Effects of Alveolar Hypoxia on Lung Fluid and Protein Transport in Unanesthetized Sheep

RICHARD D. BLAND, ROBERT H. DEMLING, SAMUEL L. SELINGER, AND NORMAN C. STAUB

SUMMARY To determine whether hypoxia directly affects pulmonary microvascular filtration of fluid or permeability to plasma proteins, we measured steady state lung lymph flow and protein transport in eight unanesthetized sheep breathing 10% O₂ in N₂ for 4 hours. We also studied three sheep breathing the same gas mixture for 48 hours. We surgically prepared the sheep to isolate and collect lung lymph and to measure average pulmonary arterial (P₂a) and left atrial (Pₐa) pressures. We placed a balloon catheter in the left atrium to elevate Pₐa. After recovery, the sheep breathed air through a tracheostomy for 2–4 hours, followed by 4 or 48 hours of hypoxia. In 13 4-hour studies, the average arterial P₂a fell from 97 to 38 torr; Pₐa rose from 20 to 33 cm H₂O; and lung lymph flow and lymph protein flow were unchanged. We also found that during 48-hour hypoxia, with a sustained elevation in P₂a and a decline in Pₐa, lymph flow and protein flow did not increase. In four sheep, we also raised Pₐa for 4 hours, followed by 4 hours of hypoxia with elevated Pₐa. Again, despite the added stress of elevated Pₐa, we found that lymph flow and lymph protein flow remained constant during hypoxia. We conclude that severe alveolar hypoxia, for 4 or 48 hours, alone or with increased pulmonary microvascular pressure, produced no change in lung fluid filtration or protein permeability, a finding supported by normal postmortem histology and extravascular lung water content.

IN 1945, DRINKER concluded that "oxygen lack is the most potent and elusive cause of abnormal leakage from the lung capillaries," based on the observations that flow from the right lymphatic duct of anesthetized dogs increased when the dogs breathed a 10% oxygen gas mixture. Courtice and Korner reported in 1952 that hypoxia predisposed rabbits to pulmonary edema induced by infusions of Ringer's solution, but the authors attributed the edema to heart failure and not altered vascular permeability to plasma proteins.

Other investigators have been unable to substantiate Drinker's conclusion. Haddy et al. showed in anesthetized dogs that hypoxia alone did not produce pulmonary edema, but only did so in the presence of elevated pulmonary venous pressure. Hemingway exposed guinea pigs to gas mixtures containing as little as 2% oxygen, but found no evidence of pulmonary edema when he killed the guinea pigs and examined their lungs.

Using isolated, perfused dog lungs, Nicoloff et al. were unable to demonstrate an increase in lung weight with extreme hypoxia. Goodale et al. found that even total absence of oxygen in the inspired gas did not alter the permeability of the alveolocapillary membrane to tracer albumin in isolated, perfused dog lungs. Fisher et al. saw no changes in the ultrastructure of the alveolar septum of intact dog lungs ventilated with nitrogen for 3–7 hours, and Teplitz et al. were unable to elicit pulmonary edema in rats with hypobaric hypoxia equivalent to 7% oxygen in the inspired gas for up to 30 hours.

Despite all of this contrary evidence, the current medical literature continues to cite hypoxia as a source of enhanced pulmonary microvascular permeability to protein and fluid. There are students of high altitude pulmonary edema who attribute that condition to transarterial leakage of plasma proteins or to increased permeability of the alveolocapillary membrane resulting from hypoxia. To our knowledge no one since Drinker has studied the effects of hypoxia on the permeability of the pulmonary microvasculature alone (as opposed to the alveolocapillary barrier) to plasma proteins.

We have reassessed the effects of alveolar hypoxia on steady state lung lymph flow and protein transport in unanesthetized sheep, breathing 10% oxygen in nitrogen for 4 hours in eight sheep and for 48 hours in three. This level and duration of alveolar hypoxia had no significant effect on pulmonary microvascular filtration of fluid or permeability to plasma proteins.

Methods

We studied nine female sheep, 45–60 kg, by isolating and collecting lung lymph and measuring vascular pressures after the sheep had recovered from surgery. Our preparative operations are described elsewhere in detail. Briefly, we first ligated and resected the systemic contributions to the caudal mediastinal lymph node, which is a large, sausage-shaped structure located adjacent to the aorta in the posterior mediastinum. In a later operation, we placed a small heparin-impregnated Silastic catheter (no. 602-015, Dow Corning) in the efferent duct of that node for collecting nearly pure lung lymph.

We also placed catheters in the left atrium, pulmonary artery, thoracic aorta, and superior vena cava, inserted an...
inflatable Silastic-coated balloon catheter in the left atrium, and put an inflatable latex cuff around the main pulmonary artery. We fastened a small metal clip adjacent to the dorsal surface of the left atrium, which we subsequently used for fluoroscopic identification of our zero reference point for pressure measurements.

Following the thoracotomies, the sheep recovered for 3–7 days, during which the lymph became clear of blood, and lymph flow stabilized. One day before the start of our experiments in each sheep, we made a tracheostomy. The sheep received halothane (Fluothane) anesthesia during the surgical procedures.

During all experiments the sheep were awake, unmedicated, and had free access to food and water. We measured vascular pressures continuously, using small, calibrated pressure transducers (MP 15, Micron Instruments) and an eight-channel amplifier-recorder (Accudata 113 bridge amplifiers and Visicorder oscillograph, model 1508A, Honeywell). We recorded lymph flow at 15-minute intervals, and pooled lymph samples every 30 minutes. We obtained blood samples hourly and measured protein concentrations in both lymph and plasma samples by the biuret method, with albumin and globulin fractionation by cellulose acetate electrophoresis (Beckman Microzone).

To be certain that we were collecting only pulmonary lymph without systemic contamination, we constricted the pulmonary artery with the inflatable cuff for at least 1 hour in each sheep. In all cases, despite an increase of 5–10 cm H$_2$O in systemic venous pressure, lymph flow remained constant or fell. This physiological test ensured that we were obtaining nearly pure lung lymph, since a pressure increase of this magnitude would be expected to increase the flow of lymph if it were of systemic origin.

During all studies the sheep breathed humidified gas, either air or 10% oxygen in nitrogen, from a 100-liter meteorological balloon through a low resistance valve (Hans Rudolph) and a 13-mm cuffed tracheostomy tube (Portex, Kent, England) in a nonrebreathing system which the sheep tolerated well. We found no appreciable difference in pressure across the valve on several measurements in both lymph and plasma samples by the biuret method, with albumin and globulin fractionation by cellulose acetate electrophoresis (Beckman Microzone).

To be certain that we were collecting only pulmonary lymph without systemic contamination, we constricted the pulmonary artery with the inflatable cuff for at least 1 hour in each sheep. In all cases, despite an increase of 5–10 cm H$_2$O in systemic venous pressure, lymph flow remained constant or fell. This physiological test ensured that we were obtaining nearly pure lung lymph, since a pressure increase of this magnitude would be expected to increase the flow of lymph if it were of systemic origin.

ACUTE HYPOXIA

In eight sheep, we did 13 studies. Following a 2-hour baseline period during which the sheep breathed room air, we changed the inspired gas mixture to 10% oxygen in nitrogen for 4 hours.

Elevated Pressure and Hypoxia Studies

In four of the above sheep, after a steady state period in which they breathed air and had normal left atrial pressure ($P_{La}$), we inflated the balloon in the left atrium to raise $P_{La}$ by 15–20 cm H$_2$O. We allowed 4 hours to reach a new steady state flow rate for lymph and protein, after which we switched the inspired gas mixture to 10% oxygen in nitrogen, maintaining a constant $P_{La}$ for 4 hours.

Extended Hypoxia Studies

We studied three sheep for 48 hours as they breathed 10% oxygen in nitrogen, after a 4-hour baseline period during which they breathed air. We measured vascular pressures with the sheep standing for at least 15 minutes of every hour. We collected lymph samples hourly and blood samples for plasma protein determinations every 2 hours and for blood gases every 12 hours. We measured cardiac output and minute ventilation daily. We discovered that one of the sheep was pregnant during the experiment when she extruded a nonviable fetus following 26 hours of hypoxia.

Postmortem Studies

At the conclusion of the final experiment in each sheep—six after 4 hours of hypoxia and three after 48 hours of hypoxia—we injected 10,000 U of sodium heparin into the vena cava, followed by thiopental (Pentothal) sodium (Abbott), 20 mg/kg. We immediately placed the sheep in the supine position, maintaining ventilation with 10% oxygen. We rapidly opened the chest by a midline sternotomy and killed the sheep by double cross-clamping each lung hilum at end-inspiration (20 cm H$_2$O pressure) with the heart still beating. We removed and quickly froze a piece of inflated lung in liquid nitrogen, freeze-dried it, and prepared it for histological study. We homogenized the remainder of both lungs (Waring blender, Dynamics Corporation of America) for determination of lung water content by our modification of the method of Pearce et al.

We killed six sheep, as controls, after 6 hours of breathing air through a tracheostomy tube and the attached breathing circuitry, as none of our previous controls had tracheostomies.

STATISTICAL ANALYSIS

To save space, we have summarized the important baseline and experimental data (average ± 1 SE) in Table 1. However, the statistical analysis was done by the two-tailed Student's t-test on individual paired differences. We accepted $P < 0.05$ as significant. Other ancillary data are given in the text as the average ± 1 SE. We analyzed the results of postmortem lung water analysis by the Wilcoxon nonparametric rank test, comparing the six control sheep with the nine hypoxic sheep. We accepted $P < 0.05$ as significant.

Results

ACUTE HYPOXIA

Figure 1 illustrates the results of a typical experiment in which the sheep breathed 10% oxygen for 4 hours following a steady state, baseline period of room air. With hypoxia, there was no change in lymph flow or lymph protein concentration, despite a substantial rise in average pulmonary arterial pressure ($P_{pa}$). There was a slight decline in $P_{La}$. The posthypoxia response, though not a stand-
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TABLE 1  Comparison of Vascular Pressures, Cardiac Output, and Indices of Transvascular Fluid and Protein Movement in the Lungs of Awake Sheep

<table>
<thead>
<tr>
<th>Condition</th>
<th>Inspired gas</th>
<th>Arterial P02 (torr)</th>
<th>P\textsubscript{pa} (cm H\textsubscript{2}O)</th>
<th>P\textsubscript{a} (cm H\textsubscript{2}O)</th>
<th>Cardiac output (liters/min)</th>
<th>Lymph flow (ml/hr)</th>
<th>Protein flow (g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>Air</td>
<td>102 ± 2</td>
<td>1 ± 0.3</td>
<td>7.18 ± 0.50</td>
<td>5.5</td>
<td>4.12 ± 0.29</td>
<td>46.01 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>Increased P\textsubscript{pa}</td>
<td>101 ± 0.5 ± 0.2</td>
<td>1 ± 0.2</td>
<td>6.44 ± 0.102</td>
<td>2.7</td>
<td>0.09 ± 0.10</td>
<td>7.09 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Increased P\textsubscript{a}</td>
<td>10% O\textsubscript{2}</td>
<td>40 ± 0.3 ± 2</td>
<td>17 ± 0.100</td>
<td>2.3</td>
<td>0.13 ± 0.10</td>
<td>5.09 ± 0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>Air</td>
<td>105 ± 2</td>
<td>17 ± 0.3</td>
<td>5.39 ± 0.59</td>
<td>6.7</td>
<td>4.46 ± 0.29</td>
<td>6.40 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>10% O\textsubscript{2}</td>
<td>38 ± 0.4 ± 0.3</td>
<td>22 ± 0.10</td>
<td>7.52 ± 0.13</td>
<td>2.1</td>
<td>0.26 ± 0.15</td>
<td>6.94 ± 0.36</td>
</tr>
<tr>
<td>24-28</td>
<td>10% O\textsubscript{2}</td>
<td>43 ± 0.4 ± 0.3</td>
<td>33 ± 0.10</td>
<td>6.68 ± 0.13</td>
<td>4.6</td>
<td>4.11 ± 0.10</td>
<td>6.59 ± 0.19</td>
</tr>
<tr>
<td>48-52</td>
<td>10% O\textsubscript{2}</td>
<td>43 ± 0.4 ± 0.3</td>
<td>33 ± 0.10</td>
<td>6.68 ± 0.13</td>
<td>4.6</td>
<td>4.11 ± 0.10</td>
<td>6.59 ± 0.19</td>
</tr>
</tbody>
</table>

P\textsubscript{pa} and P\textsubscript{a} = average pulmonary arterial and left atrial pressures; Alb = percent of the total protein concentration which is albumin; and lymph protein flow is the lymph flow rate times the lymph total protein concentration. Pressure measurements, flow rates, and protein concentrations are average values for measurements obtained over 2-4 hours of steady state conditions.

* Average ± 1 SEM.

ard part of the experimental protocol, is shown for completeness. During the 2-hour recovery period, vascular pressures returned to near baseline levels, but neither lymph flow nor protein concentration varied.

In 13 such experiments on eight sheep, the switch from air to 10% oxygen breathing was associated with a change in the pattern of ventilation. While minute ventilation was unaltered by hypoxia (20.2 ± 1.9 liters/min vs. 19.6 ± 1.8 liters/min), tidal volume increased from 234 ± 10 ml to 276 ± 10 ml, and frequency of breathing decreased from 81 ± 8/min to 65 ± 7/min. Arterial P\textsubscript{CO}2 decreased from 39 ± 1 torr to 29 ± 1 torr, and arterial pH rose from 7.47 ± 0.01 to 7.53 ± 0.01. All of these changes were significant and represent a normal adaptive response to acute arterial hypoxemia.

The important new data related to net transvascular fluid and protein flow are summarized in Table 1A. Associated with the fall in arterial P\textsubscript{O}2 from 97 ± 2 torr to 38 ± 2 torr, there was a rise in P\textsubscript{pa} to 65% above the baseline level, while P\textsubscript{a} decreased slightly. Cardiac output rose by 20% during hypoxia, and the calculated pulmonary vascular resistance increased 44%. All of these changes were significant.

Despite the cardiovascular response to hypoxia, steady state lung lymph flow and lymph protein transport remained unchanged. While plasma protein concentration increased with hypoxia, lymph protein concentration did not change. The albumin fraction of the lymph proteins was higher than that of the plasma proteins (0.49 vs. 0.38) during both baseline and hypoxia periods, showing that albumin transversed the vascular endothelium more readily than the larger globulin molecules, irrespective of the inspired gas.

ELEVATED PRESSURE AND HYPOXIA STUDIES

Figure 2 shows the time course of one experiment in which, after a 2-hour baseline period, we inflated the balloon catheter in the left atrium for 4 hours, followed by the additional stress of hypoxia with elevated vascular pressures for 4 hours. As expected, with the rise in P\textsubscript{pa} and the secondary increase in P\textsubscript{a} induced by inflating the balloon, lung lymph flow increased and lymph protein...
concentration fell. Then, with \( P_{lA} \) elevated at a constant level, hypoxia produced a further rise in \( P_{pa} \), a slight decline in lymph flow, and a comparably small rise in lymph protein concentration. With resumption of air breathing, \( P_{pa} \) declined slightly, as lymph flow and protein concentration returned to their prehypoxia levels. All indices returned to baseline when \( P_{pa} \) was reduced to normal.

Inflation of the balloon in the left atrium caused no significant change in ventilation. But when the sheep breathed 10% oxygen, with elevated \( P_{lu} \), arterial \( P_{CO_2} \) decreased from 35 ± 1 torr to 27 ± 1 torr and \( pH \) increased from 7.48 ± 0.01 to 7.56 ± 0.01, similar to the experiments with hypoxia alone. This respiratory alkalosis was associated with an increase in tidal volume from 291 ± 22 ml to 345 ± 29 ml but no change in minute ventilation (30.0 ± 5.7 liters/min vs. 31.2 ± 3.4 liters/min).

Table IB summarizes the important data relating to vascular pressures, cardiac output, and transvascular fluid and protein movement in the four experiments in which we combined hypoxia with increased pulmonary microvascular pressure. With inflation of the left atrial balloon, average arterial \( P_{O_2} \) did not change, cardiac output fell in three of four sheep, and \( P_{pa} \) rose from 18 ± 1 cm H\(_2\)O to 30 ± 1 cm H\(_2\)O. Lymph flow and lymph protein flow increased as expected. These changes were associated with a rise in plasma protein concentration and a fall in lymph protein concentration in all four experiments.

When the sheep breathed 10% oxygen for 4 hours, in the presence of a sustained elevation of \( P_{lu} \), arterial \( P_{O_2} \) fell from 101 ±5 torr to 40 ± 3 torr, \( P_{pa} \) rose by an additional 20%, and cardiac output increased by 40%. Yet average lymph flow and lymph protein transport did not change appreciably.

**EXTENDED HYPOXIA STUDIES**

Figure 3 illustrates the effects of alveolar hypoxia on lung lymph flow, protein concentration, and vascular pressures during one 52-hour experiment. The change from air to 10% oxygen breathing at 4 hours was associated with an abrupt rise in \( P_{pa} \), which was sustained over the next 48 hours. During this period, there was no appreciable change in lymph flow, though lymph protein concentration increased slightly for the first 24 hours and then fell to just below the baseline level during the second 24-hour period.

In all three 52-hour studies, hypoxia led to a sustained respiratory alkalosis resulting from an increase in tidal volume, again with no change in minute ventilation.

Table 1C summarizes the important data for the three extended-hypoxia studies. As in the 4-hour experiments, \( P_{pa} \) increased to almost twice the baseline level, while \( P_{lu} \) decreased by a smaller amount. Concurrently, cardiac output rose by 40% at 24 hours and remained 22% above the baseline output at 48 hours. At the end of the period of hypoxia, average pulmonary vascular resistance was 98% higher than the calculated resistance before hypoxia. Yet the average lymph flow and lymph protein flow did not increase.

As in the shorter experiments, plasma protein concentration increased with hypoxia (by an average of 9% at 24 hours) in all three studies. This change was accompanied by a 15% rise in mixed venous hematocrit. Lymph protein concentration, however, did not increase, yielding a fall in the lymph to plasma protein ratio from 0.70 to 0.62.

**POSTMORTEM FINDINGS**

We examined sections of fresh frozen lung taken from all sheep breathing 10% oxygen immediately before they were killed. We found normal lung architecture with no perivascular fluid cuffing or alveolar flooding.

Table 2 shows that there was no significant difference in the lung water content of sheep killed after breathing (1) air for 6 hours through a tracheostomy tube (controls), (2) air for 2 hours followed by 10% oxygen for 4 hours, or (3) air for 4 hours followed by 10% oxygen for 48 hours.

**Discussion**

Measurement of pulmonary lymph flow and protein concentration is a sensitive index of the net transvascular movement of fluid and protein in the lung. 18 20 22 24 Humphreys and associates 25 found that in the sheep approxi-
We found a persistent increase in pulmonary vascular resistance by almost 50% above baseline during 4 hours of hypoxia and by almost 100% after 48 hours. Previous studies by Reeves et al. and Grover showed that lambs have an immediate but small increase in vascular resistance during hypoxia, but that this effect is not sustained. Perhaps the fact that they studied young lambs, not adult sheep, bred at an elevation of 3,500 feet, not at sea level, and transported to 12,700 feet (equivalent to 13% inspired O₂, rather than 10%) contributed to their attenuated response. It is also possible that the return to normal vascular resistance during hypoxia requires longer than 48 hours (the maximum duration of hypoxia in our studies), since Reeves et al. restudied the lambs after several weeks at high altitude.

Previous investigators have demonstrated that the change in vascular resistance associated with hypoxia occurs proximal to the lung capillaries. Our finding of no change in lung flow with the persistent elevation of pulmonary vascular resistance is further evidence that the site of vasoconstriction is proximal to the fluid exchange vessels in the lung, since more distal constriction would have caused an increase in lymph flow by a rise in vascular hydraulic pressure, as we always see when Pₐa increases.

We considered the possibility that a superimposed stress, such as elevating Pₐa with hypoxia, or more prolonged hypoxia, might induce pulmonary edema. In neither case did this occur, nor did we find any evidence of altered vascular permeability to plasma proteins. Moreover, the fortuitous premature labor and delivery of one sheep during hypoxia did not evoke an increase in lymph flow or induce an abnormal accumulation of extravascular lung water, suggesting that pregnancy, and labor in particular, has no appreciable effect on lung water transport. Since exercise sometimes precipitates pulmonary edema at high altitudes, it would have been interesting to observe the effects of hypoxia during exercise. The sedentary nature of the sheep, however, prohibited this type of experiment and forced us to settle for observations during hypoxia with left atrial pressure elevation.

The results of this study show that alveolar hypoxia, with or without increased Pₐa, does not alter pulmonary microvascular fluid filtration or permeability to plasma proteins. Moreover, we have demonstrated that hypoxia produces a sustained increase in pulmonary vascular resistance in sheep, as in other species, and that the site of vasoconstriction is proximal to the exchanging vessels in the lung.

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**Table 2** Lung Water Content per Unit of Dry, Bloodless Lung for Sheep Killed after Breathing Air for 6 Hours (Control Group), 10% Oxygen in Nitrogen for 4 Hours, and 10% Oxygen in Nitrogen for 48 Hours

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>No. of sheep</th>
<th>Extravascular lung water/blood-free dry lung wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breathing air (6 hr)</td>
<td>6</td>
<td>4.45 ± 0.08</td>
</tr>
<tr>
<td>Breathing 10% O₂ (4 hr)</td>
<td>8</td>
<td>4.27 ± 0.08</td>
</tr>
<tr>
<td>Breathing 10% O₂ (48 hr)</td>
<td>3</td>
<td>4.42 ± 0.12</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

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The ventilatory changes that we found associated with acute hypoxia in sheep are comparable qualitatively to those which occur in humans following hypoxia, as previously described by Dripps and Comroe. Alveolar ventilation increased, the result of an expanded tidal volume, and respiratory rate diminished, but minute ventilation did not change. Relative to humans, sheep breathe at a higher frequency and minute volume, probably reflecting the need of sheep to dissipate heat by ventilation.

Despite the changes in respiratory pattern associated with hypoxia in our experiments, lung lymph flow did not change. This finding differs from that of Warren and Drinker, who noted in anesthetized dogs that an increase in tidal volume was associated with a fall in lung lymph flow. This discrepancy might reflect species variation or possibly the fact that their dogs were not awake and that they were ventilated with positive pressure.

In all of our experiments, we found that hypoxia produced a substantial rise in Pₐa, sustained for 48 hours in our three long-term experiments. In addition, cardiac output rose by an average of 19% in the 4-hour studies and by 24% during prolonged hypoxia. In the presence of this elevation in blood flow, Pₐa decreased, suggesting that pleural pressure may have decreased during hypoxia.
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