Lung Water and Vascular Permeability in Sheep
Newborns Compared with Adults
KENNETH L. BRIGHAM, HAKAN SUNDELL, THOMAS R. HARRIS, ZAK CATTERTON, ILLYA KOVAR, AND MILDRED STAHLMAN

SUMMARY To compare vascular permeability and water content in the lungs of newborn lambs with those in adult sheep, we measured extravascular water and permeability surface area products (PS) for 14C-urea and 14C-sucrose in nine 3- to 5-day-old lambs and 13 yearling sheep. In normal, unanesthetized animals, we injected a mixture of 14C-erythrocytes, 125I-albumin, 3H-water, and either 14C-sucrose or 14C-urea as a bolus into the right atrium and sampled blood from the thoracic aorta, calculating extravascular water and PS for urea and sucrose from the time-concentration curves of the tracers. We also measured extravascular lung water and dry bloodless lung weight postmortem. Normalized to dry lung weight, extravascular lung water by both techniques was similar in newborns and adults (indicator dilution values = 3.2 ± 0.03 (SEM) ml/g for lambs and 3.2 ± 0.03 for sheep; postmortem values = 4.07 ± 0.26 g/g for lambs and 4.03 ± 0.17 for sheep). In newborn lambs, PS calculated by integral extraction was 0.28 ± 0.04 (SEM) g/g dry lung for 14C-urea and 0.18 ± 0.04 for 14C-sucrose. These values were not significantly different from those for adult sheep (14C-urea PS = 0.26 ± 0.05; 14C-sucrose PS = 0.08 ± 0.03). We conclude that, when normalized to dry lung weight, newborn and adult sheep have similar lung water and vascular permeability to small hydrophilic molecules and that indicator methods for measuring PS and lung water are feasible in newborns.

PULMONARY edema is a prominent pathological finding in hyaline membrane disease. Since the edema fluid is rich in large proteins, at least part of the lesion may involve increased microvascular permeability. Techniques for measuring permeability in newborns could help to clarify the pathogenesis of this disease. There have been only a few studies of lung water and vascular permeability in newborn animals. We and others have used multiple indicator methods to measure permeability and water content in adult living animals and humans, but these methods seldom have been used in newborns. We wanted to see whether indicator dilution measurements were feasible in newborn lambs and to compare lung water and vascular permeability in normal newborn lambs with those in normal adult sheep. We used 14C-urea and 14C-sucrose as lung vascular permeability indicators and measured lung water content by both indicator dilution and postmortem methods. Newborn and adult lungs had similar amounts of extravascular water when normalized to dry lung weight. Permeability surface area products for 14C-urea and 14C-sucrose were similar in newborn and adult sheep when normalized to dry lung weight.

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Methods

We prepared yearling and 3- to 5-day-old sheep by putting 3.0-mm i.d. polyvinyl catheters through the jugular vein and carotid artery in the neck into the right atrium and thoracic aorta, respectively. We put catheters in adult animals under fluorohane inhalation anesthesia and studied them on another day while they stood unanesthetized in a cage; newborn lambs were restrained gently in the prone position with only local anesthesia (1% lidocaine) used for cut-downs.

Experimental Protocol

We did the indicator studies by injecting a bolus (1.0 ml in newborns and 3.0 ml in adults) of 51Cr-labeled erythrocytes, 125I-human albumin, 3H-water, and either 14C-urea or 125I-sucrose through the venous catheter. We collected arterial blood samples at 0.5-sec (newborns) or 1.0-sec (adults) intervals by allowing blood to flow from the arterial catheter into heparinized tubes mounted on a rotating disc collector. Erythrocytes from each animal were labeled before the experiment by incubation at room temperature for 45 minutes with 51Cr-sodium chromate and washing three times with 0.89% sodium chloride solution. The labeled cells then were resuspended in 0.89% sodium chloride solution to the original hematocrit concentration. We also calculated both 14C-urea and 14C-sucrose PS with acceptable confidence.7, 8 Of course, cardiac output, F, differs so little from the intravascular indicator curve. Sucrose curves also fall between the albumin and water curves, but lie very close to the albumin curve.

Calculations from Indicator Curves

We plotted activities for each indicator in each blood sample relative to the amount of the injected as a function of time after injection. There are several ways to calculate extravascular water volume and permeability-surface area (PS) products for "diffusion-limited" indicators from these curves. Based on extensive theoretical8, 9, 12 and experimental6, 9, 13 studies, we chose to calculate extravascular water and 14C-sucrose PS product using a curve-fitting method based on a Krogh-cylinder circulatory model. We have described the mathematical details of the method in prior publications.8, 9, 12 14C-Sucrose PS was not calculated by the model technique because its curve so closely approximates the curve of the intravascular indicators that the model cannot distinguish between the two with acceptable confidence.13

We also calculated both 14C-urea and 14C-sucrose PS using the widely accepted integral extraction technique.14

In this calculation:

\[ PS = -F \times \ln (1 - E). \]  

(1)

F is cardiac output and:

\[ E = \frac{\int_{0}^{t_{\text{peak}}} C_R - C_D dt}{\int_{0}^{t_{\text{peak}}} C_R dt}. \]  

(2)

C_R is relative concentration of the intravascular (reference) indicator. C_D is the relative concentration of the diffusion-limited indicator. For 14C-sucrose, 125I-albumin was the reference tracer. Since area distributes in both red cells and plasma, its reference curve was generated as a composite of the 51Cr-erythrocyte curve and the 125I-albumin curve weighting for hematocrit and red cell and plasma water content, as suggested by Goresky et al.15

This calculation assumes that 125I-albumin is confined to the vascular space in a single transit. Blood-to-lymph equilibration rates for labeled albumin validate this assumption for newborn lambs.6

Postmortem Measurements

Fifteen minutes after injecting a bolus of 51Cr-erythrocytes to label the blood, we anesthetized sheep with pentobarbital (25 mg/kg, iv) inflated the lungs to 25 cm H2O pressure with air, split the sternum, cross-clamped the hila, drew a blood sample from the heart, and excised the lungs. We homogenized the lungs in a blender, counted samples of homogenate and blood for 51Cr activity in a gamma spectrometer, and measured percent water in blood and lung homogenate by drying samples to constant weight. Using the formulas of Pearce et al.,16 we calculated extravascular lung water and the dry weight of bloodless lung assuming the ratio of 51Cr counts to mass of residual lung blood to be similar to that ratio in peripheral blood.17, 18

Statistics

We tested the significance of differences between newborn and adult sheep using an unpaired t-test.19

Results

Figure 1 shows representative transpulmonary dilution curves in newborn lambs and sheep when either 14C-urea or 14C-sucrose was used as the permeability indicator. None of the lambs had intravascular shunts. As reported before,7, 10 red cells cross the lung faster than plasma so that the red cell curve has a higher peak and steeper downslope. Urea curves fall between the intravascular and water curves as expected if microvascular endothelium restricts its exit from the vascular space. Sucrose curves also fall between the albumin and water curves, but lie very close to the albumin curve.

Table 1 summarizes the indicator dilution data for all of the studies. In the adult sheep, the values are similar to those we reported earlier.9, 10 Of course, cardiac output, lung water, and PS products are much smaller in the newborn lambs which were about one-tenth the size of the adult sheep. As we found before,13 the PS for sucrose is not very reproducible because the sucrose indicator curve differs so little from the intravascular indicator curve.
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Figure 1 | Typical time-concentration curves for intravascular indicators. $^{14}$C-urea, $^{14}$C-sucrose, and $^{3}H$-water in newborn lambs and sheep. The red cell-albumin composite intravascular curve is shown with the urea studies and the albumin curve is shown with the sucrose studies because they are the appropriate intravascular indicators in each case (see Methods).

Figure 2 shows the extraction of $^{14}$C-sucrose and $^{14}$C-urea in newborn lambs and sheep from the point of indicator appearance in the arterial samples to the peak of the intravascular indicator curves. In both groups, urea extraction was consistently higher than sucrose extraction. Also, in both groups, for both tracers, extraction decreased from appearance to peak. The extraction patterns for both tracers were similar in newborns and adults.

Table 2 summarizes normal postmortem measurements in newborn lambs and sheep. Although newborn body weight was about a tenth that of adults; the ratio of lung weights is much higher. That is, the lung weight-body weight ratio is much higher in newborns than adults. Newborns and adults had similar extravascular water content when this value was normalized to dry lung weight. We have found this the most reproducible expression of lung water content.17 Table 3 shows average values for cardiac output, extravascular water, and PS calculated from indicator dilution data and normalized to dry lung weight for each animal. When normalized this way, cardiac output is significantly higher in newborns.

Figure 2 | Fractional extraction of $^{14}$C-urea and $^{14}$C-sucrose from appearance to peak of the intravascular curve in newborn lambs (A) and adult sheep (B).

Discussion
Some data suggest that in newborn animals lung microvessels are more permeable than in adults. For example, Levine and associates' calculated higher filtration coefficients in isolated perfused puppy lungs than in lungs from grown dogs, and Taylor and coworkers' found more rapid clearance of plasma proteins in lymph from the right lymph duct in puppies than in adult dogs. From their studies of lung lymph in acutely anesthetized and dissected newborn lambs, Boyd and associates20 concluded that newborn lung lymph flow (net transvascular filtration) was higher than in adults. They also measured blood to lymph macromolecular transport and concluded that newborn

Table 1 | Summary of Indicator Dilution Data: Not Normalized (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Urea studies</th>
<th>Sucrose studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cardiac output</td>
<td>Extravascular lung water</td>
</tr>
<tr>
<td></td>
<td>(ml/sec)</td>
<td>(ml)</td>
</tr>
<tr>
<td>No. of studies/no. of animals</td>
<td>Body weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Urea studies</td>
<td>Newborn lambs</td>
<td>12/4</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>9/6</td>
</tr>
<tr>
<td>Sucrose studies</td>
<td>Newborn lambs</td>
<td>11/5</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>12/7</td>
</tr>
</tbody>
</table>

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lung microvessels had equivalent pore radii similar to those of adults but a much larger area for exchange per unit pore path length.

On the other hand, some reported data suggest no difference in lung vascular permeability-surface area products between newborns and adults. Levine and Bansil, using multiple indicator techniques similar to the ones we used in the present study, found lung vascular permeabilities to $^{21}\text{Na}$ in piglets similar to those in adult dogs. Bland and McMillan measured lung lymph in chronically prepared unanesthetized newborn lambs and found only a slightly higher lymph flow than in similarly prepared adult animals. They also found lower lymph-plasma protein concentrations and higher pulmonary vascular pressures in newborns. These findings are much like the response to experimental conditions. Our data agree with those of Boyd et al. and Bland and McMillan results from the former's use of body weight as the normalizing variable. Bland and McMillan normalized to dry lung weight.

Vascular permeability measured by indicator methods is mainly diffusive (as opposed to convective) transport so that it is affected very little by hydrostatic pressures. Our findings that urea and sucrose permeability surface area products normalized to dry lung weight are similar in newborn and adult sheep suggest that the porosity (pore size and total pore area per unit lung mass) of lung-exchanging vessels is similar in the two groups. In previous studies in adult sheep, we have shown that permeability estimates by indicator methods agree very well with estimates based on lung lymph data in a variety of experimental conditions. Our data agree with those of Bland and McMillan as well as those of Levine and Bansil. Since the lambs we studied were 3-5 days old, our data do not rule out the possibility of high lung vascular permeability in the early postnatal period which decreases over the first few days of life.

Most reported indicator dilution data with diffusion-limited tracers show a decrease in extraction of the tracers from appearance to the peak of the curve. For example, Yipintsoi found this pattern for $^{21}\text{Na}$ in normal adult dog lungs, and Levine and Bansil saw the same pattern in the lungs of piglets. In both newborn and adult sheep, our studies showed decreasing extraction for both $^{14}\text{C}$-urea and $^{14}\text{C}$-sucrose. Extraction may decrease because of increasing concentration of tracer in the perimicrovascular space resulting in a decreased transmural concentration gradient, and the standard extraction PS calculation does not take this into account. Several methods for dealing with this problem have been proposed. On the basis of an extensive comparison of the several methods reported elsewhere, we believe that, at least in the lung, the Krogh convolution-modeling technique is a superior analytical method for analyzing $^{14}\text{C}$-urea curves. The sucrose PS is imprecise no matter how it is calculated because its curve so closely approximates the intravascular curve in the lung.

Our earlier work in sheep strongly suggests that inaccuracies in the PS for urea which could theoretically result from red cell tracer transport do not occur in the pulmonary circulation. Sucrose, a completely extra-cellular tracer, avoids the theoretical red cell transport problem; it is a larger molecule than urea and, in both newborn and adult sheep, behaves so much like the intravascular tracers that the PS calculation is not precise. Therefore, urea seems to be a better permeability indicator in newborns as well as adults.

When normalized to dry lung weight, we found no difference between extravascular lung water in newborn and adult sheep. This is different from the results of Bland and McMillan. They found more water in newborn sheep lungs. They studied slightly larger lambs (mean body weight 6.0 kg) than we did, and their animals had two

### Table 2 Summary of Normal Postmortem Lung Water Measurements (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Body weight (kg)</th>
<th>Wet weight lung + blood (g)</th>
<th>Extravascular water (g)</th>
<th>Dry lung weight (g)</th>
<th>Extravascular water/dry lung weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn lambs</td>
<td>4.2 ± 0.3</td>
<td>102 ± 4</td>
<td>56 ± 4</td>
<td>14 ± 1</td>
<td>4.07 ± 0.26</td>
</tr>
<tr>
<td>Sheep</td>
<td>43.6 ± 2.2</td>
<td>562 ± 37</td>
<td>264 ± 21</td>
<td>65 ± 5</td>
<td>4.03 ± 0.17</td>
</tr>
</tbody>
</table>

### Table 3 Summary of Indicator Dilution Data Normalized to Postmortem Dry Lung Weight (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>No. of studies/no. of animals</th>
<th>Cardiac output (ml/sec per g)</th>
<th>Extravascular lung water (ml/g)</th>
<th>Integral extraction</th>
<th>Krogh convolution model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urea studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn lambs</td>
<td>12/4</td>
<td>1.7 ± 0.2*</td>
<td>3.1 ± 0.3</td>
<td>0.28 ± 0.04</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>Sheep</td>
<td>9/6</td>
<td>1.2 ± 0.2</td>
<td>3.3 ± 0.5</td>
<td>0.26 ± 0.05</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td><strong>Sucrose studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn lambs</td>
<td>11/5</td>
<td>1.9 ± 0.1*</td>
<td>3.4 ± 0.2</td>
<td>0.18 ± 0.04</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Sheep</td>
<td>12/7</td>
<td>1.1 ± 0.1</td>
<td>3.2 ± 0.2</td>
<td>0.08 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from sheep data, $P < 0.05$
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thoracotomies prior to the lung water measurements whereas ours had not been operated on. Also, there are some technical differences in the way postmortem residual lung blood was estimated. Perhaps all of these differences account for the differences in data. It is interesting that, although Bland and McMillan measured more extravascular lung water in newborn lambs, their histological sections show no evidence of either interstitial or alveolar edema and their blood-to-lung protein equilibration data even suggest that interstitial volume may be relatively smaller in newborns.

Choosing a normalizing variable is critical in comparing newborn and adult data. Body weight has been used frequently, but the ratio of lung weight to body weight is much higher in lambs (Table 2). Since the measurements relate to the lungs, it seems more accurate to use dry lung weight as the normalizing variable. This relates everything to lung mass, and the use of dry weight obviates any differences which might result from differences in lung water content. The best normalizing variable for the PS values would be exchanging vessel surface area, but this cannot be measured in vivo. If the ratio of surface area to lung mass were different in newborns than in adults, then normalizing to mass would not be an appropriate way to compare the two groups.

We conclude from these studies that microvascular permeability to small hydrophilic molecules in newborn lambs is similar to that in adult sheep. Normalized extravascular water content measured both by indicator dilution and postmortem also appears to be similar in the two groups. The demonstration that indicator methods can be used to measure vascular permeability in newborn lambs makes it possible to study the role of this variable in hyaline membrane disease and other newborn lung diseases in living animals. However, when there are intravascular shunts, interpretation of multiple indicator data will be more complicated.

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References


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