Lung Water and Vascular Permeability-Surface Area in Premature Newborn Lambs With Hyaline Membrane Disease

Håkan W. Sundell, Thomas R. Harris, James R. Cannon, Daniel P. Lindstrom, Robert Green, Jorge Rojas, and Kenneth L. Brigham

Extravascular lung water and vascular permeability-surface area products were measured with a multiple indicator dilution method in 6 premature lambs with hyaline membrane disease 1–5 hours following delivery by cesarean section. The indicators used were $^{51}$Cr-labelled erythrocytes, $^{125}$I-albumin, $^{3}$H-water, and $^{14}$C-urea. Results were compared with previously obtained data in newborn lambs without hyaline membrane disease also delivered by cesarean section. Extravascular lung water was significantly higher in lambs with hyaline membrane disease ($23.2 \pm 1.0$ (SEM) vs. $10.7 \pm 1.4$ ml/kg body wt). Vascular permeability-surface area products for $^{14}$C-urea were significantly lower in lambs with hyaline membrane disease ($0.30 \pm 0.10$ vs $0.78 \pm 0.11$ ml/s per kg). It is concluded that extravascular lung water is high in lambs with hyaline membrane disease. Permeability-surface area products for $^{14}$C-urea is low in lambs with hyaline membrane disease, which probably indicates a decrease in detectable surface area for exchange due to derecruitment or hypoperfusion of pulmonary exchange vessels in edematous and hypoxic areas of the lungs. (Circulation Research 1987;60:923-932)

It has been suggested that lung failure in hyaline membrane disease (HMD) involves abnormalities of fluid balance and of the pulmonary vascular bed.1 Pulmonary edema is consistently found in lungs from infants dying with HMD.3,4 The clinical relevance of increased fluid in the lungs is illustrated by the temporal relation between spontaneous diuresis and improvement in pulmonary function in newborn infants with HMD.5 Other abnormalities of the pulmonary vasculature in HMD include pulmonary capillary engorgement, early increased pulmonary vascular resistance reflecting vasoconstriction, and ventilation-perfusion imbalance causing hypoxemia.2

We have previously described the use of multiple indicator methods for serial measurements of extravascular lung water content (EVLW) and vascular permeability-surface area product (PS) for $^{14}$C-urea in newborn lambs.6,7 The use of this technique to study the pathophysiology of HMD has not been reported previously, although it has been employed in patients with the adult respiratory distress syndrome.8 The advantage of this technique is that repeated measurements of both EVLW and PS can be performed. Neither the indicator techniques nor any other single available method can, however, separate changes in permeability from changes in surface area.

The purpose of this study was to use the multiple indicator method to measure the extent of pulmonary edema and changes in PS thought to be present in lungs of lambs with HMD, to relate these measurements to other pathophysiological changes, and to compare the results with values obtained earlier from newborn lambs without HMD.7

Materials and Methods

General

Six lambs of Suffolk or mixed breed were used for these studies. A schematic diagram of the newborn lamb preparation is presented in Figure 1. Four to seven days prior to delivery, the fetal lambs underwent surgery. The anesthesia used for this procedure consisted of intravenous sodium thiopental (0.5 g) induction given to the ewe followed by halothane and nitrous oxide inhalation. Polyvinyl, intravascular catheters (i.d. 1.0 mm, o.d. 1.8 mm) were placed in the fetal brachiocephalic trunk, superior vena cava, and pulmonary artery (PA). These catheters were used for intravenous fluid administration and blood pressure measurements. An electromagnetic flow cuff (C and C Instruments, Culver City, Calif.) was placed around the main pulmonary artery close to the pulmonary valve and proximal to the transmural insertion site of the PA catheter. In 3 lambs, an additional cuff was placed around the main pulmonary artery beyond the origin of the ductus arteriosus proximal to the pulmonary arterial bifurcation.9
Lambs were delivered by cesarean section at 127-134 days of gestation. Median age at delivery was 131 days, which corresponds to 89% of the 145-150 days normal gestational period in these breeds of sheep. The ewes were anesthetized with 500-600 mg sodium thiopental intravenously, and the lambs were rapidly delivered through a hysterotomy. The lambs were intubated immediately following birth with a cuffed endotracheal tube (i.d. 5.0, o.d. 7.3 mm) and were ventilated with a Babybird Respirator (Bird Corporation, Palm Springs, Calif.). Arterial blood gases were determined frequently (IL Model 213 Blood Gas Analyzer, Instrumentation Laboratories, Lexington, Mass.) and the respirator was adjusted to keep the Pao₂ between 50 and 80 mm Hg and the Paco₂ between 35 and 50 mm Hg. It was, however, not always possible to ventilate the sickest lambs optimally.

The lambs were thoroughly dried and placed under radiant heat to maintain rectal temperature at 39.3 ± 0.2°C. An intravenous infusion of 10–15% dextrose water was given at 6–10 ml/hr to keep blood glucose concentrations in a range of 45–90 mg %, as checked by frequent Dextrostix® (Ames Miles Labs Inc, Elkhart, Ind.) determinations. Thiamethamine (0.3M solution, Abbott Laboratories, North Chicago, Ill.) was given intravenously, as needed, to correct a base deficit greater than 10 meq/l; the total dose given was 19.7 ± 9.8 ml/kg (mean ± SEM). Lambs were restrained in a prone position during the studies. Only local anesthesia (1% lidocaine) was used for catheter insertions.

After birth, catheters were placed in an umbilical artery, a carotid artery, a tarsal vein, and into the left and right ventricles (LV, RV). The position of each ventricular catheter tip was confirmed by pressure monitoring before and after each study and at autopsy. All recordings were made with an 8-channel Hewlett-Packard 8800S cardiovascular recording system. Statham P23ID pressure transducers (Gould-Statham, Oxnard, Calif.) were used for systemic and pulmonary arterial blood pressure measurements and were placed at the level of the left atrium. An electromagnetic blood flow meter (Narcomatic Model R1510, Narco Bio-Systems, Houston, Tex.) was used for pulmonary arterial blood flow measurements. The flow cuffs were calibrated beforehand in vitro, using excised blood vessels and saline. Determinations of zero flow reference for the proximal PA cuff were performed every 30 minutes using an unfiltered flow signal and high paper speed. Since this flow cuff was located close to the pulmonic valve, the polygraph pen position during diastole was used as the zero flow reference. This zero flow reference was usually not more than 50 ml/min different than the electronic zero baseline. In the presence of a left-to-right ductus shunt, a similar physiologic zero flow reference was not available for the distal PA flow cuff used in 3 of the lambs, so the electronic zero baseline was used. To avoid the possibility of interference between the flow cuffs, flows in each cuff were checked separately with the current to the other cuff turned off momentarily. Presence or absence of extrapulmonary shunts through the foramen ovale or the ductus arteriosus (DA) was evaluated with an indocyanine indicator dilution technique as previously described,10 using a densitometer and cardiac output computer (Lexington Instruments, Waltham, Mass.) as well as a Vanderbilt densitometer. Foramen shunts were evaluated with dye injections through the tarsal vein catheter and blood withdrawal through the brachial artery catheter. Right-to-left DA shunts were determined with injection through the RV catheter and withdrawal through the umbilical artery catheter. Left-to-right DA shunts were checked with injection through the LV catheter and withdrawal through either arterial catheter. Because of presence of shunts this method was not used to quantify cardiac output.

Right and left ventricular outputs, ductal flow in both directions, and pulmonary flow were measured with a radioactively microsphere technique as previously described.11,12 Gamma labelled, 15 i 53C, 169 Yb, 83 SR, and 35 Sc, 3M Company, St. Paul, Minn.) were injected simultaneously into each ventricle. Samples for blood flow calculations were withdrawn through the catheters placed in CA and PA, while an equal volume of donor blood was transfused through the UA catheter. The lungs and remaining carcass were counted separately postmortem to determine the microsphere content in order to calculate the percent ductal shunt in either or both directions. A high resolution detector and 1,024-channel spectrometer were used in counting the tissue samples and provided excellent separation between the gamma-ray peaks of the multiple tracers.

Because of the frequent need to use the flow cuff and microsphere methods together in calculating pulmonary blood flow, we reviewed our experience with these two methods in older lambs without extrapulmonary shunts.13 The correlations between 16 simultaneous determinations of pulmonary blood flow in four 1-2-week-old lambs was highly significant (p < 0.001, r = 0.86).

A multiple indicator method14 was used for deter-
ministration of cardiac output, extravascular lung water, and permeability-surface area products. A 0.7 ml bolus of $^{51}$Cr-labelled erythrocytes, $^{125}$I-human albumin, $^3$H-water, and $^{14}$C-urea was injected through the RV catheter. Arterial blood was collected at 0.5-second intervals by allowing blood to flow from the CA catheter into heparinized tubes mounted on a rotating disk collector. A stable blood volume and blood pressure was maintained during this sudden withdrawal of 25 ml arterial blood by a simultaneous injection of equal volume of donor blood at an equal rate into the umbilical arterial catheter.

Experimental Protocol

After insertion of the vascular catheters and stabilization of the lambs’ condition, systemic and pulmonary arterial pressures and arterial blood gases were determined. The microsphere, multiple indicator, and dye indicator studies were performed consecutively. The first set of studies was performed at a postnatal age of 1–3 hours and was repeated 1–2 times. The lambs were killed at 3–6 hours of age, when they could no longer be oxygenated or ventilated. The methods for determining postmortem lung water and dry weight of the lung have been described earlier. Because of energy overlap between the gamma-labelled microspheres and the $^{51}$Cr-erythrocytes, it was not possible to obtain accurate postmortem measurements of extravascular lung water with this method. Postmortem wet and dry weights are, therefore, presented as total weights of the lung including residual blood. A thorough autopsy was performed to verify the location of vascular catheters and flow cuffs. No evidence of vascular constriction was noticed from the flow cuffs or transmural PA catheter.

Lung tissue from the right upper and lower lobes was examined by light microscopy. Evidence of cell slough and hyaline membrane formation was seen in the lungs of all lambs.

LAMBS WITHOUT HMD. This group of 5 lambs has been described previously. Briefly, these lambs were delivered by cesarean section at 135–142 days of gestation and were treated according to the same principles as the lambs with HMD. The need for mechanical ventilation and supplemental oxygen tension was, however, much less, and all of them were weaned from the respirator by 5 hours of age. They received dextrose water intravenously at the same rate as the lambs with HMD. Metabolic acidosis was treated with intravenous thiamine or sodium bicarbonate. The mean volume given was 6.7 ± 3.5 ml/kg, which was not significantly different from the volume given to HMD lambs. These lambs were killed at 6–9 hours of age.

Calculations From Indicator Curves

Methods for calculating PS for $^{14}$C-urea by the integral extraction technique, EVLW, and intravascular volume in newborn lambs have been described previously. The effect of ductal shunts was accounted for by methods described by Harris. An extensive discussion of the use of this method as a measure of normal and abnormal lung microvascular function has been published. Details of the calculations are provided in the Appendix.

Statistics

The mean values for each group were calculated from the average results of 1–3 studies in each lamb. The significance of differences between the two groups of animals was determined using an unpaired t test. Paired t tests were used for comparisons of the first and last measurements performed in the same lambs. A p level of less than 0.05 was taken as significant. Data are presented as mean ± SEM.

Results

Table 1 lists gestational age and birth weight of the 6 lambs as well as postnatal age when the 10 individual studies were performed. Arterial blood gas measurements and systemic and pulmonary arterial blood pressure measurements obtained immediately before each multiple indicator study are presented, together with results of right ventricular output, foramen ovale, and ductus arteriosus shunt measurements determined with the microsphere, flow cuff, and green dye indicator dilution methods. The lambs with HMD had significantly higher mean pulmonary arterial pressures and significantly lower mean systemic arterial pressures when compared to the lambs without HMD. Arterial pH was lower, and PaCO$_2$ and base deficit were higher in the HMD lambs.

These studies showed that a left-to-right (L-R) shunt through the ductus arteriosus from the aorta to the pulmonary artery was present on 6 occasions in 5 of the 6 lambs. R-L ductal shunts were seen on 5 occasions in 3 lambs. A small shunt through the foramen ovale was demonstrated with the green dye indicator dilution technique on 6 occasions in 3 lambs. A small foramen shunt did not influence the calculations of EVLW and PS because the multiple indicators were injected into the RV, and the small amount of shunted blood that bypassed the lung during the multiple indicator study will dilute all isotopes equally in the arterial withdrawal samples. The composite determination of pulmonary blood flow used for PS and EVLW calculations is listed in Table 1. The calculated results of extravascular lung water volume, intravascular lung blood volume, and PS products are also presented in Table 1.

Table 2 compares the mean gestational age, birth weight, blood pressures, and blood gases in the HMD group with the earlier studied group of lambs without HMD. Significantly higher inspired oxygen concentrations were used for the HMD lambs. Respirator settings could not be compared since mechanical ventilation was used during only five of the studies in the non-HMD group. Arterial pH and PaO$_2$ were significantly lower and PaCO$_2$ was significantly higher in the HMD group. Mean pulmonary arterial blood pressures were higher and mean systemic arterial pressures were lower in the lambs with HMD.
Figure 2 shows representative transpulmonary dilution curves in a lamb with HMD. Table 3 summarizes the multiple indicator dilution data both as primary data and normalized to body weight. Extravascular lung water was significantly higher in the lambs with HMD. Three HMD lambs had repeated measurements, which showed significantly higher extravascular lung water at the time of the first study when compared with the last (Figure 3). Extravascular lung water content did not change significantly during the time span of 2–7 hours after birth in the non-HMD lambs. Measurements performed at the comparable time interval of 2–4 hours showed that 5 HMD lambs had significantly higher EVLW than 4 non-HMD lambs (21.0 ± 0.5 ml/kg vs. 11.5 ± 3.3 ml/kg, p < 0.001). There was a weak negative correlation between EVLW and A-aPo2 difference in the lambs with HMD \((r = -0.55)\).

Permeability-surface area products for \(^{14}\)C-urea was significantly lower in the HMD lambs (Table 3). PS for urea decreased between the first and the last study in 3 lambs with HMD as illustrated in Figure 4. PS remained essentially unchanged in 2 lambs without HMD during the first 5 hours after birth but increased slightly thereafter. Measurements performed at the comparable time interval of 2–4 hours after birth showed that 6 lambs with HMD had significantly lower PS than 4 non-HMD lambs (0.30 ± 0.25 vs. 0.72 ± 0.11 ml/s/kg, \(p = 0.03\)). PS did not correlate with A-aPo2 difference in either group of lambs.

Intravascular lung blood volumes were calculated from the indicator dilution curves. As shown in Table

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### Table 1. Individual Results of Blood Gas, Blood Pressure, Blood Flow, and Indicator Dilution Measurements in Lambs With HMD

<table>
<thead>
<tr>
<th>Lamb Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>Postnatal age (min)</td>
<td>187</td>
<td>247</td>
<td>222</td>
<td>169</td>
<td>69</td>
<td>116</td>
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<tr>
<td>Gestational age (d)</td>
<td>134</td>
<td>134</td>
<td>130</td>
<td>127</td>
<td>134</td>
<td>130</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>3,670</td>
<td>3,300</td>
<td>2,970</td>
<td>3,685</td>
<td>3,700</td>
<td>3,565</td>
</tr>
<tr>
<td>Respirator rate (bpm)</td>
<td>66</td>
<td>66</td>
<td>52</td>
<td>50</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Respirator pressures (cm H(_2)O)</td>
<td>36/6</td>
<td>36/6</td>
<td>36/2</td>
<td>40/6</td>
<td>38/4</td>
<td>40/5</td>
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<tr>
<td>FIO(_2)</td>
<td>0.80</td>
<td>1.00</td>
<td>0.65</td>
<td>0.70</td>
<td>0.60</td>
<td>0.60</td>
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<td>pH</td>
<td>7.27</td>
<td>7.24</td>
<td>7.27</td>
<td>7.21</td>
<td>7.06</td>
<td>6.95</td>
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<tr>
<td>PacO(_2) (torr)</td>
<td>42</td>
<td>74</td>
<td>53</td>
<td>49</td>
<td>55</td>
<td>48</td>
</tr>
<tr>
<td>PacO(_2) (torr)</td>
<td>60</td>
<td>41</td>
<td>52</td>
<td>82</td>
<td>51</td>
<td>46</td>
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<td>Base excess (meq/l)</td>
<td>-7</td>
<td>±0</td>
<td>-4</td>
<td>-9</td>
<td>-15</td>
<td>-17</td>
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<tr>
<td>A-a Po2 difference (torr)*</td>
<td>468</td>
<td>598</td>
<td>599</td>
<td>368</td>
<td>322</td>
<td>334</td>
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<tr>
<td>Pulmonary artery blood pressure (torr)</td>
<td>61</td>
<td>50</td>
<td>39</td>
<td>48</td>
<td>54</td>
<td>55</td>
</tr>
<tr>
<td>Aortic blood pressure (torr)</td>
<td>62</td>
<td>48</td>
<td>37</td>
<td>48</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>Right ventricular output by microspheres (ml/min)</td>
<td>1,100</td>
<td>1,100</td>
<td>800</td>
<td>600</td>
<td>560</td>
<td>400</td>
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<tr>
<td>Right ventricular output by flow cuffs (ml/min)</td>
<td>1,100</td>
<td>1,100</td>
<td>800</td>
<td>600</td>
<td>560</td>
<td>400</td>
</tr>
<tr>
<td>Ductus left to right shunt by microspheres (ml/min)</td>
<td>72</td>
<td>541</td>
<td>444</td>
<td>48</td>
<td>60</td>
<td>0</td>
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<tr>
<td>Ductus left to right shunt by flow cuffs (ml/min)</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>390</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ductus left to right shunt by green dye†</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ductus right to left shunt by microspheres (ml/min)</td>
<td>0</td>
<td>0</td>
<td>63</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductus right to left shunt by flow cuffs</td>
<td>75</td>
<td>550</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Foramen ovale shunt (% RVO)</td>
<td>14</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Foramen ovale shunt (% RVO)</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Pulmonary blood flow (ml/min)</td>
<td>1,025</td>
<td>550</td>
<td>900</td>
<td>1,141</td>
<td>966</td>
<td>500</td>
</tr>
<tr>
<td>Extravascular lung water (ml)</td>
<td>107.0</td>
<td>56.2</td>
<td>67.3</td>
<td>66.5</td>
<td>113</td>
<td>71.9</td>
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<tr>
<td>Intravascular lung blood volume (ml)</td>
<td>78.1</td>
<td>48.6</td>
<td>52.4</td>
<td>79.6</td>
<td>90.9</td>
<td>49.0</td>
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<tr>
<td>Permeability-surface area (ml/s)</td>
<td>3.17</td>
<td>0.58</td>
<td>1.89</td>
<td>1.41</td>
<td>0.59</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Alveolar arterial Po2 difference; †Presence or absence of identified shunt is indicated.
### Table 2. Comparison of Measurements of Blood Gases, Blood Pressures, and Blood Flows in Lambs With and Without HMD

<table>
<thead>
<tr>
<th>No. of studies/No. of animals</th>
<th>Lambs With HMD</th>
<th>Lambs without HMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (d)</td>
<td>131 ± 1*</td>
<td>140 ± 1</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>3.5 ± 0.1</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>Respirator rate (bpm)</td>
<td>55 ± 3</td>
<td></td>
</tr>
<tr>
<td>Respirator pressures (cm H₂O)</td>
<td>38 ± 2</td>
<td></td>
</tr>
<tr>
<td>FIO₂</td>
<td>0.75 ± 0.06*</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>pH†</td>
<td>7.16 ± 0.05*</td>
<td>7.38 ± 0.03</td>
</tr>
<tr>
<td>Paco₂ (torr)</td>
<td>51 ± 2*</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>Pao₂ (torr)</td>
<td>64 ± 10*</td>
<td>111 ± 14</td>
</tr>
<tr>
<td>BE (meq/l)</td>
<td>-9.3 ± 2.2</td>
<td>-5 ± 1</td>
</tr>
<tr>
<td>A-a Po₂ difference (torr)</td>
<td>454 ± 44*</td>
<td>92 ± 23</td>
</tr>
<tr>
<td>Pulmonary artery blood pressure (torr)</td>
<td>54 ± 3*</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Aortic blood pressure (torr)</td>
<td>55 ± 4*</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>Pulmonary blood flow (ml/min)</td>
<td>714 ± 127</td>
<td>724 ± 114</td>
</tr>
<tr>
<td>(ml/min/kg)</td>
<td>212 ± 45</td>
<td>186 ± 29</td>
</tr>
</tbody>
</table>

Results are means ± SEM.

*Significantly different from lambs without HMD, p<0.05.
†Lambs without HMD were not mechanically ventilated during all studies.
‡Hydrogen ion concentrations were used for calculating statistics.

3, lambs with HMD had significantly higher pulmonary blood volumes than lambs without HMD. Extravascular lung water normalized to intravascular volume was significantly greater in the lambs with HMD. PS normalized to intravascular volume were significantly less in the lambs with HMD.

Normalized to body weight, lambs with HMD had heavier lungs containing more water postmortem than lambs without HMD (Table 4). Figure 5 shows the correlation between the last extravascular lung water determination by the indicator dilution technique and postmortem water content in the lung with its blood (r = 0.77, p<0.01). The interval between the last indicator dilution study and death was 22–123 (mean = 66) minutes for the HMD lambs and 75–246 (mean = 157) minutes for the lambs without HMD. There was also a significant correlation between the sum of extravascular and intravascular lung water and postmortem water content of the lung with its blood (r = 0.65, p<0.05). The mean water content in the lung with its blood determined by the indicator dilution technique during the last study was 113% of that determined postmortem in the HMD group and 98% in the non-HMD group.

### Discussion

Hyaline membrane disease continues as a major cause of pulmonary morbidity and mortality in preterm infants. Presence of protein-rich pulmonary edema as a constant finding in this disorder is a basis for investigating the role of abnormalities in lung fluid balance in its pathogenesis.
Table 3. Comparison of Measurements With Indicator Dilution Method on the Lungs of Lambs With and Without HMD

<table>
<thead>
<tr>
<th></th>
<th>Lambs with HMD</th>
<th>Lambs without HMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability surface area</td>
<td>1.02±0.33*</td>
<td>2.95±0.40</td>
</tr>
<tr>
<td></td>
<td>0.30±0.10*</td>
<td>0.78±0.11</td>
</tr>
<tr>
<td>Extravascular lung water</td>
<td>80.0±5.7*</td>
<td>43.4±8.5</td>
</tr>
<tr>
<td></td>
<td>23.2±1.0*</td>
<td>10.7±1.4</td>
</tr>
<tr>
<td>Intravascular lung blood volume</td>
<td>63.9±4.2*</td>
<td>48.8±4.2</td>
</tr>
<tr>
<td></td>
<td>18.6±1.8*</td>
<td>12.7±1.2</td>
</tr>
<tr>
<td>Extravascular lung water/</td>
<td>1.23±0.10*</td>
<td>0.87±0.39</td>
</tr>
<tr>
<td>Intravascular volume</td>
<td>0.016±0.006*</td>
<td>0.061±0.005</td>
</tr>
<tr>
<td>Permeability surface area product</td>
<td>63.9±4.2*</td>
<td>48.8±4.2</td>
</tr>
<tr>
<td>Extravascular lung water/</td>
<td>1.23±0.10*</td>
<td>0.87±0.39</td>
</tr>
</tbody>
</table>

*Significantly different from lambs without HMD, p<0.05.

We used indicator dilution techniques, which have been applied in experimental animals and man to investigate lung fluid balance, to evaluate the concept that HMD may be perceived as analogous to the adult respiratory distress syndrome (ARDS). In ARDS, vascular permeability to water and protein is thought to be increased, and alterations in vascular permeability-surface area have been demonstrated by Brigham et al.8 This conceptual approach to HMD might influence therapeutic rationales.

The complex state seen in HMD can influence microvascular function and its measurement. However, the multiple indicator and microsphere methods provide some useful measures of the influence of factors associated with HMD on microcirculatory function. The presence of bidirectional shunts influences the analysis of the indicator curves. Our quantitation of these shunts with the microsphere and flow cuff methods enables us to identify PS and extravascular lung water volume at the instant of indicator injection. Of course, the microsphere measurement of shunt is not continuous and hemodynamic factors might change between the microsphere and indicator injections, but these studies were performed serially in time and alterations in condition over the small time span between injections are probably minimal. Mechanical ventilation can cause derecruitment of some capillaries under Zone II conditions. This may have occurred in parts of the lungs of these lambs. However, this would be reflected in a decreased PS for area from indicator curves taken during such events. The indicator method cannot explicitly measure heterogeneity in transport properties in the lung. However, it does give a flow-weighted average of PS and extravascular lung water that reflects overall function reasonably well. Only regions of the lung where flow is very slow will be omitted from the measurement. In these cases, recirculation of indicator obscures the first pass appearance from these regions, and the transport properties of these lung segments are not included in the organ estimate.

It is desirable to have a comparable control group of animals without the disease to interpret the results from the measurements in the lambs with HMD. Ideally such control animals should be of identical gestational age with equally advanced lung maturation. It might not be possible to obtain such a group of lambs, however, since the incidence of HMD in cesarean-section-delivered lambs of less than 135 days of gestation in our experience is 83% and only 17% in lambs above 135 days.17 Lambs delivered at the same gestational age but that do not develop HMD likewise might not be truly comparable because they might have had an accelerated lung maturation, e.g., from chronic stress. For these reasons, these lambs with HMD were compared with a group of lambs studied earlier that were delivered by cesarean section at a median gestational age of 141 days, which is approximately 1 week prior to full term.7 Lambs with HMD had severe progressive lung disease and required intensive mechanical ventilation with 100% oxygen. In contrast, the lambs without HMD, although they were initially mechanically ventilated, could be weaned to unassisted spontaneous breathing with inspired oxygen concentration of 21–25%.

Lung Water in Hyaline Membrane Disease

Using multiple indicator methods, we found that lambs with hyaline membrane disease had more lung water 1–2 hours after delivery than lambs without HMD (Figure 3). Lung water decreased with time in

![Figure 3. Comparison of first and last measurements of extravascular lung water normalized to body weight in 3 lambs with HMD (------) and 2 lambs without HMD (--------) studied at comparable postnatal age. Mean ± SEM. *First value significantly different from second; p < 0.005.]
lambs with HMD but remained elevated at time of death, when the measurements were confirmed by postmortem measurements. These data are consistent with other reports in the literature showing pulmonary edema in hyaline membrane disease, \textsuperscript{118-20} but our data also show decreasing lung water in the face of deteriorating gas exchange.

Both delayed clearance of fetal lung liquid, which fills the potential air spaces before birth, and increased transvascular filtration of fluid could account for increased lung water in hyaline membrane disease. Fetal lung liquid averages 30 ml/kg body weight in the lamb.\textsuperscript{21} Normand et al \textsuperscript{1} speculated that high surface tension in the alveoli might suck liquid into the air spaces from the interstitial tissue spaces. However, Egan et al \textsuperscript{19} found that fetal lung liquid left the air spaces faster in surfactant deficient lambs due to increased epithelial permeability. The liquid was displaced from the alveoli to the interstitial space rather than being cleared from the lung, and immature lungs retained more fluid than mature lungs after 4 hours. Our results are consistent with this explanation since extravascular lung water in the HMD lambs remained markedly higher at 1–2 hours after delivery than in the healthy lambs, which apparently had cleared most of their lung liquid by that time. The observed decrease in lung water with time does not appear to be explained by detection of a smaller fraction of actual lung water by the indicator method since at death that fraction was similar in lambs with and without HMD (Figure 5).

Lambs with HMD had significantly higher EVLW than lambs without HMD throughout the 4-hour study period (Figure 3). Additional accumulation of water in the HMD lungs might be due to high surface tension in the airways causing transudation of fluid from capillaries,\textsuperscript{20} high vascular pressures, increased microvascular permeability, or a combination of these factors. It is not likely that the difference in postmortem lung water can be attributed to the approximately 3-hour postnatal age difference since EVLW did not change significantly between 2 and 6 hours after birth in lambs without HMD. The dextrose infusion was given at the same rate to the two groups, but lambs with HMD received slightly more buffers to correct severe metabolic acidosis. Fluid administration did not explain the difference between the two groups of lambs in lung water results, obtained by indicator dilution or postmortem, since there was an overlap in the fluid volume given but not in the lung water content between the two groups, and no correlation between lung water content and administered fluid volume was seen in either group.

Egan et al\textsuperscript{22-23} have shown a functional breakdown of the alveolar-interstitial barrier with positive pressure ventilation, which is more pronounced in immature lungs. A leakage of protein rich fluid into the air spaces leading to formation of hyaline membranes can then take place. Our lambs with HMD were ventilated with high airway pressures, and they generated high transpulmonary pressure gradients during spontaneous breathing, so that a transfer of interstitial fluid across the epithelium could have occurred.

Although intuitive reasoning suggests that the amount of water in the lung should correlate with the severity of the gas exchange abnormality, that is not always the case. In adult sheep with diffuse lung injury produced by endotoxemia, there was no correlation between the severity of the gas exchange abnormality and the amount of lung water measured postmortem.\textsuperscript{24} In humans with ARDS, there was no correlation between extravascular lung water measured by indicator dilution methods and the degree of respiratory failure.\textsuperscript{4}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{Lambs with HMD} & & & & \\
\textbf{n} & \textbf{Total lung weight} & \textbf{Percent Water} & \textbf{Water in whole lung} & \\
& (g) & (g/kg) & & \\
\hline
6 & 123.3 ± 9.2 & 35.0 ± 2.1\* & 87.6 ± 0.3\* & 107.1 ± 8.1 & 30.7 ± 4.5\* \\
\hline
\textbf{Lambs without HMD} & & & & \\
\textbf{n} & \textbf{Total lung weight} & \textbf{Percent Water} & \textbf{Water in whole lung} & \\
& (g) & (g/kg) & & \\
\hline
5 & 99.5 ± 17.6 & 24.6 ± 1.9 & 85.8 ± 0.6 & 85.5 ± 15.6 & 21.2 ± 1.7\* \\
\hline
\end{tabular}
\caption{Summary of Measurements on the Lung Postmortem}
\end{table}

Results are means ± SEM
\*Significantly different from lambs without HMD, \(p<0.05\).
It is likely that failure of gas exchange results from a complex of lung dysfunctions of which pulmonary edema is only one component. This is probably true for both HMD and ARDS.

**Lung Vascular Permeability-Surface Area in Hyaline Membrane Disease**

While increased epithelial permeability is present in HMD, lung vascular permeability may also be increased explaining the histological evidence of marked interstitial edema present in this disease. \(^1\) \(^2\) However, we found that the permeability-surface area product (PS) for a small hydrophilic solute, urea, was lower in lambs with HMD than in control lambs. In addition, in lambs with HMD, PS decreased with time after birth while PS remained essentially unchanged between 2 and 4 hours after birth in lambs without HMD.

These findings are very similar to data reported earlier in adult sheep with endotoxin-induced lung injury \(^1\) \(^4\) and in patients with ARDS. \(^8\) In both situations, even severe protein-rich pulmonary edema is often accompanied by a lower than normal PS. Since the method used did not separate permeability from surface area, it seems most logical to interpret a low PS value in the face of protein-rich pulmonary edema as a low perfused microvascular surface area. \(^8\)

There are good reasons to expect that diffuse lung injury would result in alterations in distribution of perfusion to the lungs, which could alter the amount of the perfused microvasculature. Hypoxic areas of lung undergo vasoconstriction and reroute blood flow to better ventilated areas. \(^2\) Severely edematous areas of lung are also underperfused as a result of pressure exerted on the small lung vessels by the edema fluid. \(^2\)

A decrease in PS cannot unequivocally be assigned to a decrease in effective capillary surface area. However, lung injury does not appear to reduce transvascular permeability. \(^1\) \(^4\) If flow is heterogeneous in the lung, the slower areas will contribute little to the PS measurement. They are essentially “derecruited” from observation even though they may be capable of filtering fluid. The histologic evidence of microvascular congestion of capillaries and small veins in some areas of the lungs with HMD may reflect a low flow, which is consistent with a loss of identifiable microvascular exchange. \(^1\) \(^2\)

Even though PS changes indicate that microvascular surface area was reduced, total intravascular volume was increased in HMD lambs. The total intravascular volume measured by the indicator method includes large vessels and exchange vessels. We speculate that exchange vessels have been reduced, but larger vessels have increased in volume. This is consistent with the idea that precapillary vessels were constricted and larger arteries were inflated because of increased PA pressure.

In addition to derecruitment, PS could be reduced because of a mismatch between flow rate and capillary surface area. If flow is very high, urea will not have time to escape during capillary passage and extraction falls to zero. This may have occurred during some of these studies. However, Table 1 shows that the lowest PS values coincide with the lowest, not the highest, flow rates. Therefore, if it occurred, it was present in the low S situations and does not alter our conclusions regarding decreased S.

**Summary**

In the present study, a multiple indicator dilution technique was used to measure extravascular lung water and the lung vascular permeability-surface area for a small hydrophilic solute, urea, in cesarean section delivered premature lambs. We sought to determine whether abnormalities in the lung circulation and lung fluid balance in hyaline membrane disease were like those in diffuse lung injury in adult animals. Compared with lambs without HMD, lambs with HMD had increased lung water and decreased permeability-surface area product for urea. Lung water decreased with time, even though respiratory failure worsened. PS also decreased with time in the HMD lambs. These findings are very similar to those reported in adult sheep with diffuse lung injury caused by endotoxemia and in humans with the adult respiratory distress syndrome. We conclude that in HMD, as in diffuse lung injury in adults, respiratory failure is not a simple function of the amount of water in the lungs. Further, decreased exchange vessel surface area is typical of HMD, likely resulting from both vasoconstriction and the presence of large amounts of edema fluid in the lungs. In regard to abnormalities in lung vascular function and lung fluid balance, HMD is properly perceived as analogous to diffuse lung injury (ARDS) in adults.
Appendix

Multiple tracer experiments were analyzed by methods discussed by Harris et al.\textsuperscript{14,15,27} Permeability-surface area for $^{14}$C-urea product (PS) was computed from the Crone integral extraction equations shown below:

$$PS = -F \log(a(1-E))$$  (1)

$$E = 1 - \int \frac{C_D}{C_R} dt$$  (2)

where $a$ = appearance time of indicator; $C_D$ = concentration of diffusing tracer; $C_R = [0.92(1-hct)C_{sb} + 0.7(hct)C_{ne}]/(0.92(1-hct) + 0.7(hct))$; $C_{sb}$ = albumin concentration; $C_{ne}$ = $^{51}$Cr red blood cell concentration; $F = [0.92(1-hct) + 0.7(hct)]$ pulmonary blood flow; $hct$ = fractional hematocrit; and $t$ = time of peak of the reference curve.

The presence of ductal left-to-right shunts in these animals complicates the analysis of extravascular lung water. The classic mean transit-time computation is more difficult because rapid recirculation obscures the downslope of the indicator curves. However, the availability of detailed shunt-flow information from the microsphere studies allowed us to use a recirculation model of the central circulation to compute extravascular lung water volume and intravascular volume. We used the method suggested by Harris,\textsuperscript{15} in which the intravascular reference curve was described by the solution to the following equations, shown to be descriptive of adult sheep lung circulation:\textsuperscript{26}

$$\frac{\partial C_R}{\partial t} + \frac{F}{V_c} \frac{\partial C_R}{\partial x'} = \frac{D}{L^2} \frac{\partial^2 C_R}{\partial x'^2}$$  (3)

where $V_c$ = intravascular lung volume; $D/L^2$ = relative dispersion of indicator due to parallel intravascular flow paths through the lung; $L$ = mean intravascular flow path distance through the lung; and $x'$ = flow distance from injection site/L.

The diffusing tracer $^{3}$HOH was assumed to be described by the following model:\textsuperscript{26}

$$\frac{\partial C_D}{\partial t} + \frac{F}{V_c} \frac{\partial C_D}{\partial x'} = -\frac{K}{V_c} (C_D - C_D')$$  (4)

$$\frac{\partial C_D'}{\partial t} = \frac{K}{V_c} (C_D - C_D')$$  (5)

where $C_D'$ = concentration of diffusing tracer in the extravascular space; $K$ = mass transfer coefficient for water exchange; $V_c$ = lung capillary intravascular space; and $V_E$ = lung extravascular distribution volume of water.

If the principal shunt affecting indicator curves is ductal left-to-right flow, then the Fourier transform solution of equations 1 to 5 is

$$\mathcal{C}_{DA}(j\omega) = \frac{(1 - \phi)C_D(j\omega)C_R(j\omega)}{1 - \phi \cdot C_D(j\omega) \cdot C_R(j\omega)}$$  (6)

where $\mathcal{C}_{DA}(j\omega)$ = Fourier transform of the measured indicator curve; $C_D(j\omega)$ and $C_R(j\omega)$ = Fourier transforms of the diffusing and reference tracer models; and $\phi$ = left-to-right shunt flow/pulmonary blood flow.

This model can be numerically inverted to the time domain by methods discussed by Rowlett and Harris\textsuperscript{28} to provide a predicted concentration-time curve. This model was fitted to the reference and $^{3}$HOH data by the following method: Pulmonary blood flow and $\phi$ were computed directly from microsphere shunt observations. Then equation 3 was fitted to the $C_R$ data by assuming $C_D(j\omega)$ to be 1 in equation 6 and adjusting $V_c$ and $D/L^2$ and numerically inverting equation 6 into the time domain until a good fit was obtained. Then the model was fitted to the $^{3}$HOH data by equation 6 with $C_R(j\omega)$ determined by the parameters obtained in the $C_R$ data fit and the parameters $K$ and $V_c$ were adjusted for an adequate fit. $K$ for water is the smallest identifiable transport parameter for water and is much less than true water permeability surface area (see Harris et al.\textsuperscript{27} for discussion). $V_c$ however is equivalent to the extravascular lung water volume computed from the mean transit time formula in the absence of shunts. The modeling technique discussed above allows computation of $V_c$ in the presence of shunts. The integral extraction method for the computation of urea PS is not affected by shunting since left-to-right shunts only distort the indicator curves after the appearance of the reference curve peak.\textsuperscript{15,29}

Small right-to-left shunts (~5% of total curve area) were occasionally seen. These peaks occurred before the rise of the main indicator curve and exhibited no separation of diffusible and nondiffusible tracers. This probably reflects small intracardiac shunts or minor reversal of ductal shunting. They were eliminated by back extrapolation of the reference curve. Since flow was measured separately, they had no effect on indicator-dilution calculations.

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