Pure Oxygen Breathing Increases Sheep Lung Microvascular Permeability

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Sheep that breathe pure oxygen via a tracheostomy develop progressive respiratory failure and die within four days. The characteristic terminal findings include an increased water content of the lung, a decrease in lung compliance, and severe hypercapnia.

To sequentially assess alterations of lung transvascular fluid dynamics during prolonged oxygen breathing the authors measured lung lymph flow (Q lymph), protein transport (Q protein), and pulmonary vascular pressures in five sheep with chronic lung lymph fistulas. No significant changes of lung transvascular fluid dynamics occurred during the first 60 hours of oxygen breathing, although an increasing trend of Q lymph and Q protein was demonstrable. However, after 72 hours of oxygen breathing, Q lymph, Q protein, and extravascular lung water had increased significantly without any change of pulmonary vascular pressures. The authors conclude that the toxic effects of oxygen on the lungs of sheep include a delayed but marked increase of pulmonary microvascular permeability to protein and fluid. (Key words: Lung; lymph flow; vascular permeability. Oxygen: toxicity.)

For over eighty years it has been known that breathing pure oxygen can severely injure the lungs. Within a few days, breathing oxygen causes acute pulmonary edema that leads to death of all the mammalian species that have been studied.1,2 Sufficient data exists to demonstrate that pulmonary oxygen toxicity occurs in humans.3 The adult respiratory distress syndrome is characterized by an increased right-to-left shunt and intrapulmonary maldistribution of gas that requires treatment with an increased inspired oxygen concentration. This creates the physician's dilemma of treating the acutely injured lung by prolonged breathing of toxic oxygen levels.

The acute pulmonary toxicity of oxygen results in interstitial and intra-alveolar edema with vascular congestion. However, the earliest observable lesion caused by breathing pure oxygen has been shown to be swelling of the capillary endothelium and not injury to the alveolar epithelium.4,5 Since the capillary endothelial barrier preserves the normal balance of fluid and proteins within the lung, we hoped that analysis of microvascular fluid transport might provide a sensitive indicator of the capillary toxicity of oxygen. Therefore, we applied the lung lymph fistula model in sheep to assess alterations of fluid and protein transport within the lung during prolonged breathing of oxygen.

Seven years ago, we reported the sequential changes of pulmonary mechanics and central hemodynamics in sheep that breathed pure oxygen via a tracheostomy. In those sheep studies, severe pulmonary edema occurred after two to four days of breathing oxygen.6 In contrast to many other forms of acute lung injury in the sheep, there was no change of the pulmonary artery pressure or the pulmonary artery occlusion pressure despite severe pulmonary edema. Thus, this model offered an advantage; one might assess the effects of increased microvascular permeability on the lung lymph protein concentration and flow rate without any increase of pulmonary microvascular pressure.

Methods

We studied five 3- to 5-month-old Suffolk sheep weighing 30–35 kg. They were weaned at the age of 8–10 weeks, and their diet then consisted of a mixture of soybean meal, corn, and hay with a vitamin supplement containing 14 IU vitamin E/kg. The sheep were anesthetized, intubated, and ventilated mechanically with a mixture of pure oxygen and 1–2% halothane. The lungs were hyperinflated at frequent intervals to prevent atelectasis. The sheep were prepared surgically for the collection of lung lymph as described previously.7 Through three thoracotomies, heparin-coated silicone rubber catheters (TDMAC process)8 were placed into the left atrium and the efferent duct of the caudal mediastinal lymph node. The tail of this node was resected at the caudal margin of the pulmonary ligament to eliminate major contributions of systemic lymph. Sheep recovered following surgery and their lung lymph flow rate was constant and free of blood by two to four days after surgery. The sheep then were anesthetized briefly and a tracheostomy was performed. A cuffed stainless steel tracheotomy tube (8-mm internal
diameter) was inserted into the stoma. The femoral artery was cannulated with a 3-mm outside diameter (OD) polyvinyl chloride catheter for blood sampling. A #7-French flow-directed thermodi-lution catheter (Elecath Co., Rahway, New Jersey) with a proximal injection site 12.5 cm from the tip was inserted into the jugular vein and advanced into the pulmonary artery to permit measurements of mean pulmonary artery pressure (PAP). Cardiac output was determined by the thermodilution method and calculated by computer (Elecath Co., Model COC 4000). Following recovery from surgery, the sheep were placed in cages with free access to food and water. The animals were given feed pellets containing 8 IU/kg vitamin E (H. K. Webster Co., Inc., Lawrence, Massachusetts). Humidified inspired air or oxygen was administered via a non-rebreathing T-piece system. Vascular pressures were measured continuously using Hewlett-Packard® 1280C transducers and an 8-channel recorder (Hewlett-Packard® 7798A).

All animals breathed humidified room air for 24 h while hemodynamic parameters and lymph flows stabilized before 100% oxygen was administered. During the experimental period, lymph was collected in heparinized tubes at hourly intervals and the flow was averaged over each 4-h period. Heparinized blood samples were obtained every four hours for plasma protein determinations and every eight hours for the measurement of arterial blood-gas tensions. From previous experiences with sheep breathing oxygen through a tracheostomy in this laboratory, it was decided to kill these animals when the arterial P CO$_2$ reached 70 mmHg to avoid respiratory discomfort.

The lymph and blood samples were centrifuged and the concentration of total protein was measured by refractometry (American Optical Corp., Model 10400A). Albumin concentration was assessed by the Bromcresol green method. Globulin concentrations were estimated by subtracting the albumin concentration from the total protein concentration. P AO$_2$, P CO$_2$, and pH$_4$ were measured with a Corning® blood-gas analyzer Model 175.

At the end of the experiments, a reference blood sample for hemoglobin determination was drawn from the arterial cannula and the sheep was killed with an overdose of barbiturate. Following rapid sternotomy, the lungs were removed after cross-clamping both lung hila. The excised lungs were passively drained of blood, weighed, and homogenized in a blender (Waring® 5011) with an equal volume of water. The water content of the lung homogenate, the supernatant fraction of the centrifuged homogenate, and whole blood were measured by drying them to constant weight (48 h) at 80°C. The total hemoglobin concentration of the blood and the supernatant of the homogenate was measured by the cyanmethemoglobin method. Calculation of extravascular lung water after correction for the intravascular blood water content was carried out as outlined by Pearce and co-workers. Extravascular lung water was determined in eleven additional control sheep (without vascular or lymphatic cannulation) killed immediately after breathing pure oxygen via a tracheostomy for 24 h (n = 3), 48 h (n = 2), and 72 h (n = 3), or breathing room air for 3 h (n = 3).

**DATA ANALYSIS**

Pulmonary capillary pressure (PCP) was calculated using the formula,

$$PCP = LAP + 0.4(\bar{PA} - LAP)$$

where LAP and \(\bar{PA}\) are the average left atrial and pulmonary artery pressures. The constant 0.4 is the estimated fraction of total pulmonary vascular resistance that is downstream from the microvascular exchange surface. Fluid filtration from lung capillary to interstitium is described by Starling’s fluid transport equation,

$$\dot{Q}_f = K_f[(PCP - P_{in}) - \sigma(\pi_{cap} - \pi_{int})]$$

where \(\dot{Q}_f\) is the net transvascular fluid flow and \(K_f\) is the fluid filtration coefficient reflecting both the lung capillary membrane area and its permeability. The capillary and interstitial colloid osmotic pressures, \(\pi_{cap}\) and \(\pi_{int}\) were calculated from the total protein concentrations in plasma and lymph using the regression equations of Landis and Pappenheimer. The solute reflection coefficient \(\sigma\) for protein was set equal to one (completely impermeable to protein). We assumed interstitial pressure (P$_{in}$) was equal to alveolar pressure and, therefore, zero. To calculate \(K_f\), we referred the left atrial and pulmonary artery pressures to the ventral surface of the lung by adding 11 mmHg. The numerous assumptions necessary to apply Starling’s equation have been described previously.

For statistical analysis in table 1, we used the paired t test to compare baseline measurements with those obtained after 72 to 75 h of O$_2$ breathing. We used an analysis of variance and subsequently an unpaired t test to compare alterations of vascular pressure and lymph clearance among the three experimental groups in figure 2. A probability of less than 0.01 was considered statistically significant. All the data presented are mean values ± SD.

**Results**

The hemodynamic and lymph transport data measured in five sheep breathing 100% oxygen for up to
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114 h are presented in table 1. For the first 60 h of oxygen breathing there were no significant changes of lymph protein transport or flow rate, although an increasing trend was evident. After 72 h, a significant increase of lymph flow rate and lymph protein transport occurred. This was reflected by a significant increase of both the lymph-to-plasma total protein ratio and the calculated value of the filtration coefficient $K_f$. At the end of the study the cardiac output ($4.05 \pm 0.35$ l/min), mean pulmonary artery pressure ($13 \pm 2$ mmHg), and left atrial pressure ($4 \pm 1$ mmHg) were unchanged from the control values during oxygen breathing. The sheep were never hypoxic but evidenced statistically significant hypercapnia after 72 h.

Figure 1 graphically presents the changes in lymph flow rate and lymph protein clearance ($C_L$) of a single sheep breathing pure oxygen. After 60 h, there was a gradual increase of lymph flow from a stable 6.1 ml/h baseline to a peak value of 39.9 ml/h before death at 114 h. The lymph protein clearance also increased from 4 ml/h at 20 h to 30 ml/h at 114 h. Pulmonary capillary pressure was unchanged during the entire period of oxygen breathing.

In figure 2 we contrast the mean baseline and final measurements of $C_L$ and PCP of five sheep breathing oxygen with those obtained by Erdmann et al., after increasing PCP by inflating a left atrial balloon and those reported by Brigham et al., several hours after infusion of Pseudomonas bacteria. Both Pseudomonas bacteremia and oxygen breathing increase $C_L$ despite an unchanged PCP. The lymph protein clearance and pulmonary artery pressure following either Pseudomonas infusion or oxygen breathing are significantly different ($P < 0.01$) from those obtained by mechanically increasing left atrial pressure.

Extravascular lung water (EVLW) content of 13 sheep is plotted against the number of oxygen breathing hours via a tracheostomy in figure 3. The range of EVLW ($4.65–5.04$ g/g dry lung) measured in three sheep breathing air via a tracheostomy for three hours is drawn on the graph for reference. There was no difference between these normal values and the EVLW determined after 24 and 48 h of oxygen breathing. However, after 72 h the EVLW markedly increased to $5.86 \pm 0.16$, g/g dry lung, and just prior to death with severe acute respiratory failure it was $5.92 \pm 0.17$ g/g dry lung.

**Discussion**

These studies demonstrate that after 50–60 h of pure oxygen breathing by 35-kg sheep there is a marked increase of the lung lymph flow rate and protein concentration. The increases of lymph flow rate and protein

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concentration are concomitant with a marked increase of lung extravascular water. Increased lung lymph flow and protein content occurs at a constant cardiac output and without any increase of the pulmonary artery pressure or left atrial pressure (fig. 1). These changes can therefore be assumed to be due to an increase of microvascular permeability. The increase is major and progressive after 60 h of oxygen breathing in the sheep illustrated in figure 2. The increasing lung lymph protein concentration reflects an increase of interstitial lung albumin concentration reducing the colloid oncotic pressure gradient that removes water from the pulmonary interstitium. The increased interstitial protein concentration is due to a marked increase of microvascular permeability and results in heavy lungs with a markedly increased extravascular lung water measured after 72 h of oxygen breathing (fig. 3).

Recently, Drake et al. raised some concern over the validity of the sheep lymph fistula preparation for the detection of lung microvascular permeability changes. Even with the most careful surgical technique some minor degree of nonpulmonary lymph contamination probably exists. The lymph contamination from nonpulmonary sources may play a role when only small increases of lymph flow and protein content are measured after an experimental injury. We observed large increases in lymph protein clearance concomitant with a marked increase of lung extravascular water. Therefore, we consider our measurements to be a true reflection of the increased pulmonary vascular permeability following pure oxygen breathing.

Assuming a steady state of lung fluid balance, one can calculate $K_r$, the filtration coefficient (table 1) which

![Graph](image1)

**Fig. 1.** Lymph flow (ml/h) (triangles), pulmonary capillary pressure (PCP, mmHg) (squares), and lymph protein clearance (ml/h) (closed circles) plotted as a function of time in one sheep breathing 100% oxygen for 114 h.

![Graph](image2)

**Fig. 2.** Lung lymph protein clearance ($C_L$, ml/h) and pulmonary capillary pressure (PCP, mmHg) during baseline and experimental periods in three groups of sheep. Closed circles indicate the five sheep breathing oxygen reported in this paper. Squares demonstrate the effect of Pseudomonas infusion in six sheep previously reported by Brigham et al.15 Triangles show the effects of increasing left atrial pressure in six sheep with a balloon.19 All values are means.

![Graph](image3)

**Fig. 3.** Extravascular lung water (EVLW) (g water per g dry bloodless lung) as a function of time in oxygen breathing sheep. Dots indicate values of individual sheep breathing pure oxygen for 24, 48, and 72 h. Triangles are values of individual sheep breathing pure oxygen with lymph fistulas. The area between the broken lines encloses the range of EVLW values for three sheep killed after breathing humidified air for three hours.
increased progressively from 0.5 to 11.5 ml·mmHg⁻¹·h⁻¹. However, several important and possibly unjustified assumptions are required. Firstly, hydrostatic interstitial pressure was considered to be constant and equal to zero. Secondly, the microvascular reflection coefficient for protein, σ, was set equal to one. Interstitial pressure may initially be negative and then increase with enhanced transvascular fluid filtration and edema formation.¹⁸ An increase of interstitial pressure would oppose fluid filtration and Kᵣ would then be higher than we calculated. The microvascular protein reflection coefficient, σ, has been estimated at 0.74 for sheep lung,¹⁹ and would decrease with increased permeability; thus we would have overestimated Kᵣ. The calculated increase of Kᵣ might be due to an increased microvascular surface area. This is unlikely since pulmonary vascular pressures and cardiac output did not change throughout these studies. Fluid conductivity through the interstitial matrix may have increased. Recent studies by Granger et al. suggest that increased gel hydration can increase matrix hydraulic conductivity several-fold; thus, Kᵣ increases.²⁰ The absolute value of Kᵣ we estimated in our studies is probably inaccurate; however, we believe alterations of the calculated value will reflect the trend of increasing permeability.

Our results are in agreement with the studies of Bres- sack et al. of six newborn lambs (12–19 days old) breathing pure oxygen.²¹ These lambs also developed an increase of lung lymph protein concentration and flow by 72 h of oxygen breathing. Several minor differences between the studies deserve a brief comment. The newborn sheep were maintained in a Lucite® chamber and breathed pure oxygen at ambient pressure via their normal airway. The albumin and globulin concentrations reported in plasma and lung lymph by Bressack et al. were somewhat lower than those we measured in older sheep. This may be due to the differing methods used to measure these proteins or changes with age in the sheep. The diets of their lambs and our sheep were quite different; our sheep were weaned and fed on hay, grass, and soybean feed for three months and then laboratory chow (containing 8 IU/kg vitamin E) for one or two weeks before breathing oxygen. Bressack’s lambs were fed ewe’s milk until breathing oxygen and then were fed a commercial infant formula. Five of Bressack’s six lambs survived 110 h of breathing oxygen while we had four of five die by 100 h. It is possible that breathing oxygen via a tracheostomy in the older lambs provided additional lung injury (e.g., infection, loss of effective cough, etc.). We have not evaluated the extent that the vitamin E supplement in the diet influenced our results.

After 40 h of breathing oxygen the animals became progressively hypercapnic (table 1). We have previously shown this is due to a marked decrease in functional residual capacity and static lung compliance leading to alveolar hypoventilation.⁶ This study confirms the key role of endothelial injury to the lung in acute oxygen toxicity. Although oxygen may directly injure endothelial cells causing increased microvascular permeability, recent studies have shown the possible importance of humoral factors in oxygen injury of the lung. As free radical injury is probably central to oxygen-induced lung injury and white cells have been shown to release superoxide anion, leukocytes may contribute to lung damage. Recent studies in rats have demonstrated pulmonary leukocyte accumulation in the terminal stages of oxygen toxicity, apparently attracted by substances released by alveolar macrophages damaged by hyperoxia.²² Future studies will clarify the precise mechanisms and mediators of endothelial damage in oxygen breathing acute pulmonary toxicity and may suggest pharmacologic means to ameliorate or avoid acute lung injury.

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References


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