Effect of Edema on Segmental Vascular Resistance in Isolated Lamb Lungs Determined by Micropuncture

J. Usha Raj and Priscilla Chen

We have studied the mechanical effects of fluid accumulation on the pulmonary vasculature in 28 isolated blood perfused lungs of newborn lambs. Vascular resistance in the pulmonary arteries, microvessels, and veins was determined by micropuncture measurement of microvascular pressures, and regional distribution of blood flow in the lungs was determined using radiolabelled microspheres both before and after the development of varying degrees of hydrostatic edema. Edema was induced by raising venous pressure. During measurements, alveolar and venous pressures were kept constant at 7 and 8 cm H2O, respectively, as well as lung blood flow (540 ± 107 ml/min). All vascular pressures were referenced to the superior surface of the lung, site of all micropunctures. Active vasomotor changes were eliminated by addition of papaverine to the perfusate. Under baseline nonedematous conditions in the absence of vasomotor tone, 17% of the total pressure drop was in arteries, 41% was in microvessels, and 42% was in veins. With the development of alveolar edema (80 ± 13% weight gain), there was no change in total or segmental vascular resistance, but after 148 ± 97% weight gain, total pulmonary vascular resistance increased by 74%. Segmental pressure drop increased in arteries by 172% and in microvessels by 132% but decreased by 22% in the venous segment. Regional distribution of blood flow remained unchanged. Possible mechanisms for increased resistance to blood flow may be compression of small arterioles and venules (<20 μm diameter) by liquid cuffs and/or occlusion of microvessels by the weight of alveolar liquid. (Circulation Research 1987;61:236-243)

The response of the pulmonary circulation to edema has been studied extensively in adult animals, but there is very little information available about the effects of edema on the neonatal pulmonary circulation. Furthermore, in spite of the body of work on this subject, controversy still exists regarding the severity of edema that results in increased pulmonary vascular resistance and the site of increased resistance to blood flow. West and coworkers and, later, Hughes suggested that perivascular cuffs formed during the stage of interstitial edema may compress extra-alveolar vessels and cause a reduction of blood flow to the base of the lung. However, more recent studies suggest that vascular resistance does not increase until alveolar flooding occurs. Some studies have suggested that the site of increased resistance to blood flow may be the extraalveolar vessels, while other reports suggest that the weight of edema fluid in alveoli may compress alveolar wall capillaries. Conflicting results from different studies done in a variety of animal species and under different experimental conditions keep this issue unresolved.

The purpose of this study was to determine the effects of hydrostatic edema on the neonatal pulmonary circulation and, specifically, to determine the degree of edema that results in increased pulmonary vascular resistance, to identify the site and mechanism of increased resistance to blood flow, and to determine the effect of edema on the distribution of blood flow in the lungs. Determination of the pulmonary vascular pressure profile and segmental vascular resistance in the lung enables us to determine which segment(s) of the pulmonary vasculature may have undergone a change in caliber. Hence, we used isolated blood-perfused lungs of newborn lambs and determined the effect of edema on segmental vascular resistance by micropuncture measurement of microvascular pressures. Also, the effect of edema on the slope and intercept of the vascular pressure-flow relation in the lungs was determined. Papaverine was added to the perfusate to paralyze vascular smooth muscle so the mechanical effects of fluid accumulation on the pulmonary vasculature could be studied without active vasomotor changes that may be induced by hypoxia or vasoactive agents released by the lung during edema formation.

In nonedematous lungs, in the absence of vasomotor tone, only 17% of the total pressure drop was in arteries, but 41% was in microvessels and 42% was in veins. Alveolar edema developed after 80 ± 13% weight gain without any change in total or segmental vascular resistance. After a weight gain of 148 ± 97%, total vascular resistance increased by 74%, mainly because of an increase in resistance in arteries and microvessels (with a small decrease in venous resistance).
Materials and Methods

Isolated Lung Preparation

Lungs of 28 lambs, whose average age and body weight was 10 ± 4 days and 5.9 ± 1.6 kg, respectively, were isolated and perfused as previously described. Briefly, animals were anesthetized with ketamine (25 mg/kg i.m.), and catheters were placed in the carotid artery and jugular vein. An endotracheal tube was tied into the trachea, and ventilation with 100% oxygen was instituted by hand. After infusing 5% dextran 70 in Ringer’s lactate solution (20 ml/kg), heparin (1,000 IU/kg), and sodium pentobarbital (25 mg/kg) intravenously, the lambs were rapidly exanguinated through the carotid artery catheter. To obtain an adequate volume of blood for the perfusion circuit, additional 5% dextran solution was infused intravenously during exanguination. After sternotomy, the heart and lungs were removed and weighed. The superior and inferior vena cava, ascending aorta, ductus arteriosus, and transverse sinus were ligated, and a suture was tied around the atrioventricular groove to occlude the ventricular lumen. Cannulas filled with 5% dextran were tied into the pulmonary artery and left atrium and were connected to the perfusion circuit.

Time from onset of exanguination to perfusion was ~20 minutes.

The lungs were perfused with the lamb’s own blood. Papaverine hydrochloride was added to achieve a perfusate concentration of 100 μg/ml. To test whether the vasculature was effectively paralyzed, 20 μg angiotensin was injected as a bolus into the pulmonary artery both before and after adding papaverine to the perfusate. The 5-7 cm H₂O increase in pulmonary artery pressure produced by angiotensin was abolished after addition of papaverine. Perfusate was kept at body temperature (38-39° C) with a Travenol Miniprine heat exchanger and was continuously filtered of clots (Pall, Ultipore, New York). A bubble trap in the circuit acted between micropuncture containing 6% CO₂-94% O₂ and by addition of 50% glucose in water.

The lungs were ventilated with a gas mixture containing 6% CO₂-94% O₂ between micropuncture and were kept steadily inflated during micropuncture at an airway pressure of 7 cm H₂O. Blood Po₂ was kept >200 torr. During perfusion, weight gain in the lungs was assessed by the decrease in volume of perfusate in the reservoir. The lungs were weighed before starting perfusion and after the end of the experiment.

Microvascular Pressure Measurement

In 17 lungs, micropipettes and the servo-nulling technique were used to measure pressures in 20–80-μm diameter subpleural arterioles and venules. Glass micropipettes with a tip diameter of 2–4 μm were filled with 1.2 M NaCl colored with green dye and were connected electrically and hydraulically to a servo-null pressure-measuring system (model 4A, Instruments for Physiology and Medicine, San Diego). The lung surface was stabilized with a metal ring that also held a pool of normal saline for obtaining the zero reference pressure. Subpleural vessels were viewed through a Zeiss stereomicroscope at 80 × or 120 × magnification with illumination from a cold light source (Intralux 5000, Volpi Co., Auburn, N.J.). Venules were identified by observing the direction of blood flow from small to larger vessels; in arterioles, flow occurred in the opposite direction. Valid microvascular pressure measurements fulfilled the standard criteria.

Paired venular and arteriolar pressure measurements during baseline and after severe edema were obtained in 14 and 4 of the 17 lungs, respectively. However, all venular and arteriolar pressure measurements have been reported.

Pressure–Flow Curves

Vascular pressure–flow relation in the lungs was determined by adjusting blood flow rate from 80 to 680 ml/min. This range of flow rates was used because it yielded a linear pressure–flow relation (correlation coefficient >0.99). Flow rate was increased in discrete steps every 30 seconds by adjusting pump speed, and pulmonary artery pressure was recorded. Left atrial pressure was kept constant at 8 cm H₂O, and airway pressure was 7 cm H₂O.

Experimental Protocol

All lungs were perfused for 15–30 minutes during a baseline period during which blood flow was adjusted to maintain pulmonary artery pressure around 30 cm H₂O, and left atrial and airway pressures were 8 and 7 cm H₂O, respectively. Once adjusted, blood flow was kept constant for the rest of the experiment (92 ± 27 ml/kg/min). Hydrostatic edema was induced by elevating left atrial pressure to 25 cm H₂O and by continuing perfusion. Once the alveoli and airways were filled with liquid, ascertained by the appearance of edema fluid in the trachea (alveolar edema period), left atrial pressure was lowered to 8 cm H₂O for measurements during this period. Perfusate hematocrit was measured more frequently (at approximately 2–5-minute intervals) after alveolar edema had developed, and 5% dextran solution was added to the perfusate if necessary to keep hematocrit constant. The lungs were perfused again with elevated left atrial pressure, and
edema formation continued until pulmonary vascular resistance (PVR) increased significantly. Again, we ascertained that the vasculature was paralyzed by observing no response to separate bolus injections of angiotensin (200 \(\mu\)g) and prostacyclin (5–10 \(\mu\)g). Left atrial pressure was once again lowered to baseline, and measurements were obtained during this period; this will be referred to as the severe-edema-with-increased-PVR period. In 17 lungs, microvascular pressure measurements were obtained during baseline and again after alveolar and severe edema had developed, and in 6 lungs, vascular pressure flow data were obtained during all three periods: baseline, alveolar edema, and severe edema with increased PVR.

**Effect of Left Atrial Pressure Elevation on Pulmonary Vascular Resistance During Severe Edema**

We wished to determine whether elevation of vascular intraluminal pressures might oppose the external pressures and blood flow were stable, an injection of 15-\(\mu\)m diameter radionuclide microspheres was made. The turbulence created by the injection facilitated mixing of the microspheres. At the end of the experiments, blood flow was stopped, the trachea was clamped at 7 cm H₂O airway pressure, and the lungs were frozen in liquid nitrogen. The lungs were cut into 1-cm-thick horizontal slices (average height of lungs was 9 cm). At the site of micropuncture, a 1-mm-thick slice of the subpleural region was cut. Each slice was cut into 1-cm² pieces, and the anatomic location of each piece was noted. Lung pieces were weighed before radionuclide counting of the two tracers ⁴⁶Sc and ⁹⁰Sr in a multiple channel gamma counter (LKB Wallac 1282 Compu Gama, Finland). The fractional blood flow to each slice was determined and was expressed as ml/min/g dry lung, Lung wet-to-dry-weight ratio for each slice was also determined.

**Lung Morphometric Measurements**

Eight lungs were frozen in liquid nitrogen at the end of the experiments: 4 were frozen after alveolar edema and 4 were frozen after severe edema. The lungs were cut at the hilum at −30°C, the hilar surface was planed smooth with a microtome knife, and a block was cut out that included the hilar vessels. The blocks were placed on dry ice, and the hilar surface was photographed at constant magnification. The slides were projected onto a screen with a grid of equidistant points; the number of points within the cut surface of the vessel and within the perivascular liquid cuff were counted, and the ratio of points within the cuff to that within the vessel was calculated. All vessels with thick walls and lying adjacent to airways were considered as arteries, and other vessels were considered as veins.

**Data Analysis**

All data reported are mean ± SD for each group of lungs. To compare longitudinal segmental pressure drops during each condition, i.e., baseline and severe edema, we used a two-way analysis of variance and applied the Newman-Keuls test. Both a paired \(t\) test for all paired measurements and an unpaired \(t\) test were used to compare vascular pressures and segmental pressure drops between baseline and severe edema periods in the group of lungs used for micropuncture.

For pressure flow points, linear regression analysis was performed and a slope and intercept in each lung were obtained for the three experimental periods. Mean data for both slopes and intercepts were compared using a two-way analysis of variance and the Newman-Keuls test. A \(p\) value of <0.05 was accepted as indicative of statistical significance.

**Results**

Perfusate Po₂ (308 ± 98 torr), Pco₂ (38 ± 9 torr), and pH (7.38 ± 0.05) remained stable throughout the experiments. Perfusate hematocrit was 17.4 ± 4.4% at the start of perfusion and was constant during the baseline period. After development of alveolar edema, hematocrit rose rapidly to 24.2 ± 6.0%, but with addition of 5% dextran in Ringer’s lactate, hematocrit was maintained at 17.2 ± 4.5%.

Initial lung weight was 198 ± 32 g. When alveolar edema with edema fluid in the trachea developed, the lungs had gained 80 ± 13% of initial weight. With a further increase in weight gain up to 148 ± 97% of the initial weight, there was a significant increase in total pulmonary vascular resistance. All lungs had gained >100% of initial weight before total pulmonary vascular resistance increased. The time interval between the development of alveolar edema and the increase in vascular resistance was 38 ± 10 minutes.

Under baseline nonedematous conditions in lungs with no vasomotor tone, only 17% of the total arteriovenous pressure drop was in arteries and 41% was in microvessels and 42% was in veins (Table 1). With interstitial and early alveolar edema, there was no change in total or segmental vascular pressure drops. With severe edema, total pulmonary vascular resistance increased by 74% of initial baseline values. Segmental vascular resistance increased by 172% of the baseline value in arteries and by 132% in microvessels, with a small decrease (22%) in venous resis-
Table 1. Effect of Edema on Pulmonary Microvascular Pressure Profile in 17 Isolated Perfused Lamb Lungs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pulmonary artery</th>
<th>Arterioles 20-80 μm</th>
<th>Venule 20-80 μm</th>
<th>Left atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline no edema</td>
<td>29.3±2.6</td>
<td>25.7±4.0 (6)</td>
<td>17.0±2.9 (20)</td>
<td>8.0±0.0</td>
</tr>
<tr>
<td>Severe edema with ↑ PVR</td>
<td>45.0±4.9↑</td>
<td>35.2±1.2↑ (6)</td>
<td>15.0±2.1↑ (14)</td>
<td>8.0±0.0</td>
</tr>
</tbody>
</table>

Results are mean ± SD.
*Pressures in cm H₂O relative to pleural pressure (atmospheric) at level of micropuncture. Blood flow was constant at 92 ± 27 ml/kg body wt/min. Number of micropuncture pressure measurements in parentheses.
†Different from baseline by paired t test, p<0.05.
‡Different from baseline by both paired and unpaired t test, p<0.05.

Perivascular interstitial liquid cuffs at the hilum were present in all 8 lungs that were frozen after development of alveolar edema (n = 4) and severe edema with increased PVR (n = 4). The size of the liquid cuffs, however, was smaller in lungs with early alveolar edema than in lungs with severe edema (Table 3).

Discussion

Segmental Vascular Resistance in Nonedematous Lamb Lungs With no Vasomotor Tone

In the absence of vasomotor tone, the pulmonary arteries impose very little resistance to blood flow in the lung (17%), while the microvessels (41%) and veins (42%) are the major sites of resistance to flow. This is similar to the low arterial resistance (15%) reported in isolated perfused adult dog lungs with low basal vascular resistance using the micropuncture technique.14 It appears that when basal vasomotor tone is low, microvessels become a major site of resistance to flow. However, in isolated perfused lamb lungs without paralyzed vasculature, basal vascular resistance is high mainly because of increased vasomotor tone in the arteries.13

Venous resistance is higher than arterial resistance in lamb lungs with paralyzed vasculature. We believe that this is due to a lower distending pressure in veins resulting in a smaller caliber of the veins since vessel distensibility is the same in arteries and veins.13

A high basal venous resistance seems to be a feature of the neonatal lung; in 3-4-week-old rabbit lungs,

![Figure 1. Pulmonary vascular pressure profile in nonedematous and severely edematous lamb lungs. Vascular pressures (in cm H₂O, relative to level of micropuncture, pleural pressure being atmospheric) are shown as ± SD for a group of 17 lungs: *p < 0.05 by paired t test different from baseline. Severe edema resulted in significant increases in pulmonary artery and arteriolar pressures with small decrease in venular pressure.](http://circres.ahajournals.org/)

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tance (Table 1 and Figures 1 and 2). The major site of resistance to blood flow in edematous lungs was the microvasculature.

In 6 lungs, when left atrial pressure was elevated to 25 cm H₂O after total vascular resistance had increased, the total arteriovenous pressure drop decreased from 32 ± 7 to 23 ± 5 cm H₂O, a 39% decrease in total pulmonary vascular resistance. The effect of left atrial pressure elevation was to distend the vasculature and, thus, to reduce total vascular resistance.

With the development of alveolar edema, there was no change in the slope in the extrapolated pressure intercept of the vascular pressure flow lines in any of the lungs. However, after pulmonary vascular resistance had increased with severe edema, there was a significance increase in both the slope and pressure intercept of the pressure flow lines (Table 2 and Figure 3).

Blood flow distribution along a vertical gradient in 5 lamb lungs was uniform during baseline (blood flow, 0.34; height, +16.2; r = 0.43; n = 50) and remained unchanged even after the development of severe edema with increased vascular resistance (blood flow, 0.22; height, +17.1; r = 0.38; n = 50) (Figure 4). Blood flow in the 1-mm-thick subpleural region was 15 ± 6% lower than that in the middle of the lung during both baseline and severe edema.

There was a significant correlation between lung height and lung wet-to-dry-weight ratio, which were 8.3 ± 1.6 at the top of the lung and 11.2 ± 0.1 in the lowest slice (Figure 5).
Table 2. Vascular Pressure Flow Data In 6 Lamb Lungs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Slope (cm H$_2$O/ml/min)</th>
<th>Pressure intercept (cm H$_2$O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.046 ± 0.013</td>
<td>9.2 ± 2.2</td>
</tr>
<tr>
<td>Alveolar edema</td>
<td>0.048 ± 0.015</td>
<td>9.4 ± 2.4</td>
</tr>
<tr>
<td>Severe edema</td>
<td>0.062 ± 0.014*</td>
<td>12.9 ± 3.8*</td>
</tr>
</tbody>
</table>

Results are mean ± SD. *p < 0.05 by ANOVA; different from baseline and alveolar edema.

Effect of Severe Edema on Pulmonary Vascular Resistance

We have defined severe edema as a weight gain of >100% (148 ± 97%) with an associated increase in total vascular resistance. With the appearance of fluid in the trachea, a point at which alveolar flooding is thought to have occurred, there was no change in vascular resistance. It was only after another 30–40 minutes of continued perfusion and weight gain that pulmonary vascular resistance slowly increased. Thus,
it appears as if the onset of alveolar flooding does not in itself lead to an increase in vascular resistance; rather, a certain degree of weight gain must occur, and at least 30 minutes must elapse from the onset of alveolar flooding before vessels are compressed. During the period of continued weight gain after alveolar flooding, blood hematocrit tended to rise, so additional 5% dextrose in Ringer's lactate had to be added to the perfusate to keep hematocrit constant. When hematocrit was allowed to rise, pulmonary vascular resistance increased further in a stepwise manner.

Site(s) of Increased Resistance to Blood Flow

The increase in total pulmonary vascular resistance with severe edema was mainly due to an increase in resistance in arteries (172%) and microvessels (132%). It is interesting that venous resistance did not increase, but there was a small, though significant, decrease in venous resistance. It is possible that with the development of severe edema as total lung volume increased, the parenchymal attachments to the veins may have exerted radial stresses, which resulted in actual distention of the veins.

Our observations on the mechanical effects of edema on segmental vascular resistance in lamb lungs are different from those in adult rabbit lungs. In rabbits, all three vascular segments were compressed in a manner such that the fractional resistance imposed by each segment was unchanged from baseline. In lamb lungs, since only arteries and microvessels were compressed, the distribution of segmental vascular resistance changed after severe edema, and microvessels became the major site of resistance to flow (55%) with arteries imposing 26% and veins only 19% of total pulmonary vascular resistance.

We are uncertain as to the exact mechanism by which resistance in arteries and microvessels increased during severe edema. Since the pressure drop in the venous segment (from 20-80 μm venules to left atrium) changed little, we must assume that vascular compression occurred at some point downstream from the pulmonary artery up to the 20-μm venule. Further, since the large arteries have thick walls and, in our experiments, had fairly high intraluminal pressures, the most likely sites of compression are the small non-muscular vessels and capillaries. It is possible that the high arteriolar intraluminal pressures may have resulted in increased fluid filtration with larger liquid cuffs around arterioles and therefore in compression of these vessels. It is clear that the increased resistance of the arteries and microvessels is due to external compression by edema fluid since raising vascular intra-

**Table 3. Hilar Cuff to Vessel Area Ratio**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of lungs</th>
<th>Artery</th>
<th>Vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar edema</td>
<td>4</td>
<td>0.81 ± 0.42</td>
<td>0.84 ± 0.16</td>
</tr>
<tr>
<td>Severe edema with ↑ PVR</td>
<td>4</td>
<td>1.94 ± 0.41*</td>
<td>1.50 ± 0.33*</td>
</tr>
</tbody>
</table>

Results are mean ± SD.

*p < 0.05 by unpaired t test; different from alveolar edema.
luminal pressures by left atrial pressure elevation resulted in a 39% decrease in total arteriovenous pressure drop, which indicates that some degree of vascular compression was overcome by elevating intraluminal pressure in the compressed vessels.

Conhaim and coworkers\(^2\) studied the sequence of perivascular liquid accumulation in dog lungs and found that liquid cuffs formed readily and more frequently around arteries <0.5 mm in diameter than in similar size veins. After 45-300 minutes of inflation of liquid-filled lobes, they found that >90% of arteries ranging from 100-500-μm diameter had liquid cuffs that had attained maximum size and that 38% of small veins <500 μm in diameter had liquid cuffs. This pattern of perivascular liquid accumulation also appears to be present in sheep lungs (Conhaim, personal communication). Cuffs around hilar vessels were found to be larger in severely edematous lungs than in lungs with alveolar edema alone. The cuff size around small nonmuscular arteries and veins was unable to be assessed. If perivascular liquid cuffs around small nonmuscular arteries and veins can result in vascular compression, this would explain the increased resistance in arteries and microvessels with little change in venous resistance. Morphometric measurements in adult dog lungs by Smith and coworkers\(^2\) indicate that liquid cuffs do not compress large arteries and veins. However, there is little information regarding the effect of perivascular liquid cuffs on small nonmuscular arterioles and venules in neonatal lungs.

**Effect of Severe Edema on Regional Distribution of Blood Flow in the Lungs**

We did not find any change in the regional distribution of blood flow in the lungs after severe edema. This finding is different from the response of the adult lung to severe edema. Muir et al.\(^2\) reported an inversion of blood flow in adult dog lungs when both alveolar and interstitial edema were present. As hypoxia also results in a similar redistribution of blood flow from the base to the top of the lungs,\(^2\) it is possible that hypoxic vasoconstriction contributed to the redistribution of blood flow seen during edema in their experiments. Even in the presence of paralyzed vasculature, severe edema in adult rabbit lungs resulted in marked decreases in blood flow along a vertical gradient from the top toward the base of the lung, and we speculated that this may have been due to progressive derecruitment of capillaries toward the base of the lungs. Because we kept blood flow constant and allowed vascular pressures to increase, the high intraluminal pressures in the experiments with lamb lungs may have prevented total collapse of vessels. Overall, the response of the neonatal lamb lung to edema is different from that of the adult rabbit and dog lung; species- or age-related differences may be responsible.

In this study, the mechanical effects of fluid accumulation on the pulmonary vasculature in neonatal lamb lungs have been described. In vivo, the process of edema formation may result in the release of many vasoactive agents. Also, in the intact animal, changes in pleural pressure during severe edema formation may alter pulmonary hemodynamics. However, the response of the neonatal pulmonary circulation to edema under more physiologic conditions may be quite different.

**Acknowledgments**

We thank Dr. N.C. Staub for helpful discussion, L. Navazo for technical assistance, and P. Barrette for preparing the manuscript.

**References**


**Key Words** • neonatal pulmonary circulation • pulmonary vascular resistance • pulmonary edema • microvascular pressures • lung micropuncture
Effect of edema on segmental vascular resistance in isolated lamb lungs determined by micropuncture.
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Circ Res. 1987;61:236-243
doi: 10.1161/01.RES.61.2.236

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