid.

**Pressure and Flow Measurements:**

Results will be described according to the groups illustrated in figure 1.

**Group Ia.** The data of table 1 suggest that the development of pulmonary vascular lesions was associated with an increasing left pulmonary artery pressure above that present immediately after the shunt was made, but the difference is not statistically significant. Flow measurements show no significant difference between the group with vascular lesions and those without. These measurements were made during the latter part of the interval between operation and biopsy, one measurement being taken just before biopsy. The dogs without lesions may, of course, develop them after a longer time.

**Group Ib.** There were four dogs in this group, all of which developed severe pulmonary edema in the left upper lobe as soon as the shunt was opened. Only one dog survived more than 12 days after operation, and this dog developed grade 3 lesions, first looked for after seven and one-half months. No adequate pressure or flow measurements were made in this group, but it is significant that a subclavian artery shunt was poorly tolerated in the left upper lobe (16 per cent of total lung) whereas it was well tolerated in the left lower lobe (26 per cent of total lung), as in group Ic. Animals in the latter group probably had somewhere near the maximum size of shunt that could be accepted without difficulty in the immediate post-operative period.

**Group Ic.** There were 16 dogs from group Ia in which the left upper lobe was removed several weeks after the shunt was made, thus putting the dogs into group Ic. In five additional dogs, the upper lobe was removed at the time the shunt was made. Three of the dogs from group Ia had lesions in the pulmonary vessels at the time lobectomy was performed. These lesions progressed from grade 1 to grade 2 in one dog after lobectomy, and remained

<table>
<thead>
<tr>
<th>Vascular lesions*</th>
<th>Without lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular lesions*</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Without lesions</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Range</td>
</tr>
</tbody>
</table>

Numbers of animals in parentheses.

* In three dogs lesions were grade 2, and in the others grade 1.
TABLE 2.—Pulmonary Pressure and Flow Data in Dogs of Group Ic

<table>
<thead>
<tr>
<th>Vascular lesions*</th>
<th>Without lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of Dogs</strong></td>
<td><strong>7</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>26 (6)</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>18-35</td>
</tr>
</tbody>
</table>

**Pul. Art. Pres.**
- At operation: 26 (6)
- At biopsy: 34 (11)

**Pul. Blood Flow Index**
- Mean: 9.4 (6)
- Range: 4-25

**Resistance**
- Mean: 9.3
- Range: 1.2-8.9

**Duration of Shunt**
- Mean: 18 wks.
- Range: 10-30

* Lesions grade 1 in 3, grade 2 in 1, grade 3 in 3 dogs.

These dogs were not included in the data for table 2.

These data show more clearly than those in Table 1 that in association with the development of vascular lesions there is an increase in pulmonary artery pressure, an increase in resistance, and a decrease in blood flow, at least relative to conditions in dogs which did not develop lesions. Early and late flow measurements in animals in which lesions were developing were obtained in four instances, and a similar trend was even more evident. Pulmonary artery pressure in one animal with grade 3 lesions eventually was the same as aortic pressure (100 mm Hg).

In four of the animals in which the upper lobe was removed when the shunt was made, more complete pressure measurements (Table 3) show a gradual rise of pulmonary artery pressure during the few minutes immediately after the shunt was opened, with a subsequent fall in three animals. Since the systemic pressure and pulmonary venous pressure remained steady while pulmonary artery pressure rose, the latter change must reflect a change of resistance in the shunt-perfused lung. The reversibility of this change in resistance, apparent in the measurements at 10 minutes, suggests that vasomotion rather than vascular damage, hemorrhage, or edema was the mechanism involved. Of these four dogs, two died at 4 and 5 weeks, showing no pulmonary vascular lesions; one was biopsied at 4 months and had grade 1 lesions with intimal proliferation (grade 1 in table 3), and one was without lesions at three and one-half months (grade 4 in the table).

A gradual rise in pulmonary artery pressure immediately after opening the shunt was never observed in group II dogs; it was specifically looked for in only three of them, however.

**Group II.** There were no mean pulmonary artery pressures higher than 32 mm Hg at the time of biopsy in the 16 dogs of this group (Table 4). In four animals there were grade 1 lesions which appeared after 5 months or more. Two of these dogs had brachiocephalic artery instead of subclavian artery shunts. The two dogs with additional carotid-jugular shunts have not developed lesions, nor do they have the highest flow indices, although the shunts are open.

Comparison of flow indices reveals the higher flow rates at lower pressures generally prevailing in group II. The mean resistances for the various subgroups are all higher in group I, and vascular lesions appeared in about half the dogs in group I compared to one-fourth of those in group II. Lesions were more severe and appeared much earlier in group I.

**Regression of Lesions:**

In 6 group I dogs with vascular lesions, the shunt was removed 1 to 8 months after it was made and the pulmonary artery reanastomosed

TABLE 3.—Integrated mean pressures in mm. Hg from 4 dogs of Group Ic with chest open, taken shortly after the shunt was made

<table>
<thead>
<tr>
<th>Dog</th>
<th>Shunt Closed</th>
<th>Open 10-20 Sec.</th>
<th>Open 1-5 Min.</th>
<th>Open 10 Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pul. a.</td>
<td>4</td>
<td>29</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Pul. v.</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Fem. a.</td>
<td>135</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>Pul. a.</td>
<td>4</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Pul. v.</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Fem. a.</td>
<td>120</td>
<td>85</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>Pul. a.</td>
<td>4</td>
<td>30</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Pul. v.</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Fem. a.</td>
<td>135</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>Pul. a.</td>
<td>2</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Pul. v.</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Fem. a.</td>
<td>140</td>
<td>125</td>
<td>125</td>
</tr>
</tbody>
</table>
TABLE 4.—Hemodynamic Data in Dogs of Group II

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>At operation</td>
<td>At biopsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIa—No lesions</td>
<td>10</td>
<td>Mean 36 (3)</td>
<td>24 (10)</td>
<td>11.2 (7)</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range 34-40</td>
<td>19-29</td>
<td>7-18</td>
<td>1.5-3.2</td>
</tr>
<tr>
<td>IIb*</td>
<td>2</td>
<td>Mean 21 (2)</td>
<td>27 (2)</td>
<td>23.9 (2)</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range 12-29</td>
<td>26-27</td>
<td>22-26</td>
<td>1.0-1.3</td>
</tr>
<tr>
<td>Without lesions</td>
<td>5</td>
<td>Mean 26 (5)</td>
<td>26 (4)</td>
<td>11.7 (5)</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range 20-31</td>
<td>17-32</td>
<td>3-17</td>
<td>1.7-5.0</td>
</tr>
<tr>
<td>IIc' with r. lung intact. No lesions</td>
<td>6</td>
<td>Mean 20 (6)</td>
<td>26 (6)</td>
<td>16.6 (6)</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range 12-24</td>
<td>20-32</td>
<td>9-19</td>
<td>1.3-3.3</td>
</tr>
<tr>
<td>IIe† (as in Fig. 1).</td>
<td>2</td>
<td>Mean 23 (2)</td>
<td>29 (2)</td>
<td>16.0 (2)</td>
<td>1.8</td>
</tr>
<tr>
<td>Vascular lesions†</td>
<td></td>
<td>Range 20-26</td>
<td>27-31</td>
<td>15-17</td>
<td>1.6-2.0</td>
</tr>
<tr>
<td>Without lesions</td>
<td>4</td>
<td>Mean 22 (4)</td>
<td>25 (4)</td>
<td>14.1 (4)</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Range 20-23</td>
<td>22-31</td>
<td>10-17</td>
<td>1.4-3.0</td>
</tr>
</tbody>
</table>

* Same dogs as IIa, after removal of right upper and middle lobes.
† All lesions in Group II were grade 1; biopsy of right lower lobe.
‡ Same dogs as IIc', after removal of right upper and middle lobes.

in the normal relationship. Pulmonary artery pressures all returned to normal. After a further 5 to 8 months, in 4 dogs with grade 3 lesions which had developed over the same lengths of time, the lungs were again biopsied and the pulmonary artery anastomoses were shown to be open. In one dog with grade 2 lesions the artery was closed at the anastomosis but open distally, at 6 months (1 year after the original shunt). The remaining animal had grade 1 lesions (medial hypertrophy) at one month, when the shunt was removed, and was re-examined after 1 month more.

The lesions for the most part showed no evidence of regression (fig. 4), even in the animal with only medial hypertrophy. There were occasional vessels in which the areas of intimal proliferation appeared to be recanalized (fig. 3). A few vessels also showed small areas of intimal proliferation without medial hypertrophy, a combination not found in dogs with open shunts, where medial hypertrophy is the primary change.

Although most of the lesions were already grade 3, it was possible to observe that there was no further progression after the shunt was removed. Repetition of the toluidine blue and fat stain techniques again gave negative results at the end of these experiments.

DISCUSSION

There is more reaction in the pulmonary vessels when a systemic arterial shunt is directed by end-to-end suture into a branch of the pulmonary artery leading to the lung, than when a similar shunt enters into the main pulmonary artery, even though in the latter instance the amount of blood flow per unit lung is as great as or greater than in the former. If the difference in response between groups I and II were due to difference in oxygen or CO₂ content of the blood, all the dogs in group I should have been affected instead of only half of them.

A possible explanation of the difference between the groups is that the kinetic energy or pulsatile thrust of the shunt blood in group I animals is more directly transmitted to the pulmonary arterioles than it is in group II, where it is partly dissipated in the reservoir provided by the main pulmonary artery. The temporary arteriolar constriction suggested by the data in table 3 may be repeated whenever a normal increase in systemic pressure occurs due to stress. Such activity of the arteriolar muscle would provide a reasonable explanation of medial hypertrophy, which is the first change we observed.

It is not known what effect denervation of
the pulmonary vessels, as a consequence of surgical division of the left pulmonary artery or its lower branch, may have had on the experiment. Complete denervation probably did not occur, since only a short length of artery was dissected free, and the bronchus and veins remained intact.

Intimal proliferation in the present experiment is not apparently a consequence either of elastic tissue degeneration or of lipid infiltration. A subendothelial fibrous zone in human systemic vessels has been described, which appears early in life and is progressive, and has the same distribution as the atherosclerosis which appears later in life. This fibrous thickening is increased by hypertension, and may be pronounced in some individuals who have little atheroma, a situation analogous to that in our experiment.

Regression of pulmonary vascular lesions does not readily occur when the inciting factor is removed. Nevertheless, lesions do not progress further, and there is evidence of some attempt at healing, especially of vessels with incomplete obliteration. However, the medial hypertrophy and intimal changes present at birth are known to disappear completely under normal circumstances. Healing of induced lesions may continue over a longer period of time than we have observed.

**Summary**

Pulmonary blood pressure and flow have been increased in dogs by anastomosis of a systemic artery to the pulmonary artery. Measurements of pressure and flow, and histologic study of the lungs, have led to the following conclusions:

Pulmonary arteriolar medial hypertrophy and intimal proliferation are not readily induced simply by a large increase in blood flow.

An end-to-end systemic-pulmonary artery shunt is much more effective in producing vascular alterations than an end-to-side shunt producing a similar initial increase in pressure and flow.

Pulmonary vascular lesions have developed without histologic evidence of elastic tissue degeneration or of lipid infiltration.

When the inciting factor is removed, the vascular changes do not appreciably regress within the same period of time that was required for their development.

**REFERENCES**

The Relation of Blood Pressure and Flow to the Development and Regression of Experimentally Induced Pulmonary Arteriosclerosis
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