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The Flow and Composition of Pulmonary and Systemic Lymph in Dogs with Edema

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Summary

In animals with edema, pulmonary and systemic lymphatics may function to remove accumulated extravascular fluid in addition to responding to events as they occur in the exchanging vessels. We tested this hypothesis by measuring the flow rate and composition of thoracic duct and right duct lymph in anesthetized dogs made edematous by rapid fluid infusion. During a fluid challenge equivalent to 30% of body weight, thoracic and right duct lymph flow rates increased 30- and 60-fold, respectively. After the infusion, lymph flow rates rapidly decreased even though the dogs were edematous. During the postinfusion period, the decrease in right duct lymph flow rate was directly related to a decrease in estimated net pulmonary fluid filtration pressure. We conclude that lymph flow rate and composition reflect events occurring in the microvasculature whether or not edema is present.

Introduction

Several studies have demonstrated that, under steady-state conditions, the flow rate and composition of pulmonary lymph reflect events occurring in the fluid exchange vessels of the lung (1–4). Other reports have shown that the lymphatic system serves to limit and delay the accumulation of extravascular fluid, particularly under conditions leading to an increased rate of net transvascular fluid flux (5–7). However, it is not clear what role lymphatics play in the resolution of acute pulmonary and systemic edema. *Pine* and coworkers (8) measured right duct lymph flow rate in dogs after inducing pulmonary edema with α-naphthylthiourea (ANTU). They found that the lymph flow rate was increased in edematous dogs, and that the flow rate correlated with the amount of extravascular lung water. Their results suggest that lymphatics play a quantitatively important role in the removal of edema fluid. We tested this hypothesis by measuring the flow rate and composition of thoracic and right duct lymph in anesthetized dogs made edematous by rapid fluid infusion. We found that lymph flow rates decreased rapidly after the infusion even though the dogs were edematous. In contrast to the results of *Pine* and coworkers (8), our data suggest that lymph flow rate and composition reflect current microvascular events rather than past extravascular fluid accumulation.

Methods

Animal Preparation

We anesthetized 12 mongrel dogs (body weight, 12 to 20 kg, average 15.4 kg) with 30 mg/kg pentobarbital and made a tracheostomy. The dogs were ventilated on room air throughout the experiments. We placed catheters in the aorta, left ventricle, right atrium and pulmonary artery (Thermodilution Catheter, Model 1-60-536, 7F, Technology Sales Corp., Hartsdale, NY). Blood pressures were continuously recorded using the most dependent region of the lung as the zero reference level. A large bore catheter was placed in a femoral vein for fluid infusion and administration of drugs. In 6 dogs, we cannulated the right lymph duct (RD) via a cervical lymphatic as described by *Vreim* and *Ohkuda* (9). We also cannulated the thoracic duct (TD) in the cervical region near its entrance to the left jugular vein. After both cannulas were in place, we administered 3,000 units of heparin intravenously.

Protocol

We collected lymph for a 30 to 60 minute baseline period. Midway in each lymph collection, we measured cardiac output by the

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thermodilution method (Thermodilution Computer, Model 3500, KMA, Technology Sales Corp., Hartsdale, NY) and took a 2 ml aortic blood sample. We also measured left ventricular end-diastolic pressure (Plved) and mean pressures in the aorta (Pa), right atrium (Pra), and pulmonary artery (Ppa). In one dog, we continued the baseline period for 4 hours. After the baseline measurements, we began infusion of warmed Ringer's solution neutralized with bicarbonate. We infused an average of 4.7 L (i.e., 30% body weight) at an average rate of 151 ml/min. During the infusion, we used 15 minute lymph collection periods with a pressure record and blood sample taken halfway through the collection. Cardiac output was not measured during the infusion. We maintained the 15 minute data collection periods for the first hour postinfusion. For the final 90 minutes of the experiment, we used 30 minute collection periods. Two and one-half hours postinfusion, we removed the lungs and rapidly froze them in liquid nitrogen. When possible, we microsampled free interstitial fluid from perivascular cuffs as described by *Gee* and *Staub* (10). After microsampling, the lung was homogenized to measure extravascular water content (11, 12). In 6 additional dogs we measured extravascular lung water one hour after the infusion.

Assays, Calculations and Statistical Analysis

After centrifugation, we measured the total protein concentration in lymph and plasma (13) and calculated the albumin and globulin concentrations following cellulose acetate electrophoresis. We used these data to calculate the oncotic pressure in lymph (πpmv) and plasma (πmv) (14). We also calculated the pulmonary microvascular pressure (Pmv) by assuming that 60% of the total plumonary vascular resistance is precapillary (15). These data were used to estimate the net pulmonary microvascular fluid filtration pressure (ΣP) as follows: $\Sigma P = Pmv - (\pi mv - \pi pmv)$. We assumed that the interstitial hydrostatic pressure was zero. Statistical analyses were done using an unpaired "t" test and linear regression analysis (16). Unless stated otherwise, we accepted $p < 0.05$ as indicating statistical significance. Numerical data are reported and means and standard errors of the mean.

Results

In one dog studied for 4 hours without a fluid challenge, there were no significant changes in the data with time (Table 1). The TD lymph flow rate, protein concentration and calculated oncotic pressure were significantly greater than those in RD lymph. The estimated net fluid filtration pressure averaged 5.5 cm H_2O.

In the experimental group, we successfully cannulated both lymphatics in all five dogs. However, we did not include results of RD collections in one dog since the baseline lymph was chylous. In a different dog, we inadvertantly dislodged the TD cannula early in the experiment. Therefore, results given for the two lymph fluids were obtained in 5 dogs, 3 with simultaneous collection from both duct

Tab. 1 Summary of Data Obtained in One Anesthetized Control Dog. Data are Means and Standard Errors of Measurements Made in 9 Sampling Periods Over 4 Hours. Ppa, Mean Pulmonary Artery Pressure; Plved, Left Ventricular End-Diastolic Pressure; Pa, Mean Aortic Pressure; Pra, Mean Right Atrial Pressure; TD, Thoracic Duct Lymph; RD, Right Duct Lymph

Hemodynamic Data			
Vascular Pressures			
Ppa	18.6	± 0.6	cm H_2O
Plved	6.3	± 0.2	cm H_2O
Pa	116	± 2	Torr
Pra	4.0	± 0	cm H_2O
Cardiac Output	107	± 2	ml/min · kg
Plasma and Lymph Data			
Plasma Protein Concentration	5.42	± 0.08	g/dl
Lymph/Plasma Protein Ratio			
TD	0.84	± 0.01	
RD	0.72	± 0.01	
Oncotic Pressure			
Plasma	19.5	± 0.4	cm H_2O
TD	15.6	± 0.4	cm H_2O
RD	13.8	± 0.3	cm H_2O
Lymph Flow Rate			
TD	14.4	± 0.7	ml/hr
RD	0.6	± 0.1	ml/hr

Tab. 2 Hemodynamic Changes in 4 Anesthetized Dogs Subjected to Rapid Volume Expansion. Ppa, Mean Pulmonary Arterial Pressure; Plved, Left Ventricular End-Diastolic Pressure; Pa, Mean Aortic Pressure; Pra, Mean Right Atrial Pressure; $\dot{Q}$, Cardiac Output. Data are means and standard error.

Protocol	Ppa (cm H_2O)	Plved (cm H_2O)	Pa (Torr)	Pra (cm H_2O)	$\dot{Q}$ (ml/min · kg)
Baseline	18.4 ± 2.5	7.1 ± 0.5	133 ± 9	4.3 ± 0.8	154 ± 8
Infusion					
0 to 15 min	56.0 ± 8.6	48.9 ± 7.0	154 ± 11	26.0 ± 1.8	
15 to 30 min	64.0 ± 5.7	57.0 ± 3.6	149 ± 9	34.6 ± 2.7	
Postinfusion					
0 to 15 min	30.9 ± 0.6	13.3 ± 4.1	136 ± 14	9.8 ± 1.7	251 ± 29
15 to 30 min	29.7 ± 2.0	11.5 ± 2.3	139 ± 10	8.4 ± 1.7	224 ± 20
30 to 45 min	32.3 ± 0.3	10.0 ± 1.2	146 ± 9	8.0 ± 1.5	175 ± 17
45 to 60 min	29.0 ± 3.7	9.0 ± 1.2	147 ± 7	7.2 ± 1.6	161 ± 12
60 to 90 min	31.3 ± 0.9	8.3 ± 0.9	149 ± 8	6.8 ± 1.5	128 ± 16
90 to 120 min	29.3 ± 3.5	8.0 ± 0.9	149 ± 5	6.4 ± 1.5	119 ± 13
120 to 150 min	28.8 ± 3.5	7.8 ± 1.0	148 ± 5	6.6 ± 1.6	110 ± 14

Table 2 shows hemodynamic data obtained during the experiment. The infusion produced dramatic increases in Ppa, Plved and Pra. Atrial pressures rapidly decreased to baseline levels after the infusion. In contrast, pulmonary artery pressure decreased but remained significantly greater than baseline throughout the postinfusion period.

During the infusion, plasma protein concentration decreased to about 40% of baseline (Fig. 1). However, as the fluid load distributed in the total extracellular space, the protein concentration increased to about 65% of the pre-infusion value. The lymph plasma protein concentration ratios (L/P) are shown in Fig. 2. The baseline L/P ratio in the TD was significantly greater than that in the RD. The L/P ratios transiently increased during the infusion since the plasma protein concentration was falling very rapidly. However, throughout the postinfusion period, the L/P ratios were much lower than the pre-infusion levels. Figure 3 shows the protein concentrations in RD and TD lymph and in lung free interstitial fluid in one dog in which we obtained suitable microsamples from fluid cuffs. In most dogs, the cuffs were either hemorrhagic or small. The protein concentration in lung free interstitial fluid closely approximated that in the postinfusion RD lymph, but was greater than the concentration in TD lymph.

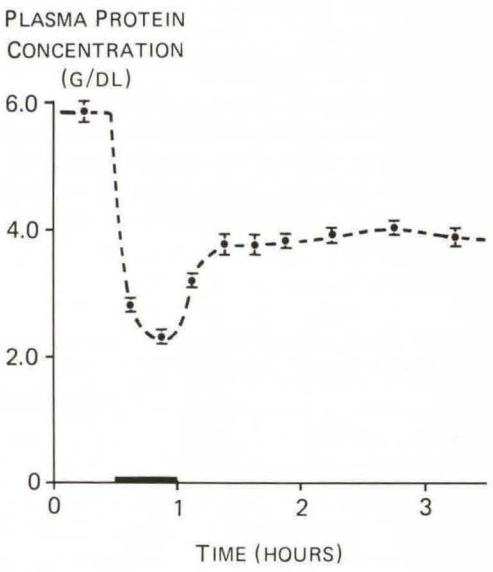
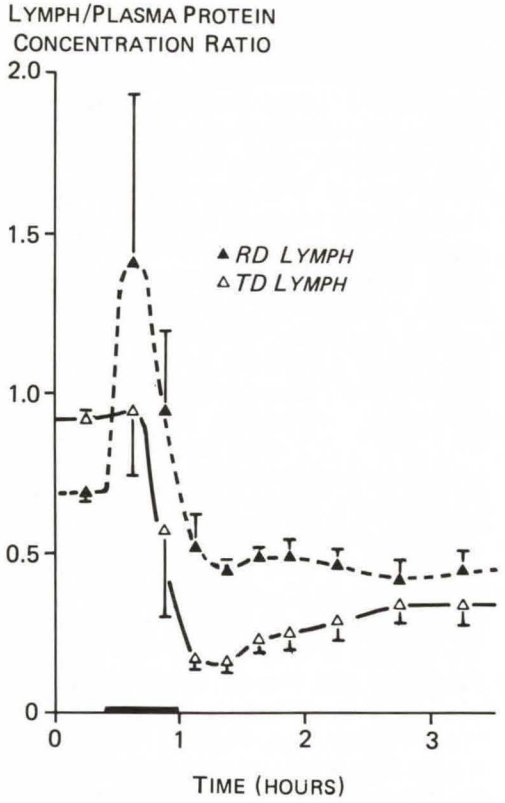


Fig. 1 Data illustrated are means and standard errors of measurements made in 5 anesthetized dogs subjected to rapid volume expansion. The bar marks the fluid infusion period

As shown in Fig. 4, the baseline lymph flow rate ($\dot{Q}_L$) in TD was 30 times that in the RD. In response to the infusion, RD $\dot{Q}_L$ increased 60 fold and TD $\dot{Q}_L$ increased 30 fold. Although the magnitude of the lymph flow response to fluid challenge was different, the pattern of the response was similar in the two lymphatics.



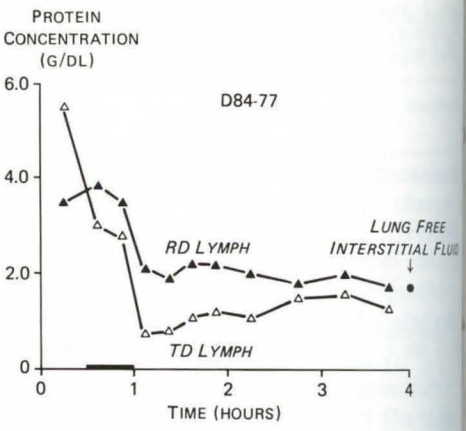


Fig. 3 Protein concentration of thoracic (TD) and right duct (RD) lymph and of lung free interstitial fluid in one anesthetized dog subjected to rapid volume expansion. The bar marks the fluid infusion period

Fig. 2 Data shown are means and standard errors of measurements made in 4 anesthetized dogs subjected to rapid volume expansion. The bar marks the fluid infusion period. RD, Right Duct Lymph; TD, Thoracic Duct Lymph

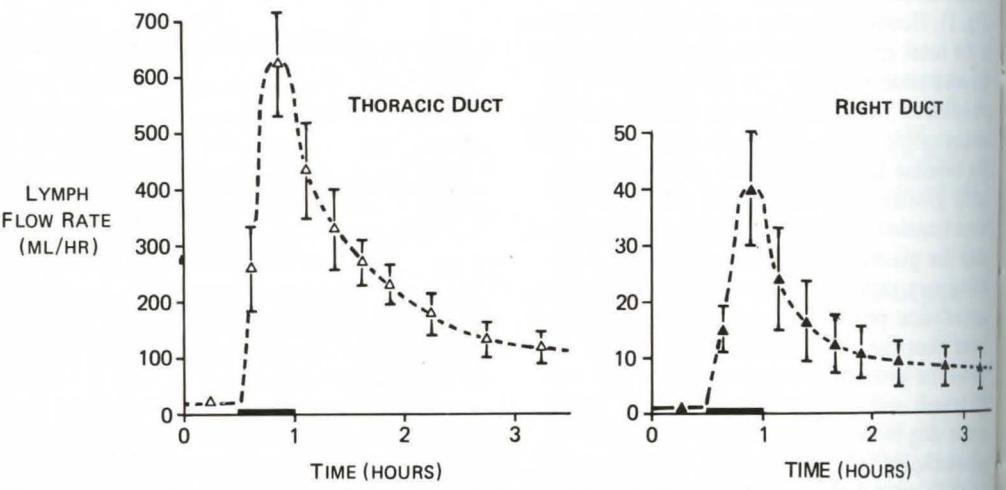


Fig. 4 Means and standard errors of thoracic and right duct lymph flow rates measured in 4 dogs subjected to rapid volume expansion. The bar marks the fluid infusion period. Standard errors for the baseline collections were too small to illustrate

In both instances, $\dot{Q}_L$ increased dramatically during the infusion and it decreased rapidly in the postinfusion period.

We do not have quantitative data on extravascular fluid accumulation in the abdomen and other regions contributing to TD lymph formation. However, we did note a marked persistent ascites in each dog. Table 3 shows extravascular lung water (Qwl/dQl) content measured in these dogs and in the 6 dogs in which we removed the lungs 1 hour after the fluid challenge. In the lungs taken 1 and 2.5 hours postinfusion, Qwl/dQl was about 70% greater than normal (3.58 ± 0.10 g H_2O/g dry lung in our laboratory (12). This increase in Qwl/dQl is similar to that reported by *Snashall* et al. (5) who fluid challenged anesthetized dogs to a volume equal to 30% body weight at an infusion rate about one fourth the rate we used. These data indicate that the quantity of extravascular fluid in the lung did not decrease during the postinfusion times we studied. The one dog in which we collected only TD lymph developed fulminant pulmonary edema with an extravascular lung water of 20.36 g H_2O/g dry lung.

Tab. 3 Extravascular Lung Water Content (Mean ± Standard Error) in Anesthetized Dogs Subjected to Rapid Volume Expansion

Number of Dogs	Postinfusion Time (Hours)	Extravascular Lung Water (g H_2O/g Dry Lung)
6	1.0	5.97 ± 0.57
4	2.5	6.00 ± 0.27

Table 4 shows the derived data necessary to estimate the net pulmonary microvascular fluid filtration pressure. We could not do a similar analysis for TD lymph since it represents a mixture of lymph formed in many different organs and we have no reasonable estimate of microvascular hydrostatic pressure. During the baseline and postinfusion periods, there was an oncotic pressure gradient (πmv–πpmv) of 6 to 7 cm H_2O favoring net fluid reabsorption. Since pulmonary microvascular pressure was greater than 7 cm H_2O, there was always a net fluid filtration pressure.

The relationship between net pulmonary microvascular fluid filtration pressure and RD lymph flow rate is shown in Fig. 5. Data used in the analysis are mean values obtained during the baseline and postinfusion sampling periods.

Tab. 4 Calculated Data (Means and Standard Errors) for Pulmonary Microvascular Pressure (Pmv), Oncotic Pressures in Plasma (πmv) and RD Lymph (πpmv), and the Estimated Net Pulmonary Fluid Filtration Pressure (ΣP)

Protocol	Pmv (cm H_2O)	πmv (cm H_2O)	πpmv (cm H_2O)	(ΣP) (cm H_2O)
Baseline	11.5 ± 1.2	20.2 ± 1.3	13.9 ± 1.4	5.2 ± 1.5
Infusion				
0 to 15 min	53.0 ± 7.3	9.0 ± 0.6	14.2 ± 4.2	61.5 ± 5.6
15 to 30 min	59.8 ± 4.2	7.2 ± 0.3	7.3 ± 1.7	61.7 ± 3.2
Postinfusion				
0 to 15 min	20.5 ± 1.8	10.5 ± 0.2	5.4 ± 0.9	15.4 ± 2.7
15 to 30 min	19.2 ± 0.9	12.3 ± 0.5	5.5 ± 0.5	11.9 ± 0.6
30 to 45 min	19.1 ± 0.6	12.4 ± 0.1	5.6 ± 0.3	12.7 ± 0.8
45 to 60 min	17.1 ± 1.4	12.6 ± 0.3	6.1 ± 0.7	10.7 ± 0.8
60 to 90 min	16.8 ± 1.3	12.9 ± 0.4	5.7 ± 0.7	9.6 ± 0.8
90 to 120 min	16.8 ± 1.8	13.6 ± 0.4	5.6 ± 0.8	8.9 ± 1.5
120 to 150 min	16.5 ± 1.6	12.9 ± 0.9	5.6 ± 0.8	9.7 ± 0.8

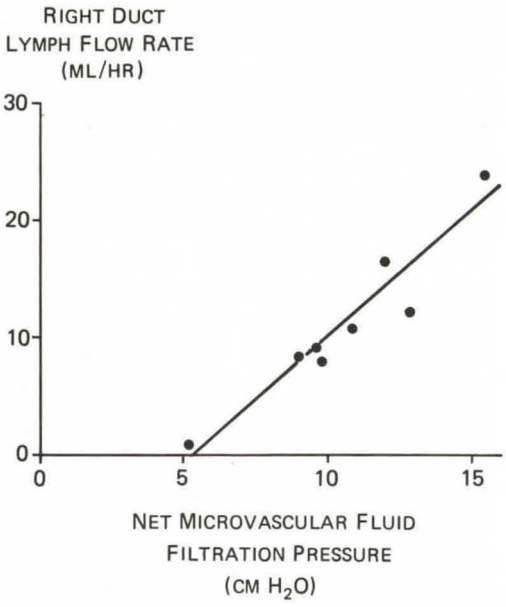


Fig. 5 Mean data obtained in 4 dogs during the baseline and postinfusion periods are illustrated. The equation and correlation coefficient for the regression line are Y = 2.18X−11.60, r = 0.96

There was a significant ($p < 0.001$) linear relationship between ΣP and $\dot{Q}_L$ described by the equation, Y = 2.18 X−11.60, r = 0.96. We also analyzed the data for each dog separately and found a significant linear relationship for every experiment. These data suggest that RD $\dot{Q}_L$ decreased in the lungs with edema, because the net fluid filtration pressure decreased.

Discussion

The baseline lymph protein concentrations and flow rates we measured are similar to those previously reported for nonedematous, anesthetized dogs (9, 18, 20, 21). However, *Pine* and coworkers (8), who used the venous pouch method (17) to collect RD lymph, reported control flow rates about 8 times greater than ours. One possible explanation for this difference is that they did not divert lymph draining into the pouch from the head, neck and foreleg. The differences we reported in RD and TD lymph protein concentrations and flow rates and the presence of chyle in TD lymph are consistent with the hypothesis that TD lymph is formed primarily in the liver and gut while RD lymph is formed in the lungs and heart (1, 2, 4, 8, 18, 19).

We reasoned that if removal of edema fluid were an important function of lymphatics, then rapid volume expansion should result in a prolonged increase in lymph flow rate that could not be explained by the net fluid filtration pressure. However, we found that lymph flow rates decreased after the fluid challenge even though the dogs were edematous. Furthermore, the decrease in RD $\dot{Q}_L$ during the postinfusion period was directly related to the decrease in the estimated net fluid filtration pressure. In calculating ΣP, we made some important assumptions. We assumed that changes in interstitial pressure that may have resulted from interstitial fluid accumulation were smaller than the measured changes in Pmv and oncotic pressures. Therefore, we did not include a value for interstitial hydrostatic pressure. We also assumed a reflection coefficient of unity for the pulmonary exchange vessels despite data that suggest it is less than one. However, using a value less than one would change the slope of the line relating ΣP and $\dot{Q}_L$ without altering the fact that a relationship exists between the variables. More important is the assumption that the reflection coefficient is constant. That is, the permeability of the pulmonary exchange vessels does not change during the postinfusion period. Given these assumptions, we interpret our data as indicating that the flow rate and composition of lymph reflect current microvascular events rather than past extravascular fluid accumulation.

Pine and coworkers (8) found that the RD lymph rate was increased in dogs with ANTU-induced edema. They could not explain the observed lymph flow rates on the basis of changes in pulmonary wedge pressure. They concluded that $\dot{Q}_L$ was elevated because of the accumulation of lung extravascular fluid not because net fluid filtration pressure increased. However, we would not expect to find the same lymph flow rate at a given Pmv in normal lungs and in lungs with increased vascular permeability (1). Furthermore, vascular

hydrostatic pressure is only one of three measureable pressures that are important in estimating the net fluid filtration pressure. However, with permeability edema it is difficult to calculate the effective transvascular oncotic gradient. For these reasons, we think it is much more difficult to study mechanisms of fluid clearance with permeability edema than with hemodynamic edema.

The lack of data on the mechanism of clearance of edema fluid necessitated, in both our experiments and those of *Pine* et al. (8), that we ask a rather naive question: Will lymph flow be maintained at an abnormally high rate in an edematous lung? Although this was a logical hypothesis to test, the different results obtained suggest that the mechanism of clearance of edema fluid may be more complex than the question assumes. For example, the slope of the line relating net fluid filtration pressure and lymph flow rate may indeed vary with the quantity of extravascular fluid in the lung. If so, then lymphatics remove accumulated edema fluid at a rate related to the net fluid filtration pressure. Certainly, we expect that the rate of direct microvascular reabsorption of edema fluid would be dependent on the sum of pressures determining transvascular fluid dynamics. This suggests that interstitial hydrostatic pressure is an important factor in determining the rate of fluid clearance from the interstitium. Furthermore, we know very little about the relative rates of clearance of interstitial fluid versus alveolar fluid. In the present study and in the ANTU experiments (8), no effort was made to quantitate the relative amount of edema fluid in the interstitial and alveolar spaces. It may well be that the mechanism of fluid clearance from these two compartments is different (22). Finally, the apparently conflicting results obtained in the ANTU and fluid overload experiments may indicate that the mechanism of fluid clearance is different in permeability and hemodynamic edemas. In this regard, it is possible that the interstitial protein concentration may affect both the mechanism and rate of fluid clearance.

Acknowledgements

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References

1 *Brigham, K.L., W.C. Woolverton, L.H. Blake, N.C. Staub:* Increased sheep lung vascular permeability caused by pseudomonas bacteremia. J. Clin. Invest. 54 (1974) 792–804

2 *Erdmann, A.J. III, T.R. Vaughan, Jr., K.L. Brigham, W.C. Woolverton, N.C. Staub:* Effect of increased vascular pressure on lung fluid balance in unanesthetized sheep. Circ. Res. 37 (1975) 271–284

3 *Vreim, C.E., P.D. Snashall, N.C. Staub:* Protein composition of lung fluids in anesthetized dogs with acute cardiogenic edema. Am. J. Physiol. 231 (1976) 1466–1469

4 *Vreim, C.E., P.D. Snashall, R.H. Demling, N.C. Staub:* Lung lymph and free interstitial fluid protein composition in sheep with edema. Am. J. Physiol. 230 (1976) 1650–1653

5 *Snashall, P.C., W.J. Weidner, N.C. Staub:* Extravascular lung water after extracellular fluid volume expansion in dogs. J. Appl. Physiol. 42 (1977) 624–629

6 *Taylor, A.E., W.H. Gibson, H.J. Granger, A.C. Guyton:* Interaction between intracapillary and tissue forces in the overall regulation of interstitial fluid volume. Lymphology 6 (1973) 192–208

7 *Cowan, G.S.M. Jr., N.C. Staub, L.H. Edmunds, Jr.:* Changes in the fluid compartments and dry weights of reimplanted dog lungs. J. Appl. Physiol. 40 (1976) 962–970

8 *Pine, M.B., P.M. Beach, T.S. Cottrell, M. Scola, G.M. Turino:* The relationship between right duct lymph flow and extravascular lung water in dogs given α-naphthylthiourea. J. Clin. Invest. 58 (1976) 482–492

9 *Vreim, C.E., K. Ohkuda:* Improved method for cannulation of the right lymph duct in dogs. J. Appl. Physiol. 43 (1977) 899–901

10 *Gee, M.H., N.C. Staub:* Role of bulk fluid flow in protein permeability of the dog lung alveolar membrane. J. Appl. Physiol. 42 (1977) 144–149

11 *Pearce, M.L., J. Yamishita, J. Beazell:* Measurement of pulmonary edema. Circ. Res. 16 (1965) 482–488

12 *Gee, M.H., P.A. Gwirtz, J.A. Spath, Jr.:* Extravascular water content of heart and lungs after acute myocardial ischemia. J. Appl. Physiol. 45 (1978) 102–108

13 *Lowry, O.H., N.J. Rosebrough, A.L. Farr, R.J. Randall:* Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1951) 265–275

14 *Landis, E.M., J.R. Pappenheimer:* Exchange of substances through the capillary walls. In: Handbook of Physiology, Circulation, Sec. 2, Vol. 2, Edited by *W.F. Hamilton* and *P. Dow.* American Physiological Society, Washington, D.C., 1963, pp. 961–1034
15 *Gaar, K.A., A.E. Taylor, L.J. Owens, A.C. Guyton:* Pulmonary capillary pressure and filtration coefficient in the isolated, perfused lung. Am. J. Physiol. 213 (1967) 910–914
16 *Snedecor, G.W., W.G. Cochran:* Statistical Methods. Iowa State University Press, Ames 1967
17 *Leeds, S.E., H.N. Uhley, J.J. Sampson, M. Friedman:* A new method for measurement of lymph flow from the right duct in the dog. Am. J. Surg. 98 (1959) 211–216
18 *Vreim, C.E., K. Ohkuda, N.C. Staub:* Proportions of dog lung lymph in the thoracic and right lymph ducts. J. Appl. Physiol. 43 (1977) 894–898
19 *Feola, M., G. Glick:* Cardiac lymph flow and composition in acute myocardial ischemia in dogs. Am. J. Physiol. 229 (1975) 44–48
20 *Meyer, E.C., R. Ottaviano:* Pulmonary collateral lymph flow: Detection using lymph oxygen tensions. J. Appl. Physiol. 32 (1972) 806–811
21 *Schad, H., H. Brechtelsbauer:* Thoracic duct lymph flow and composition in conscious dogs and the influence of anesthesia and passive limb movement. Pflugers Arch. 371 (1977) 25–31
22 *Meyer, E.C.:* Acute and chronic clearance of lung fluid, proteins and cells. In: *Lung Water and Solute Exchange,* Ed. *N.C. Staub.* Marcel Dekker, Inc., New York, 1978, pp. 277–321

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