Overperfusion, Hypoxia, and Increased Pressure Cause Only Hydrostatic Pulmonary Edema in Anesthetized Sheep

Cleland C. Landolt, Michael A. Matthay, Kurt H. Albertine, Philip J. Roos, Jeanine P. Wiener-Kronish, and Norman C. Staub

SUMMARY. Overperfusion (high pressure and flow through a restricted microvascular bed) has been suggested as the mechanism for both microembolic and high altitude pulmonary edema. In eighteen anesthetized, ventilated sheep, we measured pulmonary hemodynamics, lung lymph flow, and lymph:plasma protein concentration ratio. After a 2-hour stable baseline, we resected 65% of lung mass (right lung and left upper lobe) and gave whole blood transfusions to maintain cardiac output. During overperfusion of the left lower lobe, lymph flow increased moderately (5.8 ± 2.3 to 7.7 ± 3.8 ml/hr) and lymph:plasma protein concentration decreased (0.73 ± 0.08 to 0.64 ± 0.08). After a 2-hour stable period, we decreased inspired oxygen in 10 sheep (Pao2 = 40 ± 3 mm Hg). With added alveolar hypoxia, pulmonary artery pressure increased modestly, but lymph flow and the lymph:plasma protein concentration ratio did not change. In eight sheep (four hypoxic, four normoxic), we raised left atrial pressure approximately 12 cm H2O for 2 hours. Lymph flow rose (10.8 ± 3.8 ml/h) and lymph:plasma protein concentration decreased further (0.52 ± 0.07). At each step, lymph:plasma protein concentration decreased, as predicted for the calculated rise in microvascular pressure. There was no evidence that overperfusion, with or without alveolar hypoxia, increased lung endothelial barrier protein permeability. (Circ Res 52: 335-341, 1983)

GIBBON and Gibbon (1942) and Hultgren and co-workers (Hultgren et al., 1966, Hultgren, 1978) surgically restricted lung mass and increased blood flow by transfusion, thereby causing increased permeability edema by “overperfusion” in cats and dogs, respectively.

Acute alveolar hypoxia may lead to severe uneven pulmonary arterial constriction and to overperfusion of the remaining open circulation. This is a leading theory to explain the pulmonary edema seen at high altitude (Viswanathan et al., 1969; Hultgren, 1978). Recently, Hackett and associates (1980) provided clinical evidence in support of the theory. They reported on four people with congenital absence of the right pulmonary artery, all of whom developed pulmonary edema in the opposite overperfused lung at moderate high altitude.

When we began to study uneven pulmonary vascular obstruction caused by microemboli, we also speculated that overperfusion might contribute to the endothelial permeability injury associated with the microembolism syndrome (Ohkuda et al., 1978).

We have now made a direct test of the overperfusion theory. In anesthetized sheep with lung lymph fistulas, we examined the effects of lung mass restriction and transfusions, hypoxia, and increased left atrial pressure on lung fluid and protein exchange. Restriction and transfusion caused only high pressure interstitial edema. Addition of acute alveolar hypoxia and increased left atrial pressure did not alter microvascular fluid and protein permeability.

Methods

In several anesthetized sheep, we did preliminary studies and found that most of them could tolerate the combination of a right pneumonectomy and a left upper lobectomy. The remaining left lower lobe allowed continued lung lymph flow measurement through the pulmonary ligament and caudal mediastinal lymph node (CMN). In two sheep, we determined blood-free wet weights and the extravascular water content of each lobe. The data are shown in Figure 1. The right lung plus the left upper lobe account for about 65% of the total lung mass. The extravascular lung water contents of the individual lobes are all the same (3.8-4.0 g/g dry lung) and consistent with our values for normal lungs (Selinger et al., 1975).

Surgical Preparation

We successfully prepared 22 yearling sheep (22-50 kg, average 36 kg) to collect predominantly lung lymph from the efferent duct of the CMN and to measure pulmonary and systemic hemodynamics, as previously described (Staub et al., 1975; Ohkuda et al., 1978). All of the surgery was on the day of the experiment.

We anesthetized each sheep with thiopental sodium (20 mg/kg, iv), inserted an endotracheal tube, and ventilated it, using a controlled volume ventilator with 5 cm H2O end-expiratory pressure. We maintained anesthesia with 1-1½% halothane in 50% oxygen and induced muscle paralysis with pancuronium bromide (1-2 mg, iv, hourly). As the lungs...
were resected, we reduced the tidal volume and increased the ventilatory rate to maintain PaCO₂ between 30 and 40 mm Hg and peak inspiratory airway pressure at 25–30 cm H₂O. PaO₂ was always greater than 150 mm Hg.

Through a right neck incision, we passed polyvinyl catheters into the descending aorta via the carotid artery to measure systemic pressure and into the right atrium via the external jugular vein to use later for the measurement of cardiac output by thermodilution. Through the fourth left interspace, we placed catheters into the left atrium and pulmonary artery. A 3F thermistor (Mansfield Scientific) was attached to the pulmonary artery catheter to record body temperature and for use in determining cardiac output. Through the 10th right interspace, we resected the tail of the CMN to remove the major systemic contributions. Through the sixth right interspace, we cannulated the efferent duct of the CMN.

We obtained 1000–1500 ml of fresh heparinized donor sheep blood at the time of the surgery. The blood was given through a micropore filter (PALL) into the left atrium in 30- to 40-ml increments throughout the transfusion portion of the experiment to maintain cardiac output near baseline levels.

**Measured Variables**

We measured systemic arterial, pulmonary arterial, left atrial, and airway pressures using strain gauges (P23G, Gould-Statham Instrument Co.) connected to a direct-writing polygraph (model 7, Grass Instrument Co.). We determined thermodilution cardiac output as the average of three determinations, using a cardiac output computer (model 3500, Mansfield Scientific), by injecting 6 ml of room temperature 5% dextrose in water into the right atrium.

We recorded the hemodynamic variables continuously and calculated pulmonary vascular resistance every 15 minutes. We measured the volume flow rate of the lung lymph collected in heparinized, graduated tubes every 15 minutes. We collected the lymph every 30 minutes and a heparinized blood sample every 60 minutes for determination of total protein and albumin concentration (Ohkuda et al., 1978). Arterial blood gases and pH were measured hourly on suitable electrodes (model 168, Corning Instrument Co.).

**Experimental Protocols**

Following are the four sequences of procedures that were used.

**Control**

In four sheep, after a 2-hour steady state baseline period, we tested the effects of fresh whole sheep blood transfusions given to increase and maintain cardiac output for 4 hours.

**Resection and Transfusion**

In eighteen sheep, after a 2-hour baseline, we did a right pneumonectomy and left upper lobectomy. The resection required 1½ hours. After resection, we waited until hemodynamics and lymph flow were fairly stable (about 1 hour); then we gave the small intermittent transfusions to increase and maintain cardiac output near baseline levels with a limited increase in left atrial pressure (<4 cm H₂O). Four of the sheep were followed until we had achieved a 2-hour steady state. The total amount of blood given to any sheep ranged between 750 and 1000 ml.

**Hypoxia**

In 10 of the 18 sheep, after a stable period following resection and transfusion, we progressively lowered the inspired oxygen concentration until PaCO₂ = 40 ± 5 mm Hg. We measured lymph flow and hemodynamic variables for 2 hours.

**Increased Left Atrial Pressure**

In eight of the 18 sheep (four each with and without alveolar hypoxia) we placed a 16F Silicone-coated rubber Foley catheter with a 30-ml inflatable balloon into the left atrium at the level of the mitral valve during the preliminary surgery. At the appropriate time during the experiment, we inflated the balloon to obstruct the mitral orifice partially and raise left atrial pressure. We measured lymph flow and hemodynamic variables for 2 hours. The lymph flow was stable for at least 1 hour.

**Terminal**

In all sheep we removed the left lower lobe and froze it in liquid nitrogen for histological examination and for determination of extravascular lung water. We had preserved the resected lung lobes in a similar fashion.

**Statistics**

The data are presented in summary form as the mean ± 1 so. Each animal, however, served as its own control. If there was only one intervention, we used a paired t-test. We accepted P < 0.05 as indicating statistical significance. In the majority of experiments, there were successive interventions, so we did an analysis of variance among groups. If a significant difference was established, we applied the Bonferroni correction to the paired t-test between groups, accepting P < 0.05/n as indicating statistical significance (Wallenstein et al., 1980). We compared the two groups with elevated left atrial pressure by using an unpaired t-test.

**Results**

These were difficult long experiments to complete because of the various interventions in sequence. The duration, after the initial preparation, was 8–10 hours. Early on, we discarded several animals because we failed to achieve a 2-hour stable period in the resection and transfusion portion of the study.

**Children**

In four sheep, we measured the effects of blood transfusions alone, that is, we did no lung resection. Pulmonary artery and left atrial pressures increased...
by an average of 3.8 and 2.4 cm H2O, respectively, and cardiac output increased from 3.7 ± 1.0 to 5.6 ± 2.6 liters/min. Lung lymph flow increased from 5.1 ± 1.4 to 7.2 ± 2.0 but the lymph:plasma protein concentration ratio did not change significantly. Although we do not have a direct measure of microvascular exchange surface area, we interpret this small change in lymph flow as due to increases in exchange area, not to an increase in permeability. Postmortem lung water averaged 4.1 ± 0.1 g/g dry lung. Histologically, the lungs appeared normal.

Resection and Transfusion

The summary data for the 18 sheep are shown for the baseline period and after resection and transfusion in Table 1. Except for cardiac output and left atrial pressure, all of the changes during overperfusion are statistically significant. Moreover, the same directional change occurred in every sheep. The small increase in lymph flow is more important than appears at first glance. Since we removed the right lower lobe, which is about half of the drainage area for the CMN portion of the lung lymph, we would have expected lymph flow to decrease by about half, as we find in experiments in which we clamp one pulmonary ligament (unpublished data). Stothert et al. (1981) reported similar results in goats. The fact that lymph flow increased after lung resection, should be interpreted as meaning that left lower lobe liquid filtration more than doubled. This increase is consistent with an increase in microvascular hydrostatic pressure and in surface area for filtration.

Figure 2 shows the time course of one overperfusion experiment. After resection, pulmonary vascular resistance was increased due to the large rise in pulmonary arterial pressure, which reflected the restriction of the microvascular bed. The blood transfusions restored cardiac output to about baseline level and increased pulmonary arterial and left atrial pressures to levels similar to those shown in Table 1. These data are similar to those recently described by Snapper and co-workers (1982), who measured changes in area permeability-surface area product in anesthetized sheep, with and without increased cardiac output, as lung mass was decreased by lobe ligation. They interpreted their results as showing recruitment of exchange surface area, both with increased blood flow and with increased pressure after lobe ligation. Maseri and associates (1972) had made a similar interpretation of the effect of increasing pulmonary arterial pressure on vascular resistance and volume in the perfused dog lung.

In the four sheep killed after 5–6 hours of overperfusion, extravascular lung water was increased by 23% compared either to the lungs of the four control sheep or the resected lung of the same animal (Table 1). Histologically, the left lower lobes showed interstitial edema consisting of peribronchovascular fluid cuffs. We did not see any alveolar edema.

Interestingly, after the resection and transfusion, $P_{aO_2}$ tended to rise, although $P_{aCO_2}$ was maintained nearly constant. We attribute this to an improvement in the distribution of blood flow in the overperfused lobes compared to the baseline condition.

Hypoxia

The effect of added hypoxia following resection and transfusion in 10 sheep are shown in Table 2. Figure 3 shows the time course of one experiment. The alveolar hypoxia increased pulmonary arterial pressure and pulmonary vascular resistance modestly.

### Table 1

| Effect of Overperfusion on Pulmonary Hemodynamics and on Lung Fluid and Protein Exchange |
|---------------------------------|-----------|-----------------|-----------------|-----------------|
| **Pulmonary artery pressure (cm H2O)** | **Left atrial pressure (cm H2O)** | **Cardiac output (liters/min)** | **Calculated resistance (cm H2O/min per liter)** | **Lymph flow (ml/hr)** | **Protein concentration (lymph:plasma)** | **Lung water (g/g dry lung)** |
| No. | 16.8 ± 2.6 | 8.8 ± 2.2 | 2.9 ± 0.6 | 2.9 ± 0.5 | 5.6 ± 2.3 | 0.73 ± 0.08 | 4.0 ± 0.2 |
| Baseline | 18 | 18 | 18 | 18 | 18 | |
| Overperfusion†† | 29.9 ± 6.0 | 11.8 ± 2.8 | 2.9 ± 1.0 | 6.6 ± 1.8 | 7.7 ± 3.8 | 0.64 ± 0.08 | 4.8 ± 0.2 |

* Average ± so.
† Resected lung as normal.
‡ Resection of 65% lung mass followed by small intermittent transfusions to restore cardiac output to baseline level.
§ Significant at p < 0.05 by paired t-test.
|| Data for four animals terminated at this point.

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Time course of one experiment showing the effect of lung resection and of blood transfusion. After 65% of the lung mass was removed, the pulmonary vascular resistance was more than doubled, and lung lymph flow, after a transient increase, returned to approximately the baseline level. The L/P was decreased. Transfusion increased lymph flow, but did not significantly alter L/P.
TABLE 2
Effect of Overperfusion and Acute Alveolar Hypoxia on Pulmonary Hemodynamics and on Lung Fluid and Protein Exchange

<table>
<thead>
<tr>
<th>Pulmonary artery pressure (cm H2O)</th>
<th>Left atrial pressure (cm H2O)</th>
<th>Cardiac output (liters/min)</th>
<th>Calculated resistance (cm H2O/min per liter)</th>
<th>Lymph flow (ml/hr)</th>
<th>Protein concentration (lymph/plasma)</th>
<th>Lung water (g/g dry lung)</th>
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<td>* Subgroup of animals shown in Table 1.</td>
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<td>† Average ± so.</td>
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<td>§ Resection of 65% lung mass followed by small intermittent transfusions to restore cardiac output to baseline level.</td>
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<td>Significant by ANOVA and paired t-test (Bonferroni correction) at p &lt; 0.05/n with preceding value.</td>
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<td>\ PaO2 = 40 ± 5 mm Hg.</td>
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This indicates that active hypoxic pulmonary vasoconstriction in the sheep is able to overcome partially the high vascular distending pressures due to overperfusion. The change in resistance is, however, much less than in sheep with normal pulmonary vascular pressure (Bland et al., 1976). Lung lymph flow and L:P did not change. In three of the 10 sheep, lymph flow and L:P did increase slightly. This occurred among the first six sheep studied. We thought it indicated that some of the sheep may show an increase in microvascular permeability by the combination of over perfusion and acute alveolar hypoxia (Landolt et al., 1981). We have not, however, seen this effect in the last four sheep, even when left atrial pressure was increased, as will be described. At postmortem in six sheep, the left lower lobe extravascular water was increased an average of 24%. The lobe showed interstitial edema.

Increased Left Atrial Pressure

When we increased left atrial pressure in eight sheep to stress the microvascular barrier further, we achieved the same results whether the animals were hypoxemic or not. Figures 4 and 5 show an example of each. Left atrial pressure was increased by an average of 12 cm H2O. Lung lymph flow always increased (10.8 ± 5.1 ml/hr) and L:P always decreased (0.52 ± 0.07) from the levels in Tables 1 and 2 (last lines). At postmortem examination, the left lower lung lobe extravascular water was increased an average of 24%. The lobe showed interstitial edema.

Discussion

The control group of four sheep shows that our preliminary surgery together with blood transfusion did not affect microvascular permeability. Although the transfusions increased cardiac output and vascular pressures modestly, there was only a slight increase in lymph flow which we believe was due mainly to an increase in filtration area, since L:P did not change.

Our method for detecting changes in microvascular...
permeability depends on the effect of each maneuver on the L:P. Therefore, in Figure 6, we have summarized the sequential changes in L:P as a function of calculated microvascular hydrostatic pressure (Garr et al., 1967). It is clear that the lymph protein concentration falls linearly as the microvascular pressure rises. When we compared our new data with our published data obtained in unanesthetized sheep with increased left atrial pressure, we found the slopes to be the same (Erdmann et al., 1975; Ohkuda et al., 1981).

Our first conclusion is that we were unable to find evidence for an increase in lung microvascular permeability following restriction of the circulation and transfusions to increase microvascular hydrostatic pressure and the linear velocity of blood flow. These results are different from what we had expected (Ohkuda et al., 1978) and what others had claimed occurred in cats (Gibbon et al., 1942) and in dogs (Hultgren et al., 1978).

Gibbon and Gibbon (1942) resected 70% of the lungs of cats. Resection alone did not lead to any obvious pulmonary edema. Thus, our results are similar to theirs, although the extent of our lung resection appears to be a little less than they achieved. Gibbon did, however, produce severe edema in 8 of 11 cats with airway fluid having a high protein concentration when he gave plasma transfusions (15 ml/kg) to increase flow and pressure. We did not obtain this result. Our results following resection and transfusion are consistent with increased filtration and interstitial edema of the high pressure variety. The total amount of blood we gave is more on a body weight basis than that used by Gibbon, although we gave it more slowly. The cardiac output we achieved did not exceed the baseline value for our anesthetized, open-thorax animals. Gibbon did not measure cardiac output or pulmonary vascular pressures.

Hultgren and his associates (1966) did not fully describe their overperfusion experiments until recently (Hultgren et al., 1978). Since they tied off the hila of both lower lobes of the dog lung, it appears they blocked blood flow to approximately 80% of the lung mass. This degree of vascular restriction was too much for the dog's right ventricle. Hultgren was only able to sustain blood flow by imposing a mechanical pump to force blood through the lung. He described the pulmonary edema fluid he produced as having a high protein concentration—but it was also hemorrhagic. In our sheep, neither the lymph nor the interstitial edema fluid we saw in frozen lung sections was bloody. Apparently, our pressures were not so high as to cause microvessel rupture.

In the experiments of Ohkuda and associates (1978), the sheep were prepared in the same manner as described here, but the restriction of the lung's circulation was achieved by microemboli, not lung resection. At the time, we were not clear as to what the microemboli did, other than to obstruct the microcirculation. In order to achieve a synthesis as to causality, we speculated that the high pressure and linear blood flow velocity had physically injured the microcirculation. In order to achieve a synthesis as to causality, we speculated that the high pressure and linear blood flow velocity had physically injured the microcirculation. Our calculations of shear stresses within the microcirculation supported that possibility.

It is now clear that the basis of microembolic lung vascular injury is entirely different. We have found that circulating neutrophils are necessary for the response, both in the anesthetized and unanesthetized sheep (Flick et al., 1981). Interestingly, in the neutropenic sheep, the embolic microvascular obstruction did cause a rise in fluid filtration but with a decrease in the L:P, indicating high pressure edema, just as we have found here.

As Figure 6 shows, the L:P ratios change in a manner entirely predictable from the data obtained in
sheep with an increase in left atrial pressure alone. In addition, we have recently reported that when left atrial hypertension is added to increased lung permeability from air embolus, the L:P ratio does not decline (Ohkuda, 1981). Thus, we cannot confirm our own speculation, nor the previous experimental claims that overperfusion increased microvascular distending pressure and increased linear blood flow velocity measurably damages the microvascular barrier.

Our second conclusion is that the combination of alveolar hypoxia and overperfusion (with or without left atrial hypertension) does not cause any significant increase in microvascular barrier permeability. Acute alveolar hypoxia caused a small increase in pulmonary artery pressure and calculated resistance indicating active pulmonary vasoconstriction, even though pulmonary artery pressure was already substantially elevated by the resection and transfusion procedures.

The data shown in Table 2 confirm and extend the findings of Bland and coworkers (1976) that acute alveolar hypoxia in the sheep does not cause any increase in lung lymph flow or lymph protein concentration. It appears that the vasoconstriction is in the small pulmonary arteries proximal to the fluid exchange sites (Kato et al., 1966). Because three of our sheep did show a slight increase in L:P (Landolt et al., 1981), we used increased left atrial pressure to stress the microcirculation. Although lymph flow increased, the L:P ratio always decreased.

High altitude pulmonary edema in man is an enigma that is somehow related to acute alveolar hypoxia. Viswanathan et al. (1969), Hultgren et al. (1978), and Hackett et al. (1980) have attributed the primary pathogenesis of high altitude edema to overperfusion of a markedly restricted microvascular bed. We had hoped to achieve a comparable result in our restricted sheep lungs with alveolar hypoxia and elevated left atrial pressure. This did not occur.

Perhaps it is time to reassess our concept of high altitude pulmonary edema. Classical pathologists (Arias-Stella et al., 1963) reported that the edema fluid seen in the lungs of people dying of high altitude edema was “proteinaceous.” This led to its being classified as an increased permeability edema (Hultgren et al., 1978). However, the L:P ratio or its equivalent, the edema fluid:plasma protein ratio, had not been measured in man. Recently, Dr. Rodman Wilson (personal communication) obtained edema fluid from a climber on Mt. McKinley, Alaska. The edema fluid:plasma protein concentration ratio of that man was 0.6, which is above the ratio predicted for high pressure edema but below the ratio predicted for increased permeability edema (Fein et al., 1979).

Contrary to the hypothesis with which we began this study, namely, that high distending pressure and increased blood flow velocity in the restricted pulmonary microcirculation would cause endothelial barrier damage, we have found instead that the microvascular barrier in the sheep lung is remarkably resistant to physical injury. We already knew that the endothelial barrier was resistant to hypoxia, since there are data to indicate that the endothelium functions quite adequately in the absence of oxygen (Fisher et al., 1972) and that, in the unanesthetized sheep, acute and chronic alveolar hypoxia do not lead to changes in microvascular permeability (Bland et al., 1976). Nevertheless, in view of the recent reports of patients with congenital absence of a pulmonary artery, who developed signs and symptoms of pulmonary edema at high altitude (Hackett et al., 1980), we also tested the hypothesis that physical stress on the hypoxic endothelium might lead to endothelial barrier injury. We have not been able to cause any significant endothelial barrier injury by the combination of increased physical distending pressure, increased linear blood flow velocity, and acute alveolar hypoxia. We wonder whether the hypothesis that high altitude pulmonary edema is a form of increased permeability edema is tenable.

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Dr. Landolt was supported by (GM2019) Surgical Training Grant. His current address is Beth Israel Hospital, Department of Surgery, 330 Brookline Avenue, Boston, Massachusetts 02215.

Dr. Albertine was supported by HL66168 (NRSA).

Dr. Roos was supported by HL7185 (PFTG) Pulmonary Faculty Training Grant and HL536. His current address is Jerry L. Pettis Memorial Veterans Hospital, Pulmonary Section, 11201 Benton Street, Loma Linda, California 92357.

Dr. Wiener-Kronish was supported by HL07185 (PFTG).

Address for reprints: Michael A. Matthay, M.D., Cardiovascular Research Institute, 1315-M, University of California, San Francisco, San Francisco, California 94143.

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References


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