

TISSUE PROTEIN WASHOUT IN SHEEP LUNG LYMPH

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ABSTRACT

At high microvascular filtration rates, the lung lymph protein concentration (C_L) may be higher than the filtrate protein concentration due to protein washed into the lymph from the lung tissue space. To test that hypothesis, we increased the microvascular filtration rate in 5 anesthetized sheep and determined the relationship between C_L and the plasma protein concentration (C_P). Then we extrapolated the data to estimate C_L at $C_P = 0$. Because the filtrate protein concentration should be zero at $C_P = 0$, we recorded the extrapolated C_L as the concentration of tissue protein in the lymph (C_t). Our C_t estimate ($0.92 \pm 0.38 \text{g/dl}$) was significantly greater than zero ($P < 0.05$). This result is important because tissue protein in lymph may cause errors when investigators use lung lymph to study microvascular permeability. However, our technique to estimate C_f may allow investigators to correct for the tissue protein problem.

The pulmonary microvascular reflection coefficient to protein (σ) may be estimated from the lung microvascular filtrate protein concentration (C_f) at high filtration rates as:

$$\sigma = 1 - C_f / C_P \quad (1)$$

where C_P is the plasma protein concentration (4,6,7,9). Because C_f cannot be measured directly, investigators have used the lung lymph protein concentration (C_L) as an

estimate of C_f . To cause the high microvascular filtration rates necessary for this technique (the lymphatic washdown technique), investigators usually increase the left atrial pressure. After 2-3 hours, C_L decreases to a plateau and investigators substitute that C_L for C_f to estimate σ from equation 1 ($\sigma = 1 - C_L / C_P$).

The accuracy of the washdown technique depends on the assumption that C_L equals C_f . However, there are several grams of protein sequestered within the lung tissue space. After investigators increase the microvascular filtration rate, some of this protein is swept into the lymphatic vessels. Until that tissue protein is cleared through the lymphatics, C_L is higher than C_f . Although most investigators have assumed that the tissue protein is cleared within 2-3 hours, Parker and his associates (8) have suggested that it may 24 hours or more to completely clear the tissue protein. If they are correct, then 2-3 hours after investigators increase the filtration rate, C_L must equal the filtrate protein concentration plus the concentration of tissue protein in the lymph (C_t). That would cause investigators to underestimate σ with equation 1. The purpose of the present study was to estimate C_t in sheep lungs. Our technique was based on a rearranged form of equation 1.

$$C_f = (1 - \sigma) C_P \quad (2)$$

If lymph contains protein washed from the lung tissue, then C_L should be the sum of C_f and C_t :

$$C_L = (1-\sigma)C_P + C_t \quad (3)$$

According to equation 3, at high microvascular filtration rates, C_L should decrease linearly to C_t as C_P is decreased to zero. Thus, in this study, we determined the relationship between C_L and C_P for sheep lung lymph. Then, to estimate C_t , we extrapolated the relationship to $C_P=0$. Our results indicate that C_t is high enough to cause a significant error in reflection coefficient estimates in sheep lungs.

METHODS

We anesthetized 5 sheep with thiopental sodium, placed a tracheostomy tube and ventilated the sheep with 30% O_2 in air. We cannulated a jugular vein and infused warmed Ringer solution. A 30cc Foley balloon cannula and a polyethylene cannula were placed into the left atrium through a left thoracotomy, and a cannula was placed into the pulmonary artery. Through a right thoracotomy we placed a loose ligature around the inferior cava.

We cannulated an efferent lymphatic vessel from the caudal mediastinal lymph node (CMN) as described by Staub (11). The CMN receives afferent lymph from the lungs and from nonpulmonary tissues. We took several steps to eliminate the nonpulmonary lymph. First we ligated and resected the CMN 2-3 cm above the lower border of the right pulmonary ligament. Most investigators ligate the CMN at the border of the pulmonary ligament but some nonpulmonary lymph vessels enter the node above the ligament border (2). By resecting the node 2-3 cm above the ligament, we blocked flow from those vessels. Second, we cauterized the surface of the diaphragm (10). Third, we previously found that many nonpulmonary lymphatics enter the CMN either in or near the left pulmonary ligament (1). Accordingly, we cut the left ligament along the length of the node and cauterized the underlying tissue. We also resected the tissue above the CMN (between the node and the aorta).

Thus, when we completed our preparation the CMN was virtually isolated; the only visible afferent lymphatics were in the right pulmonary ligament and the CMN received lymph from only a portion of the right lung. We estimate that the CMN isolation procedure reduced the lung lymph flow to the CMN by ~75%. We began the experiments immediately after we isolated the CMN.

We used pressure transducers to monitor the aortic pressure, pulmonary arterial pressure and left atrial pressure, and we estimated the pulmonary microvascular pressure (P_c) as the average of the left atrial and pulmonary arterial pressures (5). The right atrium was used as the zero reference level for pressure measurement. We measured lymph flow rate by timing the flow of lymph into a calibrated pipette placed level with the right atrium.

During the ~2 hours required to place cannulas and isolate the CMN, we infused 2-3 L of Ringers solution into each sheep. We gave that much fluid because we wanted to decrease C_P before we began the experiments. There were two advantages to a low C_P . First, our plan was to determine the C_L vs. C_P relationship and extrapolate the relationship to $C_P=0$. The accuracy of this extrapolation would be improved if our C_L vs. C_P data were at low C_P 's. The second advantage of a low C_P is that microvascular filtration is increased at low C_P 's (low plasma protein osmotic pressures). Equations 1-3 are accurate only at high filtration rates. After the infusions, the plasma protein concentration (4.5_0.6 g/dl) was much lower than the $C_P=6.8\pm 0.6$ g/dl for awake sheep in our laboratory (5). Furthermore, the lymph flow rate (101 \pm 34 μ l/in) was relatively high for the small percent of the lung drained by the cannulated lymphatic. We attribute the low C_P and high lymph flow rate to the volume infusions.

The Experiments

Our aim was to determine the

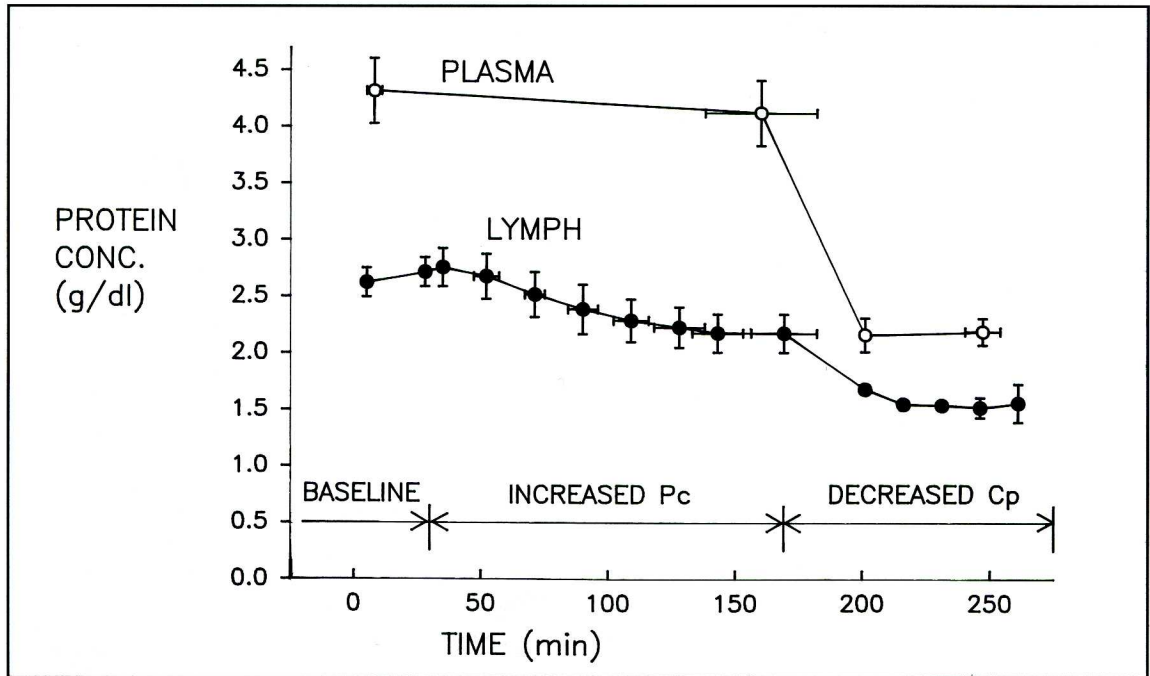


Fig. 1. Lymph and plasma protein concentrations in 4 sheep at baseline, with increased pulmonary microvascular pressure (increased P_c) and with decreased plasma protein concentration (decreased C_p).

relationship between C_L and C_p at high microvascular filtration rates without causing pulmonary edema. We did not want to cause edema because edema fluid may dilute the tissue protein and thus cause us to underestimate C_t (6). After a 20-30 minute baseline period, we inflated the left atrial balloon and increased P_c to 20-25 mmHg. We did not increase P_c above 25 mmHg because we did not want to cause edema. We determined C_L each 10-15 minutes until it decreased to a steady level (less than 0.1 g/dl change in 30 minutes). Once C_L was stable, we took samples of plasma and lymph and decreased C_p .

We have previously used rapid infusions of Ringer solution to decrease C_p in dogs (6). In this study we infused 2-4 L of warmed Ringer solution in ~20 minutes. Rapid infusions usually cause substantial increases in pulmonary vascular pressures and the combined effect of increased P_c and decreased plasma protein osmotic pressure

may cause pulmonary edema. To prevent the P_c increase, we deflated the left atrial balloon and partially tightened the inferior vena caval ligature. After the rapid infusion period, we slowed the Ringer's infusion rate and adjusted P_c to prevent a decrease in lymphatic flow rate. Once C_L decreased to a steady level, we took plasma and lymph samples. We euthanized the sheep with 30 ml of saturated KCl solution and removed the lower right lung lobe. We used our modification of the method of Pearce (5) to determine the lung extravascular fluid/blood free dry weight ratio.

Protein Concentrations

We used an American Optical refractometer to determine C_L and C_p . We calibrated our refractometer for protein concentrations from 0-8 g/dl. However, some investigators doubt that the American Optical refractometer is accurate at low protein

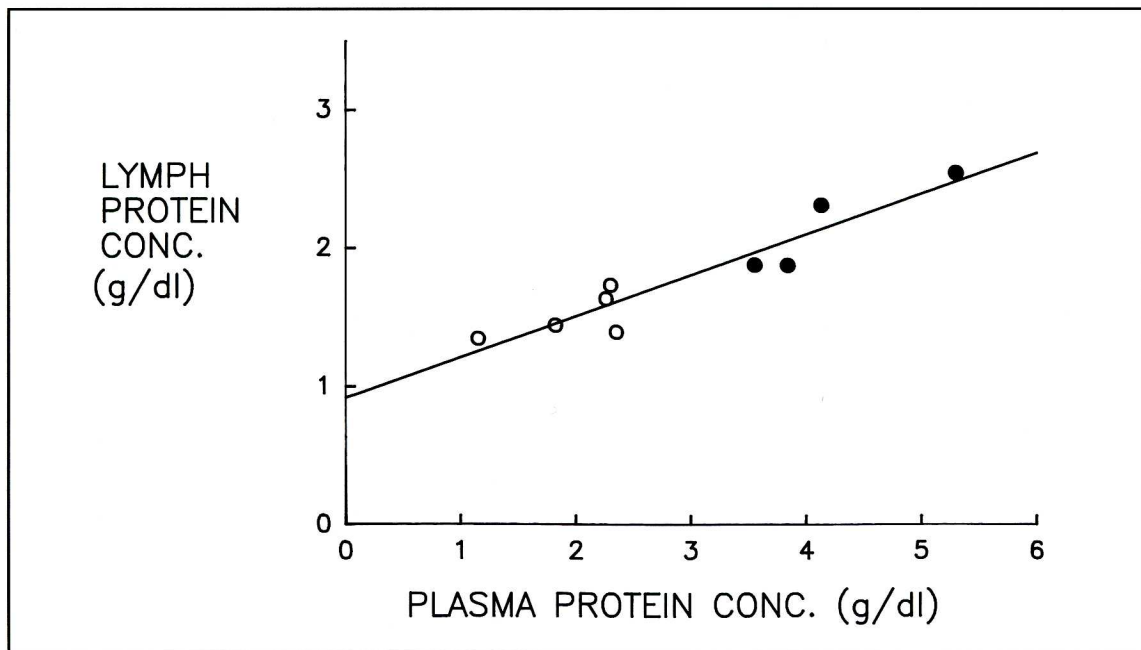


Fig. 2. Lymph vs. plasma protein concentration data with increased pulmonary microvascular pressure (solid circles) and decreased plasma protein concentration (open circles). Protein concentrations were estimated with a refractometer. Regression line: $C_L = 0.29C_P + 0.92$ ($r = 0.94$).

concentrations. Thus, we used the Bio-Rad technique to confirm our data for the steady state lymph and plasma samples (6).

Statistics

Data are summarized as mean \pm SD in the text and mean \pm SE in the figures. We used the method of least squares to determine the relationship between variables, and we used Student's t-test to test for significant differences between data. $P < 0.05$ was accepted to indicate significance.

RESULTS

Fig. 1 shows the lymph and plasma protein concentrations vs. time for 4 of the group I experiments. In one experiment, we did not establish a steady state after we elevated P_c so we did not include that experiment in Fig. 1. For the experiments of Fig. 1, the lymph flow rates at increased P_c

and after we decreased C_P were 136 ± 11 and 163 ± 43 $\mu\text{l}/\text{min}$, respectively ($P > 0.05$).

The solid circles in Fig. 2 show the C_L vs. C_P data with increased P_c and the open circles show the data with decreased C_P . The regression line in Fig. 2 was fit to all the data. The $C_P = 0$ intercept of the regression line ($C_L = 0.92 \pm 0.38$ g/dl) was significantly greater than zero. The slope of the regression line was 0.29 ± 0.10 . As shown in Fig. 3, the C_L vs. C_P relationship for the protein concentration data determined with the Bio-Rad technique was very similar to the relationship for the refractometer data (Fig. 2). The postmortem lung extravascular fluid to blood free dry weight ratio was 4.8 ± 0.7 for the 5 sheep of this study.

DISCUSSION

According to the results, during acute increases in microvascular filtration rate, the sheep lung lymph protein concentration was

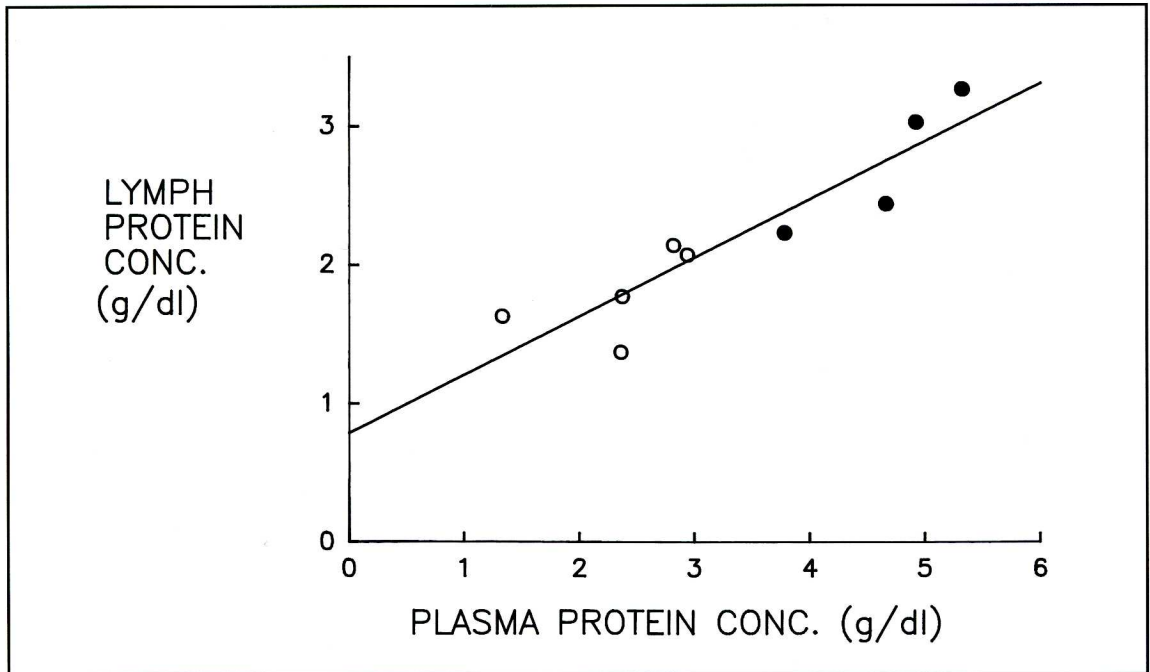


Fig. 3. Data for protein concentrations estimated with the Bio-Rad technique. Solid circles represent data with increased pulmonary microvascular pressure and open circles represent data after we decreased plasma protein concentration.

significantly higher than the filtration protein concentration. Although we could not measure the filtrate protein concentration, there can be no protein in the filtrate with $C_p=0$. Thus from the $C_p=0$ intercept of the C_L vs. C_p regression line (Fig. 2), we estimate that the lung lymph protein concentration was 0.92 ± 0.38 g/dl higher than the filtrate protein concentration. We believe that the excess lymph protein came from the lung tissue and the concentration of tissue protein within the lymph (C_t) was 0.92 ± 0.38 g/dl.

Adair et al (1) have shown that, due to fluid or protein exchange across the blood-lymph barrier, C_L may increase within lymph nodes. However, with our procedure to isolate the caudal mediastinal lymph node, we interrupted blood flow to the node. Consequently, we do not believe that the lymph protein concentration was increased within the node. Nevertheless, we cannot be certain that we completely eliminated all blood flow to the node. If the lymph protein concen-

tration was increased within the node, then we probably overestimated C_t from our post-nodal lymph data.

Although we attempted to minimize pulmonary edema formation in our sheep, we could not totally prevent it. Accordingly, the lung extravascular fluid/flood free dry weight ratio (4.8 ± 0.7) was higher than the ratio of 4.0 ± 0.2 for control sheep in our laboratory (4). The excess lung tissue fluid may have diluted the tissue protein and decreased C_t . Consequently, our C_t estimate may be lower than C_t for nonedematous lungs.

If our results are correct and C_L is higher than the filtrate protein concentration during acute increases in microvascular filtration rate, then equation 1 will yield an underestimate of σ . However, it should be possible to account for the C_t problem. Investigators could increase the left atrial pressure to determine the washdown C_L , then use our technique to estimate C_t . The filtrate protein concentration could be estimated as:

$$C_f = C_L - C_t \quad (4)$$

Then the C_f from equation 4 could be used to calculate σ from equation 1. For example, Parker et al (9) increased the left atrial pressure for several hours in sheep and determined the washdown $C_L=1.57$ g/dl with $C_p=5.95$ g/dl. By assuming $C_L=C_f$, they calculated $\sigma=0.74$ with equation 1. However substituting C_L and our C_t estimate into equation 4, we calculate $C_f=1.57-0.92=0.65$ g/dl. Substituting that C_f and C_p into equation 1, we calculate $\sigma=0.89$. Thus, our σ estimate corrected for C_t is 20% higher than the $\sigma=0.74$ Parker et al (9) calculated.

Another way to correct for tissue protein washout may be to increase the filtration rate long enough to clear all the excess tissue protein through the lymphatics. Then $C_L=C_f$. Parker et al (8) used that technique to improve their σ estimate in sheep. This time, Parker and his associates elevated the left atrial pressure for 24 hours and C_L decreased much more than in their previous study with acute left atrial pressure elevation. The σ estimated from the 24 hour washdown C_L data ($\sigma=0.89\pm 0.02$) is not different from the σ we calculated from our C_t and the Parker et al (8) acute washdown C_L data. In other words, we obtained the same σ estimate by accounting for tissue protein washout as Parker et al (8) obtained by depleting the tissue protein.

We conclude that during acute increases in microvascular filtration rate, sheep lung lymph may contain a significant concentration of protein washed from the tissue space. This tissue protein could cause errors in estimates of the microvascular membrane reflection coefficient with the lymphatic protein washdown technique. However, there are several techniques which may allow investigators to account for tissue protein washdown.

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