Effect of Increased Vascular Pressure on Lung Fluid Balance in Unanesthetized Sheep

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ABSTRACT

In 20 unanesthetized sheep, we measured lung lymph flow and lymph and plasma protein concentrations during steady-state baseline conditions and during steady-state elevations of pulmonary microvascular hydrostatic pressure (range 3 to 23 cm H2O). In every sheep there was a baseline lung lymph flow (average 5.7 ± 2.5 (SD) ml/hour), demonstrating that net fluid filtration occurred. The baseline lymph-plasma total protein ratio averaged 0.69 ± 0.05, indicating a high protein osmotic pressure in the interstitial fluid at the filtration site. Lymph flow increased and lymph protein concentration decreased approximately linearly whenever hydrostatic pressure rose. A new steady-state condition was reached in 1-2 hours. The difference in plasma-to-lymph protein osmotic pressure increased by half the hydrostatic pressure increment (50% negative feedback regulation). Extravascular lung water content, measured post-mortem, did not change significantly until microvascular hydrostatic pressure more than doubled, indicating a large safety factor that protects the lungs against fluid accumulation normally. The major contributions to the safety factor appeared to be a sensitive and efficient lymph pump coupled to a washout of interstitial protein. The fluid filtration coefficient, whose calculation required many assumptions, averaged 1.64 ± 2.65 ml/(cm H2O x hour) in the baseline condition and did not change significantly over the pressure range studied.

Several investigators have studied fluid filtration in the lung in acute experiments in anesthetized dogs by raising pulmonary microvascular pressure or lowering plasma protein osmotic pressure (1-3). They have calculated the rate of fluid accumulation by comparing the water content of each dog’s lungs at the end of the experiment with their predicted normal value. They have variously concluded that (1) no fluid filtration or accumulation occurs until vascular pressures exceed plasma protein osmotic pressure, (2) perimicrovascular (interstitial) protein osmotic pressure has little or no influence as a force related to fluid filtration, and (3) the lung’s lymphatic system affords no significant protection against edema.

At least the first of these conclusions appears to be contradicted by measurements of lymph flow from the right lymph duct in dogs (4-6) and of the pulmonary contribution to thoracic duct flow in sheep (7, 8). Staub (9) has developed a steady-state, unanesthetized sheep preparation that permits continuous measurement of net fluid and protein flow from the lung. He has confirmed (9) that there is a continuous flow of lymph from the lung, indicating net fluid filtration, even under baseline conditions.

In the present paper, we used this sheep model to demonstrate that (1) every steady-state rise in pulmonary microvascular pressure leads to an increase in steady-state lung lymph flow, (2) perimicrovascular (interstitial) protein osmotic pressure is of major importance in regulating lung fluid balance, and (3) fluid accumulation in the lung is largely prevented by the sensitivity and the efficiency of the lung’s lymphatic system until high vascular hydrostatic pressures are reached.

Methods

PREPARATION OF SHEEP

In 33 female Suffolk sheep weighing 24-86 kg (average 39 kg), we made three thoracotomies over a 2-3-week period to obtain nearly pure lung lymph, to implant catheters for measuring pulmonary arterial, left atrial, aortic, and superior vena cava pressures, and to allow us to change left atrial pressure or to constrict the main pulmonary artery.

All of the sheep were fasted for 48 hours, and fluid was...
withheld for 12 hours before surgery. After sodium thiopental anesthesia (30 mg/kg, iv) and endotracheal intubation, the sheep were maintained on 0.5–1.0% halothane and 50% oxygen using a positive-pressure ventilator (Ventimeter, Air Shields) set at 15 cycles/min and a peak airway pressure of 30 cm H₂O.

Through a right thoracotomy, we identified the large caudal mediastinal lymph node (CMN) (10), which, in the sheep, receives most of the lung lymph (9, 11) and conveys it to the thoracic duct via a single efferent duct. The CMN also receives some systemic lymph. To eliminate this input, we resected the portion of the node posterior to the free margin of the pulmonary ligament and tied all of the identifiable diaphragmatic surface and chest wall lymph afferent vessels.

A week later, we made a left thoracotomy and placed Silastic catheters (no. 602–281, Dow Corning) in the main pulmonary artery and the left atrium, a 16F silicone-coated rubber Foley catheter with a 20-ml inflatable balloon (no. 334–16, Dow Corning) through the left atrium to the level of the mitral annulus, and an inflatable latex cuff (no. 10407, Davol Rubber Co.) around the main pulmonary artery. We brought all of the catheters out onto the flank and sewed them to the skin.

Finally, after another week, we made a second right thoracotomy and cannulated the efferent duct of the CMN with a small-bore Silastic catheter (no. 602–151 or no. 171, Dow Corning) that had been impregnated with heparin to retard clotting (TDMAC process, Gulf South Medical Research, Australian National University, Canberra, Australia). We studied them in the standing position, unrestrained, with free access to food and water.

For experiments in the other 20 sheep, lymph flowed for 7–28 days. This fact accounts for some of the variability in the number of studies per sheep. Data from all studies are reported. Several sheep were studied only once and were then used for other experiments (12, 13).

PREPARATION FOR EXPERIMENTS

Each sheep lived in a mobile metabolism cage (based on an original design of the John Curtin School of Medical Research, Australian National University, Canberra, Australia). We studied them in the standing position, unrestrained, with free access to food and water.

We measured pressures in the left atrium (Pₚₐ), the pulmonary artery (Pₚₚ), the superior vena cava or the right atrium (Pₚₐ), and the aorta (Pₚₕ) using miniature strain gauges (no. MP-15, Micron Instrument Co.) and recorded them on a multichannel ultraviolet polygraph (Visicorder 1508-A, Honeywell Test Instrument Co.). We initially zeroed the strain gauges level with a clip on the dorsal surface of the left atrium, visualized fluoroscopically, but we have referred all pressures in the text to the bottom of the lung by adding 9 cm H₂O to each (14). We calculated the pulmonary microvascular pressure, Pₚₚ, relative to the bottom of the lung using the formula

\[ P_{\text{mv}} = P_{\text{io}} + 0.4(P_{\text{pa}} - P_{\text{ra}}) \]  

where \( P_{\text{io}} \) and \( P_{\text{pa}} \) are the average left atrial and pulmonary artery pressures, respectively, referred to the bottom of the lung and 0.4 is the fraction of total pulmonary vascular resistance that is downstream from the microvascular exchange surface (9, 15, 16).

We examined the sheep daily and only used them for experiments if their rectal temperature was in the normal range (39-39.5°C) and they were eating, drinking, ruminating, defecating, and urinating normally. We flushed the vascular catheters daily with heparin (1000 units/ml); otherwise, we gave no anticoagulants.

LYMPH PURITY TEST

To be certain that the lymph source was pulmonary and not systemic, we always did a preliminary physiological test. We inflated the pulmonary artery constrictor cuff to increase \( P_{\text{pa}} \) approximately 10 cm H₂O and measured lymph flow for 1 hour. Any significant systemic source of lymph should have been manifested by an increase in flow. In our experience over 4 years, we have found evidence of persistent systemic lymph contributions in 5% of the sheep. If the CMN had not been resected, every sheep would have shown a systemic contribution (unpublished observation). After we deflated the pulmonary artery cuff, we tested the left atrial balloon by inflating it to raise \( P_{\text{io}} \) and obtain an increase in lymph flow. All of the sheep reported on in this paper passed the lymph purity test, which means that they had no measurable systemic component to their lung lymph.

THE BASIC EXPERIMENT

Each experiment lasted at least 6 hours. After all pressures had been calibrated and were recording satisfactorily, the base-line observation period began. We measured electronically averaged pressures continuously and pulsatile pressures hourly. We collected lymph in graduated centrifuge tubes containing a small amount of dried heparin. We read lymph flow to the nearest 0.1 ml at 15-minute intervals and collected samples over 30-minute periods for protein determinations. We drew heparinized blood samples every hour. After pressures and lymph flow had been stable for at least 2 hours, we raised left atrial pressure by inflating the balloon quickly, adjusting the pressure to the desired level, and maintaining it as constant as possible for at least 4 hours. We then deflated the balloon and allowed the sheep to recover.

During the experiment, we took bubble-free systemic arterial blood samples for the determination of oxygen tension (P₀₂), carbon dioxide tension (Pₐₜ₉), and pH every 2 hours.

We also measured cardiac output by the indicator-dilution method using \(^{131}\text{I}\)-albumin as the tag. We made injections into the right atrial catheter and obtained samples in a rotating collector by free flow from the aortic catheter. We counted the \(^{131}\text{I}\)-albumin in 0.5-ml samples of whole blood in a gamma scintillation spectrometer (Autogamma 3002, Packard Instrument Co.). We normalized the indicator-dilution curves and plotted the points on semilogarithmic paper to correct for recirculation. Usually, we measured cardiac output once
in the base-line period and once in the final steady-state condition after increasing $P_{ia}$.

**THE FINAL EXPERIMENT**

To relate the quantity of lung water to lymph flow and to the microvascular pressure, $P_{mv}$, we killed 20 sheep (7 with lymph flow) at the conclusion of a 4-hour steady-state pressure period. We gave sodium thiopental intravenously, placed the sheep in the supine position, intubated the trachea with a cuffed catheter, inflated the lungs to an airway pressure of 20 cm H$_2$O, opened the thorax by a sternum-splitting incision, doubly clamped the thorax by a sternum-splitting incision, doubly clamped the lung hilae, and removed the lungs. The total elapsed time from anesthesia to hila clamping did not exceed 5 minutes.

**ANALYTIC METHODS**

We measured the polygraph records of average $P_{mv}$ and $P_{ia}$ over 30-minute periods to calculate $P_{mv}$. We centrifuged blood and lymph samples and measured total proteins in duplicate by the Biuret method and the albumin and globulin fractions in duplicate by cellulose-acetate electrophoresis (Microzone 110, Beckman Instrument Co.).

We used large-vessel plasma protein concentration and its albumin fraction to calculate microvascular protein osmotic pressure, $p_{mv}$, from the regression equations of Landis and Pappenheimer (17), allowing for protein-protein interaction. Likewise, we calculated the lymph protein osmotic pressure, $p_{lym}$, from the protein concentration in steady-state lung lymph.

We passively drained the excised lungs of blood, weighed them, and homogenized them in a blender (Polytron 45, Brinkman Instrument Co., or Waring 5011) with an equal volume of water. We measured the water content of quadruplicate samples of the homogenate, the supernatant fraction of the centrifuged homogenate, and whole blood by drying them to constant weight (48 hours) at 80°C. We measured the hematoctrit of the whole blood and the total hemoglobin of the blood and the homogenate supernatant fraction by the cyanmethemoglobin method after clarifying the lung homogenate supernatant fraction by the cyanmethemoglobin method (18).

In several experiments, we tagged the plasma with $^{125}$I-albumin 10 minutes prior to killing the sheep. In these studies, we measured the radioactivity of quadruplicate samples of blood and lung homogenate in the gamma counter.

The calculations of lung hemoglobin content (red cell mass), plasma volume, extravascular lung water, and dry lung weight required successive application of the conservation of mass law (12) as outlined by Pearce et al. (18). We calculated the fluid filtration coefficient, $K_r$, relative to the bottom of the lung using the general fluid transport equation:

$$
\dot{Q}_r = K_r [(P_{mv} - P_{pme}) - \sigma(p_{mv} - p_{pme})] 
$$

where $\dot{Q}_r$ is the net transvascular fluid flow (volume/time), $P_{mv}$ and $P_{pme}$ are the microvascular and perimicrovascular (interstitial) hydrostatic pressures, respectively, $\sigma$ is the solute reflection coefficient, which is a measure of the effectiveness of the microvascular membrane as a barrier to solute flow relative to water flow (if $\sigma = 1$, the barrier is impermeable to the specified solute; if $\sigma = 0$, the specified solute flows freely through the barrier with fluid), and $p_{mv}$ and $p_{pme}$ are the microvascular and perimicrovascular (interstitial) solute osmotic pressures, respectively.

In the steady state, we assumed that $\dot{Q}_r$ equals lung lymph flow ($Q_{lym}$). For the range of steady-state pressures that we used, the only solutes contributing to osmotic fluid flow are the plasma proteins (19, 20). This situation is equivalent to setting $\sigma$ equal to zero for all other solutes. For protein, we assumed that $\sigma$ was unity (complete barrier impermeability) and that $p_{lym} = p_{pme}$. Finally, we assumed that $P_{pme}$ was constant and set it equal to the average alveolar pressure, that is zero.

The numerous assumptions necessary to apply Eq. 2 have been discussed extensively by Staub (21). The absolute value of $K_r$ is questionable, but the relative values in individual sheep are meaningful.

**STATISTICS**

Where summary data are given, we show the average ± 1 SD. The significance of paired data was compared by the nonparametric sign test or the paired t-test. We accepted $P < 0.05$ as indicating significant differences.

We fitted least-squares regression lines to selected relationships to determine the closeness of correlation and to obtain an indication of the predictive value of the relationships.

**Results**

Figure 1 shows the time course of one experiment. The lymph-plasma ratios for albumin and globulin were initially 0.84 and 0.57, respectively.


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<th>Table 1: Steady-State Parameters in Every Experiment in 20 Unanesthetized Sheep with Lung Lymph Flowing</th>
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**Proteins**

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LUNG TISSUE FLUID BALANCE

All baseline studies for each sheep were grouped as the average ± standard deviation of the sample. A column of baseline studies is listed for each experiment in Table 1. The results of each experiment are presented in the table. The average body weight of the 20 sheep was 43 ± 13 kg. The average baseline primary hemodynamic data were \( P_{pa} = 29.8 ± 3.41 \) cm H\(_2\)O, \( P_{la} = 13.2 ± 2.8 \) cm H\(_2\)O, and, in 13 sheep, cardiac output = 4.23 ± 1.14 liters/min (0.12 liters/min kg\(^{-1}\)). Baseline steady-state \( Q_{lym} \) was 5.7 ± 2.5 ml/hour (0.13 ml/hour kg\(^{-1}\)). Total plasma protein concentration averaged 6.59 ± 0.49 g/100 ml, albumin 2.76 ± 0.31 g/100 ml, and globulins 3.83 ± 0.44 g/100 ml. The total lung lymph protein averaged 4.51 ± 0.46 g/100 ml, albumin 2.43 ± 0.37 g/100 ml, and globulins 2.08 ± 0.39 g/100 ml. The average baseline lymph-plasma ratio for total protein was 0.69 ± 0.05, for albumin 0.88 ± 0.08, and for globulins 0.54 ± 0.06.

Table 2 lists the derived data for all 47 experiments. The average baseline \( P_{mv} \) was 19.9 ± 2.7 cm H\(_2\)O, \( r_{mv} \) was 31.9 ± 3.7 cm H\(_2\)O, and \( r_{lym} \) was 19.6 ± 2.6 cm H\(_2\)O. The average filtration coefficient, \( K_f \), was 1.64 ± 2.65 ml/(cm H\(_2\)O x hour).

In every experiment, when \( P_{mv} \) increased, \( Q_{lym} \) increased and \( K_{lym} \) decreased. Figure 2 shows \( Q_{lym} \) as a function of \( P_{mv} \) for the six sheep with multiple pressure elevations. We did not plot all of the data because they would have crowded the figure. Figure 3 is a plot of \( r_{mv} \) and \( r_{lym} \) as functions of \( P_{mv} \) for each of the five experiments with lymph in sheep no. 6. These experiments were done over a period of 2 weeks. The line of best fit has a slope of -0.54 and a correlation coefficient of -0.95.

Figure 4 is a plot of \( r_{lym} \) normalized as a percent of simultaneous \( r_{mv} \) against \( P_{mv} \) for all 47 experiments. The straight line of best fit for \( r_{lym} \) as a percent of the protein osmotic pressure of plasma, is given by the equation

\[
\pi_{lym} = 87 - 1.36P_{mv} \text{ (cm H}_2\text{O)}
\]  

and has a correlation coefficient of -0.83.
# Table 2

Derived Data in Every Experiment in 20 Sheep

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Table 2 parallels Table 1. All base-line studies for each sheep were grouped as the average ± SD of sample. $P_{mv}$ = pulmonary microvascular pressure calculated by the formula $P_{mv} = P_{va} + 0.4 (P_{pa} - P_{na})$. $\pi_p$ and $\pi_{lym}$ are plasma and lymph protein osmotic pressures, respectively, calculated by the equations of Landis and Pappenheimer (17). $K_r$ is the fluid filtration coefficient calculated according to Eq. 2 in the text.
LUNG TISSUE FLUID BALANCE

Steady-state lung lymph flow as a function of calculated average hydrostatic pressure in the pulmonary microvascular bed. Data for 16 experiments in six sheep are shown. Solid circles = sheep no. 2, open circles = sheep no. 4, solid squares = sheep no. 6, open triangles = sheep no. 7, solid triangles = sheep no. 16, and open squares = sheep no. 17.

Since $ir_{mu}$ remained nearly constant and $7r_{vm}$ decreased linearly as $P_{mv}$ increased in our experiments, we compared the steady-state change in the plasma-to-lymph protein osmotic pressure ($ir_{mv} - T_{vm}$) with the change in $P_{mv}$ for each of 27 experiments in 16 sheep. The results are shown in Figure 5. The equation for the line of best fit is

$$A(7T_{in} - 1y_{im}) = 0.51AP_{TM} - 0.38 \text{ (cm H}_2\text{O) (4)}$$
with a correlation coefficient of +0.75. The y-intercept is not significantly different from zero. Thus, over the 3-23-cm H₂O range of changes of \( P_{mv} \) that we used, the plasma-to-lymph protein osmotic pressure difference increased at half the rate at which the hydrostatic pressure increased.

Table 3 lists the hemodynamic data in the 13 sheep whose lung lymphatic flow stopped before any experiments could be done. Their body weight averaged 33.6 ± 5.8 kg. The base-line pulmonary artery and left atrial pressures averaged 30.7 ± 3.7 cm H₂O and 14.1 ± 4.3 cm H₂O, respectively. Cardiac output averaged 4.84 ± 0.81 liters/min (0.14 liters/min kg⁻¹), and the average base-line \( P_{mv} \) was 20.5 ± 3.8 cm H₂O.

Of the measured data, the only significant difference between the 20 sheep with lymph flow and those without was the lower average body weight in the latter group. These data are consistent with the interpretation that within a week of major efferent lung lymph obstruction alternate pathways for lymph-flow developed and the animals are comparable to base-line sheep with functioning lymph cannulas (22).

Table 4 lists all of the postmortem lung compartment data. The four sheep killed at base-line \( P_{nv} \) had a wet lung weight \( (Q_{lb}) \) averaging 506 ± 90 g (14.82 ± 2.92 g/kg). The residual blood content averaged 20% of the total wet weight, leaving 404 ± 76 g of blood-free lung \( (Q_{fl}) \). The extravascular lung water \( (Q_{ev}) \) averaged 324 ± 62 g (9.51 ± 2.05 g/kg). Extravascular lung water per unit of dry blood-free lung...
Lung Tissue Fluid Balance

Table 4

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Condition at Death</th>
<th>(P_{mv}) (cm H₂O)</th>
<th>(Qlb) (g)</th>
<th>(Ql) (g)</th>
<th>(dQl) (g)</th>
<th>(Qwl) (g)</th>
<th>(Qwl/dQl)</th>
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</tr>
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<td>306</td>
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<td>507</td>
<td>375</td>
<td>68</td>
<td>317</td>
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</table>

\(P_{mv}\) = pulmonary microvascular pressure for 4 hours prior to the time of death, \(Qlb\) = total weight of lungs and blood, \(Ql\) = wet lung weight after correction for residual blood content, \(dQl\) = dry weight of blood-free lungs, \(Qwl\) = quantity of water in the lungs determined by the formula \(Qwl = Ql - dQl\), and \(Qwl/dQl\) = fractional water content per gram of dry lung. Histology was based on rapidly frozen inflated lung biopsies taken at autopsy. Sheep 8-20 (Tables 3 and 4) were used for other experiments and killed under conditions not related to this study.

* \(P_{mv}\) was chronically increased for 2 weeks prior to death.

Figure 6 is a plot of \(Qwl/dQl\) for the 20 sheep considered in Tables 3 and 4 as a function of microvascular pressure. The line of best fit is a hyperbola (23) whose equation is

\[
Qwl/dQl = 2.79 - \left\{73.75/(P_{mv} - 81.51)\right\}. \tag{5}
\]

Lung extravascular water content and pulmonary microvascular pressure in 20 sheep. The two open circles are for sheep with chronic pressure elevations lasting for 2 weeks; the solid circles are for sheep with pressure elevations lasting 4 hours. The statistical line of best fit is a hyperbola. The stippled area represents the 95% confidence limits for the individual points.
as $P_{mv}$ decreases to zero, whereas the hyperbola predicts that $Qwl/dQl$ will equal 3.69 at $P_{mv} = 0$. Also, the hyperbola has asymptotes that predict a minimum water content of 2.79($Qwl/dQl$) and an infinite water content at $P_{mv} = 81.5$ cm H$_2$O. Experimental tests of these predictions may be feasible.

Comparing Figure 2 to Figure 6, it is clear that steady-state fluid filtration, as represented by lymph flow, increased approximately linearly with $P_{mv}$ but that fluid accumulation was insignificant until $P_{mv}$ had doubled.

There are two open circles in Figure 6. These circles show the lung water contents of the two sheep with chronic elevations of $P_{mv}$ lasting for 2 weeks each. These points lie on the line of best fit, further supporting the evidence such as that shown in Figure 1 that four hours of increased $P_{mv}$ is sufficient to establish a new steady-state condition. The point at $P_{mv} = 65$ cm H$_2$O may be an exception, because lung water increased 77% and may have caused some alveolar flooding. In all of the other sheep, $Qwl/dQl$ increased less than 40% which is compatible with interstitial edema only (21).

**Discussion**

**THE EXPERIMENTAL ANIMAL**

Compared to the anesthetized, acutely-operated dog, the unanesthetized, chronically instrumented sheep is a celestial creature. Female sheep are large, docile animals. Most of our sheep were Suf- folks, in the commercial category known as feeder lambs; they were approximately 1 year old and weighed between 30 and 40 kg. The few larger sheep were older ewes. The exact breed is not critical to the term capillary hydraulic pressure, $P_{mv}$, rather than left atrial pressure in our graphs, and we considered steady-state lung lymph flow, $Q_{lym}$, to be equal to net transvascular fluid flow. In addition, making several assumptions, we calculated the fluid filtration coefficient, $K_{lym}$. Although these three quantities have been discussed extensively elsewhere (21), their measurement remains controversial, and we feel that these determinations should be briefly discussed to place their reliability in proper perspective.

**Calculation of Microvascular Hydrostatic Pressure.**—We prefer the term microvascular hydrostatic pressure, $P_{mv}$, to the term capillary hydrostatic pressure, because of uncertainty as to the exact site of the major net fluid exchange in the lung. Iliff (24) has suggested that under some conditions a large portion of fluid exchange occurs in the pulmonary venules. It is more likely, considering the vast extent of the alveolar capillary network surface area, that the exchange is within the capillaries themselves (21). Even in the lung capillaries, however, there may be a longitudinal gradient of protein permeability as has been suggested in systemic capillary beds (25-27).

The estimation of $P_{mv}$ from average pulmonary artery and left atrial pressures is based on estimations of pre- and postpulmonary microvascular resistances. Some investigators (1, 3) have assumed that $P_{mv}$ is approximately equal to left atrial pressure. In addition, all workers have disregarded the height of the lung, which causes regional variations in $P_{mv}$. They have assumed a calculated value relative to some hydrostatic level. The equation we used to calculate microvascular pressure assumed a Zone III lung with 40% of the total pulmonary vascular resistance downstream from the filtration bed.

Several workers have tried to analyze the distribution of pre- and postalveolar vascular resistance. Agostoni and Piiper (28) estimated the distribution of resistance as 60% downstream, whereas Brody et al. (29) calculated it to be 46% upstream and 20% downstream with the remainder within the pulmonary capillaries. If the resistance in the capillaries

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1A more detailed description of the lymph surgery and testing procedure has been published elsewhere (Staub et al. J Surg Res, in press).
LUNG TISSUE FLUID BALANCE

is redistributed equally upstream and downstream, about 40% of the total vascular resistance is downstream. Gaar and associates (15) used isolated, perfused dog lung lobes and reported that approximately 40% of the pulmonary vascular resistance was downstream from the alveolar walls. A similar value in isolated, perfused lungs was found by Üter and associates (16). We used the value of 40% for the downstream resistance.

Measurement of Net Fluid Filtration.—The assumption that \( Q_{\text{lym}} \) equals net fluid filtration, \( Q_f \), in the fluid transport equation must be true in the steady-state if we have collected all of the pulmonary lymph.

There is no single efferent lymphatic that drains the entire lung. The outflow from the CMN is nearly pure lung lymph as our lymph purity test showed, but anatomically this duct drains only the posterior two-thirds of the lung. Staub (9) has shown that some lung lymph drains from the left hilum through a small duct. Humphreys et al. (7) have found that about one-third of the lung lymph drains from the right lymph duct.

We tried to determine the fraction of total lung lymph drained via the CMN by comparing the tracer albumin wash-in volume with the \(^{14}\)C-sucrose–measured total interstitial fluid volume (11). The tentative results indicated that we collected about 70% of the total lung lymph through the CMN. In a recent paper, Meyer and Ottaviano (30) used the same approach in the dog to determine the fraction of total lung lymph in the right lymph duct outflow with similar results.

Unfortunately, the data are not as convincing as we originally thought, because the size of the wash-in volume is extremely sensitive to the model chosen for the protein tracer transport (convective or diffusive flow). At best the 70% value appears to be an upper limit for the fraction of total lung interstitium drained by the CMN.

A reasonable anatomic estimate of the fraction of lung drained by the CMN is 50% (see the discussion in the following section). Consequently, the filtration coefficient may be underestimated by a factor of two on this basis.

Still, we could use our lymph flow to calculate relative filtration coefficients if we could be assured that we always collected the same fraction of net fluid filtered. In view of our good \( Q_{\text{lym}} \) reproducibility from day to day (especially in sheep no. 6, [Tables 1 and 2, and Fig. 2] in which we did five complete studies over a 2-week period), we believe that it is likely that the CMN fraction of total lung lymph flow is constant. At the maximum lymph flow rate we obtained (27 ml/hour), the calculated resistive pressure drop through our Silastic lymph catheter did not exceed 1 cm H\(_2\)O.

In spite of these favorable data, we cannot prove, by any means we know of, that we always collected a constant fraction of total lung lymph.

**The Fluid Filtration Coefficient, \( K_f \).—**One of our original goals was to compare \( K_f \) under base-line conditions with \( K_f \) after elevation of \( P_{m_v} \) (9, 31). As we became more experienced with our sheep model, we became less confident that we could make a meaningful absolute calculation of \( K_f \) without much more information (21), but we feel the relative values are interesting.

We have listed \( K_f \) in Table 2 for all 47 experiments. The average of all base-line \( K_f \) calculations in 20 sheep was 1.64 ± 0.265 ml/(cm H\(_2\)O × hour). The large sample standard deviation was mainly due to 3 sheep with high apparent \( K_f \) values of 11.70, 4.00, and 4.38 ml/(cm H\(_2\)O × hour) and 1 sheep with a very low value of 0.07 ml/(cm H\(_2\)O × hour). We have no justification for eliminating these experiments; however, it is fair to point out that all of the other 16 base-line \( K_f \) values lie in a much narrower range (0.35 to 2.71 ml/(cm H\(_2\)O × hour), average 0.78 ± 0.58 ml/(cm H\(_2\)O × hour)).

The \( K_f \) values of all 27 experiments with increased \( P_{m_v} \) averaged 0.89 ± 0.54 ml/(cm H\(_2\)O × hour). Compared with the overall base-line value, \( K_f \) decreased slightly, and the variation decreased markedly. If we used only the 16 “reasonable” values of base-line \( K_f \), there was no change in average \( K_f \) or its variance with increased \( P_{m_v} \). More important statistically are the paired comparisons of \( K_f \) for individual sheep. There was no significant trend.

**REGULATION OF LUNG FLUID BALANCE**

In the figures, we have displayed the relationships we think are most important. But we have also included the data for individual sheep. As far as we know, these determinations are the first quantified measurements of net lung fluid and protein exchange under steady-state hemodynamic conditions in intact animals. We believe the sheep preparation is a sensitive model that has wide usefulness (12, 13, 32, 33).

**Base-Line Lung Lymph Flow.**—In every sheep there was always a net outward flow of lung lymph in the base-line condition. The sum of forces in Starling’s equation does not equal zero. It is always positive, that is, directed outward from the vascu-
lar lumen. No other method can detect or measure this base-line transvascular filtration rate.

Some investigators have regarded normal lung lymph flow as trivial (3). It is true that basal lung fluid filtration is an infinitesimal fraction of pulmonary plasma flow (0.005% in our sheep). But the fluid transport equation (Eq. 2) involves pressures, not flows. Fluid filtration is not affected by plasma flow except where the filtration fraction is large (renal glomeruli) or the plasma flow is stopped.

Lymph flow is clearly necessary for the maintenance of normal lung fluid content. The average base-line lymph flow in our sheep was 5.7 ml/hour or about 130 ml/24 hours. Nakahara et al. (34) have reported a 25% increase in extravascular lung water 24 hours after lymph blockade in dogs, and Cowan et al. (35) have found a 50% increase 3 days after lung reimplantation.

Lung lymph flow is high when it is computed on an organ-weight basis. The blood-free lung weight in four base-line sheep (Table 4) plus others we have measured (21) is only about 1% of the body weight. If other organs had similar $Q_{lym}$-weight ratios, total body $Q_{lym}$ would exceed 500 ml/hour. This flow rate is several times that measured for the thoracic duct (36).

**Effects of Increased Left Atrial Pressure on Lung Lymph Flow.**—Every rise in left atrial pressure caused a rise in lung lymph flow to a new steady-state level within 1–2 hours. The rise in flow was approximately linear with the increase in $P_{mv}$ over the range we used. The data should not be extrapolated to higher pressures, because the condition of alveolar flooding occurs, opening a large extravascular space (the alveolar gas space) in parallel to the lymphatic system.

Again, there is no other method, except for continuous weighing of isolated, perfused lungs that is as sensitive as the lymph flow method. Not only can we measure small changes in transvascular fluid flow in response to small changes in filtration forces, but we can see that a change in extravascular fluid volume (net fluid accumulation) accompanies each change in $P_{mv}$. For example, in the experiment shown in Figure 1, lymph flow did not reach its new steady-state level until 2 hours after the microvascular pressure elevation. The lymph debt (stippled area in the figure) is 6 ml and is a measure of net fluid accumulation in the lung. Since we did not obtain all of the lung's lymph, the value we calculated was an underestimate. An alternate prediction, based on Eq. 5, is that net fluid accumulation was about 20 ml. By either measure, it was too small to detect by any of the standard water content methods (21).

How do we know we have reached a new steady state? Our best evidence is that both $Q_{lym}$ and the lymph-plasma albumin and globulin ratios were at steady levels for at least 2 hours. All sheep in Tables 1 and 2 achieved these conditions.

We need to follow some sheep for much longer periods to prove conclusively that we, indeed, have a steady-state condition. Unfortunately, the two sheep in Tables 3 and 4 with elevated $P_{mv}$ for 2 weeks did not have lymph flowing, although the fact that their increases in lung water were the same as those for the short-term experiments is indicative that the steady state we measured at 4 hours continued unchanged for 2 weeks.

**Sensitivity of Lung Lymph Flow.**—Every increase in $P_{mv}$ caused some increase in interstitial fluid volume. The estimates of 6–20 ml calculated for the example shown in Figure 1 are 2–6% of the predicted lung water for that sheep's body weight. Since the $\Delta P_{mv}$ was 17 cm H$_2$O and $Q_{lym}$ increased 8.5 ml/hour (236% increase), we conclude that the lung's lymphatics are a highly sensitive system responding vigorously to small changes in interstitial fluid volume.

The overall relationship between lung water content and $P_{mv}$ shown in Figure 6 bears this idea out. The microvascular hydrostatic pressure had to rise to 50 cm H$_2$O before lung water accumulation rose 25%. In experimental and clinical pulmonary edema with alveolar flooding, lung water is usually increased 50–100%.

A 25% increase in extravascular lung water is well within the capacity of the lung's interstitium. Thus, in terms of the lung's edema safety factor (37), a substantial portion is accounted for by the lymphatic pump or a rise in interstitial fluid pressure ($P_{pms}$). We have previously found that the lung's lymphatics are actively contractile so that we believe some of the edema safety factor resides in this characteristic. It is likely that some increase in interstitial pressure occurred in the loose connective tissue where the lymph capillaries are situated. But a small rise in fluid pressure at this point need not be reflected back to the filtration site, that is, $P_{pms}$ need not rise (21).

**Lymph Protein Concentrations and Transport.**—The base-line steady-state lymph total protein concentration averaged 0.69 of the plasma concentration. This relationship is similar to that for other visceral organ lymph (36). The high lymph-plasma ratio of albumin (0.88) relative to that of globulin (0.54) is clearly indicative of molecular sieving. We also have data on fibrinogen lymph-plasma ratios for three base-line sheep. The average value is 0.25.
The total base-line steady-state protein flow measured as $\dot{Q}_{lvm}$ multiplied by lymph total protein concentration averaged 0.26 g/hour. Of that, albumin accounted for 54% (0.14 g/hour). Based on our measurements of the total extravascular albumin pool in base-line sheep lungs (21, and Pietra and co-workers (39) to detect electron-dense tracer protein flow out of the lung’s vascular bed in brief experiments.

When $P_{mv}$ was increased, the total base-line steady-state protein flow through the lung increased. When we determined the percent change in total protein flow ($\dot{Q}_{prot}$) as a function of the percent change in lymph flow for all 27 experiments in which we increased $P_{mv}$, we found a good linear relationship.

$$\Delta \dot{Q}_{prot} = 0.39 \Delta \dot{Q}_{lvm} + 6.4\%.$$  \hspace{1cm} (6)

Protein flow increased 39% when lymph flow doubled. The correlation coefficient of 0.89 is significant. Furthermore, there was no tendency for total protein flow to deviate from linearity at the highest pressures. This finding affords additional evidence for substantial molecular sieving at the plasma-tissue barrier and suggests that within the range of our experiments increasing $P_{mv}$ has little effect on endothelial membrane porosity—neither pore stretching (39, 40) nor pore constriction (41).

Changes in Lung Protein Osmotic Pressure.—The linear decrease in lymph protein osmotic pressure with increasing $P_{mv}$ is remarkable but not predictable for any endothelial barrier showing molecular sieving (42). Theoretically, the protein concentration in the lung’s interstitium and lymph will bottom out at some high value of $P_{mv}$ if the hindrance to protein flow and diffusion at the microvascular membrane is not complete, that is, if the reflection coefficient, $\sigma$, does not equal exactly 1 (17). We did not try to push $P_{mv}$ to extremely high values because the non-steady-state conditions of capillary rupture and alveolar flooding could have occurred.

At the other extreme, as $P_{mv}$ decreases, net filtration ought to decrease toward zero. At $\dot{Q}_{lvm} = 0$, interstitial and lymph protein osmotic pressures will equal that of plasma. We did not try to lower $P_{mv}$ in our sheep.

Within the experimental range we tested, the magnitude of the change in $\pi_{lvm}$ was striking. As Figure 5 shows, the change in plasma-to-lymph protein osmotic pressure was one-half (slope of the line of best fit) the change in $P_{mv}$, indicating a 50% negative feedback regulation of $\Delta P_{mv}$ by $\pi_{lvm}$. In terms of the lung’s edema safety factor (37), half of it is accounted for by the change in $\pi_{lvm}$.

Such feedback regulation is only possible if the microvascular barrier is somewhat permeable to protein. If $\sigma = 1.0$, the perimicrovascular (interstitial) fluid and lymph protein concentrations would be zero and no interstitial osmotic pressure safety factor could exist unless the tissue produced its own extracellular proteins at comparable concentrations. Obviously, if the microvascular membrane is readily permeable to proteins, then the total protein flow will increase in proportion to $\dot{Q}_{lvm}$; no feedback effect can occur in this case either. We have documented such effects elsewhere (12).

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