Effects of Dextran 70 on Hemodynamics and Lung Liquid and Protein Exchange in Awake Sheep

M. Arakawa, E.H. Jerome, K. Enzan, M. Grady, and N.C. Staub

We studied the effect of intravenous dextran 70 infusion on lung liquid and protein exchange to determine whether its effects were due to altered hemodynamics or to altered microvascular permeability. In each of six instrumented awake sheep with chronic lung lymph fistulas, we performed three experiments: 1) control, 2) a 30-minute infusion of 1 l of 6% dextran 70, and 3) an infusion of 1 l of 0.9% NaCl. In addition to pulmonary hemodynamics and lymph dynamics, we measured the plasma-to-lung lymph equilibration rate of [125I]albumin. We followed all the sheep for 10 hours, including a 2-hour baseline period. Dextran was more effective in expanding plasma volume (63±15% [mean±SD]) than saline (11±6%) at the end of the 30-minute infusion. Pulmonary vascular pressures increased after dextran and remained elevated for 8 hours, whereas after saline the pressures returned to baseline within 1 hour. After dextran, lung lymph flow increased and remained elevated. It was only transiently increased after saline. We confirmed that dextran equilibrated rapidly with lung lymph (half-time, <0.6 hour), even though it maintained plasma volume expansion for the whole body (half-time, 11.1±2.7 hours). The dextran increased both plasma and lymph total macromolecular osmotic pressure but did not increase the plasma-interstitial (lymph) osmotic pressure difference in the lung, except transiently during the infusion. The lymph/plasma protein concentration ratio increased after dextran due mainly to plasma protein dilution. There were no differences in the half-time of tracer albumin equilibration between plasma and lung lymph (control, 2.2±0.6 hours; saline, 2.0±0.6 hours; dextran, 2.3±0.6 hours). Dextran 70 increased liquid filtration mainly by increasing microvascular pressure and possibly filtration surface area. There was no evidence for a change in the leakiness of the lung microvascular barrier to albumin. (Circulation Research 1990;67:852–861)

Transvascular liquid balance in the lung is governed by several factors as described by the Starling equation.1 One important factor is the normal protein osmotic pressure difference across the microvascular endothelial barrier. During resuscitation from hemorrhagic, traumatic, or cardiogenic shock, it may be useful to infuse macromolecular substances to increase or maintain plasma volume and cardiac output.2–4 The increased plasma macromolecular osmotic pressure may also decrease liquid filtration into the lung. Likewise, prompt treatment of shock with these agents may be effective in preventing the development of Adult Respiratory Distress Syndrome. Among macromolecular substances, dextran 70 is widely used to expand plasma volume.5,6 Kramer et al5 compared resuscitation by lactated Ringer’s solution with that by dextran 70, after third degree burns in sheep. Their goal was to restore and maintain central venous pressure and pulmonary wedge pressure to baseline levels. Dextran 70 caused a 50% decrease in lung lymph flow, and its lymph concentration remained less than 0.5 of that in plasma for up to 12 hours. Although the lymph-to-plasma total protein concentration after dextran increased, Kramer et al concluded that the initial lung results could be explained by dehydration of the lung due to the high plasma macromolecular osmotic pressure. No change in lung microvascular permeability was required. Toyofuku et al,7 however, concluded from their studies in sheep that there may be an increase in lung microvascular permeability after dextran 40 infusion, although recruitment of micro-

From the Cardiovascular Research Institute and Departments of Physiology and Anesthesia, University of California at San Francisco, San Francisco, Calif.
Supported in part by program project grant HL-25816. M.A. was supported in part by a grant from the Japanese Ministry of Education.
Address for correspondence: Michio Arakawa, MD, Second Department of Internal Medicine, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500, Japan.
Received February 6, 1990; accepted May 21, 1990.
vascular surface area was probably more important. Rutili et al\textsuperscript{8} used dextran 70 in moderate doses (15 ml/kg) in anesthetized dogs whose lungs had been injured by \(\alpha\)-naphthylthiourea. When they maintained a constant left atrial pressure, lung lymph flow did not rise during the first hour, but if the left atrial pressure was allowed to rise as a result of plasma volume expansion, lymph flow increased to high levels within 30 minutes.

Albumin (66 kDa)\textsuperscript{9} and transferrin (78 kDa)\textsuperscript{10} equilibrate between plasma and lung lymph in normal sheep with a half-time of 2–3 hours, whereas for the body as a whole, the plasma escape rate of labeled albumin is much slower (half-time, 10–12 hours).\textsuperscript{11,12} Since infusion of dextran 70 increases plasma macromolecular osmotic pressure and pulmonary microvascular pressure, the net effect of dextran 70 on normal lung liquid and protein exchange is not known. In chronically instrumented sheep, we compared a 1:1 infusion of dextran 70 with a 1:1 infusion of saline. We compared the volume infusions in terms of their effectiveness for altering plasma volume, pulmonary hemodynamics, and lymph dynamics. We asked the following questions: What are the relative effects of saline versus dextran 70 on plasma volume, pulmonary microvascular pressure, and lung lymph flow? What is the net effect of saline or dextran on the macromolecular osmotic pressure difference across the microvascular barrier in the lung? What are the rates of dextran and of tracer protein equilibration between plasma and lung lymph in sheep?

Our results in unanesthetized sheep confirm that dextran is much more effective in expanding plasma volume than is saline. But dextran 70 equilibrates rapidly with lung perimicrovascular interstitial liquid (lung lymph), does not effectively increase the plasma-to-lung lymph macromolecular osmotic pressure difference, and, thus, acts to increase net liquid filtration by raising lung microvascular pressure. Further, dextran 70 had no effect on the equilibration rate of tracer albumin between plasma and lung lymph.

**Materials and Methods**

**Animal Preparation**

We used six sheep weighing 38.5±3.5 kg (mean±SD) and prepared them for hemodynamic and lung lymph dynamic studies according to standard procedures developed in our laboratory and extensively described elsewhere.\textsuperscript{13,14} Briefly, in the halothane-anesthetized, tracheally-intubated, ventilated animal we made a sterile left thoracotomy and placed polyvinyl catheters in the main pulmonary artery and left atrium. We attached a 3.5F thermistor (Baxter Healthcare Corp., Deerfield, Ill.) to the pulmonary artery catheter. We also placed an ultrasonic flow probe cuff (Parks Medical Electronics, Aloha, Ore.) around the pulmonary artery, resected the tail of the caudal mediastinal lymph node, and cauterized the left surface of the esophagus and diaphragm to remove extrapulmonary lymphatics. A week later, we reanesthetized the animal, made a right thoracotomy, and cannulated the efferent duct of the caudal mediastinal node with fine polyvinyl tubing impregnated with heparin by the triodecy methyl ammonium chloride process. We also cauterized across the right side of the esophagus and diaphragm. In addition, we placed polyvinyl catheters into an external jugular vein for the volume infusions and into the carotid artery for blood sampling and pressure measurements.

We allowed 4–6 days for recovery, after which we did experiments with the sheep awake in its mobile metabolism cage with free access to food and water. Average body temperature at the time of our experiments was 39.3±0.3°C, \(\text{PaO}_2\) was 85.8±8.2 mm Hg, and \(\text{PaCO}_2\) was 37.3±3.4 mm Hg.

**Experimental Protocol**

Three studies were done in each sheep. After a 2-hour baseline period, the appropriate intervention was made (see below), and the animal was followed for 8–10 hours. Experiments were done 2–3 days apart, so that the animal had time to recover fully between experiments.

The first experiment was always a control (no liquid infusion). The second and third experiments were alternated between 1 l sterile 6% dextran 70 in 0.9% sodium chloride solution (Macrodex, Pharmacia Inc., Piscataway, N.J.; Gentrans-70, Baxter Healthcare) and 1 l sterile 0.9% sodium chloride solution. The liquid infusions were made intravenously over 30 minutes. Dextran 70 is a long chain neutral polymer of glucose with relatively little branching. The 6% solution we used is supplied commercially in saline at pH 5.0. Although the average molecular weight is 70 kD, 90% of the particles lie between 20 and 115 kD.

We measured all hemodynamic and lymph dynamic variables every 15 minutes for 10–12 hours, including a 2-hour baseline, 30-minute infusion, and 8–10 hour follow-up. Vascular pressures were measured using disposable pressure gauges (Medex, Inc., Hilliard, Ohio) attached to the sheep's vest and zeroed relative to atmospheric pressure at the most dependent part of the chest. The transducer outputs were recorded on a direct writing polygraph (model 7, Grass Instrument Co., Quincy, Mass.).

**Measurements**

Lymph was collected every 15 minutes, measured by volume, and pooled every 30 minutes for measurements of total protein, albumin fraction, dextran concentration, or osmotic pressure. Likewise, blood samples were taken every half hour during and up to 4 hours after the infusion and every hour thereafter for similar measurements. Total protein and albumin were measured on an automated analyzer (model A1H, Miles Technicon Instrument Co., New York).

There is a positive error in the standard biuret total protein analysis due to turbidity caused by
precipitation of dextran. Turbidity can be largely avoided by using the modifications of Flack and Woollen. Up to a concentration of 3 g/dl, which exceeded the maximum concentration we found in blood or lymph, dextran 70 caused less than 5% interference with the measurement of total protein concentration. Albumin was assayed by the bromcresol green method. It was not affected by dextran 70.

For measurement of macromolecular osmotic pressure, we used a commercial osmometer (model 4100, Wescor Inc., Logan, Utah) with a 20,000 molecular weight cutoff membrane (type C, Nucleopore Corp., Pleasanton, Calif.). We calibrated the osmometer with a water manometer and with a pooled sheep plasma protein standard. The osmotic pressure of 6% dextran 70 in our osmometer averaged 78 cm H₂O at 39º C, which is similar to that reported by Haraldson et al.

The plasma and lymph concentrations of dextran were determined in duplicate by the anthrone method. All samples were measured simultaneously after the end of an experiment. In the anthrone reaction, it is important to control the time of color development before measuring reactant absorbance. Therefore, as soon as the reaction was complete at 100º C, all tests were moved to a room temperature water bath and then read immediately.

**Tracer Protein Equilibration**

To measure the rate of equilibration between plasma and lung lymph for [¹²⁵I]human serum albumin, we followed the procedure of Vaughan et al. We injected 8–15 µCi of [¹²⁵I]albumin intravenously, increasing the dose in successive experiments because of the residual background radioactivity. We collected lymph every 15 or 30 minutes and blood every 30 or 60 minutes up to 8 hours.

We measured weighed samples (approximately 0.5 g) of plasma and lymph for radioactivity in a well-type gamma spectrometer (Autogamma 3002, Hewlett-Packard Co., Downers Grove, Ill.). Radioactivity was recorded as total counts/(minutes×gram sample) and also as specific activity [counts/(minutes×gram albumin)]. From the accumulated data, the half-time for the plasma-to-lymph equilibration was determined by computer, using a least-squares method for fitting an exponential function. In addition, we used the plasma radioactivity during the first 30 minutes after each injection of isotope to calculate circulating plasma volume. The plasma dilution immediately after the saline and dextran 70 infusions was also calculated from the change in the total protein concentration in all sheep.

**Statistics**

The data are shown in the tables as the group mean±1 sample SD. Within groups, we used a two-way analysis of variance (animal, time), and for statistical differences we compared selected time periods with the baseline by a paired t test on the individual animal data, allowing for repeated measurement of the same variable. For the half-time of albumin equilibration, we used a one-way analysis of variance (experiment) to compare the plasma escape rates or the plasma-to-lymph equilibration rates. We applied the Bonferroni correction for multiple comparisons of the same data.

**Results**

**Control Experiment**

The group data are summarized in Table 1, and selected variables are graphed in Figures 1–4 (open diamonds). One of the outstanding features of the unanesthetized sheep is its stability. There were no significant changes during the 10-hour experiment. In addition, arterial blood gases were stable. The mean PaO₂, PaCO₂, and pH for the 2-hour baseline and last hour were 85.3±5.9 and 84.3±10.9 mm Hg, 36.4±3.0 and 36.9±3.7 mm Hg, and 7.50±0.04 and 7.50±0.02, respectively.

**Saline Experiment**

The data are summarized in Table 2, and selected variables are plotted in Figures 1–4 (solid triangles). The infusion had little effect on plasma volume (Figure 1). Lung lymph flow tended to increase in the
first hour (Figure 2) but was within normal limits thereafter. The lymph/plasma total protein concentration ratio (Figure 3) tended to decrease (but not significantly). Finally, the macromolecular osmotic pressure difference (Figure 4) was not significantly affected except for a small transient fall during the infusion, due principally to the decrease in the lymph protein concentration, as shown in Table 2.

Dextran Experiment

The data are summarized in Table 3, which has the same format as the other tables, with the addition of the dextran concentration in plasma and lymph. Selected variables are plotted in Figures 1–4 (solid squares). Although the volume and rate of the dextran 70 infusion were the same as for saline, the effects were greater and longer lasting. Pulmonary vascular pressures were significantly increased for 2 hours and tended to remain elevated throughout the experiment. Figure 1 shows that plasma volume was increased by 60 ± 16% at 1 hour after the dextran infusion, as measured by protein dilution, and remained significantly increased by about 30% at 8 hours. Lymph flow increased during the first 2 hours after the infusion and tended to remain elevated throughout the experiment.

The lymph/plasma total protein concentration ratio, as shown in Figure 3, rose during the infusion, mainly because of the dilution of the plasma proteins.

**Figure 1.** Plot showing time course of relative plasma volume changes summarized for all sheep for each experiment. Symbols are group mean ±1 SD. Open diamonds are control experiment, solid triangles are 1-1 saline infusion, and solid squares are 1-1 dextran infusion. *Statistically significant at p < 0.05; **statistically significant at p < 0.01.

**Figure 2.** Plot showing time course of lung lymph flow (ml/15 min). All baseline data are averaged over 2 hours; otherwise, for interval indicated. Symbols are group mean ±1 SD. Open diamonds are control experiment, solid triangles are 1-1 saline infusion, and solid squares are 1-1 dextran infusion. *Statistically significant at p < 0.05; **statistically significant at p < 0.01.

**Figure 3.** Plot showing time course of lymph/plasma total protein concentration ratios. Baseline data are mean for 2 hours; otherwise, for interval indicated. Symbols are group mean ±1 SD. Open diamonds are control experiment, solid triangles are 1-1 saline infusion, and solid squares are 1-1 dextran infusion. *Statistically significant at p < 0.05; **statistically significant at p < 0.01.

**Figure 4.** Plot showing macromolecular osmotic pressure difference (plasma minus lymph). All baseline data are averaged for 2 hours; otherwise, for interval indicated. Symbols are group mean ±1 SD. Open diamonds are control experiment, solid triangles are 1-1 saline infusion, and solid squares are 1-1 dextran infusion. *Statistically significant at p < 0.05; **statistically significant at p < 0.01.
The ratio decreased exponentially with a half-time of 2.25 hours but remained elevated throughout the experiment. The lymph total protein reached its minimum concentration at 2 hours and remained constant thereafter; the plasma protein concentration increased slowly, as shown in Table 3. The plasma dextran concentration averaged 2.4 g/dl at the end of the infusion and then declined slowly. The lymph dextran concentration increased rapidly, achieving a peak at 1 hour.

The total macromolecular osmotic pressure difference (protein plus dextran) across the lung (Table 3, row 3 minus row 4) remained essentially constant, except for a large transient rise during the 30-minute infusion, as shown in Figure 4.

**Escape of Dextran and Tracer Albumin From Plasma**

The rate of escape of dextran 70 from plasma, based on the data in Table 3, has a half-time of 11.1±2.7 hours, which is about the same as that for albumin. On the other hand, the concentration of dextran in caudal mediastinal node efferent lymph (Table 3) indicates rapid equilibration (<1 hour) with the lung perimicrovascular interstitial liquid, as represented by caudal mediastinal node efferent lymph.

Since the total protein concentration in lung lymph also remained high relative to plasma (Figure 3), a small increase in microvascular protein leakiness may have occurred. To test this, we measured the plasma-to-lymph equilibration of [125I]albumin in every experiment. The albumin specific activity of plasma, lymph, and the plasma-to-lymph difference in one sheep is plotted in Figure 5. The data for all six sheep showing the half-times of plasma escape and the plasma-to-lymph equilibration are summarized in Table 4. The escape rate from plasma tended to be faster after the volume expansion, especially with the dextran, but by analysis of variance, none of these results are statistically significant. We believe the trend toward a faster escape rate is due to a rise in plasma volume and microvascular pressure rather than any effect on systemic microvascular protein leakiness.

The more interesting feature of Figure 5 and Table 4 is that the half-time of labeled albumin equilibration between plasma and lung lymph was not affected by dextran. This is a sensitive test for measuring microvascular protein leakiness. In other words, the

**Table 2. Time Course of Main Variables After 1-l Saline Infusion in Six Sheep**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>End of infusion</th>
<th>Time elapsed after start of infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr</td>
</tr>
<tr>
<td>Ppa (cm H2O)</td>
<td>32.5±7.0</td>
<td>35.2±6.8</td>
<td>34.1±7.2</td>
</tr>
<tr>
<td>Pla (cm H2O)</td>
<td>11.2±2.4</td>
<td>16.4±3.4*</td>
<td>12.0±2.7</td>
</tr>
<tr>
<td>τp (cm H2O)</td>
<td>24.9±1.4</td>
<td>21.0±1.7*</td>
<td>22.8±1.6*</td>
</tr>
<tr>
<td>τl (cm H2O)</td>
<td>17.6±1.6</td>
<td>14.8±1.9</td>
<td>13.2±1.4*</td>
</tr>
<tr>
<td>L (ml/15 min)</td>
<td>2.1±0.6</td>
<td>3.1±0.8</td>
<td>4.8±1.2*</td>
</tr>
<tr>
<td>(TP)p (g/dl)</td>
<td>5.5±0.5</td>
<td>4.9±0.6*</td>
<td>5.2±0.5*</td>
</tr>
<tr>
<td>(TP)l (g/dl)</td>
<td>3.9±0.4</td>
<td>3.6±0.3</td>
<td>3.1±0.3*</td>
</tr>
</tbody>
</table>

Values are group mean±1 SD. Baseline was an average period of 2 hours; infusion period was 30 minutes. Ppa and Pla, mean pulmonary arterial and left atrial pressure, respectively; τp and τl, macromolecular osmotic pressure in plasma and lymph, respectively; L, lymph flow from caudal mediastinal node efferent duct; (TP)p and (TP)l, total protein concentration in plasma and lymph, respectively; (D)p and (D)l, total dextran concentration in plasma and lymph, respectively.

*Significant by analysis of variance and paired t test with respect to baseline at p<0.05.

**Table 3. Time Course of Main Variables after 1-l Dextran 70 Infusion in Six Sheep**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>End of infusion</th>
<th>Time elapsed after start of infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr</td>
</tr>
<tr>
<td>Ppa (cm H2O)</td>
<td>31.4±4.7</td>
<td>44.6±7.7*</td>
<td>41.0±6.8*</td>
</tr>
<tr>
<td>Pla (cm H2O)</td>
<td>11.1±2.5</td>
<td>28.8±6.8*</td>
<td>24.6±4.2*</td>
</tr>
<tr>
<td>τp (cm H2O)</td>
<td>24.3±2.1</td>
<td>35.6±2.5*</td>
<td>32.6±2.3*</td>
</tr>
<tr>
<td>τl (cm H2O)</td>
<td>16.8±2.3</td>
<td>19.0±2.1*</td>
<td>22.8±1.4*</td>
</tr>
<tr>
<td>L (ml/15 min)</td>
<td>2.4±0.9</td>
<td>3.3±1.1</td>
<td>6.3±1.6*</td>
</tr>
<tr>
<td>(TP)p (g/dl)</td>
<td>5.5±0.5</td>
<td>3.4±0.6*</td>
<td>3.4±0.6*</td>
</tr>
<tr>
<td>(TP)l (g/dl)</td>
<td>3.8±0.6</td>
<td>3.8±0.5</td>
<td>3.5±0.5</td>
</tr>
<tr>
<td>(D)p (g/dl)</td>
<td>0</td>
<td>2.5±0.2</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>(D)l (g/dl)</td>
<td>0</td>
<td>0.4±0.3</td>
<td>1.3±0.2</td>
</tr>
</tbody>
</table>

Values are group mean±1 SD. Baseline was an average period of 2 hours; infusion period was 30 minutes. Ppa and Pla, mean pulmonary arterial and left atrial pressure, respectively; τp and τl, macromolecular osmotic pressure in plasma and lymph, respectively; L, lymph flow from caudal mediastinal node efferent duct; (TP)p and (TP)l, total protein concentration in plasma and lymph, respectively; (D)p and (D)l, total dextran concentration in plasma and lymph, respectively.

*Significant by analysis of variance and paired t test with respect to baseline at p<0.05.
FIGURE 5. Plots showing examples of plasma escape and plasma-to-lymph equilibration of tracer albumin for three experiments in one sheep. Top panel: Control. Middle panel: One-liter saline infusion. Bottom panel: One-liter dextran 70 infusion. The y axis is a logarithmic scale of specific activity of \( ^{125}I \) albumin \( \text{counts/(min x g albumin)} \). The plasma escape rate is slightly faster in successive panels, due, we believe, to the elevation of plasma volume (increased systemic microvascular pressure or surface area). The plasma-lymph line was generated by subtracting the lung specific activity from the plasma specific activity at each time. The correlation coefficients for the least-squares regression lines were \(-0.988\) for panel A, \(-0.978\) for panel B, and \(-0.964\) for panel C. The x axis represents hours after the tracer albumin was injected intravenously, which was 1 hour after the beginning of the volume infusion.

higher than expected protein concentration of lung lymph and the rapid equilibration of dextran in lymph must be explained on a hemodynamic basis.

Discussion

General

The results confirm the effectiveness of dextran 70 and the ineffectiveness of saline as a plasma expander. For example, the plasma volume of a sheep is approximately 5% body weight,\(^{19}\) that is, 1.9 l for our 38.5 kg sheep. At the end of 1 l dextran 70 infusion, we calculated that the plasma volume increased by 63% based on protein dilution. Even after 8–10 hours, the plasma volume was still elevated by 30%. The half-time for plasma dextran escape was about 11 hours (Figure 1), which corresponds to the slow decline in plasma volume and the slow recovery of the plasma protein concentration toward baseline level.

The saline infusion decreased plasma protein concentration by 11% at the end of the infusion. This, of course, is readily explained by the rapid redistribution of saline, according to the normal plasma/interstitial volume ratio (1:3). These results for dextran 70 and saline are in agreement with calculations of the distribution of extracellular volume in other published reports.\(^{20-22}\)

Since the saline infusion did not raise pulmonary vascular pressures, it had only a minimal effect on lung lymph flow (Figure 2) and that only during the first 2 hours. This result is consistent with the experiments of GEE and SPATH,\(^{23}\) who rapidly infused large volumes of saline into dogs and showed rapid recovery of right duct lymph flow toward normal thereafter.

There was a large increase in pulmonary arterial and left atrial pressures associated with the dextran 70 infusion. Using the standard formula for calculating pulmonary microvascular pressure under zone 3 conditions in the sheep,\(^{24,25}\) we estimate that the mean pulmonary microvascular pressure increased from 19 cm H\(_2\)O (relative to the bottom of the lung) in the baseline period to 31 cm H\(_2\)O at 1 hour and was still slightly above the baseline microvascular pressure at 10 hours. Thus, dextran 70 is an effective plasma expander, but by being so, it increases microvascular pressure, which is the main driving force for filtration.\(^{26}\) Indeed, the time course of the lung lymph flow increase in the sheep after dextran 70 (Figure 2) reflects almost exactly the time course of the elevated pulmonary microvascular pressure. It is also probable that, due to the increased pressures, more microvascular filtration surface area was recruited, but we have no specific data on this point.

Plasma-to-Lymph Dextran and Tracer Albumin Equilibration

The equilibration of the neutral dextran 70 with lung lymph was exceedingly rapid with a half-time of about 24 minutes (lower line in Figure 6). This is

<table>
<thead>
<tr>
<th>Half-times</th>
<th>Control</th>
<th>Dextran</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (hr)</td>
<td>12.0±1.1</td>
<td>8.3±1.7</td>
<td>10.4±1.1</td>
</tr>
<tr>
<td>Plasma-to-lymph equilibration (hr)</td>
<td>2.2±0.6</td>
<td>2.3±0.6</td>
<td>2.0±0.6</td>
</tr>
</tbody>
</table>

Values are mean±SD.
much faster than the plasma-to-lymph equilibration rate for protein molecules of about the same mean molecular weight and size as dextran, such as albumin or transferrin. Since the whole body plasma escape rate of dextran 70 is the same as for albumin, dextran is behaving in the pulmonary microvascular bed in a manner different from its behavior in the systemic vascular bed, which is dominated by the skeletal muscle mass. This difference contrasts with the general view of electron microscopists that histologically the lung microvascular endothelial barrier is similar to that of skeletal muscle.

Since dextran behaves in the kidney glomerulus differently from comparably sized plasma proteins, the effect may be charge-related but may also be related to the long flexible linear nature of dextran 70, which may allow it to behave as a smaller particle during filtration.

Pietra et al. and Glauser et al. found that neutral dextran in tracer quantities equilibrated much more slowly with lung lymph than did anionic (negatively charged) dextran. Parker et al. made an extensive study of seemingly contradictory data on equilibration of lactate dehydrogenase isozymes between plasma and afferent lung lymph in the anesthetized dog. They reported that the lymph-to-plasma lactate dehydrogenase concentration ratios of two isozymes were equal at 24 hours but the more anionic (more negatively charged) lactate dehydrogenase isozyme equilibrated with lymph faster and initially had a greater lymph protein concentration, whereas the less negatively charged lactate dehydrogenase isozyme escaped faster into lung tissue but its appearance in lymph was delayed by a larger distribution volume (smaller excluded volume). Thus, one must consider not only the microvascular barrier leakiness but also the interstitial available volume. The latter is important for tracer quantities but less so for bulk quantities, which ought to overwhelm the interstitial delaying effects, just as a gel filtration column separates small quantities of material but fails with large quantities.

Commercial dextran 70 is a neutral molecule and therefore should equilibrate with lung lymph slowly. Ultimately, its steady-state lymph/plasma protein concentration ratio is approximately the same as that of the albumin. However, we found a rapid rise in dextran concentration in lymph reaching 0.76 of the plasma concentration by 2 hours. Haraldson et al. suggested that the loosely coiled dextran 70 moved more rapidly than predicted from its molecular weight, due to its ability to wriggle through channels by the process known as reptation. This phenomenon is known to occur for very large linear molecules, such as DNA, in gel filtration columns, and the increased microvascular hydrostatic pressure in the pulmonary circulation may well be the explanation for the rapid plasma-to-lymph transport we found. This seems to be a reasonable explanation for our findings for the rapid rise in the lymph-to-plasma dextran concentration when the microvascular pressure was elevated, compared with the slow rise reported when pressure was not elevated.

The equilibrium lung lymph/plasma ratios for dextran 70 and albumin are approximately 0.76 and 0.80, respectively, which suggests that, when hydrostatic pressure is not elevated, both molecules are sieved by molecular size almost equally. Due to the rapid escape of dextran into the lung, we must conclude that dextran 70 has only transient effectiveness as an osmotic agent in the pulmonary microvascular bed.

Another important point concerning plasma-to-lymph equilibration is that the microvascular surface area/interstitial volume ratio in the lung is much larger than that for any other organ, and therefore, the washout of the lung's interstitium ought to proceed more rapidly than for skeletal muscle. That is why plasma proteins equilibrate more rapidly in the lung than in the whole body (see Table 4, Control). Toyofuku et al. thought that increased microvascular surface area largely explained the more rapid equilibration of dextran 40 in their experiments, but that does not explain the difference between dextran and tracer albumin, because the exchange surface area/interstitial volume ratio should have been the same for both, unless the lung interstitial excluded volume for dextran is much different from that for albumin. We did not measure lung interstitial distribution volume for dextran or albumin in these experiments because that requires removing the lungs for analysis.

The equilibration of [125I]albumin is shown in Figure 5 and in Table 4. There was no significant effect of the dextran or saline infusions on either systemic or pulmonary microvascular protein leakiness. Thus, we confirm the conclusion of Kramer et al. that dextran 70 does not increase microvascular permeability to protein.

We conclude that, in the systemic circulation, tracer albumin and dextran 70 behave similarly in terms of microvascular leakiness. In the lung microcirculation, there is a marked difference between the equilibration rates of these molecules. Thus, molec-
ular flexibility, microvascular pressure elevation, interstitial distribution volume, or charge effects may be more significant in the pulmonary than in the systemic circulation.30-34

The Macromolecular Osmotic Pressure Difference Across the Pulmonary Microvascular Barrier

As Figure 4 shows, neither saline nor dextran had any lasting effect on the macromolecular osmotic pressure difference ($\pi_{mv} - \pi_{pmv}$). Dextran during the infusion period opposed the increased microvascular pressure, which may explain why lymph flow was not significantly increased until 30 minutes after the infusion was completed.

There was no sustained effect of saline on lung lymph flow because the quantity of saline infused did not increase lung microvascular pressure or alter the osmotic pressure difference, even though most of the saline was distributed to the interstitium. The volume of the interstitium, which we estimate to be 15% of body weight, or 5.8 l in our 38.5 kg sheep, was only expanded by 17% (1/5.8). If this expansion had increased perimicrovascular interstitial pressure, we would have expected it to increase lung lymph flow considerably. Since the lymph flow did not increase, we must conclude that the perimicrovascular pressure did not rise. There are two possible explanations: either the lung perimicrovascular interstitial distensibility is high, as suggested by the direct micro-puncture measurements of Bhattacharya et al.,35 or the expanded interstitial volume does not affect the parenchymal interstitium of the alveolar wall but only the loose, binding connective tissue of the bronchovascular cuffs and interlobular septae,36,37 which do not have ready access to the lung lymphatics.38

Dextran, on the other hand, caused a rise in microvascular pressure without a sustained increase in the transvascular macromolecular osmotic pressure difference. Therefore, filtration and lung lymph flow increased substantially, declining slowly toward baseline levels over 8-10 hours. We conclude that filtration and lymph flow are directly related to the net filtration pressure, not to overall interstitial volume.

The Extracellular Distribution of Protein

Since the saline was distributed proportionally between plasma (25%) and interstitium (75%), one might expect the plasma and interstitial protein concentrations to be diluted by the same amount. However, we must make allowance for the interstitial excluded volume for macromolecules. The lung interstitium has an excluded volume for albumin averaging about 60-70%.33,39 Suppose, on the average, only 35% of the interstitial volume was available for protein. When the saline was infused, the interstitial volume increase was not distributed equally between the excluded and available compartments but went predominantly to the available compartment. This is consistent with data in the dog lung obtained by Parker et al.,39 who found that during saline loading the fractional excluded volume decreased markedly but that the absolute excluded volume decreased only modestly, until the infusion volume was large. Thus, the dilution of lymph total protein is greater than the estimated 17% dilution of total interstitial volume. If 65% of the interstitial volume is excluded from protein dilution, we can almost exactly account for the dilution of total protein in lymph and for the modest decrease in the lymph/plasma total protein concentration ratio seen immediately after the saline infusion.

Initially, most (63%) of the infused dextran 70 volume remained in the plasma compartment. During the infusion period, the macromolecular osmotic pressure difference within the lung was nearly doubled, which opposed the rising microvascular pressure. However, the rapid leak of dextran into the perimicrovascular space, as seen 30 minutes after the infusion, eliminated this protection. The increased lung microvascular pressure increased filtration, which also decreased the perimicrovascular interstitial protein concentration according to the sieving coefficient $(1-\sigma)$, where $\sigma$ is the total protein reflection coefficient. Thus, the lymph total protein concentration decreased, as it does after any elevation of microvascular pressure.25 Since the plasma was markedly diluted by the dextran, the plasma total protein fell by 38% by the end of the infusion (Table 3). The lymph/plasma protein concentration ratio rose to a high level, exceeding unity, and remained elevated throughout the experiment because of the persistent plasma protein dilution.

Since lung lymph is derived principally from the perimicrovascular parenchymal interstitium,39 the modest fall in the lymph total protein concentration reflects sieving at the microvascular barrier without implying much dilution of the lung interstitium, which at best would have occurred slowly over the course of the experiment. Indeed, Erdmann et al.25 showed that there was minimal increase in extravascular lung water content until microvascular pressure doubled (due to the lymph flow safety factor against edema1,40). Using Figure 6 in the study of Erdmann et al.,25 we estimate that the 12 cm H$_2$O increase in microvascular pressure at 1 hour would have caused an increase in extravascular water of 10% after about 2 hours, which would adequately account for the decrease in the lymph protein concentration.

The final problem is to explain why the lymph/plasma total protein concentration ratio remained above the baseline level after the dextran infusion. Early on, it is easy enough to explain the high ratio as being due to the dilution of the plasma compartment, but the half-time of albumin and, indeed, total protein equilibration in the sheep lung is about 2-3 hours (Table 4).25 We anticipated that the lymph/plasma protein concentration ratio would recover to the baseline level in a few hours. Instead, it slowly recovered over many hours, as shown in Figure 3. We can only speculate about an explanation for this phenomenon. One possibility is that the high dextran concentration in the lung interstitium osmotically
concentrated the lymph as it flowed along collecting lymphatics in the lung\textsuperscript{41} or within the caudal mediastinal node.\textsuperscript{42}

After the dextran infusion, lymph flow was increased 162\% at 1 hour and 46\% at 10 hours. If one applies the Starling equation assuming the reflection coefficient of dextran is equal to that of albumin, namely, about 0.7,\textsuperscript{43,44} then the lymph flow increases should have been much less. On the other hand, if the effective microvascular reflection coefficient for dextran is less than that of albumin, as we surmise on the basis of the rapid plasma-to-lymph equilibration, then we can use the Starling equation to calculate it to be approximately 0.4.

**Conclusion**

Dextran 70 is an excellent plasma expander. But the 1-l volume we infused (30 ml/kg) also raised lung microvascular pressure and increased fluid filtration. Therefore, care must be taken in the use of dextran 70 as osmotic therapy, especially in patients who have injured lungs or edema. The administration of dextran in small increments could maintain an effective plasma volume, venous return, and cardiac output without increasing lung microvascular pressure significantly.

**Acknowledgments**

We thank Oscar Osorio for his surgical skill in preparing the instrumented sheep with long-term lung lymph catheters and Bernie Baccay for preparing the manuscript.

**References**


**KEY WORDS** • lung • lymph flow • microvascular filtration • tracer protein equilibration • plasma expanders • lymph protein concentration • permeability • pulmonary edema
Effects of dextran 70 on hemodynamics and lung liquid and protein exchange in awake sheep.
M Arakawa, E H Jerome, K Enzan, M Grady and N C Staub

Circ Res. 1990;67:852-861
doi: 10.1161/01.RES.67.4.852

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/67/4/852

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/