The Effect of Fluid Volume Loading on Exclusion of Interstitial Albumin and Lymph Flow in the Dog Lung

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SUMMARY The excluded volume fraction for interstitial albumin (F_E) was estimated in the lungs of seven mongrel dogs during steady state conditions following intravenous infusions of Ringer's solution, amounting to 0, 5, 10, and 15% of body weight (BW). We estimated the tissue blood volume with 51Cr red cells, extracellular space with 99mTc-diethylenetriaminepentaacetic acid (99mTc-DTPA), and the albumin pool with 125I human serum albumin. A prenodal tracheobronchial lymphatic was cannulated for recording lymph flow (QL), total protein (CL), and albumin [C_L(A)] concentrations. From these measurements, we calculated the extravascular albumin content (Q_A) and 99mTc-DTPA space (V_i) of lung tissue samples collected at the successive volume expansions. The apparent tissue concentration of albumin (C_{App} = Q_A/V_i) decreased from a control value (mean ± SE) of 0.89 ± 0.06 to 0.46 ± 0.04 g/dl following the 15% BW infusion, whereas C_L(A) decreased from 1.43 ± 0.16 to 0.50 ± 0.07 g/dl for the same volume expansion. By assuming that pulmonary lymph represented tissue fluid, we calculated a control FE of 0.38 ± 0.02 using the equation, FE = 1 - [C_{App}/C_L(A)]. FE decreased following successive infusions to 0.28 ± 0.03, 0.16 ± 0.02, and 0.10 ± 0.02. These data indicate a significant contribution by the decrease in FE to the total decrease in tissue albumin concentration as interstitial fluid volume increased. Somewhat unexpectedly, the mean steady state Q_L increased by only 2.1-fold following the 5% BW expansion, but did not further increase following subsequent volume expansions. This has been attributed to a nonlinear interstitial compliance, sequestration of interstitial fluid, or possible deterioration of the experimental preparation.

GUYTON and Lindsey (1959) found in mongrel dogs that pulmonary edema was formed in significant amounts only when the left atrial pressure was elevated above a level of approximately 23 mm Hg. This resistance of the lung to edema formation has been attributed to a normally low pulmonary capillary hydrostatic pressure, the colloid osmotic pressure of the plasma proteins, and a readjustment of various tissue "safety forces" (Guyton et al., 1971). Although presently there is disagreement among investigators as to the relative contribution of the individual factors opposing edema formation, the overall safety factor generally is considered to include three basic components: (1) an increase in interstitial fluid pressure as interstitial fluid volume is increased (Guyton, 1965); (2) an increase in pulmonary lymph flow that removes a portion of the excess fluid filtered from the pulmonary exchange vessels (Erdmann et al., 1975); and (3) a decrease in the colloid osmotic pressure of the tissue fluid during edema. If the plasma colloid osmotic pressure remains unchanged, the greater osmotic gradient across the capillary endothelium will increase the rate of fluid reabsorption by the pulmonary capillaries (Staub, 1974). This decrease in tissue protein concentration may result from convective removal of proteins by the lymphatic system or dilution of tissue proteins in an expanded interstitial fluid volume. The glycosaminoglycans (mucopolysaccharides), proteoglycans (mucoproteins), and collagen in the interstitial ground substance also interact with plasma proteins present in the interstitium to influence their effective colloid osmotic pressure (Comper and Laurent, 1978). These polysaccharides and collagen form a densely tangled matrix that fills the interstitial spaces and can exclude large protein molecules, such as albumin and globulin, from a portion of the total interstitial water volume. Exclusion properties for a given solute depend upon the molecular size of the solute, as well as the concentrations of the various components of the interstitial matrix (hyaluronic acid, mucoprotein, and collagen). When proteins are excluded from a large fraction of the interstitial fluid volume, their effective concentration is increased (Comper and Laurent, 1978). Therefore, any change in the interstitial excluded-volume fraction would modulate the effective colloid osmotic pressure of the tissue proteins.

In the present study, we measured the effect of a reduced albumin-excluded volume fraction on the effective tissue albumin concentration during the formation of interstitial pulmonary edema. To do this, Ringer's solution was infused in increments...
equal to 5% of the animal’s body weight (BW). This increased the pulmonary interstitial fluid volume and produced changes in the tissue safety factors against edema without causing alveolar flooding (Taylor et al., 1973). The reduced excluded volumes observed in these experiments accounted for a portion of the safety factor that resulted from a decrease in the colloid osmotic pressure of the tissue proteins.

Methods

One day prior to the experiment, mongrel dogs weighing between 16.8 and 21.0 kg were injected with radiolabeled red blood cells (I\textsuperscript{31}Cr) and albumin (I\textsuperscript{125}I). To label these red cells, 20 ml of whole blood were withdrawn from a cephalic vein into a 75-ml vial containing 5 ml of acid-citrate-dextrose (ACD) solution (E. R. Squibb & Sons, Inc.), and I\textsuperscript{59}Cr (10 mCi/kg BW) (E. R. Squibb & Sons, Inc.) was added to the blood. The lymph was agitated gently over a 1-hour period, after which 100 mg of ascorbic acid (USP) were added to retard further entry of chromium into the red cells. The red cell wash procedure consisted of centrifuging the blood, discarding the plasma, and resuspending the red cells in sterile saline. The cells were centrifuged again, the saline discarded, and the saline wash procedure repeated. Then, I\textsuperscript{125}I-labeled human serum albumin (7 mCi/kg BW) (E. R. Squibb & Sons, Inc.) was added to the red cell suspension, and the mixture was infused into the cephalic vein.

The experiments were performed either 24 or 48 hours after the red cell tagging procedure. All dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and ventilated with a Harvard respirator. The kidneys were isolated through bilateral flank incisions and the renal vessels ligated. An external jugular vein was catheterized, and 400 mCi of Tm\textsuperscript{Tc}-DTPA (E. R. Squibb & Sons, Inc.) labeled diethyleneetriaminepentaacetic acid (DTPA), mol. wt. 403, (Diagnostic Isotopes, Inc.) was infused through the jugular catheter. The left carotid artery was catheterized to record systemic arterial pressure, and the remaining jugular vein was catheterized with a no. 5 Swan-Ganz balloon catheter. This was floated into the pulmonary artery to monitor continuously pulmonary artery pressure. The left chest was opened through an intercostal incision, and a catheter was sutured into the auricle of the left atrium. An afferent lymphatic to the left tracheobronchial lymph node was cannulated with polyethylene tubing (P.E. 20-60) and fixed in place with a ligature and cyanoacrylate adhesive (Superglue). The use of a prenodal lymphatic eliminates the possibility of contamination by cardiac and chest wall lymph and also any concentration changes that may occur as lymph passes through a lymph node (Taylor et al., 1973). We assumed that lymph collected from the cannulated lymphatic was representative of lymph from all of the lung tissue, since it was impossible to collect total lung lymph. Lymph flow was measured using calibrated micropipettes (Accupipette, American Hospital Supply), and lymph protein concentration was estimated using a refractometer (American Optical Co.). Blood samples were withdrawn periodically to determine total plasma protein, hematocrit, and isoctote activity. Lung tissue was sampled by ligating and excising small portions of lung parenchyma from the lung borders. These tissue samples were blotted to remove excess blood and weighed quickly. Their weights ranged between 0.15 and 0.5 g. When possible, samples of both right and left lungs were taken at each postinfusion state to reflect overall lung hydration. This tissue sampling procedure produced no observable effect on lymph protein composition or flow during control measurements.

The basic protocol consisted of a period of control measurements beginning approximately 2 hours after the Tm\textsuperscript{Tc}-DTPA injection. Lymph flow rate and protein concentration were monitored for approximately 45 minutes. When these parameters were stable, control samples of plasma, lymph, blood, and tissue were collected. A volume of lactated Ringer’s solution equal to 5% BW was warmed to body temperature and then infused over a 20- to 25-minute period. A period of at least 1 hour following termination of the infusion was allowed for the volume load to redistribute from the blood to the tissues. The criteria for reaching a steady state following the infusions were: (1) constant vascular pressures, (2) a constant lymph flow rate, and (3) constant lymph and plasma protein concentrations for at least 20 minutes. At each postinfusion steady state, samples of lymph, plasma, and tissue were collected. A volume of lactated Ringer’s solution equal to 5% BW were infused to a total of 15% BW. Samples were collected after each infusion as described above using the same steady state criteria. These samples were placed in preweighed plastic counting tubes, weighed on an analytical balance, and counted on a three-channel y counter (Packard model 3000). Appropriate formulas were used to correct for overlap in the energy spectra between the three isotopes. Also, it was necessary to correct the technetium counts for decay during the counting period because of the relatively short half-life (6.03 hours) of technetium. The tissue and blood samples then were dried to a stable weight at 60°C, and the tubes were reweighed to obtain total tissue and blood water. The albumin concentration of the lymph and plasma samples were determined using gradient gel electrophoresis (Brigham and Owen, 1975).

To compensate for the non-ideal properties of isotopic-labeled indicators, it was necessary to apply various correction factors to the measured counts. To estimate the free I\textsuperscript{125}I and I\textsuperscript{59}Cr in plasma, additional plasma samples were obtained in each experiment and mixed with cold trichloroacetic acid in a 1:5 ratio to precipitate the plasma proteins. Then the supernatant was counted for free I\textsuperscript{125}I and
$^{51}$Cr. Since the amount of free chromium was always less than 0.5%, no correction was made. However, approximately 1% of the iodine was not bound to the plasma albumin, and this percentage ranged as high as 1.9% free iodine in some cases. To correct for this error, the percentage of unbound isotope was subtracted from the total iodine label of the samples. The acid precipitation technique was not considered a suitable test for estimating free technetium because of the change in binding characteristics that could occur with an increase in the cationic valence of the technetium (Eckelman and Levenson, 1977).

Technetium is a polyvalent cation, which emits a $\gamma$ ray of 0.4 meV. This ion is tightly bound by the chelating agent, DTPA, mainly in the $+3$ valence state. $^{99m}$Tc-DTPA is known to be cleared by the kidneys at a rate that approaches the inulin and creatinine clearances (Klopper et al., 1972), but a tendency of $^{99m}$Tc-DTPA to bind to plasma proteins does lead to an underestimation of the glomerular filtration rate (Kempi and Persson, 1975). Binding has been estimated to range between 2 and 4% of the injected dose, but the rapid clearance of $^{99m}$Tc-DTPA by the kidneys indicates that $^{99m}$Tc-DTPA passes freely into the extracellular fluid.

The plasma binding of DTPA could be a source of error in our estimates of tissue and plasma albumin content, so a correction factor was estimated in each experiment using the small differences between the steady state technetium counts of lymph and plasma samples. The higher technetium counts usually observed in plasma compared to lymph were attributed to binding to the greater quantity of protein found in plasma. Restriction of $^{99m}$Tc-DTPA by the capillary membrane is unlikely, because transient analysis of equilibration of a bolus injection of $^{99m}$Tc-DTPA indicated such a rapid transit that lymph concentration always exceeded plasma concentration from shortly after injection until equilibration occurred. From the differences in protein and $^{99m}$Tc-DTPA, an estimate of $^{99m}$Tc-DTPA binding to protein of from 1.5 to 4.3% of the total technetium counts was estimated. The total technetium counts for each sample then were corrected by this binding factor in each experiment.

The quantity of extravascular fluid and albumin in the tissue samples was calculated by subtracting the quantities of blood albumin and water in each tissue sample from the total amounts in each tissue sample. First, the weight of blood (WB) in each tissue sample was calculated by dividing the counts per minute (CPM) of $^{51}$Cr in the tissue sample by the CPM in 1 g of whole blood, according to the formula:

$$\text{tissue CPM } ^{51}\text{Cr} = \frac{\text{WB(g) } ^{51}\text{Cr}}{\text{CPM } ^{51}\text{Cr/g wet weight blood}}$$  \hspace{1cm} (1)

For this calculation, the same hematocrit was assumed for blood in the lung tissue samples as measured in the mixed venous blood sample.

The quantity of extravascular albumin ($Q_a$) per gram blood-free dry weight (BFDW) in each of the tissue samples was calculated by subtracting the blood $^{125}$I CPM in the tissue sample from the total $^{125}$I CPM using the following formula:

$$Q_a(\text{mg/g BFDW}) = \left(\frac{125\text{I CPM/g wet weight tissue}}{\text{WB (g wet weight)}} \times \frac{125\text{I CPM/g wet weight blood}}{\text{WB (g wet weight)}}\right) + \left(\frac{125\text{I CPM/mg albumin}}{\text{BW (g BFDW/g wet weight tissue)}}\right)$$  \hspace{1cm} (2)

where $^{125}$I CPM/mg albumin is the iodine count per mg albumin obtained from the $^{125}$I CPM of lymph and plasma samples and their albumin concentrations as determined by gradient gel electrophoresis. The quantity of BFDW/g tissue wet weight was calculated by equation no. 9 below. The extravascular $^{99m}$Tc-DTPA space ($V_i$) was calculated using the equation:

$$V_i = \left(\frac{99m\text{Tc CPM/g wet weight tissue}}{\text{WB (g wet weight)}} \times \frac{99m\text{Tc CPM/g wet weight blood}}{\text{WB (g wet weight)}}\right) + \left(\frac{99m\text{Tc CPM/ml lymph}}{\text{BW (g wet weight blood)}}\right) + \left(\frac{125\text{I CPM/g wet weight tissue}}{\text{WB (g wet weight)}}\right) + \left(\frac{125\text{I CPM/mg albumin}}{\text{BW (g wet weight blood)}}\right)$$  \hspace{1cm} (3)

The extravascular $^{99m}$Tc-DTPA space was used to estimate the interstitial volume. The fraction of $V_i$ that excludes albumin ($F_E$) was calculated from the relationship between the apparent interstitial albumin concentration in the tissue ($C_{App} = Q_a/V_i$) and the concentration of albumin in pulmonary lymph ($C_L(A)$) using the following equation:

$$F_E = 1 - \frac{Q_a}{V_i} \cdot \frac{C_L(A)}{C_{App}}$$  \hspace{1cm} (4)

The quantity of extravascular water ($Q_w$) and BFDW/g wet weight of tissue were calculated, using the weight of blood in the tissue samples (WB), the fractional water content of blood (FW), the tissue blood water (BW), the dry weight of blood in the tissue (BDW), total tissue water (TW), tissue wet weight (WW), and tissue dry weight (DW), using the following relationships:

$$BW = WB \times FW \text{ (blood)},$$
$$BW = WB - BW,$$  \hspace{1cm} (5)
$$TW = WW - DW,$$  \hspace{1cm} (6)
$$Q_w = TW - BW, \text{ and}$$  \hspace{1cm} (7)
$$BFDW = DW - BDW.$$  \hspace{1cm} (8)

All of the above are expressed per gram wet weight of tissue, but can be expressed per gram BFDW by dividing by BFDW.

Statistics

The data are expressed as means ± standard errors in the text and graphs. A paired $t$-test was used to test for statistically significant differences.
between the control and postinfusion states for each parameter and between infusion states. When multiple tissue samples were taken at one postinfusion state, the mean value for that state was used for the paired statistical comparison. Differences between states were considered statistically significant when \( P < 0.05 \) was obtained. In addition, correlations between some variables were tested using a standard regression coefficient and standard deviation of the regression coefficient for the two variables (Snedecor and Cochran, 1976). Means of tissue parameters are based on \( n = 16, n = 16, n = 19, \) and \( n = 24 \) samples for the steady states following the 0, 5, 10, and 15% body weight infusions, respectively. The plasma, lymph, and vascular pressure parameters are means based on \( n = 7 \) for each of the four hydration states.

## Results

### General

The acute effects of a typical infusion on the various lung fluid balance parameters are shown in Figure 1. During the infusion period, systemic arterial pressure, pulmonary arterial pressure, and left atrial pressures all increased but returned approximately to control levels following the infusion. Lymph flow increased to a peak value but returned towards control. The protein concentration in both plasma and lymph decreased during the infusion, but the plasma protein concentration tended to recover slightly in the postinfusion period as the hypoosmotic fluid was redistributed between blood and tissues. As indicated in the figure, the lymph-to-plasma concentration ratio of total protein (\( C_l/C_p \)) usually increased during the infusion period because the decrease in lymph protein concentration lagged behind that of plasma, but the \( C_l/C_p \) ratio was always below the control value during the postinfusion period. The example shows one of the highest peak lymph flows obtained during an infusion (19 \( \times \) control). In this example, the lymph flow did not reach a new steady state until 1 hour after termination of the infusion. Following most infusions, the parameters returned to steady state values within 1 hour after termination of the infusion.

Although the vascular pressures transiently increased during the infusions, these pressures were not significantly different from control during the postinfusion periods, except that the systemic arterial pressures following the last infusion were significantly below the control pressures. For the control and successive postinfusion states, the mean pulmonary artery pressures were 17.3 ± 2.9, 15.3 ± 1.6, 17.3 ± 1.7, and 17.3 ± 1.8 cm H\(_2\)O; whereas, the mean left atrial pressures were 3.3 ± 0.5, 3.4 ± 0.4, 4.7 ± 0.7, and 4.1 ± 0.8 cm H\(_2\)O, respectively.

### Effect of Volume Load on Parenchymal Hydration

The increase in tissue hydration following each 5% BW infusion is shown graphically in Figure 2. These tissue fluid volumes and all of the other tissue data are based on \( n = 16, 16, 19, \) and 24 samples for the control; 5, 10, and 15% BW postinfusion states. The extravascular \(^{99m}\)Tc-DTPA space (\( V_i \)) increased to 191% of control following the last infusion, leading to an increase in the total extravascular water volume (\( Q_W \)) to 133% of control. Both fluid volumes were significantly higher than their control values at each infusion state. However, not every postinfusion volume was significantly higher than the immediately preceding volume. The difference between \( Q_W \) and \( V_i \) represents the mean cellular water volume. The mean cellular volumes at the four volume-loaded states were 1.79 ± 0.19, 1.50 ± 0.09, 1.41 ± 0.10, and 1.11 ± 0.09 ml/g BFDW. There was a significant decrease in cellular volume after the 15% BW volume load when compared with control \( (P = 0.026) \). This reduced cellular volume may represent a reduced excluded volume for \(^{99m}\)Tc-DTPA, cellular uptake of the tracer, or entry of the tracer into some other tissue fluid pool rendered accessible by the increased tissue hydration. The overall increase in tissue hydration also was re-

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**Figure 1** A graph showing the acute effects of a 5% BW infusion of Ringer's solution on various hemodynamic parameters, including systemic arterial pressure (SAP), pulmonary artery pressure (PAP), left atrial pressure (LAP), pulmonary lymph flow (\( Q_l \)), plasma (\( C_p \)) and lymph (\( C_l \)) total protein concentration, and the ratio of these concentrations (\( C_l/C_p \)).
The effects of fluid volume loading on total extravascular water ($Q_W$) and extravascular $^{99m}$Tc-DTPA space ($V_I$) in the lung.

Effect of Volume Loading on Lymph and Plasma Protein Concentrations

The effect of successive volume loads on the lymph and plasma total protein concentrations is shown in Figure 3. As expected, lymph and plasma protein concentrations decreased significantly at each postinfusion state, indicating the dilution of protein by the infused fluid volume. However, the decreased L/P ratio at each state indicates that lymph protein concentration decreased by a greater amount than would be expected from the dilution effect alone. There was a significant decrease in the lymph and plasma concentrations of total protein and albumin, as well as the $C_L/C_P$ and $C_L(A)/C_P(A)$ ratios at each postinfusion state when compared with the previous state. It is of interest that the difference in total protein between lymph and plasma remained remarkably constant, being 1.67, 1.70, 1.62, and 1.49 g/dl for the respective hydration states. However, because of the curvilinear relationship between total protein concentration and colloid osmotic pressure, the transcapillary colloid osmotic pressure differences ($\pi_T - \pi_L$) were 9.5, 7.3, 5.4, and 5.0 cm H$_2$O (Navar and Navar, 1977), respectively. Therefore, there was a net reduction in the colloid osmotic pressure gradient favoring reabsorption of fluid into the capillaries, even though the $C_L/C_P$ ratio decreased with each increase in hydration. There was also a trend for the albumin fraction to increase with increased hydration in lymph (0.318 ± 0.033, 0.351 ± 0.040, 0.330 ± 0.031, 0.382 ± 0.067), but not in plasma (0.296 ± 0.036, 0.297 ± 0.037, 0.281 ± 0.030, 0.277 ± 0.032). This may suggest an increased sieving of the larger protein molecules.

Relationship of Lymph Flow Rate to Interstitial Fluid Volume

The relationship of mean steady state postinfusion lymph flow ($Q_L$) to extravascular $^{99m}$Tc-DTPA space ($V_I$) is shown in Figure 4. The mean lymph flow rate increased significantly to 2.1 times control following the 5% BW infusion but, surprisingly, did not increase further following subsequent infusions. This modest increase is in sharp contrast to the acute effects of the volume infusion on lymph flow (Fig. 1). Lymph flows as high as 21 times control were observed during one infusion, whereas the maximum steady state lymph flow observed during a postinfusion period was only 7.3 times control. The postinfusion lymph flows at each steady state were significantly above control, but mean lymph flow decreased significantly following the 15% BW volume load when compared with the peak lymph flow rate by paired analysis. In four experiments,
ALBUMIN-EXCLUDED VOLUME IN THE LUNG/Parker et al.

Figure 4 The relationship of pulmonary lymph flow (Q_L) to extravascular 99mTc-DTPA space (V_i) at different states of lung hydration.

The peak lymph flow occurred following the 5% BW volume load; whereas in two experiments, the peak flow was observed after the 10% BW volume load. Using a regression analysis, we found that there was a significant correlation (P < 0.05) between lymph flow and extravascular 99mTc-DTPA space between control hydration and the 5% BW postinfusion state, but there was no significant correlation between these parameters in the V_i range of 3.1 to 4.1 ml/g BFDW, which represents successive infusions of 5, 10, and 15% BW.

Excluded Volume Fraction

The inverse relationship of the excluded volume fraction (F_E) for albumin to the extravascular 99mTc-DTPA space is shown in Figure 5. There was a highly significant inverse relationship between F_E and V_i using a regression analysis. The excluded volume fraction decreased significantly at each postinfusion state, from a 38% control value to 10% following the 15% BW infusion. This represents an increase in the available volume fraction for albumin, from 62% at control to 90% following the last infusion.

Excluded and Available Volumes

The effect of volume loading on the excluded (V_E) and available (V_Av) volumes for albumin is shown in Figure 6. These volumes were calculated from the mean extravascular 99mTc-DTPA space and the excluded volume fractions. The excluded volume decreased significantly (P < 0.05) to 53% of control following the 15% BW infusion compared with the decrease in excluded volume fraction to 26% of control. A decrease in the excluded volume, F_E • V_i, has the effect of increasing the available volume, (1 – F_E) • V_i, for albumin because V_i = V_E + V_Av. Although V_i increased by 1.97 ml/g BFDW to 191% of control between the control state and the 15% BW volume load, the actual fluid volume available to dilute tissue albumin increased by 2.42 ml/g BFDW. This produced an effective fluid volume available to dilute tissue albumin that was 23% greater than the total increase in interstitial fluid volume.

Effective Albumin Concentration in Tissue and Lymph

The effects of volume loading on the effective (C_Eff) and apparent (C_App) concentrations of albumin in the interstitium are shown in Figure 7. The albumin concentration in pulmonary lymph, C_L(A), was assumed to represent the effective concentration of protein in the interstitium, or C_Eff = C_L(A). The effective concentration of albumin is depen-
The apparent albumin concentration in the tissue was calculated by assuming that interstitial albumin was distributed in the total extravascular \textsuperscript{99m}Tc-DTPA space without restriction. This would be the case if there were no interstitial matrix to exclude proteins. The apparent interstitial concentration was calculated using the following equation:

\[ C_{\text{App}} = \frac{Q_A}{V_i}. \]  

As shown in the graph, the apparent concentration was considerably lower than the effective concentration under control conditions, but these concentrations approached each other during successive volume expansions. The ratio of the two concentrations can be used to calculate the available volume fraction for albumin \((1 - F_E)\), according to:

\[ 1 - F_E = \frac{C_{\text{App}}}{C_{\text{Eff}}}. \]  

The effective concentration is dependent upon the quantity of interstitial proteins as well as the available interstitial volume, so the effective concentration may be decreased either by a reduction of \(Q_A\), an increase in \(V_i\), or a reduction in \(F_E\). The quantity of tissue protein in turn may be reduced by convective removal at increased lymph flows.

Since each variable in Equation 10 was measured during the different hydration states, the relative contribution of each variable to the observed decrease in the effective tissue albumin concentration could be calculated. After one of the three variables was set as a constant at its control value, an effective concentration was calculated for each hydration state. These calculations were repeated for each variable in turn. The relative contribution of each of the three variables to the observed change in lymph concentration then was estimated by comparing the calculated albumin concentration to the measured albumin concentration of lymph. Figure 8 shows the relative contribution of each factor expressed as a percent of the total decrease in tissue albumin concentration. Apparently the major factor affecting tissue albumin concentration during fluid volume expansion is simple dilution \((D)\) of the albumin by an increased interstitial fluid volume. The decrease in excluded volume fraction \((E)\) at each postinfusion state accounted for 25-32% of the decrease in tissue protein concentration. Surprisingly, the convective removal, or washout \((W)\), of tissue albumin did not contribute significantly to the decreased tissue albumin concentration. In fact, there was a net "wash in" of protein into the tissue following the 5% BW infusion. This has been represented by a negative 18% contribution at this volume state.
Discussion

Effect of Fluid Volume Expansion on Interstitial Albumin Exclusion

The physiochemical properties of the interstitial glycosaminoglycans and proteoglycans, as well as their role in transcapillary fluid balance, recently have been reviewed (Comper and Laurent, 1978). Briefly, the ground substance surrounding tissue cells consists of a collagen network, interspersed with a gel of glycosaminoglycans and proteoglycans that forms a dense meshwork. The major glycosaminoglycan is hyaluronic acid, a linear polyanionic polymer of N-acetylglucosamine and glucuronic acid. This macromolecule has a high affinity for water and serves to immobilize interstitial fluid within the gel matrix. However, there is no actual chemical bonding of water to the matrix. The mucopolysaccharides offer little resistance to the diffusion of water molecules, but they do restrict the diffusion of larger molecules, such as proteins. In addition, albumin and globulin are excluded from a fraction of the matrix fluid volume in proportion to their molecular size and the hyaluronic acid concentration of the matrix. Apparently, many of the interstices between the ground substance molecules are too small for entry by proteins but readily admit water molecules or small ions (Comper and Laurent, 1978).

The phenomenon of macromolecular exclusion by a gel has been demonstrated in a number of in vitro experiments. Laurent (1964) found that a 0.5% hyaluronic acid solution excluded albumin (36 Å) from 25% and globulin (56 Å) from 40% of the total fluid volume. When the hyaluronic acid concentration was increased to 1.5%, the excluded volume for albumin increased to 75% of the total solution volume. Solutions of interstitial proteoglycans were found to have essentially the same exclusion properties as hyaluronic acid. Weiderhelm and Black (1976) found similar exclusion properties in hyaluronic acid solutions, but also found a significant exclusion of albumin by collagen mixtures of various concentrations. Granger et al. (1975) used the actual interstitial gel matrix obtained from human umbilical cord for exclusion studies. The normally hydrated Wharton’s jelly excluded albumin from 50% and globulin from 70% of the matrix fluid volume. Conversely, sucrose molecules were excluded from only a few percent of the matrix volume. The excluded volumes in hyaluronic acid solutions and tissue gels were attributed to steric hindrance of the protein molecules by the polysaccharide molecules, rather than any charge interactions between molecules.

Hallén (1974) found that the excluded volume for polyethylene glycol (radius 32 Å), a molecule approximately the size of albumin (radius 36 Å), was related directly to polysaccharide content in several tissues. Various cartilagenous tissues, with a polysaccharide concentration above 5 g/dl, had excluded volumes of greater than 90%; whereas, skin and tendon, with a polysaccharide concentration less than 1 g/dl, excluded polyethylene glycol from less than one-third of the extracellular fluid volume (Comper and Laurent, 1978). Excluded volumes for albumin between 20 and 34%, have been estimated for skin, tendon, and muscle, using tissue lymph albumin concentrations (Bell et al., 1978).

We used the assumption that pulmonary lymph represented fluid at equilibrium with the interstitial matrix and calculated an excluded volume for albumin which was 38% of the interstitial fluid volume of normally hydrated dog lungs. The ratios we obtained for albumin of C_App/C_P(A) = 0.62, C_App/C_P(A) = 0.49, and C_P/C_P = 0.79 during control conditions are comparable to the ratios of C_App/ C_P(A) = 0.50, C_App/C_P(A) = 0.44, and C_P/C_P = 0.68 obtained using whole homogenized sheep lungs and caudal mediastinal node lymph (Vaughn et al., 1971). Our interstitial volume fraction (V_i/Q_w) of 0.56 was somewhat higher than the 0.36 obtained from the whole sheep lungs using sucrose spaces.

An alternative explanation for the differences between the apparent interstitial albumin concentration and the higher concentration of albumin in pulmonary lymph is some mechanism for concentrating interstitial fluid within the lymphatic vessels (Guyton et al., 1978). We cannulated a prenodal lymphatic to prevent concentration changes associated with transit through lymph nodes. Quin and Lascelles (1975) found a higher protein concentration in postnodal than prenodal popliteal lymph at low lymph flow rates in sheep. Conditions may exist at the terminal lymphatics for concentrating lymph proteins, but this has not been demonstrated experimentally (Casley-Smith, 1969). A more likely explanation for the observed difference between the apparent tissue concentration and lymph concentration of albumin is exclusion of albumin by the glycosaminoglycans and proteoglycans present within the interstitial spaces. However, we must emphasize that the apparent tissue concentration of protein should approach the lymph concentration at increased lymph flow rates for a lymph concentrating mechanism, as well as a reduced excluded volume fraction.

The physiochemical properties of the interstitial gel are altered markedly when matrix hydration is increased. As shown diagrammatically in Figure 9, overhydration increases the distances between the randomly dispersed hyaluronic acid molecules and collagen strands and allows access of protein molecules to a larger percentage of the total interstitial fluid volume. Collagen tends to disperse with difficulty in vitro, so a constant portion of the excluded volume may be expected that is related to the collagen content (Comper and Laurent, 1978). Alveolar flooding would lead to an underestimate of
the true excluded volume fraction, but would not change the absolute excluded volume. Therefore, the significant reduction in excluded volume we observed suggests an increase in gel hydration, rather than simply an accumulation of alveolar fluid. As an added precaution, we used extravascular volume expansions within a range thought to produce interstitial edema, rather than alveolar flooding.

We used the extravascular \(^{99m}\text{Tc-DTPA}\) space as an indicator of interstitial fluid volume. The mean "interstitial" fraction of extravascular fluid volume was 0.56 for \(n = 16\) during baseline conditions (range, 0.38–0.60). In general, a sucrose space measures approximately 50% of the total extravascular water in the dog lungs, whereas sodium and chloride spaces range between 0.42 and 0.69% of the total extravascular water (Staub, 1974). A simultaneous sucrose space measured 64% of the chloride space in normally hydrated sheep lungs. This indicates some leakage of chloride into the cells and an overestimation of the true interstitial fluid volume by the chloride space (Sellinger et al., 1975). In addition, sodium ions may also bind to the polyanionic hyaluronic acid of the interstitial matrix (Granger et al., 1975). The \(^{99m}\text{Tc-DTPA}\) complex closely resembles the \(^{51}\text{Cr-EDTA}\) complex in molecular weight and chemical structure. To the extent that these compounds are similar, the diffusive properties and capillary permeability of \(^{99m}\text{Tc-DTPA}\) should be similar to those of sucrose (Trap-Jensen and Lassen, 1970). \(^{99m}\text{Tc-DTPA}\) should permeate throughout the interstitial fluid volume because sucrose is not significantly excluded from the gel matrix (Granger et al., 1975). However, the interstitial fractions calculated from the \(^{99m}\text{Tc-DTPA}\) spaces were larger than those obtained using sucrose spaces for dog lungs of 0.47, \(n = 5\) (Snashall et al., 1977), and in sheep lungs of 0.36, \(n = 7\) (Vaughan et al., 1971). The following factors may have contributed to these larger volumes. First, the most likely explanation is simply differences in initial lung hydration between experimental animals, since the interstitial hydration is the result of the total balance of filtration forces across the capillary (Guyton et al., 1971; Staub, 1974). Second, the small cationic charge of the \(^{99m}\text{Tc-DTPA}\) complex may produce some binding interaction with the anionic hyaluronic acid of the interstitium. This charge accounts for some 2% binding of \(^{99m}\text{Tc-DTPA}\) to plasma proteins. Third, we used small parenchymal samples from the periphery of the lung to calculate the fluid volumes, compared to homogenized whole lungs used in the other studies. These lung homogenates include larger bronchi and blood vessels, which could contribute significantly to the BF DW of the lung and lead to lower estimates of fluid volumes when referred to BF DW.

Another possible source of error in calculating the interstitial volume and excluded volume fraction of the tissue samples was the assumption that blood in the peripheral regions of lung has the same hematocrit as has the mixed venous blood sampled from the right atrium. The well-known effects of plasma skimming, axial streaming of red cells, and surface area-to-volume ratio have been shown to produce a lower hematocrit in small vessels compared to that found in the large veins (Barbee and Cokelet, 1971). Rappaport et al. (1956) reported a 5% lower hematocrit in pulmonary vessels than that found in large peripheral vessels. Brigham et al. (1975) demonstrated an extra plasma volume in the lung using plasma and red cell transit times. This suggests a pulmonary small vessel hematocrit only 93% of that of the large vessels. If the hematocrit of lung blood was in fact lower than the mixed venous hematocrit, the excluded volumes for albumin would be underestimated. However, when we assumed a lung hematocrit of 95% of that measured in the mixed venous blood, the excluded volumes calculated for albumin in the normally hydrated dog lungs were only 2% greater than those calculated without hematocrit correction. Therefore, hematocrit differences within a probable range could account for only a small underestimation of the true excluded volume.

Our control values for albumin exclusion in dog lung may be extrapolated to in vitro exclusion data obtained from hyaluronic acid and collagen mixtures in order to estimate an "equivalent matrix concentration" of polysaccharides. Using data presented by Comper and Laurent (1978), we estimated an interstitial hyaluronic acid concentration of approximately 0.5 g/dl for normally hydrated dog lungs and a concentration of 0.6 g/dl for normal sheep lungs. This is a reasonable concentration for tissue hyaluronic acid, and the decrease in excluded volume fraction observed in the dog lungs at maximum hydration was only slightly greater than
would be predicted for a comparable dilution of a 0.5 g/dl hyaluronic acid solution.

Role of Excluded Volume in Transcapillary Fluid Exchange

It is evident from these data that a change in excluded volume contributes significantly to the decrease in interstitial protein concentration. The change in excluded volume served to amplify the effects of simple dilution, and tissue oncotic pressure was reduced without significant transport of protein from the interstitial spaces.

Even though the transcapillary protein concentration gradient remained relatively constant, the colloid osmotic pressure gradient for fluid absorption by the capillaries actually decreased following each infusion. The decreased concentration of tissue protein was not sufficient to prevent the redistribution of the excess intravascular fluid into the tissue spaces. Therefore, this osmotic buffering mechanism was less effective than that observed during conditions of elevated pulmonary vascular pressures, where successively higher capillary pressures increase the net colloid osmotic pressure gradient between lymph and plasma. Erdmann et al. (1975) estimated that the increase in this colloid osmotic pressure gradient was sufficient to compensate for some 50% of an increase in capillary filtration pressure in unanesthetized sheep. However, Drake (1975) estimated that this increased gradient compensated for only 33% of the vascular pressure increase in isolated dog lungs. Therefore, infusions of crystalloid solutions tended to reduce the transcapillary colloid osmotic pressure gradient even though the $C_i/C_p$ ratios were reduced. This can be attributed to the curvilinear relationship of colloid osmotic pressure to plasma protein concentration (Navar and Navar, 1977). Therefore, infusion of physiological crystalloid solutions for the treatment of pulmonary edema necessarily must reduce the net colloid osmotic pressure gradient for fluid absorption (Marty, 1974) and would lead to greater transcapillary filtration rates for any given increase in capillary hydrostatic pressure (Granger et al., 1978).

Response of Pulmonary Lymph Flow to an Increased Extravascular Fluid Volume

The failure of lymph flow rate to increase, except following the 5% BW infusion, was unexpected. The high lymph flows obtained during the volume infusions (Fig. 1) indicate a lymph flow capacity much greater than that of the flow rates obtained during the steady state periods. This apparent dissociation between pulmonary interstitial fluid volume and lymph flow rate has been observed by other investigators (Gee and Siegman, 1978). The interstitial fluid volume is not in itself a determinant of the net filtration pressure, but may affect filtration only indirectly by changing the interstitial fluid hydrostatic pressure (Guyton et al., 1971). Interstitial fluid pressure changes are related to volume changes by the tissue compliance, or $C = \Delta V_t/\Delta P_t$ (Guyton, 1965). An interstitial compliance of from 2 to 2.5 ml/mm Hg per 100 g wet weight has been calculated for isolated lungs (Drake, 1975), whereas estimates in intact dogs' lungs (Parker et al., 1978) and isolated lobes (Goldberg, 1978) suggest a compliance of approximately two-thirds of this value. Moreover, compliance may increase by some 10-fold at interstitial fluid pressures several mm Hg above the control value (Parker et al., 1978; Taylor and Drake, 1978). This type of nonlinear tissue compliance has been reported for Wharton's jelly (Granger et al., 1975) and subcutaneous tissue (Guyton, 1965). The higher interstitial compliance observed in lung at increased interstitial fluid pressures may be a characteristic of the interstitial space itself, or may simply indicate alveolar flooding. Certainly the alveolar spaces would constitute a tremendous volume sink for free interstitial fluid when compared to the interstitial fluid volume.

The increased lymph flow observed after the 5% BW infusion suggests a relatively low tissue compliance; that is, the initial increase in interstitial volume produced a significant increase in the interstitial fluid pressure driving fluid into the lymphatics (Taylor et al., 1973). Subsequent increases in interstitial volume did not further increase interstitial fluid pressure and lymph flow by a comparable amount. A decrease in fluid driving pressure could be caused by rupture of the alveolar membranes and bulk flow of fluid into the alveolar spaces (Egan et al., 1977), such as occurs in the intestine at the onset of filtration secretion following saline infusions (Granger et al., 1977). In the present study, the maximum interstitial fluid volumes were approximately those associated with the onset of alveolar flooding (Staub et al., 1967).

Other possible mechanisms for a reduced lymph flow at high interstitial fluid volumes are as follows: (1) Sequestration of edema fluid at a site remote from the terminal lymphatics could occur within perivascular and peribronchial cuffs (Staub et al., 1967), or within the alveolar air spaces (Staub, 1974). (2) Accumulations of fluid may also inhibit lymph flow by extramural compression of larger lymphatic vessels (Permutt and Riley, 1963), but such a mechanism has not been demonstrated. (3) Deterioration of the experimental preparation is also a possibility. The mean systemic arterial pressure was significantly decreased following the last volume infusion, although pulmonary artery and left atrial pressures were not significantly different from control. However, a reduced vascular surface area due to reduced perfusion of the lung would result in a reduced lymph flow even at normal pulmonary vascular pressures. Thus, the rate of pulmonary lymph flow was determined by several interrelated driving pressures and was not a simple function of the interstitial fluid volume.
In summary, a decrease in the excluded volume (together with simple dilution) was a significant factor in reducing the effective concentration of tissue albumin following expansion of the pulmonary interstitial fluid volume. However, neither a reduced interstitial protein concentration nor other tissue safety factors prevented interstitial edema in these studies. Although a considerable amount of interstitial fluid was removed by the increased rate of lymph flow, the lymph flow rate was not maintained sufficiently high to prevent accumulation of excess interstitial fluid, but instead was determined by the factors that affect the net filtration pressure.

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