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Equilibration of Intravascular Albumin with Lung Lymph in Unanesthetized Sheep

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Summary

In 16 unanesthetized sheep with chronic lung lymph fistulas we measured pulmonary vascular pressures, lymph flow, lymph and plasma total protein and albumin concentration. We determined the rate of equilibration of radioiodinated albumin between plasma and lung interstitial fluid (lung lymph) in three steady-state conditions; baseline (n = 14), increased pulmonary microvascular pressure (n = 9) and increased microvascular permeability (n = 4). The tracer protein equilibration proceeded according to single compartment wash-in kinetics in all experiments. Lung lymph flow averaged 5.3 ± 2.8 (S.D.)ml/h under baseline conditions, 16.1 ± 10.6 ml/h during increased pressure and 37.3 ± 29.4 ml/h during increased permeability. The half time of equilibration averaged 2.9 ± 1.0 h, 2.2 ± 1.0 h and 0.7 ± 0.2 h, respectively. Lung interstitial fluid equilibrates with plasma proteins more rapidly than most other organs. The marked difference between increased permeability and the other conditions demonstrates the sensitivity of this method. No evidence was obtained that any tracer protein entered lung lymph within the caudal mediastinal lymph node.

The importance of protein osmotic pressure in the determination of transvascular fluid balance in the lung (as in other organs) depends on two factors; namely, the concentration of protein in plasma and interstitial free fluid, and the restriction of the microvascular barrier to protein convection (bulk flow during filtration) and diffusion (along its concentration gradient) (1). These factors are included in the equation for transvascular fluid flow (Starling's equation),

$$\dot{Q}f = K\,[(Pmv - Ppmv) - \sigma\,(\pi mv - \pi pmv)],$$

where $\dot{Q}f$ is the net transvascular fluid flow, K is the fluid filtration coefficient, P is the hydrostatic pressure in the microvascular lumen (mv) and perimicrovascular interstitial fluid (pmv), respectively; σ is a weighted plasma protein reflection coefficient which determines the "effective" transvascular protein osmotic pressure difference, and π is the protein osmotic pressure in the plasma and perimicrovascular free fluid compartments, respectively.

Although it is a simple matter to measure plasma protein concentration, it is not easy to measure the lung interstitial fluid protein concentration nor the reflection coefficient, which is an estimate of the microvascular barrier restriction to protein movement (2).

One question of considerable clinical as well as fundamental interest is: Can the lung transvascular fluid filtration rate be reduced significantly by suddenly increasing the protein concentration in plasma (for example, by infusion of concentrated serum albumin)? The word, 'significantly', includes the duration of the change in filtration rate as well as the absolute amount of the change.

In order to answer the question under various conditions which affect fluid balance in the lung, we have determined the rate of equilibration of radioactively labelled albumin between

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plasma and lung lymph in unanesthetized sheep with chronic lung lymph fistulas. These sheep are used extensively by us for investigations into the pathophysiology of pulmonary edema (3–5).

Methods

We prepared yearling female sheep (weight 25–48 kg) in order to collect nearly pure lung lymph and to measure pulmonary hemodynamics as previously described (5, 6). Briefly, at preliminary surgery, we resected the distal portion of the caudal mediastinal lymph node (CMN) to remove essentially all of its systemic lymph input. At a second thoracotomy, we placed catheters in the main pulmonary artery and left atrium in order to measure pressures and an inflatable balloon in the left atrium for later use in obstructing the mitral valve orifice in order to raise pulmonary microvascular pressure.

Finally, at a third thoracotomy, we identified and cannulated the efferent duct of the CMN with a heparin-impregnated silicone rubber catheter.

We gave the sheep several days to recover from the final operation before starting experiments. The recovery period was long enough to allow lymph flow to stabilize and become clear of red blood cells. All experiments were done with the sheep awake and standing in a mobile metabolism cage with free access to food and water. We used a total of 16 sheep. We did 14 experiments under baseline conditions, 9 experiments after elevating pulmonary microvascular pressure by inflating the left atrial balloon and 4 experiments after increasing pulmonary microvascular permeability with *Pseudomonas Aeruginosa* bacteria. In 6 sheep we did both baseline and increased pressure experiments and in 4 sheep we did both baseline and increased permeability experiments. We did all 3 experiments in 1 sheep.

In each experiment, we determined the rate of equilibration of intravascular albumin with interstitial lung fluid (lung lymph) in the following way. We injected 10–15 μCi of ^{125}I-human serum albumin intravenously. We measured the specific activity (concentration of protein-bound radioactivity as counts per minute/g protein) of albumin in plasma and lymph by counting the ^{125}I radioactivity in a gamma spectrometer (Autogamma 3002, Packard Instrument Co.). We measured total protein in plasma and lymph by the biuret method and the albumin fraction by cellulose acetate electrophoresis (Microzone 100, Beckman Instrument Co.). We measured the specific activity of albumin in plasma every 30 min and in lymph every 15 min until the plasma/lymph equilibration was at least 75% complete in each experiment.

We estimated pulmonary microvascular pressure (Pmv) from the average pulmonary artery and left atrial pressures recorded relative to the hydrostatic level of the left atrium and assuming that 40% of the total pulmonary vascular resistance was on the venous side of the fluid exchange vessels (5). Although lymph flow is measured every 15 min, it is reported as ml/h. The albumin fraction in lymph is reported as the lymph/plasma ratio.

The rate of equilibration of the tracer albumin was obtained by plotting the specific activity of protein in plasma and lymph over the time course of the experiment and the difference between plasma and lymph specific activities on a logarithmic scale. An example of the results for one experiment is shown in the Figure. The plasma to lymph difference decreased linearly on the logarithmic scale. We determined the straight line of best fit over the time course of the experiment by least squares regression analysis and then calculated the half time of the process.

The data are presented as group means and standard deviation. The data between groups were compared by an unpaired t-test. We also compared subgroup data in the 6 sheep with both baseline and increased microvascular hydrostatic pressure and in the 4 sheep with both baseline and increased permeability using a paired t-test. We accepted $p < .05$ as indicating statistical significance.

Results

The condition of the sheep and the time course of their response to increased left atrial pres-

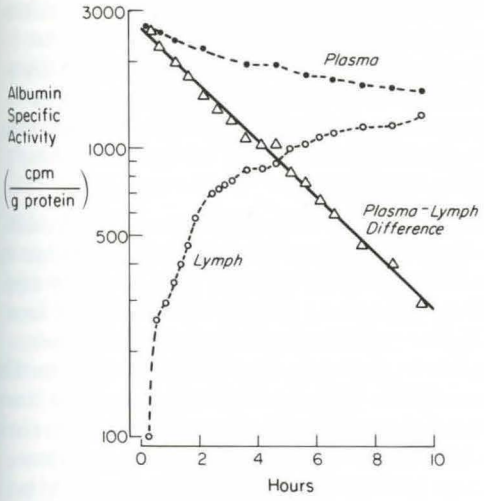

Fig. 1 Time course of the equilibration of intravenously injected ^{125}I-Albumin specific activity (counts per min/g protein) on a logarithmic scale. Plasma (•), lymph (○) and the plasma to lymph difference (Δ) are shown for the entire 10 h experiment. The straight line of best fit is shown. The half time is 3.1 h

sure and the increased microvascular permeability due to *pseudomonas* bacteremia have been described in detail elsewhere (4, 5). In this paper, we are only reporting results of the equilibration rate of tracer albumin. During the tracer studies, the variability in lung lymph flow expressed as the coefficient of variation in any single experiment averaged 15%; that is, lymph flow was steady. We followed the equilibration process through at least two half times in every sheep, ranging from 73% in the baseline experiments to 93% in the *pseudomonas* experiments.

The result of one baseline experiment is shown in the Figure. Plasma to lymph specific activity difference decreased along a single straight line compatible with single compartment wash-in kinetics. The correlation coefficient for the least squares regression line drawn through the data points was 0.96 and the equilibration half time was 3.1 h.

The results of all the experiments are summarized in the Table. Under baseline conditions, the albumin concentration in lung lymph was high, averaging 87% of the plasma concentration. This is for the albumin fraction only, not total protein. The half time for equilibration between plasma and lung interstitial free fluid averaged about 3 h. That is faster than for the body as a whole which, from the plasma decay data, had an equilibration half time of approximately 10–12 h. In every experiment the equilibration proceeded according to single compartment wash-in kinetics; that is, the logarithmic plot of the plasma-lymph specific activity difference against time was a single straight line.

In the increased hydrostatic pressure group, lung lymph flow was approximately three times the baseline rate. The restriction of the endothelial barrier to albumin flow is shown by the fall in the lymph albumin concentration. The difference between the baseline and increased hydrostatic pressure group equilibra-

Tab. 1 Effects of increased microvascular pressure or permeability on lung lymph flow, albumin concentration and tracer albumin equilibration in unanesthetized sheep

Condition*	No.	Microvascular Pressure (cm H_2O)	Lymph Flow (ml/h)	Albumin Lymph Concentration (lymph/plasma)	Albumin Tracer Equilibration (half-time, h)
A. Baseline	14	10.7 ± 2.6 †	5.3 ± 2.8	0.87 ± 0.06	2.9 ± 1.0
B. Increased Pressure	9	25.4 ± 4.1 (s) §	16.1 ± 10.6 (s)	0.63 ± 0.14 (s)	2.2 ± 1.0
C. Increased Permeability	4	12.9 ± 1.3 (s)	37.3 ± 29.4 (s)	0.77 ± .24	0.7 ± 0.2 (s)

* Duration of experiments up to 10 h. Pressure increased by balloon obstruction of mitral valve orifice. Permeability increased by *Pseudomonas* bacteremia.

† Average ± standard deviation.

§ (s) = Statistically significant at $p < 0.05$ by unpaired t-test between experimental and baseline groups.

tion half times is not significantly different because of the large variance within each group. But in the paired subgroup, all six sheep had more rapid albumin equilibration half times when the microvascular hydrostatic pressure was increased. The difference is statistically significant.

When the microvascular barrier permeability was increased by *pseudomonas* bacteremia, the lymph flow rose to about seven times the baseline level. The microvascular hydrostatic pressure was increased only an average of 2 cm H_2O. The lymph/plasma albumin concentration ratio decreased slightly but not significantly. The half time of tracer albumin equilibration decreased significantly to approximately one-fourth the baseline value.

Discussion

The lymph we obtained from the CMN efferent duct after appropriate surgical preparation of our sheep (6) is almost entirely lung lymph; however, it is not all of the lung lymph (3, 7). But, for studies of tracer equilibration, we believe it is fair to say that the time course we have observed is representative of the whole lung.

The measurement of plasma to lymph equilibration rates for various molecules to assess vascular permeability and changes in vascular permeability has been made in several organs (8–11). Studies of molecular equilibration for permeability determinations are done principally with macromolecules since small molecules (electrolytes and sugars) equilibrate rapidly (12).

In fetal and newborn lambs, *Boyd* and associates (13) found that polyvinylpyrrolidine, with a molecular radius similar to albumin, equilibrated in lung lymph with a half time of under 30 min, whereas *Bland* and *McMillan* (14) in anesthetized newborn sheep measured a tracer albumin equilibration rate of 100 min. In our laboratory, using anesthetized adult sheep, *Demling* and coworkers (15) measured a tracer albumin equilibration half time averaging 2.8 h in nine sheep and *Gorin* and coworkers (16), using Indium-labeled transferrin (a molecule slightly larger than albumin, but with less negative charge), found an equilibration half time of 2.4 h in ten sheep under baseline conditions compared to an equilibration half time of 0.95 h in six sheep after *pseudomonas* bacteremia. Thus, our new data in unanesthetized sheep are similar to these prior findings.

Studer and *Potchen* (17) measured individual organ equilibration rates for tracer albumin in rats. They found that the lung was fully equilibrated in about 1 h. It was one of the most rapidly equilibrating extravascular albumin pools in the body. Our evidence confirms their result. The lung equilibrates about three times faster than the body as a whole. Indeed, *Gorin* (16) made use of this differential equilibration rate between the lung and the body in order to measure lung protein turnover by counting suitably labeled tracers externally over the chest.

On the other hand, *Meyer* and *Ottaviano* (18) found a very slow equilibration rate for tracer albumin in right duct lymph in anesthetized dogs; average half time of 5.6 h. *Gorin* (16) in three anesthetized sheep with increased microvascular pressure, found a fast equilibration rate for transferrin; average 1.3 h. These discrepant data, one very slow and one rather fast, cannot readily be reconciled with the other published data on lung protein equilibration.

Studies of protein equilibration in human lung are fragmentary. *Robin* and coworkers (19) studied the equilibration of graded dextrans in two patients with increased permeability edema. In one patient, the equilibration rate of dextran 70 (m.w. = 70,000) had a half time of 1 h between plasma and airway fluid. In the other patient, the edema fluid dextran concentration was nearly equal to that of the plasma after 2 h.

Albumin Equilibration with Increased Hydrostatic Pressure

The fact that the protein equilibration rate was slightly faster when microvascular hydrostatic pressure was increased is consistent with the multiple pore theory of *Blake* and *Staub* (20) as recently modified by *McNamee*

and *Staub* (21). These authors did not find it necessary to invoke "pore-stretching" to explain their results. The old phenomenologic concept of "pore-stretching" introduced by *Shirley* and associates (22) especially as applied to the lung by *Pietra* and coworkers (23) is not supported by our new data.

That there is some decrease in the equilibration half time for albumin can easily be explained by the fact that the tracer protein equilibration occurs simultaneously by convection and diffusion. Clearly, the increased filtration under pressure increased the convective protein flow (solvent drag) and the decrease in the interstitial free fluid protein concentration, as manifested by the decreasing lymph/plasma ratio (L/P), increased the diffusive protein flow. Although the Table shows only a modest decrease in the lymph/plasma protein ratio for albumin with increased microvascular hydrostatic pressure, the concentration gradient from plasma to perimicrovascular free interstitial fluid (1-L/P) increased from 0.13 in the baseline conditions to 0.37 with increased pressure, a threefold increase.

Although in the paired experiments, the half time for equilibration during increased pressure was always less than for the baseline experiment, the individual variation in equilibration rates was large so that a single determination cannot reliably separate the two groups.

Albumin Equilibration with Increased Permeability

The rapid equilibration of tracer after *pseudomonas* bacteremia (increased microvascular permeability) required larger pore sizes in order to explain the marked augmentation in lymph flow, the high L/P ratio and the fivefold increase in transvascular protein flow (4). By either paired or unpaired statistics, the equilibration half time was significantly less than in the baseline condition. A single study of protein equilibration rates should detect a difference from the normal population with a high reliability. It is a sensitive test for altered microvascular permeability.

Clinical Usefulness of Concentrated Albumin Solutions

Returning to the question of the clinical usefulness of concentrated albumin solutions or other colloid osmotic agents in the treatment of pulmonary edema, it is clear that these substances may be effective in high pressure edema because the microvascular membrane is performing its normal barrier function (24). Not only will the increased osmotic pressure difference exert an immediate action to decrease the sum of pressures (in the Starling equation) causing fluid filtration, but the effect will persist for some hours according to the time course of protein equilibration between plasma and lung tissue fluid.

In increased permeability edema, however, the rapid equilibration rate means that, in addition to a reduced reflection coefficient (decreased effective osmotic difference because of increased pore size) the duration of any effect will be correspondingly brief. This has been confirmed by us in the isolated, perfused dog lung (25). We conclude on the basis of available evidence that the use of osmotic agents in patients with increased permeability edema is unwarranted; at least, in so far as its use is predicted on its osmotic action in decreasing fluid filtration rate.

Lung Lymph as a Measure of Perimicrovascular Interstitial Free Fluid Protein Concentration

An important corollary of our study was the finding that equilibration proceeded as a single exponential under all conditions, but particularly in the baseline group (see Figure).

Meyer and *Ottaviano* (18) also found a single exponential wash-in in right duct lymph in dogs. These data are at odds with the recent work of *Quin* and associates (26, 27). They claim that in the popliteal lymph node and other lymph nodes of sheep 30–50% of the total protein and fluid output in the efferent lymph is due to leakage within the node. They believe the mechanism of leakage is that a small amount of plasma enters the efferent lymph in proportion to the lymphocyte flux. While it is true that lymphocytes and small

amounts of immunoglobulins are added to efferent lymph during passage through nodes, including the CMN, our data do not support the concept that there is any osmotically significant addition of protein or fluid within the node.

Since our injected tracer protein equilibrated in the circulating plasma throughout the body with a half time of less than 2 min, any significant contribution from that source to CMN efferent lymph should have shown up in our experiments as a rapid initial equilibration phase, the Y-intercept of the plasma-lymph specific activity difference should have been below that of the plasma concentration. This did not happen. The correlation coefficients for the single exponential are very high (exceeding 0.95 in every experiment) so that it is unlikely that we missed any significant second compartment contributing labeled protein at a different rate.

These new data confirm and extend the findings of *Vreim, McNamee* and *Staub* (28) as to the effects of anesthesia on sheep lung lymph composition. They found no relationship between lymphocyte concentration in CMN efferent lymph and either lymph or lymph protein flow.

Whatever may be occurring in the popliteal lymph node of sheep is certainly not contributing any significant quantity of fluid or protein to the efferent lymph from caudal mediastinal node draining the lung in the unanesthetized sheep.

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