

Noninvasive Measures of Radiolabeled Dextran Transport in In Situ Rabbit Lung

Valerie J. Abernathy, N. Adrienne Pou, Teresa L. Wilson, Richard E. Parker, Scott N. Mason, Jeffery A. Clanton, Lawrence J. Baudendistel and Robert J. Roselli

Department of Biomedical Engineering, School of Engineering, Center for Lung Research, School of Medicine and Department of Nuclear Medicine, Vanderbilt Medical Center, Vanderbilt University, Nashville, Tennessee; and Department of Anesthesiology, School of Medicine, St. Louis University, St. Louis, Missouri

Dextran is nontoxic and can be obtained in a wide variety of molecular weights. The purpose of this study was to label 6-kDa and 40-kDa dextrans with gamma- (^{99m}Tc) and positron- (^{18}F) emitting radioisotopes and monitor their transport across the pulmonary microvascular barrier. **Methods:** External scan measurements for radiolabeled uncharged dextrans, albumin and red blood cells were obtained in eight blood-perfused in situ rabbit lung preparations. After 3 hr of external scanning, the lungs were removed for postmortem and extravascular distribution volume calculations. Extravascular distribution volumes were obtained in six additional rabbits following 4 hr of dextran perfusion to compare the effect of time. The normalized slope index (NSI), a measure of transvascular transport rate, was calculated for each diffusible tracer. **Results:** The mean NSI for albumin ($0.001676 \pm 0.000537 \text{ min}^{-1}$) was significantly lower than NSI for the 40-kDa dextran ($0.002303 \pm 0.0005426 \text{ min}^{-1}$) as well as the 6-kDa dextran ($0.004312 \pm 0.001134 \text{ min}^{-1}$). The difference between the 6-kDa and the 40-kDa dextrans was also significant. After 4 hr of equilibration, distribution volumes were not significantly different than those obtained at 3 hr. **Conclusion:** Dextrans can be radiolabeled with gamma and positron emitters and small dextrans traverse the lung microvascular barrier more rapidly than albumin. Our results suggest that the use of small dextrans rather than albumin can reduce scan times in clinical applications and minimize motion artifact associated with the noninvasive gamma detection method.

Key Words: radiolabeled dextran; gamma scan; in situ rabbit lung; microvascular permeability

J Nucl Med 1995; 36:1436-1441

External gamma detection has been used to monitor lung transvascular protein flux for a number of different situations in experimental animals and humans. Results from these studies indicate that the rate of protein accumulation is not altered appreciably by changes in blood flow (1) and positive end-expiratory pressure (2,3) or by

infusion of iloprost or noradrenaline (4). Protein flux is clearly elevated in adult respiratory distress syndrome (5,6) and renal failure (7) and is elevated in some patients after hemodialysis (8). Intravascular infusions of a number of substances in experimental animals have increased transvascular protein flux, including oleic acid (5,9), *E. Coli* endotoxin (10), *Pseudomonas aeruginosa* (6,11), alloxan (12), thiourea (13) and perilla ketone (2,14).

Transferrin and albumin are the only macromolecules that have been used in lung external gamma detection studies. Both albumin and transferrin normally move very slowly across the microvascular barrier, requiring long scan times of 45 min to 1 hr before slopes can be accurately measured. Furthermore, only a single diffusible macromolecule is injected per study. Scan times can be significantly reduced if labeled macromolecules with faster exchange rates are developed, and lung permeability characteristics can be much better defined if tracers of different size and/or electrical charge are injected simultaneously.

Dextrans appear to be a logical choice to replace slowly exchanging proteins in noninvasive measurements of macromolecular flux. They can be obtained in a wide variety of molecular weights. They are also easily sulfated to produce anionic macromolecules or diethylaminated to produce cationic molecular probes (15). They do not bind to proteins, and dextrans 40 kDa and below do not bind to red cells (16,17). Dextrans are nontoxic and are used extensively for plasma volume expansion (18). Dextrans with molecular weights below 70 kDa are cleared relatively fast from the blood stream (19). Infusions of moderate amounts of dextrans do not affect vascular pressures (20) or permeability (21), and dextrans are not trapped within the interstitial spaces of the lung (22).

The purposes of this study were to label 6-kDa and 40-kDa dextrans with gamma- and positron-emitting radioisotopes and monitor their transport across the pulmonary microvascular barrier of a blood-perfused in situ rabbit lung preparation using gamma scintillation detectors. The accumulation of 6-kDa and 40-kDa uncharged dextrans labeled with ^{99m}Tc or ^{18}F in addition to ^{125}I albumin and ^{51}Cr red blood cells were monitored for 3 hr after injection

Received Dec. 6, 1993; revision accepted Sept. 20, 1994.
For correspondence or reprints contact: Robert J. Roselli, MD, B-1318 Medical Center North, Vanderbilt Medical Center, Nashville, TN 37232-2155.

of the radiolabeled dextrans in eight rabbits. The lungs were removed at the end of each experiment for postmortem and extravascular distribution calculations. In six additional rabbits, postmortem distribution volumes were determined after 4 hr of perfusion to compare the effect of time.

MATERIALS AND METHODS

Experimental Protocol

New Zealand white rabbits weighing 4–5 kg were injected intramuscularly with a mixture of rompin (3 mg/kg) and ketamine (50 mg/kg). Approximately 10 min after this mixture was administered, 1 mg/kg acepromazine maleate was injected subcutaneously. Once the rabbit was fully anesthetized, it was immediately placed in a supine position and a tracheotomy was performed. The carotid artery was cannulated, and 3000 units of heparin were administered to prevent clotting. Immediately after the heparin administration, 20 ml of blood were withdrawn from the carotid catheter and the red blood cells (RBCs) were labeled with 100 μ Ci ^{51}Cr . The jugular vein was cannulated and the rabbit was then rapidly exsanguinated through the carotid artery catheter. The blood was drained into a sterile water-jacketed, perfusion system reservoir where it was maintained at 38°C. Approximately 60–80 ml normal saline were administered to the rabbit through the jugular vein catheter during the exsanguination. This was done to ensure sufficient perfusate for the perfusion system.

Immediately following exsanguination, a midline incision was made to a point below the xiphoid process. A parasternal incision was performed, allowing the extirpation of costal cartilages. The pericardium was opened, and the main pulmonary artery was ligated with a loosely tied suture. The inferior vena cava, descending aorta and the esophagus were ligated and the abdominal viscera were removed. The heart was incised immediately proximal to the pulmonary valve and the main pulmonary artery was cannulated. An outflow cannula was inserted through the mitral valve into the left atrium. The inflow and outflow cannulas were then connected to the perfusion system, the loose tie around the pulmonary artery removed, and a low flow established. Flow was slowly increased over a 10–20-min period until a flow rate of 100 ml/min was achieved. This flow was maintained throughout each experiment. The lungs were hyperinflated to open atelectatic areas. They were then ventilated with a mixture of 5% CO_2 in air with a tidal volume of 15 ml at a rate of 24 breaths per minute. Once the system was stable, ^{51}Cr -RBCs were added to the perfusion system reservoir. Iodine-125-albumin (10–15 μ Ci) was given approximately 10–15 min after the labeled RBCs (23). Administration of these radiolabeled tracers was followed by 10 μ Ci ^{99m}Tc dextran and then 5 μ Ci ^{18}F dextran. In eight experiments, the accumulation of ^{99m}Tc - and ^{18}F -dextrans, ^{125}I -albumin and ^{51}Cr -RBCs were monitored for 3 hr. In six of the eight experiments, 6-kDa dextrans were labeled with ^{99m}Tc and 40-kDa dextrans were labeled with ^{18}F . The labels were reversed in the remaining two experiments. In six additional rabbits, postmortem distribution volumes were determined after 4 hr of perfusion to compare the effect of time. At the end of each experiment, a blood sample was obtained and the lungs removed for postmortem analysis.

Dextran Purification

The 40-kDa (37–43 kDa) dextrans used in this study were purchased from Sigma (St. Louis, MO) and were not further purified before labeling. The 6-kDa (5.5–6.5 kDa) dextrans were

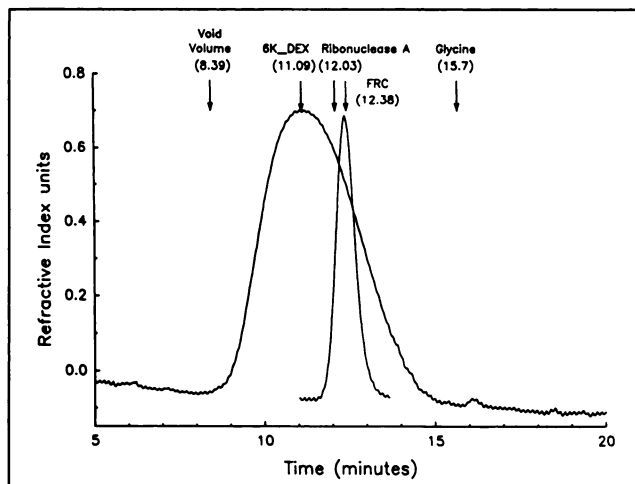


FIGURE 1. Chromatograms and retention times of 100 μ l 0.5% solution of dextrans before (6K-Dex) and after (FRC) purification, and retention times of glycine and ribonuclease A on a GS320 column.

obtained from Fluka-BioChemika (Ronkohlkoma, NY). The molecular weight range of the 6-kDa dextrans was narrowed using a gel filtration fractionation procedure. A Pharmacia XK50/100 Piscataway, NJ) SEC column (volume = 1960 ml) was packed with Sephacryl S-100 HR to a bed height of 96 cm (bed volume = 1890 ml). A 1% solution of the 6-kDa dextrans in eluent (0.10 M NaCl, 0.10% NaN_3 , 0.05 M Tris, pH 7.4) was separated on the column with a flow rate of 8 ml/min. A fraction equal to 20% of the total quantity of dextran solution was collected. This collected fraction was centered approximately about the apex of the concentration versus time curve (1245–1375 ml). Fractions from a number of identical runs were pooled, concentrated, dialyzed against ultrapure water on an Amicon spiral wound ultrafiltration unit (Beverly, MA) and lyophilized. A 1% solution of this product from the previous step in eluent was separated on the same gel column again with a flow rate of 8 ml/min. A fraction equal to 50% of the total quantity loaded onto the gel was collected. Fractions obtained from a number of identical runs were pooled, concentrated, dialyzed against ultrapure water and lyophilized. A 0.5% solution of this product in eluent was again separated on the column using the same flow rate of 8 ml/min. A fraction equal to 50% of the total quantity was collected. Fractions obtained from a number of identical runs were pooled, concentrated, dialyzed against ultrapure water and lyophilized. This final product was analyzed using high-performance liquid chromatography (HPLC) and detected with a differential refractive index. Results are shown in Figure 1 in which the purified fraction (FRC) is compared with the commercial mixture (6K-DEX). The dextran fraction FRC was eluted on a HPLC Asahipak GS320 column (Asahi Chemical Inc., Kawasaki, Japan) very near ribonuclease A (mol wt: 13,700, Stokes Einstein radius (R_s): 1.64 nm). The R_s near 1.64 nm for this dextran is greater than predicted by molecular weight alone because it has a randomly coiled structure compared to a more globular structure of proteins.

Dextran Radiolabeling

Dextrans were labeled with ^{99m}Tc in a manner similar to that described by Zanelli et al. (24). Initially, 20 mg stannous chloride dihydrate ($\text{SnCl}_2 \cdot \text{H}_2\text{O}$) was dissolved in 1 ml 12 N HCl and allowed to stand at room temperature until clear (20–30 min).

During this time, 100 ml sterile water was degassed by passage through a 0.45- μ m nylon filter using a vacuum and collection in a sterile container. The water (20 ml) was then transferred into a 30-ml sterile vial and a sufficient amount of para-aminobenzoic acid (PABA) was added to obtain a concentration of 2 mg/ml. A 10% solution of the 6-kDa or 40-kDa dextrans were prepared using a portion of the degassed water. A sufficient volume of the 10% solution was added to the 30-ml vial to obtain a concentration of 1 mg/ml. A dose of 0.1 ml $\text{SnCl}_2 \cdot \text{H}_2\text{O}$ was then added to the dextran solution and mixed under a N_2 atmosphere. The final solution was transferred in 1-ml aliquots to 10-ml glass vials, stoppered under N_2 , and frozen at -20°C . These kits were lyophilized, stoppered under vacuum and stored at 4°C . Labeling was performed by adding 5 ml sterile citrate-buffered saline to the lyophilized kit and dissolving the dextrans. Approximately 1–2 mCi $^{99\text{m}}\text{Tc}$ in a volume of 0.2–0.4 ml was added to the vial and allowed to incubate at room temperature for 10 min. Labeling efficiency was determined by instant thin-layer chromatography (ITLC) on silica gel and developed with methyl ethyl ketone. The labeled dextrans remained at the origin and free pertechnetate migrated to the solvent front.

Dextrans were labeled with ^{18}F in the following manner. Dextrans were allowed to react with one equivalent of tosyl chloride and triethylamine in a mixed solvent (1:1) of methylene chloride and tetrahydrofuran (dextran concentration 0.1 M) under a dry N_2 atmosphere. After 48 hr, the solvents were removed under reduced pressure. This process was repeated two additional times. The resultant white solid was used as the precursor for ^{18}F labeling experiments.

Fluorine-18-fluoride was produced by the (p, n) reaction using H_2^{18}O as the target material. The aqueous solution of ^{18}F was transformed into anhydrous K^{18}F through a process of mixing the fluoride with an acetonitrile solution of Kryptofix K[222] and K_2CO_3 . The acetonitrile was removed by distillation and the resultant mixture was made anhydrous through two azeotropic distillations of 1 ml acetonitrile. The dextran precursor (20 mg) was suspended in 1 ml acetonitrile, mixed well and then filtered. The resultant solution was then added to the anhydrous K^{18}F mixture and heated at 85°C for 15 min. The resultant solution (200 μ l) was then placed on an Econo-Pak P6 cartridge (Bio-Rad) and eluted with saline. One-milliliter fractions were collected and analyzed for radiochemical purity using a Bioscan System 200 Imaging Scanner (Washington, DC). Thin-layer chromatography was performed on glass-backed silica plates (Whatman, 250 μ m) using methanol/concentrated ammonium hydroxide (9:1). Ninety percent of the total activity was bound to the dextrans.

Flow System

The flow system consisted of a bubble trap, a Monostat vari-staltic pump (New York, NY), a 300-ml water-jacketed reservoir, silicone tubing and other small fittings (Fig. 2). The tubing was autoclaved the day before the experiment. Any components that could not be autoclaved were soaked in ethyl alcohol 16–20 hr before use. The system was assembled on the morning of the experiment and rinsed twice with sterile normal saline before it was primed with blood.

As blood was pumped from the reservoir into the lung, it flowed through a tube which passed through protective lead shielding where a NaI scintillation probe was mounted to monitor radioactivity in the blood. A second NaI scintillation probe was recessed 0.5 inch in a 2-cm thick lead cylinder and positioned approximately 2 inches above the lungs. Blood exiting the lungs

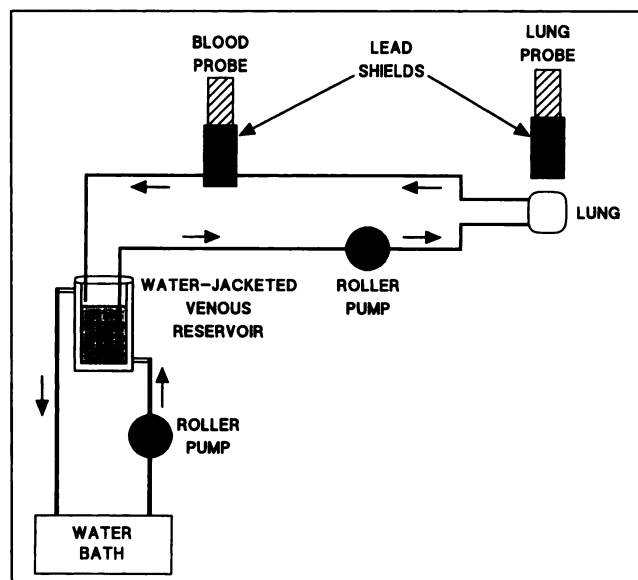


FIGURE 2. Schematic diagram of blood perfused in situ during rabbit lung preparation.

was returned to the water-jacketed reservoir. Pressure taps were placed on both the inflow and outflow sides of the lung. The following variables were recorded: pulmonary artery (inflow) pressure P_{pa} , left atrial (outflow) pressure P_{la} , airway pressure P_{air} , and blood flow rate in ml/min, determined by timed collection of outflow.

External Detection of Radioactivity

Radioactivities from the shielded 2-inch NaI scintillation detectors were sampled every 60 sec with a PC-based multichannel pulse-height analyzer (PCA-8000, The Nucleus, Oak Ridge, TN). The sampled activities were corrected for background, overlap, time decay and system deadtime. The normalized index (NI) for albumin, 6-kDa and 40-kDa dextrans were then calculated from the corrected count rates over the duration of each experiment using the equation below:

$$NI = \frac{[L_d(t)/L_d(0)] [B_r(t)/B_r(0)]}{[B_d(t)/B_d(0)] [L_r(t)/L_r(0)]},$$

where L_d is the radioactivity of diffusible tracer detected by the lung probe, B_d is the radioactivity of diffusible tracer detected by the blood probe, L_r is the radioactivity of intravascular tracer detected by the lung probe and B_r is the radioactivity of intravascular tracer detected by the blood probe. The slope of NI over 75 min was determined and reported as the normalized slope index (NSI).

Postmortem Calculation

The lungs, whole blood and plasma samples for all experiments were weighed and counted for radioactivity with a Hewlett-Packard 5000 series auto-gamma counter (Wilmington, DE). All samples were corrected for spectral overlap and time decay. Extravascular lung water, pulmonary blood volume, bloodless dry lung weight (BFDLW) and the bloodless wet-to-dry lung weight ratio were calculated as previously described (25,26). Residual blood volume for each lung was determined by dividing the counts per minute (cpm) for ^{51}Cr in the tissue by the cpm in 1 ml of mixed venous blood. The cpm due to blood were then subtracted from

TABLE 1
Normalized Slope Index for Radiolabeled Albumin, 40-kDa and 6-kDa Dextran in In Situ Rabbit Lung

Study no.	Albumin (min ⁻¹)	40-kDa Dextran (min ⁻¹)	6-kDa Dextran (min ⁻¹)
05-05-93	0.001635	0.003351	0.007411
06-10-93	0.005227	0.005396	0.010656
09-22-93B	0.000924	0.001326	0.001882
09-28-93B	0.000886	0.001886	0.002873
09-29-93A	0.000724	0.000326	0.002432
09-29-93B	0.000469	0.001468	0.001071
05-19-94A	0.001748	0.002129	0.004203
05-19-94B	0.001796	0.002543	0.003964
Mean ± s.e.m.	0.001676 ± 0.000537	0.002303 ± 0.000542	0.004312 ± 0.001134

each tissue sample to obtain tracer tissue cpm (cpm_{tissue}) for each macromolecule (albumin, 6-kDa and 40-kDa dextran). Extravascular distribution volumes for albumin and both dextrans were calculated based on plasma concentration:

$$V_{Es}(\text{ml}) = \text{cpm}_{\text{tissue}} / (\text{cpm}/\text{ml})_{\text{plasma}}$$

All extravascular volumes were then normalized to bloodless dry lung weight.

Statistics

The data are expressed as mean ± standard error. A paired Student's t-test was used to determine differences in external scan data. A two-way analysis of variance (ANOVA) and Student-Newman-Keuls test were used to compare differences in time and molecular weight for extravascular volume data. The difference was considered significant when $p < 0.05$.

RESULTS

Outlet tubing was adjusted so that the venous pressure was approximately 2–4 cm H₂O. Arterial pressure ranged from 8 to 15 cm H₂O depending on the experiment. Both arterial and venous pressures remained constant throughout all experiments.

External Detection of Macromolecular Flux

We obtained external scan measurements for 3 hr in eight rabbits. Normalized slope indices for albumin and 6-kDa and 40-kDa dextrans are listed for each study in Table 1. The mean NSI for albumin ($0.001676 \pm 0.000537 \text{ min}^{-1}$) was significantly lower than NSI for the 40-kDa dextran ($0.002303 \pm 0.0005426 \text{ min}^{-1}$), as well as the 6-kDa dextran ($0.004312 \pm 0.001134 \text{ min}^{-1}$). The difference between the 6-kDa and the 40-kDa dextrans was also significant. Figure 3 shows normalized index for each diffusible tracer over the course of experiment 05-05-93. Figure 4 shows radioactive counts per minute in the blood and over the lung for experiment 09-28-93B. Count rates in the blood were constant throughout the ⁵¹Cr-RBC, ¹²⁵I-albumin and ¹⁸F 40-kDa dextran studies. Counts rates of ^{99m}Tc-labeled 6-kDa dextran in the blood decreased because of the rapid transport into extravascular spaces of the lung. As one might expect from differences in molecular size, ^{99m}Tc 6-kDa dextran count rates over the lung increased at a much higher rate than those of ¹⁸F 40-kDa dextran and ¹²⁵I-albumin.

Extravascular Distribution Volume

We obtained extravascular distribution volumes for six rabbits after 3 hr of dextran perfusion (scan data presented for these rabbits). We also determined extravascular distribution volumes on six additional rabbits after 4 hr of perfusion with radiolabeled dextrans. Scan data were not obtained for these six rabbits due to a defective NaI detector.

The wet-to-dry weight ratio was similar in both groups (3 hr: 4.47 ± 0.17 , 4 hr: $4.37 \pm 0.21 \text{ ml/g BFDLW}$) as was blood volume (3 hr: 1.18 ± 0.12 , 4 hr: $1.11 \pm 0.07 \text{ ml/g BFDLW}$). As seen in Figure 5, postmortem calculation of extravascular volume for albumin ($1.01 \pm 0.15 \text{ ml/g BFDLW}$), 40-kDa dextran ($2.52 \pm 0.15 \text{ ml/g BFDLW}$) and 6-kDa dextran ($3.00 \pm 0.22 \text{ ml/g BFDLW}$) were significantly different from one another after 3 hr of external scanning. After 4 hr of equilibration, distribution volumes were not significantly different than those obtained at 3 hr.

DISCUSSION

We measured accumulation of albumin, 6-kDa and 40-kDa dextran in the in situ rabbit lung using noninvasive gamma scanning. Rabbits were chosen for this study because they are small and require much less radioisotope

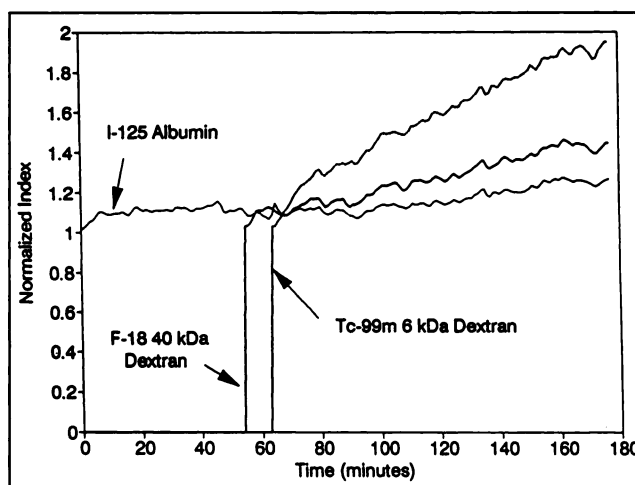


FIGURE 3. Normalized index (calculated using Eq. 1) for ¹²⁵I albumin, ^{99m}Tc 6-kDa dextrans and ¹⁸F 40-kDa dextrans for experiment 05-05-93.

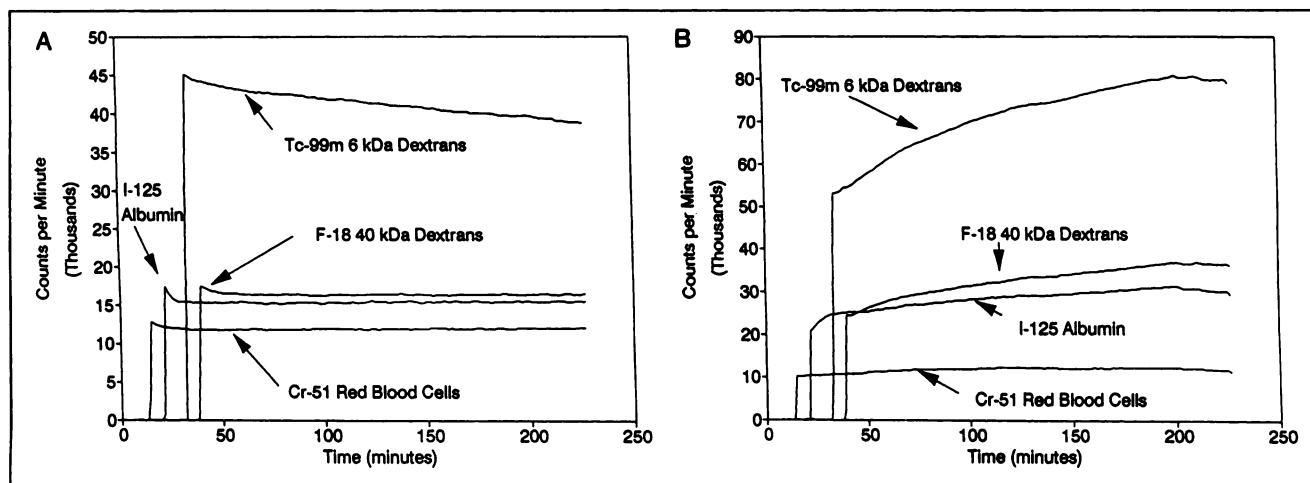


FIGURE 4. (A) Counts per minute of ^{125}I -albumin, $^{99\text{m}}\text{Tc}$ 6-kDa dextran, ^{18}F 40-kDa dextran and ^{51}Cr -RBCs detected by the blood detector (A) and the lung detector (B). Count rates were corrected for overlap, radioisotope decay and deadtime.

and preparation time than larger animals. An in situ rabbit lung preparation eliminates radioactivity from organs other than the lung, as well as the chest wall. Chest wall accumulation is neglected by most investigators, however, contributions to external count rates and NSI have not been determined. We wanted to eliminate chest wall contributions since we have not previously used external gamma scanning to monitor transport of radiolabeled dextrans.

The NSI for albumin, in addition to pulmonary artery and left atrial pressures, were constant throughout the ex-

perimental period for every rabbit lung preparation. The mean wet-to-dry lung weight ratio for all experiments was 4.43 ± 0.13 . Thus lung permeability was considered normal for all experiments.

As one might expect, smaller macromolecules accumulate in the interstitium more rapidly than larger macromolecules. The average values of NSI found in the current study were consistent with the size of the macromolecule. The albumin NSI has not been previously reported for in situ rabbit lungs. In this study, the average ^{125}I albumin NSI was $0.001676 \pm 0.000537 \text{ min}^{-1}$. This value is comparable to baseline values ($0.00142 \pm 0.00054 \text{ min}^{-1}$) reported by Abernathy et al. (14) for in situ sheep lungs. Albumin had the lowest NSI followed by ^{18}F 40-kDa dextran ($0.002302 \pm 0.000542 \text{ min}^{-1}$) and $^{99\text{m}}\text{Tc}$ 6-kDa dextran ($0.004312 \pm 0.001134 \text{ min}^{-1}$), respectively. The 40-kDa dextrans used in this study had a molecular weight range of 37–43 kDa when purchased and were not further purified before labeling. The range for the 6-kDa (5.5–6.5 kDa) dextrans was narrowed using a gel filtration fractionation procedure. Thus, the labeled 40-kDa dextrans were more polydisperse. This may have added to the variability for the 40-kDa dextrans.

We also found that proper shielding was extremely important when using a positron emitter such as ^{18}F . The ^{18}F 40-kDa stock was tested for stability over 6 hr using thin-layer chromatography on glass-backed silica plates with methanol and ammonium hydroxide (9:1) as the eluting solvent. The amount of unbound ^{18}F remained constant at $6\% \pm 2\%$ for 6 hr. At the time of these studies, however, we were unable to determine the in vivo stability of the labeled dextrans. Overall dextrans exhibit an increase in slope with a decrease in molecular weight and should be investigated further.

Extravascular distribution volumes were calculated at the end of the scan time to aid the characterization of dextrans in the lung interstitium. Extravascular distribu-

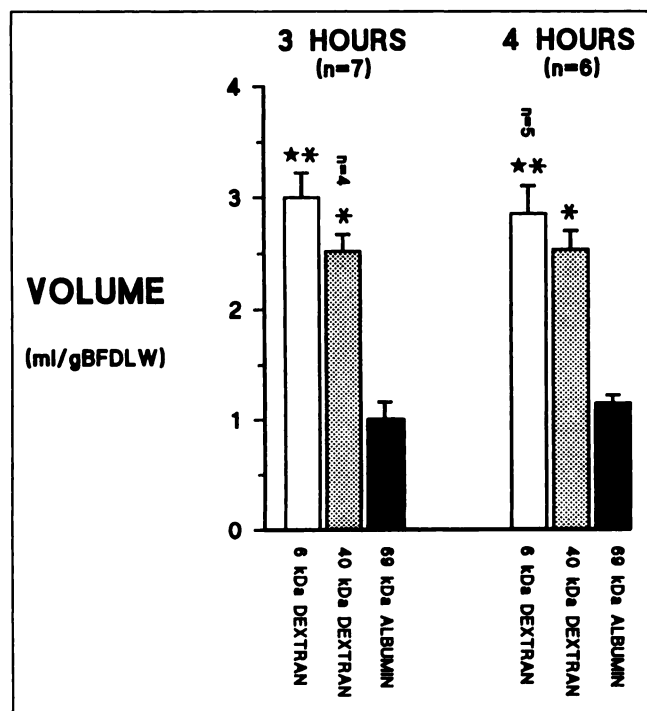


FIGURE 5. Extravascular distribution volumes (ml/g BFDLW) for ^{125}I -albumin, ^{18}F 40-kDa dextran and $^{99\text{m}}\text{Tc}$ 6-kDa dextran after 3 and 4 hr of circulation. * $p < 0.05$ compared to albumin. * $p < 0.05$ compared to 40-kDa dextran.

tion volumes of proteins in sheep have been calculated using lymph concentration (23,27), while distribution volumes in rabbits (28) and isolated lung preparations (29) use plasma concentration. Selinger et al. (27) compared extravascular distribution volumes for albumin in sheep based on plasma, as well as lung lymph concentration and found no significant differences. Thus, plasma concentration was used to calculate distribution volumes in the current study.

As expected, we found an increase in extravascular distribution volume as macromolecular size decreased. The difference between extravascular distribution volume for the 6-kDa and the 40-kDa dextran was not as great as one might have expected, but dextran tissue distribution has not been studied extensively. Solute shape as well as charge influence extravascular distribution of a molecule. In this study, albumin is charged while the dextrans are uncharged. This may have contributed to the fact that 40-kDa dextran had a larger distribution volume than expected simply based on size. The fact that there was no difference between 3 and 4 hr for both dextrans and albumin suggest near-equilibrium for these tracers. Kern et al. (29) predicted the equilibrium extravascular albumin distribution volume in isolated rabbit lungs to be 0.253 cm³/g blood-free wet lung. Based on their calculation, equilibration of the radiolabeled albumin occurs after 4.5 hr of perfusion. When the extravascular albumin distribution volume for the current study was normalized to bloodless wet weight, a value of 0.22 ± 0.01 ml/g BFDLW was calculated. This value agrees with Kern's predicted value. Experiments with longer perfusion times would be needed to confirm this hypothesis.

CONCLUSION

We have shown that dextrans can be radiolabeled with gamma- (^{99m}Tc) as well as positron-emitting radioisotopes (¹⁸F) to measure transvascular solute flux. We observed an increase in both transvascular flux and extravascular distribution volume as the molecular weight decreased. We thus conclude that small dextrans traverse the microvascular membrane more readily than albumin. Use of small dextrans rather than albumin or transferrin should shorten scan times in clinical studies and minimize motion artifact. The sequential injection of several dextrans of different size or charge should enhance the ability of the noninvasive gamma detection method to characterize the permeability of the lung microvascular barrier.

ACKNOWLEDGMENTS

This work was supported by grants HL 51234, HL 19153 (SCOR in Pulmonary Vascular Diseases), HL 08478-01 (NRSA Individual Postdoctoral Fellowship) and Biomedical Research Support grant S07-RR07201.

REFERENCES

1. Hamvas AR, Palazzo L, Kaiser J, et al. Inflammation and oxygen-free radical formation during pulmonary ischemia-reperfusion injury. *J Appl Physiol* 1992;72:621-628.

2. Roselli RJ, Abernathy VJ, Riddle WR, Parker RE, Pou NA. Evaluation of lung vascular permeability by external scanning of gamma emitter activity. In: Epstein MAF, Ligas JR, eds. *Respiratory biomechanics*, Springer-Verlag; 1990:155-161.
3. Sugerman HJ, Strash AM, Hirsch JI, et al. Scintigraphy and radiography in oleic acid pulmonary microvascular injury: effects of positive end-expiratory pressure (PEEP). *J Trauma* 1982;22:179-185.
4. Hultkvist-Bengtsson U, Martensson L. External detection of pulmonary accumulation of indium-113m-labeled transferrin in the guinea pig. *Thorax* 1990;45:688-693.
5. Mintum MA, Dennis DR, Welch MJ, Mathias CJ, Schuster DP. Measurements of pulmonary vascular permeability with PET and gallium-68 transferrin. *J Nucl Med* 1987;28:1704-1716.
6. Tatum JL, Strash AM, Sugerman HJ, Hirsch JI, Fratin MJ. Computerized scintigraphic technique for the evaluation of adult respiratory distress syndrome: initial clinical trials. *Radiology* 1982;143:237-241.
7. Rocker GM, Morgan AG, Pearson D, Basran GS, Shale DJ. Pulmonary vascular permeability to transferrin in the pulmonary oedema of renal failure. *Thorax* 1987;42:620-623.
8. Gorin AB, Weingarten J, Leblanc A, Stevens P. External radioflux detection: noninvasive measurement of protein leakage in assessing lung microvascular injury. *Ann NY Acad Sci* 1982;384:411-415.
9. Sugerman HJ, Hirsch JI, Strash AM, et al. Preliminary report. Gamma camera detection of oleic acid alveolar-capillary albumin leak. *J Surg Res* 1980;29:93-99.
10. Welsh CH, Dauber IM, Weil JV. Endotoxin increases pulmonary vascular protein permeability in the dog. *J Appl Physiol* 1986;61:1395-1402.
11. Gorin AB, Weidner WJ, Demling RH, Staub NC. Noninvasive measurement of pulmonary transvascular protein flux in sheep. *J Appl Physiol* 1978;45:225-233.
12. Aviado DM, Schmidt CF. Pathogenesis of pulmonary edema by alloxan. *Circ Res* 1957;5:180-186.
13. Dauber IM, Pluss WT, VanGrondelle A, Trow RS, Weil JV. Specificity and sensitivity of noninvasive measurement of pulmonary protein leak. *J Appl Physiol* 1985;59:564-574.
14. Abernathy VJ, Roselli RJ, Parker RE, Pou NA. Effects of perilla ketone on the in situ sheep lung. *J Appl Physiol* 1992;72:505-514.
15. deBelder AN, Granath K. Preparation and properties of fluorescein-labelled dextrans. *Carbohydrate Res* 1973;30:375-378.
16. Razavian SM, Guillemin M, Guillet R, Beuzard Y, Boynard M. Assessment of red blood cell aggregation with dextran by ultrasonic interferometry. *Biorheol* 1991;28:89-97.
17. Snabre P, Grossmann GH, Mills P. Effects of dextran polydispersity on red blood cell aggregation. *Colloid Polymer Sci* 1985;263:478-483.
18. Arakawa M, Jerome EH, Enzan K, Grady M, Staub NC. Effects of Dextran 70 on hemodynamics and lung liquid and protein exchange in awake sheep. *Circ Res* 1990;67:852-861.
19. Arturson G, Groth T, Grotte G. Human glomerular membrane porosity and filtration pressure: dextran clearance data analyzed by theoretical models. *Clin Sci* 1971;40:137-158.
20. McNamee JE. Restricted dextran transport in the sheep lung lymph preparation. *J Appl Physiol* 1982;52:585-590.
21. Michel RP. Lung microvascular permeability to dextran in α -naphthylthiourea-induced edema. *Am J Pathol* 1985;119:474-484.
22. Mayerson HS, Patterson RM, McKee A, LeBrie SJ, Mayerson P. Permeability of lymphatic vessels. *Am J Physiol* 1962;203:98-106.
23. Pou NA, Roselli RJ, Parker RE, Clanton JC. Effects of air embolism on sheep lung fluid volumes. *J Appl Physiol* 1993;75:986-993.
24. Zanelli GD, Ellison D, Barrowcliffe MP. The preparation of kits for labelling macromolecules of biological interest with technetium-99m. *Nucl Med Commun* 1987;8:199-206.
25. Collins JC, Newman JH, Wicksham NE, et al. Relation of blood-free to blood inclusive postmortem lung water measurements in sheep. *J Appl Physiol* 1985;59:592-596.
26. Peterson BT, Brooks JA, Zack AG. Use of microwave oven for determination of postmortem water volume of lungs. *J Appl Physiol* 1982;52:1661-1663.
27. Selinger SL, Bland RD, Demling RH, Staub NC. Distribution volumes of [¹³¹I]albumin, [¹⁴C]sucrose, and ³⁶Cl in sheep lung. *J Appl Physiol* 1975;39:773-779.
28. Kwong MS, Egan EA. Validation of [⁵⁷Co]cyanocobalamin as an extracellular fluid marker and measurements of albumin exclusion from the interstitium in the rabbit. *Microvasc Res* 1985;29:152-160.
29. Kern DF, Levitt D, Wangenstein D. Endothelial albumin permeability measured with a new technique in perfused rabbit lung. *Am J Physiol* 1983;245:H229-H236.