Correlations of Microradiographic and Histological Findings in the Pulmonary Vascular Bed

Technique and Application in Pulmonary Hypertension

By John T. Reeves, M.D., Duane Tweeddale, M.D., Jacqueline Noonan, M.D., James E. Leathers, B.S., and Mervyn B. Quigley, D.D.S.

Patients with congenital intracardiac defects, pulmonary hypertension, and high pulmonary vascular resistance show widespread pulmonary arteriolar obstruction from medial hypertrophy and intimal proliferation. Postmortem radiograms show enlargement of the proximal pulmonary arteries but relatively few distal branches. Vascular channels remaining patent in such patients are often dilated. Dilated thin-walled vessels are found far distant in the pulmonary arterial bed, but the vascular segments involved have not been established. These dilated segments have been considered to be dilated arterioles bronchopulmonary anastomoses, or microscopic arteriovenous (A-V) fistulas. Examination of dilated vessels in pulmonary hypertension requires reconstruction of the lesion from serial sections. Changes in capillaries have not been emphasized; yet we have expected these vessels to be altered by the profound vascular disturbances within such lungs. Capillaries have thin walls and small diameters and are difficult to study unless they are filled by injection media, such as Berlin blue, India ink, or barium sulfate. Study of capillaries so identified should also demonstrate vessels which supply and drain them, but we have not found reports in which special injection techniques were applied to the capillary beds of patients dying from congenital heart disease. We attempted to examine by microradiography the lungs of patients with pulmonary hypertension by injecting barium sulfate into the pulmonary arteries and capillaries. Convincing correlation of microradiograms with stained tissue sections was retarded, however, until single vessels could be visualized by both methods. To date 12 patients have been studied by the techniques to be described correlating histological, radiographic, and microradiographic data. The present report (a) describes a technique for correlating radiograms with stained sections, and (b) uses the technique to demonstrate normal capillary bed in one patient and prominent dilation of capillaries in two patients with pulmonary hypertension.

Patients Studied

Patient with Normal Lungs

J. R., a 47-year-old white man, had acute myocardial infarction complicated by complete heart block. He died suddenly 36 hours after insertion of an intravenous pacing electrode. At postmortem examination his lungs were normal by gross and microscopic study.

Patient with High Pulmonary Artery Pressures and Increased Pulmonary Blood Flow (Normal Pulmonary Vascular Resistance)

J. B., a 6-year-old Negro female, had evidence on physical examination of a large left-to-right shunt with pulmonary hypertension. At cardiac catheterization the catheter passed through both a patent ductus arteriosus and ventricular septal defect. Pulmonary blood flow was more than five times systemic flow, and there was severe pulmonary artery hypertension (table 1). At operation the large membranous defect and

From the Departments of Medicine, Pathology, Pediatrics, and Anatomy of the Schools of Medicine and Dentistry, University of Kentucky, Lexington, Kentucky.
Table 1

Summary of Data on Two Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>J.B.</th>
<th>J.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>Pressures (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>95/8</td>
<td>110/5</td>
</tr>
<tr>
<td>AO</td>
<td>95/54</td>
<td>110/70</td>
</tr>
<tr>
<td>PA</td>
<td>78/46</td>
<td>80/35</td>
</tr>
<tr>
<td>RV</td>
<td>78/8</td>
<td>90/5</td>
</tr>
<tr>
<td>LA</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>( C_{O2} - C_{V2} ) (cc/L)</td>
<td>Pulmonary</td>
<td>9</td>
</tr>
<tr>
<td>Systemic</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td>Eff. pulm.*</td>
<td>55</td>
<td>67</td>
</tr>
<tr>
<td>( \dot{V}_{O2} ) (cc/min/m²)</td>
<td>(assumed)</td>
<td>160</td>
</tr>
<tr>
<td>Flow (L/min/m²)</td>
<td>Pulmonary</td>
<td>17.0</td>
</tr>
<tr>
<td>Systemic</td>
<td>2.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Eff. Pulm.</td>
<td>2.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Resistance (units/min/m²)</td>
<td>Pulmonary</td>
<td>2.5</td>
</tr>
<tr>
<td>Systemic</td>
<td>26.3</td>
<td>34.8</td>
</tr>
</tbody>
</table>

*Effective pulmonary arteriovenous oxygen difference is oxygen content of pulmonary venous blood less than that in the vena cavae.

Abbreviations: \( C_{O2} - C_{V2} \) = difference in concentration of oxygen arterial and venous blood; \( \dot{V}_{O2} \) = oxygen consumption per minute.

large patent ductus arteriosus, diagnosed at catheterization, were repaired; in addition, two smaller muscular defects and a second membranous defect beneath the tricuspid valve were found. The patient died 8 hours after the long surgical procedure required to correct the multiple defects. Postmortem examination confirmed the cardiac lesions noted at surgery.

Pulmonary Hypertension with Normal Pulmonary Blood Flow (High Pulmonary Vascular Resistance)

J. M., a 29-year-old white woman, was known to have had heart disease since early childhood. Three years prior to her death cyanosis and clubbing developed. Clinical examination suggested severe pulmonary hypertension without a large pulmonary blood flow (table 1). Cardiac catheterization revealed a bidirectional shunt at the atrial level with a low systemic blood flow, normal pulmonary blood flow, and severe pulmonary hypertension with elevated pulmonary vascular resistance. Surgical correction was attempted, but the patient died of right ventricular failure with a low cardiac output 6 days after operation. At postmortem examination no additional intracardiac lesions were noted.

Cardiac catheterization was carried out with the patients awake but under mild seconal sedation. Systemic and pulmonary pressures were recorded simultaneously using Statham pressure transducers and an 8-channel recorder.* In calculating pulmonary and systemic flows the Fick principle was utilized with oxygen as the indicator. Oxygen uptake was estimated assuming a basal state. Oxygen content of blood for samples drawn from left and right heart chambers was determined by the Van Slyke manometric method. Blood oxygen capacity was by the Sendroy method.

Radiographic and Histological Techniques

Lungs removed at postmortem examination were perfused via the pulmonary artery with a warm (40°C) mixture of barium sulfate and gelatin at a constant pressure equal to the assumed (or measured) systolic pulmonary arterial pressure which had been present in life. Use of high pressures in normal lungs disrupts the vessels with loss of barium into the alveoli. Use of low pressures in hypertensive lungs does not

*Electronics for Medicine, White Plains, New York.
Figure 1

Macroradiogram, $\times$ 2. Normal lung slice, 1 cm thick, from J. R., age 47 years.

Figure 2

Macroradiogram, $\times$ 2. Lung slice, 1 cm thick, from J. B., age 6 years, having high pulmonary arterial pressure and flow.

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achieve adequate filling of the microcirculation. The lungs were distended with Formalin via the trachea and after 1 month were sliced on a commercial meat slicer (Horton Co.) at 1 cm thickness. The whole lung slices were then radiographed at 80 cm, 40 kv, and 50 ma (microradiogram). To this point the technique was like that previously described.\(^2\)\(^,\)\(^12\)

A cube, approximately 1 by 1 by 1 cm, was then removed from the sliced lung and processed in the usual fashion for embedding in paraffin. Sections 150 \(\mu\) thick were cut with a Spencer rotary microtome. The sections were deparaffinized in xylene, and hydrated progressively to water. They were gently floated to a 1 by 3 inch glass slide (Kodak high resolution spectroscopic plates, type 649-0) and radiographed as previously described\(^12\) (microradiogram) with a Phillips CMR 5 Microradiograph. The same 150 \(\mu\) thick tissue sections were floated free, next they were placed between two pieces of ordinary memo pad paper and then between two perforated aluminum screens. The paper prevented the tissue from bulging through the mesh of the screens. The two screens were bound together gently at their edges with thread. This unit was placed in 80% alcohol for 15 minutes, and then processed by hand through alcohol, chloroform, xylene, and paraffin—a process requiring 2 hours. Flat embedding is essential if microtome sections from such thin tissue are to include representative portions of all processed material. The block was then sectioned serially at 7 \(\mu\) on the microtome. Approximately 20 satisfactory sections were obtained. After mounting, they were stained alternately with Harris’ hematoxylin and Verhoeff’s elastic and van Gieson stains.

**Results**

We have not used the term “pulmonary arteriole” because its definition varies among authorities\(^14\) and designations according to size and presence of smooth muscle are difficult where the disease process alters the normal anatomy. We have followed Naeye and Venhart\(^6\) in using the more general term “artery” to specify inflow channels. We utilize the term “precapillary” to designate the short (10 to 20 \(\mu\)), right-angle capillaries\(^4\) which connect small arteries and capillary network. This term seems justified here in that these vessels demonstrate a characteristic microradiographic appearance,\(^13\) and they are specifically involved in the changes to be described below.

**Macroradiograms**

**Normal Lung**

Macroradiogram of lung from J. R. (fig. 1) illustrates the normal vascular pattern with
gradually tapering smooth pulmonary arteries. Diffuse background haze represents contrast within vessels too small (less than 200 μ) to be resolved by this technique.

**Pulmonary Hypertension with High Flow (Normal Resistance)**

Macroradiogram from J. B. (fig. 2) shows several greatly enlarged pulmonary arteries, a profusion of small vessels well into the periphery of the lung, and a prominent background haze of opacified microcirculation. Many small discrete vessels and prominent background haze are seen.

**Pulmonary Hypertension and Normal Blood Flow (High Resistance)**

The patient J. M. (fig. 3) has enlarged pulmonary arteries which also taper abruptly at the periphery. Background haze is strikingly diminished and that which is present forms a coarse reticulum. A few small bronchial arteries are seen (arrows).

**Correlation of Microradiograms and Histology**

**Normal Lung**

Microradiogram from J. B. (fig. 4) shows right-angle branching of a terminal pulmonary artery (PA). Short (10 to 20 μ) right-angle branches, the precapillaries (P), connect artery and capillary bed (C). The capillary networks are seen well and the capillary lumina are 8 to 12 mm in diameter. Histological section of this artery (fig. 5) shows a precapillary and part of the capillary network. Note the thin wall of the small pulmonary artery.

**Pulmonary Hypertension and High Flow (Normal Resistance)**

Microradiogram from lung of J. B. (fig. 6) shows a small pulmonary artery with dilated
(20 μ) precapillary segments, and dilated (30 μ) capillaries adjacent. Other dilated vessels in the field give the microcirculation a typical knobby appearance. Histological section of these vessels (fig. 7) shows the small artery, the dilated parallel capillary, and a short broad thin-walled channel (precapillary) connecting them. Capillaries and precapillaries are dilated to three times normal size. Other capillaries at greater distance from the artery are more nearly normal size.

Pulmonary Hypertension and Normal Flow (High Resistance)

Microradiogram from J. M. (fig. 8) shows a 100 μ artery with an 80 μ branch. Above is a 50 μ diameter tortuous vessel which communicates with the artery below it through several short, right-angle branches and with dilated capillaries above it and to the left. This adjacent parallel channel has the appearance of a “caterpillar” on a twig. Histological section of these vessels (fig. 9) demonstrates that the “caterpillar” has a thin irregular wall, is near the arteriole, and communicates distally with capillaries of near normal diameter. Thus dilated thin-walled vessels occupy an intermediate position between the arteries and the pulmonary capillary bed.

A complex vascular maze (fig. 10) was commonly found in patient J. M. Shown are a relatively normal capillary network, right, and greatly dilated capillaries with a vestige of a network appearance, left. Histological section of this vascular maze (fig. 11) illustrates the relatively thick-walled arteries and the thin-walled dilated channels bulging into the airways. Figure 12 demonstrates segmental narrowing within a pulmonary artery. The microradiogram (fig. 12) demonstrates connections...
distal to the obstruction of this artery with another pulmonary arterial vessel. Dilated capillaries frequently provided collaterals which bypassed obstruction in small arteries, as suggested by Harris and Heath. Hypertensive pulmonary vascular lesions, grade 2 (fig. 13), were frequently seen in the lung of J. M. and account for much of the high pulmonary vascular resistance found in this patient.

Discussion

The normal human pulmonary circulation is characterized by branching thin-walled pulmonary arteries which taper smoothly as they go to the periphery, short (10 to 20 μ) precapillaries which arise at right angles to the arteries and which supply a richly anastomosing capillary bed. The macroradiograms, microradiograms, and histological sections permit identification of each of these components of the pulmonary vascular bed.

Patient J. B. had pulmonary arterial hypertension and a high pulmonary blood flow without increased vascular resistance. There was dilatation of the grossly visible pulmonary arteries as well as generalized mild dilatation and medial hypertrophy of the small pulmonary arteries and capillaries. A dilated pulmonary arterial bed in the presence of a sustained high flow was indicated by Wagenvoort and associates. In addition, this patient showed capillary dilatation, particularly in the proximal capillaries. Yet there was little distortion of the microvascular pattern since capillaries could be readily distinguished from small arteries.

Patient J. M. with pulmonary hypertension and high vascular resistance showed gross enlargement of the large pulmonary arteries but
few small vessels distributing the arterial inflow to the capillaries. Microradiography showed the segmental obstruction, and histological examination confirmed the intimal proliferation within some small pulmonary arteries. Other small pulmonary arteries were patent and fed dilated and distorted precapillaries and capillaries. The dilatation lesions in this patient assumed two general forms. One we dubbed the “capillary caterpillar” because it was found beside an arterial twig and had multiple short attachments to it. The other we called the “vascular maze.” The evidence that dilated capillaries were responsible for both these lesions may be summarized as follows: (1) The dilated vessels were of irregular outline and had thin walls. (2) The dilated thin-walled vessels communicated distally with other capillaries some of which formed dilated networks. (3) The “caterpillars” had a parallel position close to the artery with short multiple right-angle connections suggesting the arrangement of the normal capillary joined to the artery by precapillaries. (4) The “caterpillars” showed various degrees of dilation (diameters ranging from 10 to 100 μ) in different areas within the lung. (5) The network appearance within the “vascular maze” indicates the dilated remnant of interconnecting alveolar capillaries. Presumably one consequence of capillary dilation in this patient would be to assist pulmonary perfusion. An unfavorable consequence could be pulmonary hemorrhage from thin-walled dilated vessels which bulge into airways and which carry blood under high pressure.

Pulmonary A-V fistulas seem an unlikely cause of the dilated vessels since pulmonary

Figure 7

Photomicrogram. Lung slice, 7 μ thick, showing the vessel in figure 6. Verhoeff’s elastic and van Gieson’s stains; × 360.
capillary networks, not pulmonary venous channels, are seen distal to the lesions.6 Bronchopulmonary anastomoses must be considered because complex anastomoses between bronchial and pulmonary arteries have been described14; bronchial arteries run parallel to pulmonary arteries; bronchial arterial supply increases in areas of focal pulmonary arterial obstructions8; and bronchial arteries were seen in the gross radiograms of patient J. M. However, the bronchial arteries we identified were small, and the dilated vessels with their thin walls and network configuration suggested that dilation of capillary channels, not bronchial pulmonary anastomoses, had occurred. Plexiform lesions have been described in irreversible pulmonary hypertension and are associated with thin-walled dilated vessels,4 which communicate distally with capillaries.6,15 As reviewed by Wagenvoort and associates,4 most authorities agree that the initial portion of the plexiform lesion is found in arterioles showing intimal fibrosis and medial hypertrophy. Microradiography could provide a fresh approach to the study of the pathogenesis of these lesions.

The structure of the small pulmonary vessels may render them particularly vulnerable to the effects of sustained high pressure and flow. The pulmonary arteries are thin-walled, short, and broad. Because the capillary bed is but 10 to 20 microns distant from the arterial inflow (fig. 4), high pressure within pulmonary arteries could easily be transmitted to precapillaries and to the proximal capillaries. Because of the large capacity of the pulmonary capillary bed with the rich intercommunication between capillary networks, velocity of flow and pressure should decrease rapidly as inflowing blood penetrates farther and farther

Figure 8

Microradiogram, × 400. Lung slice, 150 μ thick, from J. M., age 29 years. High pulmonary arterial pressure and high pulmonary vascular resistance.
into the capillary bed. The precapillaries and the proximal capillary segments would then be subjected to higher pressures and flow rates than the more distal capillaries. This hypothesis is in agreement with the findings in the patients presented. Our studies do not, however, separate the relative roles of pressure and flow in dilating the microcirculation.

Wagenvoort and co-workers indicated that high flow alone caused arteriolar dilation in patients with atrial septal defects, but he did not present data relating to capillaries. Further work is necessary to establish how the changes shown relate to increased pressure, flow, and the duration of each.

**Figure 10**

Microradiogram, × 450. Lung slice, 150 µ thick, from J. M., age 29 years. High pulmonary arterial pressure and high pulmonary vascular resistance.
The combination of microradiography with histological examination provides an additional approach to the study of such problems in pulmonary hypertension. For example, Lindgren has recently used it to demonstrate the membrane surrounding capillaries in hyaline membrane disease. The correlation of stained sections with microradiograms of the same tissue is superior to comparison of a radiogram with the histological features from separate slices. The difficulty which had to be surmounted to obtain direct comparison, was that tissue only 150 μ thick did not lie flat during conventional processing for paraffin embedding. The solution found was to process the tissue after it was pressed flat between two screens. The present report emphasizes a microradiographic technique of potential value in the study of the pulmonary circulation. We have presented preliminary results visualizing the microcirculation in three patients to illustrate this technique and to raise questions which deserve further investigation.

**Summary**

Obstruction within pulmonary arteries is a well-known cause of pulmonary hypertension in patients with congenital heart disease, but dilatation of the pulmonary vessels is less well understood. The present report has shown that the technique of microradiography is well suited to the study of dilation of the pulmonary microcirculation because (1) vessels as small as capillaries are resolved, (2) whole capillary networks are seen in relation to the arteries feeding them and to the alveoli, and (3) the radiographed tissue may

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*Photomicrogram. Lung slice, 7 μ thick, show vessels in figure 10. Verhoeff’s elastic and van Gieson stains; × 450.*
then be sectioned and stained for direct comparison of the histological and microradiographic aspects. The present study demonstrated the technique in a case of normal lungs, one of pulmonary hypertension and a high pulmonary blood flow, and in one of pulmonary hypertension and high pulmonary vascular resistance. Dilated capillaries were demonstrated in both patients with pulmonary hypertension. Greater distortion of the microvascular pattern was seen in the patient with high vascular resistance. Correlation of radiographic and histological examination provides an additional tool for study of the normal and abnormal pulmonary circulation.

Acknowledgment

The authors acknowledge the technical assistance of Mr. John Starrs and Mrs. Janet Moberly.

References

PULMONARY VASCULAR BED

Figure 13

Photomicrogram, × 390. Lung slice, 7 μ thick, from J. M. shows partial obstruction of an arteriole by intimal fibrosis.

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