Effect of concentration on albumin diffusion in lung interstitium

XIAO L. QIU, LAURA V. BROWN, SANDHYA PARAMESWARAN, GEOFFREY S. IBBOTT, AND STEPHEN J. LAI-FOOK
Center for Biomedical Engineering and Department of Radiation Medicine, University of Kentucky, Lexington, Kentucky 40506-0070

Qiu, Xiao L., Laura V. Brown, Sandhya Parameswaran, Geoffrey S. Ibbott, and Stephen J. Lai-Fook. Effect of concentration on albumin diffusion in lung interstitium. J. Appl. Physiol. 85(2): 575–583, 1998.—The transport of macromolecules through the lung interstitium depends on both bulk transport of fluid and diffusion. In the present study, we studied the diffusion of albumin. Isolated rabbit lungs were inflated with silicon rubber via airways and blood vessels, and two chambers were bonded to the sides of a 0.5-cm-thick slab that enclosed a vessel with an interstitial cuff. One chamber was filled with either albumin solution (2 or 5 g/dl) containing tracer 125I-albumin or with tracer 125I-albumin alone; the other was filled with Ringer solution. Unbound 125I was removed from the tracer by dialysis before use. The chamber with Ringer solution was placed in the well of a NaI(Tl) scintillation detector. Diffusion of tracer through the interstitium was measured continuously for 60 h. Tracer mass (M) showed a time (t) delay followed by an increase to a steady-state flow (dM/dt constant). Albumin diffusion coefficient (D) was given by L2/(6T), where T was the time intercept of the steady-state M-t line at zero M, and L was interstitial length. Interstitial cuff thickness-to-vessel radius ratio (Thy/R) was estimated by using Fick's law for steady-state diffusion. Both D and Thy/R were independent of albumin concentration. D averaged 6.6 × 10–7 cm2/s, similar to the free D for albumin. Values of Thy/R averaged 0.047 ± 0.024 (SD), near the values measured histologically. Thus pulmonary interstitial constituents offered no restriction to the diffusion of albumin.

METHODS

The procedure used to fill isolated rabbit lungs with silicon rubber and to isolate a length of blood vessel with surrounding interstitial cuff has been described (26). New Zealand White rabbits (3- to 4-kg body weight, n = 7) were tranquillized with 130 mg ketamine and 4 mg xylazine injected intramuscularly. After heparinization (3,000 U), each animal was anesthetized and killed by an overdose of pentobarbital sodium injected through an ear vein. After the chest was opened, cannulas were tied into the trachea, pulmonary artery, and left atrial appendage. A tie was secured around the heart. The lungs and heart were left within the thorax, which was separated from the body by transection across the neck and below the diaphragm. After the lungs were degassed by vacuum, the airways and vessels were filled with silicon rubber compound (Microfil, Flow-Tek) through reservoirs connected to the artery, vein, and trachea. The artery was filled first, followed by the vein and airways. White rubber was used to fill the airways; pink and yellow rubber was used to fill the arteries and veins, respectively. The reservoirs were set to 15–20 cmH2O relative to the lung base to ensure a fully inflated lung. With the use of a catalyst (5%), the rubber solution hardened within 2 h. After the rubber had set, each caudal lobe was cut into 0.5-cm-thick slabs transverse to the main dimension of the vessel tree, starting from the hilum.
Each rabbit provided at least six slabs; four slabs were chosen for each experiment.

Figure 1 is a diagram of the experimental assembly used to measure the diffusion of the radiolabeled tracer $^{125}$I-albumin through the lung interstitium. Two chambers were bonded to the opposite faces of each slab with cyanoacrylate adhesive to enclose the largest arterial segment. The (outside) chamber containing the test albumin solution with radioactive tracer was constructed of a plastic tube (0.7-cm length, 13-mm outer diameter, and 3-mm wall thickness) bonded to one side of a plastic plate (6 × 8 × 0.3 cm) adjacent to a 0.7-cm-diameter hole in the plate. The plate was oriented to the lung slab so that the hole enclosed the arterial segment. The other (inner) chamber was constructed of a plastic tube (4.7-cm length, 7.3-mm outer diameter, and 0.22-mm wall thickness) bonded to a plate similar to that used for the outer chamber. The inner chamber was designed so that a 4-cm length of the 4.7-cm-long tube would fit into a well-type NaI(Tl) scintillation detector (model no. TB-2L, Oxford Instruments, Oak Ridge, TN). The tube passed through a 0.9-cm-diameter entry hole made in a lead cap that covered the opening of the detector. The inner chamber was bonded to the face of the arterial segment with the smaller diameter, consistent with the procedure used to measure hydraulic conductivity (26). After the two chambers were bonded to the lung slab, the entire assembly was stabilized by bolts and nuts located at the corners of the plates. The interstitium surrounding the artery was prevented from drying by applying Ringer solution to both ends of the arterial segment during the setting-up procedure.

The inner chamber was filled with 1.8 ml Ringer solution, and its end was sealed with a plastic cap. The cap had a tiny hole that prevented compression of the liquid within the chamber when the end of the chamber was covered with the cap. The hole was subsequently sealed with vacuum grease. A small magnetic bar (6.5 mm in length), used to mix the chamber liquid during the diffusion experiment, was located ~1 cm from the end of the arterial segment via a string attached to the cap. The inner chamber was then placed inside the scintillation counter. The background radioactivity from the chamber was measured for 1 min. The outer chamber was filled with 0.2 ml of a test solution of albumin that contained tracer $^{125}$I-albumin, and the chamber was sealed with a rubber cap. The rubber cap had a tiny hole which prevented compression of the liquid within the chamber during insertion. The hole was subsequently sealed with vacuum grease. To prevent radiation from the outer chamber from reaching the scintillation detector, the outer surface of the detector was covered with a 2-mm-thick lead sheet. In addition, the bottom surface of the detector well was lined with lead to prevent the radiation emitted in an axial direction by the outer chamber from reaching the detector. The detector was oriented with its cylindrical axis horizontal. A thin-walled plastic tube (3-cm length, 1-cm inner diameter) was bonded to the inner surface of the lead cap to maintain the chamber axis in a horizontal position and at a fixed orientation relative to the detector. The plastic material used to construct the two chambers and supporting plate was tested to ensure that it did not absorb the radioactive tracer.

The diffusion of the radioactive tracer through the interstitium was measured continuously in the chamber located in the counter. The solution was stirred continuously by slowly rotating the magnetic bar placed within the chamber by using an external electromagnetic source (Thermix stirrer, model 1205; Fisher Scientific, Pittsburgh, PA). The emitted radiation was measured during consecutive 30-min periods for 60 h.

We measured the diffusion of albumin through lung interstitium at different Ca. Stock solutions (4 ml) were made with albumin (bovine serum albumin, batch no. A9647; Sigma Chemical, St. Louis, MO) of the test concentration in lactated Ringer solution and ~10 µCi $^{125}$I-albumin (~0.5 µCi/0.5 µl; cat no. NEX-076, New England Nuclear, Boston, MA). All solutions were filtered (0.45-µm pore diameter) and adjusted to pH of 7.35–7.45. The outer chamber was filled with 0.2 ml of a test solution (0, 2, or 5 g/dl albumin solution) containing the radioactive tracer (~4 × 10^{-7} g/dl). Before the solution was used for the diffusion experiment, any unbound $^{125}$I was separated from the stock solution by dialyzing the 4 ml of stock solution for 24 h with the use of an SS-030 wet-pack membrane (Wescor model 4000 series, Collod Osmometers). The pore size (20,000 Da) of the membrane allowed the passage through the membrane of $^{125}$I but not $^{125}$I-albumin (66,000 Da). The 4 ml of stock solution containing the $^{125}$I-albumin were placed on one side of the membrane, with 200 ml of solution of the same Ca as the stock solution on the other side. The similar Ca on both sides of the membrane prevented passive diffusion through the membrane. The radiation emitted by the unbound $^{125}$I that passed through the membrane was measured and compared with the radiation emitted by the stock solution. For these and other calibration measurements, we used a chamber identical in volume (1.8 ml) and dimensions to the inner chamber used for the diffusion of albumin through lung interstitium. Unbound $^{125}$I was <2% of the stock solution, thus verifying the manufacturer’s specification. After dialysis for 24 h, the unbound $^{125}$I was reduced to ~0.04% of that from the stock solution. Any stock solution not used in one experiment was used in the next experiment after being dialyzed again for 24 h to remove any unbound $^{125}$I. Consequently, the unbound $^{125}$I in the diffused sample at the end of the diffusion experiment was often immeasurably small.

After the diffusion experiment, the solution in the inner chamber was collected and dialyzed for 6 h to measure the amount of unbound $^{125}$I present in the tracer that diffused through the interstitium. We accepted the results of an experiment only if the radioactivity of the unbound $^{125}$I was <10% of the radioactivity of the tracer that diffused through the interstitium. In occasional experiments, the steady-state rate of tracer diffusion was considerably greater than that from the rest of the experiments, resulting in a considerably greater value of A. This was attributed to a leak from outer to inner chamber via spaces between the vessel wall and the...
silicon rubber used to filled the vessel. Also, on occasion, a leak occurred from a chamber because of improper bonding of the lung slab to the chamber. The results of those experiments were not reported. At the end of the diffusion measurements, we measured the diameter of each end of the arterial segment through a macroscope (×18). Segment length was measured by calipers. The entire experiment, including the dialysis of the test solutions, was conducted at room temperature (22–24°C).

Calibration of the scintillation counter. The system consisted of four NaI(Tl) scintillators, each connected to a photomultiplier tube (used to detect the light emitted from the scintillator when subjected to radiation), and a preamplifier and amplifier with a single high-voltage power supply (model 5040, Oxford Instruments). The output of the amplifier was connected to a computer (PC-AT 386, AT&T) via a pulse-height selector and a count-rate meter for automatic data collection. The four single-channel analyzers were calibrated in turn by measuring the spectrum of X- and gamma-rays from a 1-µCi 125I-albumin sample. The voltage applied to the photomultiplier tubes and the amplifier gain were adjusted so that the threshold voltage of 0.2–10 V corresponded approximately to gamma-ray energies of 2–100 KeV. Figure 2 shows a representative energy spectrum obtained by moving a 0.04-V window incrementally through the range from 0 to 10 V. The principal gamma-ray peak at 35 KeV was observed, as well as a coincidence peak at 70 KeV. Measurements of 125I-albumin in the experiments were made with the lower discriminator set at 0.2 V to eliminate detector noise and low-energy background radiation, and the upper discriminator was set at 10.2 V to accept pulses from both the 35- and the 70-KeV peaks.

We determined the mass of tracer that diffused through the interstitium by comparing the radioactivity detected in the inner chamber to that measured from the stock solution used in the outer chamber. Before its use in the diffusion experiment, the stock solution that was dialyzed for 24 h (as previously described) was diluted with a solution of similar Ca concentration (22–24°C). Dialysis of the test solutions, was conducted at room temperature.

However, a count rate of 60,000 counts/s fell in the nonlinear range of the detector, as indicated by the calibration curve of radiation count rate vs. relative concentration (Fig. 3). Therefore, in every experiment, the stock solution was diluted 20-fold to reduce the count rate to a level that fell on the linear part of the calibration curve of the detector.

The calibration curve was developed by measuring the radiation from a 1.8-ml sample of a stock solution containing 25 µCi 125I-albumin, after dilution of the stock solution with lactated Ringer solution by a factor of 2, followed by 10-fold increments in dilutions up to a dilution of 2 × 10^6. To ensure accuracy at the greater dilutions, it was necessary to increase the counting time. In the situation where the sample radioactivity was a small fraction of background, it was necessary to accumulate sufficient background counts so that the SD of the background measurement was negligibly small compared with the sample count rate. Thus, for the highest dilution of 2 × 10^6, the sample count rate was reduced to ~3 counts/s (Fig. 3); with a background of ~100 counts/s, the count-rate period was increased to 30 min to accumulate 10^6 counts of background radioactivity with a SD for the measurement of 0.3% of background or 10% of the diluted sample. For the same reason, we used a period of 30 min for the accumulation of the radiation counts because of diffusion (1–5 counts · s^−1 · h^−1) to ensure that the error in measurement of the background radioactivity was small compared with the diffused radioactivity.

Effect of time on interstitial hydraulic conductivity. To determine the effects of tissue deterioration, in separate experiments, we measured the flow of lactated Ringer solution (Qr) followed by the Qa (5 g/dl, bovine serum; Sigma Chemical) under conditions of 5 cmH2O driving pressure and 7.5 cmH2O mean interstitial pressure. We followed the method used previously (26). Each flow measurement required 1 h. The Qr and Qa measurements were repeated after 24 and 48 h. Finally, the flow of 0.02% hyaluronidase (Qh; hyaluronidase from bovine testes; Sigma Chemical) was measured. Between the periods when flows were measured, the driving and mean pressures were reduced to 0 cmH2O (ambient) to approximate the conditions of the diffusion experiment. The pressure conditions were chosen because they previously produced a positive hydraulic conductivity response to albumin and hyaluronidase (26).

Statistics. Results were reported as mean values ± SD. We used a linear-regression analysis to test the correlation between two parameters and an analysis of variance to test...
the correlation among more than two parameters. We used an unpaired t-test or paired t-test, where appropriate, to evaluate statistical differences between two groups of measurements. Significance was accepted at the P < 0.05 level.

THEORY

We analyzed the experimental results within the framework of unidirectional diffusion of a solute across a membrane of uniform length (L) and uniform surface area (A) after a constant solute concentration (C1) is applied at time 0 to one side of the membrane (x = 0). The membrane concentration (C0) is initially zero, and the solute concentration (C2) on the other side of the membrane (x = L) is maintained at zero. The solution C(x, t) of the one-dimension diffusion equation at any distance x within the membrane and at time t is given by (3)

\[ C = C_1 - C_1 x/L - (2/\pi) \sum_{n=1}^{\infty} (C_i/n) \sin(n\pi x/L)e^{-Dn^2\pi^2t/L^2} \]  

(1)

The mass of solute (M2) that differentiates through the membrane (at x = L) is given by

\[ M_2/ALC_1 = -A \int_0^L (C_1 - C_1 x/L - (2/\pi) \sum_{n=1}^{\infty} (C_i/n) \sin(n\pi x/L)e^{-Dn^2\pi^2t/L^2}) dx \]  

(2)

As t → ∞, Eq. 2 approaches the straight line

\[ M_2 = (DAC_1/L)[t - L^2/(6D)] \]  

(3)

which has an intercept (T) on the t-axis given by

\[ T = L^2/(6D) \]  

(4)

The steady-state mass flow (dM2/dt) of solute from Eq. 3 is then

\[ dM_2/dt = DAC_1/L \]  

(5)

The latter is Fick’s law for steady-state diffusion. Figure 4 shows a plot of the dimensionless solute mass M2/(ALC1) vs. dimensionless time DT/L2 of Eq. 2 (3). The intercept DT/L2 on the DT/L2-axis of the straight line given by Eq. 3 is 1/6. D is then equal to L2/(6DT). A good approximation to within 5% of the actual value of T is obtained by using the straight line through the solution from a time of ~3T to ~4T. Following the work of previous investigators (3, 4), we used Eqs. 3–5 to determine the D for albumin through lung interstitium and cross-sectional A of the interstitial cuff. From experimental measurements of M2 vs. t, the intercept T is measured, and D follows from Eq. 4. With the steady-state value of dM2/dt and the value of D, A follows from Eq. 5.

RESULTS

Calculation of albumin D. Figure 5A shows a representative example of the radioactivity (counts/30 min) measured in the inner chamber by the scintillation counter over the period of 60 h. In this example, the test solution in the outer chamber consisted of lactated Ringer solution with ~10 µCi of 125I-albumin. The inner chamber was filled with lactated Ringer solution. Note that the measured behavior was similar to the theoretical curve of unidirectional diffusion through a membrane (Fig. 4). The measured curve showed a characteristic delay before any radioactivity above baseline (background) was detected, followed by a continuous monotonic rise to a steady-state increase after ~40 h. To determine the steady-state line needed to obtain the intercept on the time axis passing through the background count rate (R), we used a linear regression of the data (dashed line) between 40 and 60 h: R = 7.368 t + 29.694; r2 = 0.999, n = 40. R is in counts/30 min; t is in h. The dashed line was extrapolated to intercept the t-axis through the background count rate (1.35 × 105 counts/30 min). This resulted in an intercept T of 14.3 h on the t-axis, passing through the background activity (solid horizontal line). This value of T and L of 0.45 cm, when substituted in Eq. 4, resulted in a value of D of 6.6 × 10−7 cm2/s. The pooled values for L averaged 0.48 ± 0.056 cm.

Table 1 summarizes mean values (± SD) of D obtained by using the above method at test Ca of ~0, 2, and 5 g/dl in the outer chamber. The values of D (Table 1 and Fig. 6) were independent of C a (in g/dl) by linear-regression analysis: D = 6.58 × 10−7 − 6.24 × 10−10 C a (n = 15, r2 = 0.0002, P = 0.96). The pooled data provided a mean value of D of 6.6 ± 0.97 × 10−7 (SD)
cm$^2$/s (n = 15), within 10% of the value for free diffusion of albumin (6 × 10$^{-7}$ cm$^2$/s; Ref. 28).

The pooled values of count rate minus the background radiation (mean ± SE) vs. time for the 15 experiments of Table 1 are shown as Fig. 5B. The linear-regression equation of the steady-state response between 40 and 60 h was as follows: $R = 4,700 t - 77,100$; $r^2 = 0.102$, $n = 165$, $P < 0.0001$. The time intercept ($T$) for $R = 0$ was 16.4 h. Based on this value of $T$ and the mean value of $L$ (0.48 cm), the value of $D$ from the pooled $R$-$t$ data was 6.5 × 10$^{-7}$ cm$^2$/s, consistent with the mean value calculated from individual experiments.

Calculation of interstitial area and thickness. An estimate of interstitial surface (cross-sectional) $A$ for diffusion was obtained from Fick’s law (Eq. 5). The mass flow of albumin ($dM_2/dt$) through the interstitium into the inner chamber was calculated by using the following equation

$$\frac{dM_2}{dt} = \frac{dR_2}{dt} \frac{K}{R_1}$$

(6)

Here $M_1$ was the mass of albumin present in the 1.8 ml of test solution, from which 0.2 ml were used to fill the outer chamber. $R_1$ was the radioactivity measured in the calibration chamber containing 1.8 ml of test solution diluted 20-fold to reduce the count rate to the linear part of the calibration curve. $K$ is the dilution factor (= 20) that corrected for the nonlinearity of the calibration curve. $R_2$ was the radioactivity measured in the inner chamber, and $dR_2/dt$ was the slope of the measured $R_2$-$t$ curve in the linear range (Fig. 4) after correction for the decay of the background radiation (see Correction for background radiation). The use of an outer chamber of relatively small volume was necessary to reduce the background radiation that emanated from the outer chamber into the well counter through

### Table 1. Summary of time intercept, albumin-diffusion coefficient, and thickness-to-vessel radius ratio at various albumin concentrations

<table>
<thead>
<tr>
<th>Albumin Concentration, g/dl</th>
<th>n</th>
<th>$T$, h</th>
<th>$D_i$, 10$^{-7}$ cm$^2$/s</th>
<th>$Th/R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>~4 × 10$^{-7}$ *</td>
<td>5</td>
<td>15.2 ± 2.3</td>
<td>6.52 ± 0.68</td>
<td>0.063 ± 0.014</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>18.7 ± 6.3</td>
<td>6.66 ± 0.87</td>
<td>0.032 ± 0.022</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>15.9 ± 5.2</td>
<td>6.51 ± 1.44</td>
<td>0.045 ± 0.027</td>
</tr>
<tr>
<td>Mean</td>
<td>15</td>
<td>16.6 ± 4.8</td>
<td>6.56 ± 0.97</td>
<td>0.047 ± 0.024</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of experiments; $T$, time intercept; $D_i$, albumin-diffusion coefficient; $Th/R$, thickness-to-vessel radius ratio. *Tracer $^{125}$I-albumin in Ringer solution.

Fig. 5. A: example of diffusion of radioactive tracer $^{125}$I-albumin through 0.45-cm-long lung interstitial segment. Test solution consisted of lactated Ringer solution with radioactive tracer. Radioactive count rate ($R$, counts/30 min) is plotted at 30-min intervals for 60 h. Dashed line, linear regression equation of steady-state response of $R$ between 40 and 60 h, extrapolated to an intercept value ($T = 14.3$ h) on time axis passing through background-count rate (1.35 × 10$^5$ counts/30 min). Linear-regression equation was $R = 7,368 t - 29,695$ ($r^2 = 0.999; n = 40$). $R$ is in counts/30 min; time ($t$) is in h. Steady-state rate of diffusion through interstitium is the slope (7,368 counts·30 min$^{-1}$·h$^{-1}$). See text for details. B: pooled values of $R$ − background radiation (means ± SE) are plotted at 2-h time intervals from 15 experiments given in Fig. 6. Dashed line, linear-regression equation of steady-state response between 40 and 60 h: $R = 4,700 t - 77,100$ ($r^2 = 0.102; n = 165$, $P < 0.0001$). Intercept $T$ on time axis was 16.4 h.

Fig. 6. Albumin $D$ (means ± SD) vs. albumin concentration (g/dl) in test solution. Nos. in parentheses, nos. of experiments. Values at 0 g/dl represent test solution with $^{125}$I-albumin tracer alone (~4 × 10$^{-7}$ g/dl). Dotted line, value (6 × 10$^{-7}$ cm$^2$/s) for free diffusion of albumin.
the hole in the lead cap through which the inner chamber was located. This significantly reduced the background correction to dR₂/dt (see Correction for background radiation).

Implicit in Eq. 6 is the assumption that the radioactivity measured by the counter scales directly with the dilution of ¹²⁵I-albumin in the range between R₂ and R₁. Figure 3 (log-log plot) shows radioactivity (R, in counts/s) measured in the calibration chamber vs. relative concentration (C). Note that R scales linearly with relative C below ~30,000 counts/s, but it becomes nonlinear at higher count rates. Because M₁ = C₁V₁, where V₁ is the volume of the calibration chamber, the use of Eq. 6 with Fick’s law (Eq. 5) results in the following solution for A

\[ A = L V₁ (dR₂/dt)/(K R₁ D) \]  

(7)

Note that Eq. 7 shows that C₁ does not enter into the calculation of A. Thus A can be calculated even if C₁ is not exactly known, as is the case with the test solution containing only the tracer ¹²⁵I-albumin (~4 × 10⁻⁷ g/dl).

For the example shown in Fig. 5A, dR₂/dt evaluated from the slope of the linear regression line in the range of 40–60 h was 4.09 counts·s⁻¹·h⁻¹, after a small correction for background decay (see Correction for background radiation). The radioactivity of the test solution used in the outer chamber gave a count rate in the calibration chamber of 59,141 counts/s. A 20-fold dilution of the test solution resulted in a value of R₁ of 5,145 counts/s, well within the linear range for the detector (Fig. 3). The value of A (calculated from Eq. 7 with K of 20, V₁ of 1.8 ml, and L of 0.45 cm) was 0.014 cm². We assume that the interstitium surrounding the blood vessel of uniform radius R was an annular cuff of uniform thickness (Tₐ/₂). Then from geometry, the ratio of interstitial cuff thickness-to-vessel radius (Tₐ/R) is as follows

\[ Tₐ/R = A/(2πR²) \]  

(8)

In this example, R was 0.185 cm, so that Tₐ/R was 0.064. Table 1 summarizes values of Tₐ/R. Values of Tₐ/R did not vary significantly with C₀ by a linear-regression analysis: Tₐ/R = 0.054–0.0030 C (n = 15, r² = 0.069, P = 0.34). The pooled values (n = 15) of R and Tₐ/R averaged 0.19 ± 0.018 cm and 0.047 ± 0.024, respectively.

Correction for background radiation. The background radiation measured during the diffusion experiments was mainly emitted from the outer chamber containing ¹²⁵I-albumin, and this background radiation decayed during the experiment. Thus the rate of increase in radioactivity measured in the inner chamber due to diffusion was accordingly reduced. The activity of ¹²⁵I-albumin of initial value R₀ at time t is R₀e⁻λt, where the decay constant λ, based on a half-life of 60 days, is 4.81 × 10⁻⁴ h⁻¹. For a background-count rate R₀ from Fig. 5 of 1.35 × 10⁵ counts/30 min, the rate of decay of background radiation evaluated between 40 and 60 h would be 0.035 counts·s⁻¹·h⁻¹, amounting to 0.85% of the measured steady-state value of dR₂/dt. In the experiments reported, the correction for background radioactivity averaged 3.8 ± 3.0% of steady-state values of dR₂/dt. The correction for background radiation to the value of T and D is considerably more complex and was not implemented. However, based on the small background correction to the steady-state values of dR₂/dt, the effect of background on T would be small and in a direction that results in an underestimation of T and overestimation of D.

Effect of time on interstitial hydraulic conductivity. Table 2 summarizes values (means ± SD; n = 10) of Q₁, Q₂, and Q₁/Q₂, as measured in response to 5 cm H₂O driving pressure and 7.5 cm H₂O mean interstitial pressure at 0, 24, and 48 h after the setting-up procedure. Linear-regression analyses and an analysis of variance showed no significant (P > 0.08) change in Q₁, Q₂, or Q₁/Q₂ with time. Q₁/Q₂ values measured at 0, 24, and 48 h (1.47 ± 0.53, 1.18 ± 0.19, 1.18 ± 0.22, respectively) were significantly greater than Ringer solution-to-albumin solution viscosity ratio (0.81), indicating that the Q₁ was viscosity independent (26). The flow of Q₁ was always greater than the Q₂ measured at 48 h (Table 2), with Q₁/Q₂ values (1.66 ± 0.58) significantly >1 (P = 0.001), similar to previous values measured at 0 h (26). Thus the interstitial hydraulic-conductivity response to albumin and hyaluronidase was still present after 48 h, suggesting little tissue deterioration with time.

**DISCUSSION**

The major result of this study is that the diffusion of albumin caused by concentration differences up to 5 g/dl through pulmonary interstitium was similar to values reported for the free D of albumin. This indicated that the pores through which albumin molecules diffused through pulmonary interstitium were essentially filled with free liquid, were much larger in dimension than the size of an albumin molecule, and offered no restriction to albumin. The estimate of the interstitial cross-sectional A available for diffusion was of the same order of magnitude as the total cross-

<table>
<thead>
<tr>
<th>Flows, ml/h</th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringer</td>
<td>0.12 ± 0.17</td>
<td>0.094 ± 0.094</td>
<td>0.19 ± 0.19</td>
</tr>
<tr>
<td>Albumin solution</td>
<td>0.18 ± 0.29</td>
<td>0.11 ± 0.11</td>
<td>0.23 ± 0.24</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>0.30 ± 0.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Flow ratios |  |
|-------------|-----|-----|
| Albumin/Ringer | 1.47 ± 0.53 | 1.18 ± 0.19 |
| v₁/vₕ | 0.81 | 0.81 |
| Hyaluronidase/Ringer | 1.86 ± 0.58 | 1.0 |

Values are means ± SD; n = 10 experiments. v₁/vₕ, Ringer-to-albumin viscosity ratio; v₁/vₕ, Ringer-to-hyaluronidase viscosity ratio. *Significantly greater than Ringer solution flow. †Significantly greater than viscosity ratio.

Table 2. Effect of time on interstitial hydraulic conductivity response to albumin (5 g/dl) and hyaluronidase (0.02%)
sectional A measured histologically in other studies (9, 13), indicating that the volume of pulmonary interstitium that excludes albumin was immeasurably small by this technique.

Potential errors in the method. The method for determining the D for albumin and cross-sectional A for diffusion entailed several assumptions and details that would affect accuracy. First, we assumed that the unbound 125I present in the 125I-albumin tracer was negligibly small at the start of the experiment and did not increase during the course of the experiment. The former was ensured by removing 98% of the unbound 125I from the 125I-albumin tracer by dialysis before the experiment. In preliminary experiments in which the unbound 125I was not removed (11), the estimate of the D was overestimated due to the 23-fold faster diffusion rate of 125I compared with albumin, because D is proportional to $M^{-1/2}$, and the molecular weights (M) of albumin and 125I are 66,000 and 125, respectively. In other preliminary experiments, we found that the unbound 125I suddenly increased in every experiment after ~60 h. Hence we limited the experimental time to 60 h. Nevertheless, in ~50% of the experiments conducted for 60 h, the unbound 125I accounted for >10% of the total radioactivity measured from the diffused tracer. We rejected the results of these experiments on this basis. In the experiments reported, the unbound 125I averaged $1.6 \pm 2.9\%$ (range, 0–8.5%) of the total radioactivity measured. Thus the errors in the estimate of D due to unbound 125I were deemed to be small.

Second, bulk flow caused by $C_a$ gradients (osmotic flow) that would occur because of the spatial differences in $C_a$ was eliminated by using a closed system with a constant liquid volume.

Third, the solution of the diffusion equation used to evaluate the experimental results assumed an initial $C_a$ of zero in the interstitium. However, the $C_a$ in the interstitium was not initially zero, so that an additional diffusion process must occur in conjunction with the response to the $C_a$ in the outside chamber. We assumed that the interaction between these two diffusion processes did not substantially contribute to the diffusion of the tracer. This assumption was supported by the fact that the change in $C_a$ in the outside chamber from nearly 0 to 5 g/dl did not change the diffusion response of the tracer.

Fourth, the method of continuous measurement by using a well counter ensured that the diffusion process was not disturbed during the course of the experiment. This was evident from the smoothness of the measured R-t curve (Fig. 5). Because the diffusion rate of radioactivity through the interstitium was extremely small, amounting to only 1–5 counts s$^{-1}$ h$^{-1}$, the alternative approach of sampling and subsequent remote measurement was deemed to be too disruptive. However, in the present method, only part of the inner chamber (~80%) was within the well counter, so that only part of the tracer that diffused was actually counted. A correction for the reduced volume within the well counter was effectively eliminated by ensuring a uniformly mixed solution in the inner chamber. This was achieved by continuous stirring and by measuring the radiation from the test solution in a chamber of a volume and dimensions identical to the inner chamber used to measure the diffused tracer.

Fifth, the application of Fick’s law for steady-state diffusion to calculate interstitial cross-sectional A assumed that the $C_a$ and tracer concentrations acting across the interstitium were similar to those acting in the two chambers. The presence of an unstirred layer adjacent to the membrane would reduce the actual concentration difference acting across the interstitium (19) and result in an underestimation of A (Eq. 5). Furthermore, any concentration gradient in the inner chamber would result in an underestimation of the diffused tracer measured in the part of the chamber within the well counter. To minimize errors due to the thickness of the unstirred layer and to any nonuniformity in tracer concentrations and $C_a$, the liquid in the inner chamber was stirred continuously. The outer chamber with the test solution was not stirred because of its small volume, and this would result in an underestimation of A. However, our measurements of interstitial area were somewhat higher than values measured histologically (9, 13), so unstirred layer effects could not account for these differences.

Sixth, the experiments were conducted at room temperature (22–24°C) that was below body temperature. Thus, the measured D was most likely less than that existing in vivo. In vitro measurements in mesentery indicated that values of D measured at 25°C were ~20% less than values measured at 37°C (21).

Seventh, changes in interstitial diffusional properties due to ischemia might occur during the 60-h time period of the experiment. Because the $D$ was measured from a dynamic response that required ~40 h to reach a steady state, it was not possible to determine whether the diffusion properties changed over the course of the experiment. Instead, we showed that the interstitial hydraulic conductivity measured in response to albumin and hyaluronidase was constant over 48 h (Table 2). However, the absence of a change in hydraulic conductivity might not rule out a change in diffusion. This issue warrants further study.

Finally, damage to the interstitium at the two ends of the vessel segment in cutting the lung slab would reduce the effective length of the interstitial segment and increase the effective interstitial cross-sectional A. The use of a reduced effective length would result in a smaller $D$ from the measured intercept T (Eq. 4). Thus damage to the interstitial segment might result in an overestimation of both $D$ and A. These effects could be reduced by using a thicker lung slab, as in previous studies with a 1-cm-thick slab (26). The use of the thinner slab of 0.5 cm was mandated in the present experiments to reduce the time frame of the experiments. If we had used a 1-cm-thick slab, the time intercept T (Fig. 5 and Eq. 4) would have increased fourfold, resulting in a T value of ~60 h and a prohibitively long experimental time of ~8 days. Within this longer time frame, the diffusion of unbound 125I that...
became unbound from the tracer $^{125}$I-albumin after 2 days would most likely dominate the diffusion of $^{125}$I-albumin, as measured in the preliminary experiments.

Comparison with other studies. The D for albumin measured in lung interstitium in this study was similar to values reported for the free D for albumin ($6 \times 10^{-7}$ cm$^2$/s) measured with a variety of techniques (28). Like the present results, these studies also showed that the D was independent of $C_a$.

Diffusion studies in tissue preparations do not provide an unequivocal value for tissue D for albumin. Some studies in isolated subcutaneous tissue (7), human umbilical cord (6), rat diaphragm (23), and rat mesentery (21) indicated D values for albumin (30–100%) near to the free D. By contrast, other studies in the mesentery produced values that were $>10$-fold smaller than those of the free D (5, 14, 16). The latter study of rabbit mesentery showed that the D for albumin increased with $C_a$ and was reduced in the presence of hyaluronidase, indicating that the diffusion of albumin in mesentery increased with hyaluronan concentration (16). Both these effects were attributed to an albumin-hyaluronan osmotic interaction that caused a reduced excluded volume for albumin as either $C_a$ or hyaluronan concentration increased. However, this interaction between albumin and hyaluronan cannot explain why albumin diffusion in lung interstitium is relatively high, because lung tissue has a relatively low hyaluronan concentration ($10^{-4}$ g/g; Ref. 8). The effect of hyaluronidase on the D for albumin in lung interstitium remains to be evaluated. Preliminary results with hyaluronidase indicated an apparent greater diffusion of albumin through lung interstitium (11), but this study contained artifacts from the diffusion of unbound $^{125}$I that dominated the diffusion of the tracer $^{125}$I-albumin.

The estimate for interstitial cross-sectional A for diffusion showed a mean $\Theta_{DL}$ value (0.05) that was of the same order of magnitude as that estimated for lung perivascular interstitium in rabbit lungs (mean value of 0.02, range 0.004–0.06; Ref. 9). Similar values have been measured in dog lungs (13). The relatively high diffusion area in conjunction with the measured albumin D close to the free D would imply that the volume excluded from albumin in lung interstitium is negligibly small. This prediction is in contrast to the relatively high values of excluded volume fraction measured for albumin in vivo (17, 18). This disparity between the in vitro and in vivo estimates for albumin-excluded volume might be caused by differences in interstitial constituents (for example, hyaluronan) between the perivascular interstitial cuffs of the largest pulmonary vessels in the present study and those of the smaller exchange vessels associated with the in vivo study. Accordingly, the differences in the diffusion properties and hyaluronan concentration of interstitial cuffs as a function of vessel size deserve evaluation, in particular as they pertain to the inability of a significant fraction of relatively small vessels (diameter $<0.5$ mm) to form interstitial cuffs and the virtual absence of interstitial cuffs around vessels $<0.1$ mm diameter in liquid-inflated isolated lungs (1, 2).

In summary, the diffusion of albumin through lung interstitium was close to the free diffusion for albumin. This implies that the pathways for the diffusion of albumin in lung interstitium are completely filled with liquid and have an equivalent pore radius much larger than that of the albumin molecule. Thus lung interstitial constituents offered no restriction to the passage of albumin, with a reflection coefficient virtually equal to zero. This conclusion is at odds with spatial gradients in $C_a$ measured previously in lung interstitial segments (22). This discrepancy needs further study. Direct measurements of reflection coefficient close to zero have been made recently for the rabbit mesentery (15). This result, in conjunction with the 10-fold smaller D measured for rabbit mesentery (16), implies a 10-fold smaller surface area for diffusion and a much larger volume excluded from albumin in mesentery than in lung interstitium.

This research was supported by National Heart, Lung, and Blood Institute Grant HL-40362. Address for reprint requests: S. J. Lai-Fook, Center for Biomedical Engineering, Wenner-Gren Research Laboratory, Univ. of Kentucky, Lexington, KY 40506-0070 (E-mail: bme006@ukcc.uky.edu).

Received 23 December 1997; accepted in final form 2 April 1998.

REFERENCES