Respiratory Clearance of Aerosolized Radioactive Solutes of Varying Molecular Weight

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To determine the influence of varying molecular weight (mol wt) on respiratory clearance of aerosolized solutes, we studied eight radiopharmaceuticals, each administered to four dogs: sodium ^{99m}Tc pertechnetate (TcO_4), ^{99m}Tc glucoheptonate ([^{99m}Tc]GH), ^{51}Cr-ethylenedinitrotetraacetate ([^{51}Cr]EDTA), ^{99m}Tc diethylenetriaminepentaacetate ([^{99m}Tc]DTPA), ^{111}In diethylenetriaminepentaacetate ([^{111}In]DTPA), ^{67}Ga desferoxaminemesylate ([^{67}Ga]DFOM), ^{99m}Tc dextran ([^{99m}Tc]DX) and ^{111}In transferrin ([^{111}In]TF). After aerosolization (0.8 m MMD, 2.4 GSD), clearance was determined for 30 min and then corrected by intravenous injection for nonairspace radioactivity. In-TF clearance (−0.11 ± 0.10 %/min) was lower than TcO_4 (6.32 ± 0.62 %/min), [^{99m}Tc]GH (1.50 ± 0.37 %/min), [^{51}Cr]EDTA (2.38 ± 1.02 %/min), [^{99m}Tc]DTPA (3.51 ± 0.40 %/min), [^{111}In]DTPA (2.35 ± 0.42 %/min), [^{67}Ga] DFOM (1.99 ± 0.49 %/min) and [^{99m}Tc]DX (1.81 ± 0.75 %/min) clearances ($p < 0.001$). TcO_4 clearance was higher than others ($p < 0.001$). Technetium binding to DX was unsatisfactory; aerosolization caused unbinding from DTPA. We conclude that respiratory clearance of large mol wt solutes within 30 min is negligible and, that clearance of molecules between 347–5,099 daltons differs greatly, suggesting that binding and/or intrapulmonary retention affect transfer.

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During the last few years, considerable effort has been directed toward measuring the rate of removal (clearance) of a micronic sized aerosol of technetium-99m diethylenetriaminepentaacetate ([^{99m}Tc]DTPA) from the lungs. Because the chief barrier to diffusion of solutes from the alveolar surface to the pulmonary capillaries is believed to be the alveolar epithelium (*1*), the results of increases of [^{99m}Tc]DTPA clearance have been interpreted as reflecting increases in respiratory epithelial permeability (*2–6*). But as experience with this method has broadened, even if the results do assess membrane permeability, the method is too nonspecific to be a clinically useful indicator of acute lung injury. For example, equivalent increases in [^{99m}Tc]DTPA clearance have been reported in: (a) normal persons breathing at high lung volumes (*7*); (b) otherwise healthy persons who smoke cigarettes (*2,3,5*); (c) patients with chronic interstitial lung disease (*8–11*); and (d) patients with adult respiratory distress syndrome (*12*). Clearly, there are enormous diagnostic, therapeutic, and prognostic differences among these four conditions that are not reflected in their [^{99m}Tc]DTPA clearance.

We wanted to find some other radiolabeled solute that would be a more suitable marker than [^{99m}Tc] DTPA of the increase in respiratory epithelial permeability that accompanies various acute lung injuries. We began our search by testing the hypothesis, based on previous observations, that there should be a continuum of clearance from alveolus to bloodstream that depends in large part on the molecular weight of the particular solute (*1,8,13,14*). Accordingly, we studied the pulmonary clearance in dogs of eight different ra-

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diolabeled solutes whose molecular weights varied from 163 to 76,111 daltons.

METHODS

Because our goal was to identify a marker of permeability that could be used in future studies of patients, we tested solutes that met certain criteria: (a) they had proved safe when administered to humans; (b) they were commercially available or easily prepared; and (c) they could be conveniently labeled with gamma-emitting isotopes that were suitable for external detection. An identical experimental protocol was used to test each of the eight solutes.

Animal Preparation

Studies were conducted in 32 mongrel dogs that weighed 12.7–32.0 kg. The animals were anesthetized with i.v. pentobarbitol sodium (30 mg/kg body weight), and given small supplementary doses as needed to maintain satisfactory anesthesia. A cuffed endotracheal tube was placed in the trachea, and mechanical ventilation* was begun at a tidal volume of 15 ml/kg and a respiratory frequency of 15 breaths/min. Approximately every 15 min, the animals' lungs were briefly hyperinflated to prevent atelectasis. To ensure normal respiratory function, we monitored airway pressure continuously during the experiment, and we measured arterial Po_2, Pco_2 and pH just before and 30 min after aerosolization. Acceptable values were deemed to be as follows: airway pressure < 9 cm H_2O at the outset and change < 1 cm H_2O during the experiment; Po_2 > 80 mmHg; Pco_2 30–40 mmHg; and pH 7.32–7.50. At the end of the experiment, the thorax was opened so that the right lung could be removed for inspection and measurement of the ratio wet weight to dry weight by methods to be described.

With the animals supine, a scintillation probe with a 3 in sodium iodide crystal† was directed from the right mid-thorax downward toward the right posterior lung field, taking care to avoid the heart. The results of a previous study have shown good correlation (r = 0.95) between respiratory clearance values determined by a portable sodium iodide probe and a gamma camera (*15*). A similar probe was positioned over the right thigh but pointed away from the bladder. Neither probe was collimated, to avoid problems with gamma emitters of different energies, but both were surrounded by a 2-mm malleable layer of lead to decrease emissions not directly beneath the probe. This lead cylinder could be adjusted to create a "seal" between the crystal and the skin of the chest wall or thigh. Another piece of lead was mounted between the two probes to further separate their counting fields. After positioning both probes, the animals were paralyzed with pancuronium bromide (0.06 mg/kg) to facilitate administration of the aerosol and to keep the probe-field relationships constant throughout the experiment.

Radioactive Solutes

We used the eight radioactive solutes whose abbreviations, molecular weights, and amounts added to the nebulizer are listed in Table 1. Technetium-99m was used immediately after generation; the other three isotopes were administered within three half-lives after receipt. The compounds were prepared as follows.

$^{99m}TcO_4$. We eluted $^{99m}TcO_4^-$ from a molybdenum generator‡ as sodium pertechnetate. The isotope was either administered in the uncombined form (TcO_4) or was used to bind to three other solutes as described below.

[^{99m}Tc]GH. To prepare ^{99m}Tc glucoheptonate ([^{99m}Tc]GH), we added oxidant-free $^{99m}TcO_4^-$ to an oxygen-free vial containing 200 mg lyophilized glucoheptonate sodium, 0.07 mg of tin and 0.06 mg of stannous chloride.‡

[^{51}Cr]EDTA. We used commercially produced chromium-51 ethylenedinitrotetraacetate which had a pH of 5.0.

[^{99m}Tc]DTPA. We prepared ^{99m}Tc diethylenetriaminepentaacetate by adding $^{99m}TcO_4^-$ to an oxygen-free kit¶ containing 4 mg of sodium salt of DTPA and 0.25 mg of stannous chloride.

[^{111}In]DTPA. We used commercially available [^{111}In] DTPA¶ which is an isotonic, aqueous solution buffered to pH 7–8.

[^{67}Ga]DFOM. We prepared gallium-67 desferoxaminemesylate by adding [^{67}Ga]citrate¶ to a vial containing 4 mg of desferoxamine mesylate.**

[^{99m}Tc]DX. To an oxygen-free vial containing 20 mg dextran (mol wt 5,000) and 5 mg stannous chloride, we added $^{99m}TcO_4$ as described by Henze et al. (*16*).

TABLE 1
Abbreviations, Molecular Weights, Amounts Aerosolized, and Initial Chest Probe Counts of Eight Radiolabled Solutes Used to Measure Respiratory Clearance

Solute	Abbreviation	Mol wt (daltons)	Amount (mCi)	Initial counts (counts/min ± 1 s.d.)
[^{99m}Tc]pertechnetate	[^{99m}Tc]O_4	163	5	434,511 ± 22,209
[^{99m}Tc]glucoheptonate	[^{99m}Tc]GH	347*	5	850,120 ± 126,842
[^{51}Cr]diethylenedinitro tetraacetate	[^{51}Cr]EDTA	372	2.5	1431 ± 216
[^{99m}Tc]diethylenetriamine pentaacetate	[^{99m}Tc]DTPA	492†	5	428,866 ± 470,090
[^{111}In]diethylenetriamine pentaacetate	[^{111}In]DTPA	504	1	8,475 ± 1,771
[^{67}Ga]desferoxamine mesylate	[^{67}Ga]DFOM	624	2	66,510 ± 28,838
[^{99m}Tc]dextran	[^{99m}Tc]DX	5,099	5	10,310 ± 2,162
[^{111}In]transferrin	[^{111}In]TF	76,111	1	33,401 ± 12,547

* [^{99m}Tc]GH may also exist as a dimer (*32*).
† [^{99m}Tc]DTPA may also exist in other states.

[^{111}In]TF. We made indium-111 transferrin ([^{111}In]TF) by adding [^{111}In]chloride†† to a vial containing 2 mg transferrin‡‡ buffered to pH 7 with 2 ml of 0.1 *M* sodium bicarbonate.

Aerosol Generation and Administration

Before each experiment, the aerosol delivery system (Fig. 1) was carefully checked to ensure there were no leaks. To the various radioactive solutes, prepared as described above, we added sufficient 0.9% saline to make a final volume of 4–5 ml and placed the solution in the jet nebulizer with an attached particle separator§§ and then to a 1-l plastic reservoir bag. The aerosol was generated by compressed air (6 l/min) and delivered to the animal by manually compressing the 3-l anesthesia bag in the inspiratory circuit. The recoil of the anesthesia bag permitted it to fill between compressions with aerosol from the aerosol generator and reservoir bag. One-way valves and a mushroom expiratory valve directed the aerosol either to the animals' lungs or to a filter trap.¶¶ All components of the system, except the anesthesia bag, were lead-shielded. To compress the bag, the operator wore lead gloves. After administering the aerosol, we reconnected the animal to the ventilator and the nebulizer, bags, tubing, and filter trap were removed from the room.

The size distribution of particles generated by this system was determined using a previously calibrated 7 stage Mercer cascade impactor*** (*17*). Aerosol samples were collected through a T-connector at the output of the aerosol generator. After analyzing the radioactivity on each glass plate and using log-probit plots, we calculated the mass median aerodynamic diameter 0.79 ± 0.06 μm, and the geometric s.d. as 2.4 ± 0.08 (*18*).

Respiratory Clearance

We administered the aerosol for 2 to 4 min, stopping delivery after sufficient radioactive counts were detected by the lung probe to ensure statistical accuracy (see initial count rate, Table 1). Thereafter, counts from both probes were obtained during successive 1-min intervals for the two 30-min segments of each experiment and stored separately. Before any calculations, all counts were corrected for radionuclide decay of the particular isotope, and the counts detected by the thigh probe were adjusted by back extrapolation for the radioactivity in the counting field contributed by the strong signal from the lungs. To calculate respiratory clearance, radioactive counts from only the lung probe during the first 30 min were plotted semilogarithmically against time; clearance is the negative slope of the regression line of these values and is expressed in percent per min (%/min). We call this value the "uncorrected" respiratory clearance because it is not adjusted for the increasing contribution of circulating and tissue radioactivity to the total counts detected within the lung field during the experiment.

To compare the respiratory clearances of the eight different radiopharmaceuticals, independent of their rates of accumulation in tissues and blood, we calculated "corrected" clearance according to the method of Jones et al. (*2*). Thirty minutes after the end of aerosolization, an aliquot of the radioactive solute being studied (0.15 mCi ^{99m}Tc, 0.07 mCi ^{51}Cr, 0.03 mCi ^{111}In or 0.06 mCi ^{67}Ga) was injected intravenously. This produced an increase in signal in both the lung and thigh probes proportional to the amount of radioactive solute in the nonairspace zones in the two detector fields. Counts were again obtained for the next 30 min, and the ratio of the increase in count rate in the lung field to the increase in count rate in the thigh field after equilibration was calculated. For each minute during the first 30 min after the end of aerosolization of the solute, the thigh count was multiplied by this ratio and the product subtracted from the lung count. The corrected lung counts were then used to construct a semilogarithmic regression line and respiratory clearance was calcu-

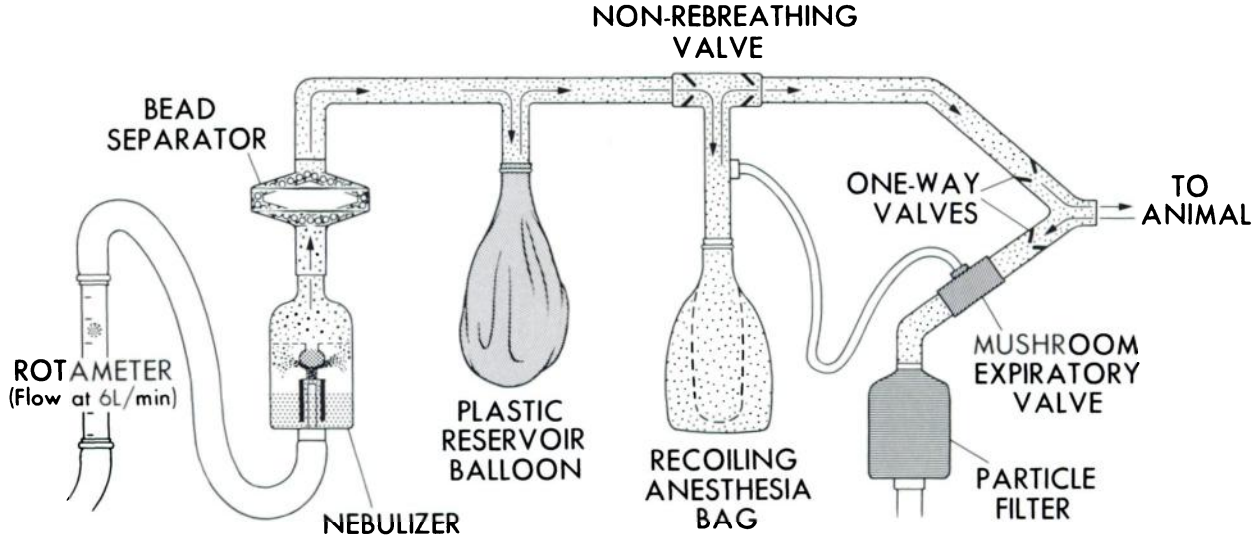

FIGURE 1
Schematic diagram of aerosol generator and delivery system. From pressurized air source, air flows through nebulizer at 6 l/min; aerosolized droplets pass through bead separator to reduce particle size, then are temporarily stored in a plastic balloon. While the aerosol generator is in action, the aerosol is delivered by manually compressing a recoiling anesthesia bag which pumps aerosol through a rebreathing valve into the dog's lungs. During inspiration, expiratory limb from animal is closed by mushroom expiratory valve actuated by pressure on anesthesia bag. During exhalation, undeposited aerosol is collected in particle filter.

lated as described for the uncorrected value. Signals from the probes were amplified and energy windows optimized for each isotope,[†††] and the data were stored in and the calculations were performed by a PDP 11-23 computer.[‡‡‡]

Binding

To assess the binding of ^{99m}Tc with ^{99m}Tc-labeled compounds before and after aerosolization, we took samples of the solute from the reservoir of the nebulizer before and immediately after 5 min of aerosolization; the latter sample represents a collection of liquid that had been aerosolized and remained in the reservoir and proximal tubing. Electrophoresis was performed to determine binding of $^{99m}TcO_4^-$ with glucoheptonate, diethylenetriaminepentaacetate and dextran by a modification of the method of Baker et al. (*19*). In a nitrogen environment, aliquots of $^{99m}TcO_4^-$ and ^{99m}Tc-labeled solutions were applied on cellulose acetate plates[§§§] and placed in a chamber. Electrophoresis was performed at 500 V for 9–11 min, using a buffer of HEPES adjusted to a pH of 8.5. The plates were cut into 5 mm lengths and counted in a well-type gamma counter.[¶¶¶] The activity of each segment was plotted against distance of migration, and the percent of ^{99m}Tc binding was defined as the number of counts in the peak of the ligand divided by the sum of counts in the peaks of $^{99m}TcO_4^-$ pertechnetate and Tc-ligand.

Liposolubility

We determined the lipid/water partition coefficient of all eight radioactive solutes by allowing each compound to distribute itself in equal volumes of saline and oil. After the solute was diluted in 2 ml saline, 2 ml soybean oil was added and the mixture agitated for 10 min. Then the water and lipid phases were allowed to separate for 1 hr at room temperature. One milliliter of each phase was carefully collected and counted. Four determinations were made for each solute; the results are expressed as percent of counts in oil relative to the counts in saline, and percent of total counts in water.

Gravimetric Lung Measurement

The extravascular water volume and the dry weight of the right lung were measured by a modification of the method of Pearce et al. (*20*). In most experiments, we used ^{51}Cr-tagged red blood cells to measure blood content, but when [^{51}Cr]EDTA was aerosolized, we used hemoglobin instead; details of both methods have been reported previously (*21,22*). Briefly, after the animal was killed, the chest was quickly opened, the hila of both lungs were clamped, and the right lung was removed. The cut surface of the lung and the airways were examined for the presence of pulmonary edema and other disease. The hilar structures were removed, and the lung was weighed, homogenized with added water in a high-speed blender, and then reweighed. Antemortem blood and lung homogenate were stored in a lead-lined freezer until the activity of the radioisotope used to label the solute was nearly null; then, ^{51}Cr activity was measured in both specimens, using a gamma counter. When ^{51}Cr had been aerosolized, the hemoglobin concentration and hematocrit ratio were measured in the supernatant fluid of centrifuged lung homogenate and blood. Weighed samples of blood and lung homogenate were desiccated in an oven at 65°C until no weight change occurred on two weighings 24 hr apart; this usually required 72 hr. The ratio of wet weight to dry weight was used to derive the percentage of water in the blood and lung homogenate, and to calculate extravascular lung water content.

Statistical Analysis

Results are expressed as mean ± 1 s.d. Between group comparisons were done by analysis of variance and means were compared by the Newman-Keuls test when the F statistic was significant; uncorrected and corrected respiratory clearance values and pre- and postaerosolization values were compared by the paired Student's t-test (*23*). We considered a p value <0.05 as significant.

RESULTS

Means and s.d. of uncorrected and corrected respiratory clearances of each solute, and the results of comparisons between these values are shown in Table 2. Analysis of variance demonstrated a significant effect of solute on clearance ($F7/24 = 13.83$; $p < 0.001$). The results of comparisons among the means of the various solutes are shown in Table 3. A plot of respiratory clearance according to the molecular weight of each aerosolized solute is shown in Figure 2.

Typical electrophoresis tracings are illustrated in Figure 3. As shown in Table 4, aerosolization did not affect binding of [^{99m}Tc]GH or [^{99m}Tc]DX, but did affect that of [^{99m}Tc]DTPA. The oil/water partition coefficients of each solute are shown in Table 5. Analysis of variance revealed no differences among these values.

DISCUSSION

We have shown that the rate of clearance from the lungs into the bloodstream of an aerosolized low mo-

TABLE 2
Means (%/min), s.d.s, and Results of Statistical Comparisons of Uncorrected and Corrected Respiratory Epithelium Clearances of Aerosolized Solutes of Various Molecular Weight*

		$^{99m}TcO_4$	[^{99m}Tc]GH	[^{51}Cr]EDTA	[^{99m}Tc]DTPA	[^{111}In]DTPA	[^{67}Ga]DFOM	[^{99m}Tc]DX	[^{111}In]TF
Uncorrected	$\bar{x}$	3.40	1.30	1.39	2.97	1.89	1.32	1.34	−0.11
	s.d.	0.38	0.41	0.47	0.17	0.33	0.27	0.47	0.09
Corrected	$\bar{x}$	6.32	1.50	2.38	3.51	2.35	1.99	1.81	−0.11
	s.d.	0.62	0.37	1.02	0.40	0.42	0.49	0.75	0.10
p		<0.001	<0.01	<0.05	<0.05	<0.01	<0.025	N.S.	N.S.

* Abbreviations as defined in Table 1

TABLE 3
Statistical Comparisons Among Respiratory Clearance Values Obtained with Different Solutes*

	$^{99m}TcO_4$	[^{99m}Tc]GH	[^{51}Cr]EDTA	[^{99m}Tc]DTPA	[^{111}In]DTPA	[^{67}Ga]DFOM	[^{99m}Tc]DX	[^{111}In]TF
[^{111}In]TF	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	−0.11†
[^{99m}Tc]DX	<0.001	N.S.	N.S.	<0.001	N.S.	N.S.	1.81	
[^{67}Ga]DFOM	<0.001	N.S.	N.S.	<0.005	N.S.	1.99		
[^{111}In]DTPA	<0.001	<0.05	N.S.	<0.01	2.35			
[^{99m}Tc]DTPA	<0.001	<0.001	<0.025	3.51				
[^{51}Cr]EDTA	<0.001	<0.05	2.38					
[^{99m}Tc]GH	<0.001	1.50						
$^{99m}TcO_4^-$	6.32							

* Abbreviations as defined in Table 1.
† Underlined values are means (%/min).

lecular weight solute, TcO_4 (163 daltons), is considerably faster than that of a high molecular weight solute, [^{111}In]TF (76,111 daltons), which did not appear to clear at all during the 30-min period of study. In contrast, the respiratory clearances of six other solutes with molecular weights ranging from 347 to 5,099 daltons, although lying in between the fastest and slowest values obtained with TcO_4 and [^{111}In]TF, varied with respect to each other and were not uniquely related to molecular weight.

Other investigators who have studied the rate of transfer of solutes from air spaces to blood or vice versa, usually in fluid-filled lungs, have observed, for the most part, an inverse relationship between clearance and molecular weight. For example, Taylor et al. (*14*) reported that the pulmonary membrane was selectively permeable to different solutes: transfer of urea (60 daltons) was seven times greater than transfer of glucose (180 daltons). In subsequent experiments using urea, glucose and sucrose (342 daltons), Taylor and Gaar (*1*) concluded that small molecules moved across the endothelial-epithelial barrier more rapidly than large molecular weight solutes. Similarly, Enna and Schanker (*13*) found that absorption rates from lung to blood usually ranked in inverse order to the molecular weights of the compounds they studied, which ranged from 60 daltons (urea) to 75,000 daltons (dextran). Finally, Theodore et al. (*24*) documented decreasing rates of transfer from plasma to alveolar liquid of urea (60 daltons), sucrose (342 daltons), insulin (5,000 daltons) and dextran (60,000 to 90,000 daltons). Thus, the general correspondence noted by all these investigators between increasing molecular weight and decreasing rate of transfer between airspaces and bloodstream suggests that, in our study, factors other than molecular weight probably affected respiratory clearance of the solutes we tested.

Technical Factors

One obvious difference between our study and all of those cited in the preceding paragraph is that we ad-

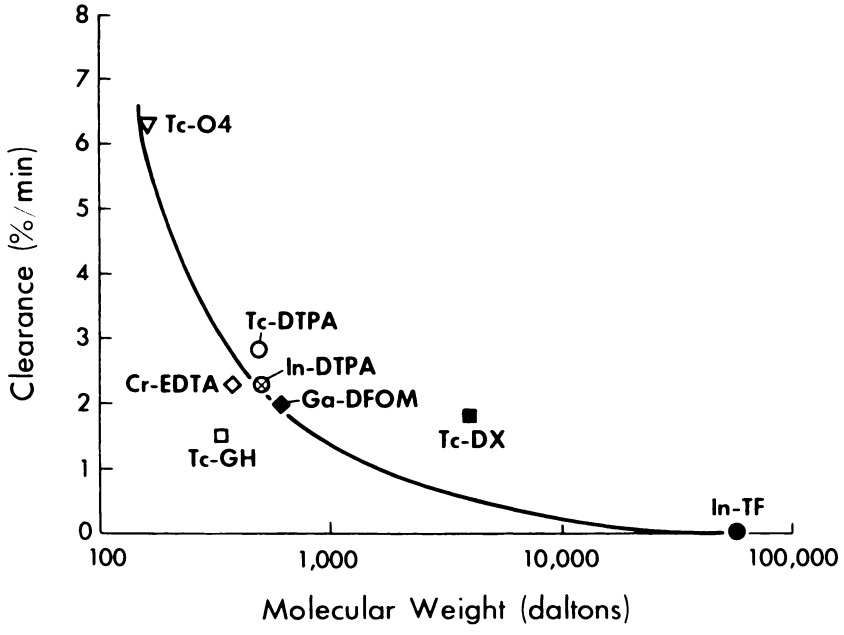

FIGURE 2
Mean values (%/min) of the respiratory clearance of eight solutes according to their molecular weight (daltons). Solid line is line of best fit.

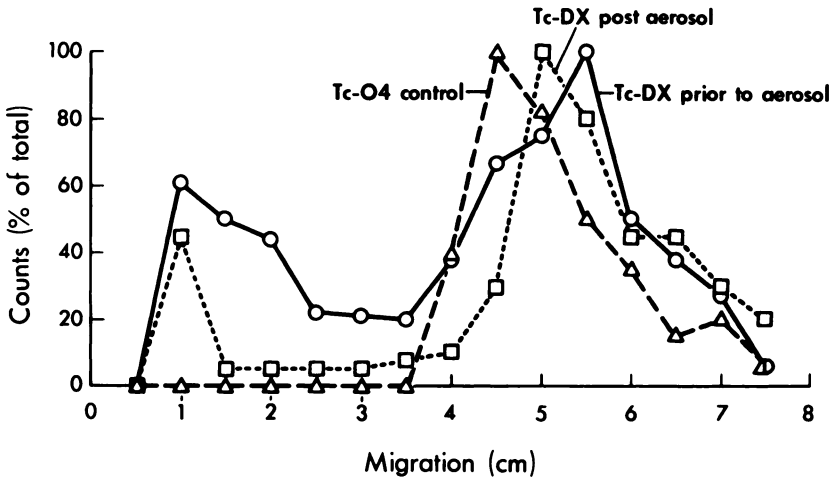

FIGURE 3
Plots of cpm from 5-mm strips versus migration toward the cathode obtained from electrophoresis of solution containing TcO_4^- and [^{99m}Tc]DX; unbound TcO_4^- in solution could be recognized as it migrated under the 5 cm peak, and bound [^{99m}Tc]DX under the 1-cm peak.

ministered the solutes by aerosolization, not by instillation or i.v. injection. Because we used a small particle generating system (0.8 μm mass median aerodynamic behavior), the bulk of the solute should have deposited chiefly on the respiratory epithelium (*25*); previous physiological observations have indicated that clearance of aerosolized particles about the size we administered is indeed from alveolar surface to pulmonary capillary blood (*26*). Moreover, we used the same aerosol generating system in each experiment so there is no reason to believe that differences in the site of deposition of the solutes account for the differences in respiratory clearances that we observed.

Increases in lung volume (*7,9,26*) and various types of lung disease (*9,10,12*) increase respiratory clearance of [^{99m}Tc]DTPA. To avoid these possibilities, we were careful to keep the mechanical ventilation settings, including tidal volume per kg ratio, constant in all experiments, and we ensured that the right lung, the one over which we counted, was normal as assessed by serial studies of arterial Po_2, Pco_2, and pH and inflation pressure, gross inspection of the lung at postmortem examination, and measurement of wet weight to dry weight ratios.

Correction for Nonairspace Activity

Before comparing respiratory clearance values of the eight different solutes, we corrected the counts obtained from the lung probe during the first 30 min for the steadily increasing counts contributed by nonairspace radioactivity, as detected by the thigh probe; the corrected and uncorrected clearances are shown in Table 2. The effect of the correction process on clearance varies, depending on the rate of transfer out of the airspaces and on the subsequent site of accumulation and volume of distribution of the particular solute. In general, the faster the clearance and the smaller the volume of distribution, the greater the difference between corrected and uncorrected values. Indium-111TF did not cross the respiratory membranes so its clearance was not changed by the correction; on the other hand, the corrected mean clearance of TcO_4, which crossed the alveolo-capillary membrane rapidly, was 86% faster than the uncorrected value. In addition, the solutes we used are known to equilibrate in varying volumes of distribution: [^{99m}Tc]DX probably remained mainly in the bloodstream during the first 30 min (*16*), [^{99m}Tc]DTPA and [^{111}In]DTPA equilibrated in the extracellular space (*4*), and TcO_4 diluted in an even larger volume (*27*). But it is clear that the correction procedure per se cannot account for the apparent inconsistencies in solute clearance that we observed.

Binding

At the outset of our study, we considered that, of all the factors that might affect respiratory clearance of the

TABLE 4
Means (%) and s.d. of ^{99m}Tc-Binding of [^{99m}Tc]GH, [^{99m}Tc]DTPA and [^{99m}Tc]DX Before and After Aerosolization

	[^{99m}Tc]GH	[^{99m}Tc]DTPA	[^{99m}Tc]DX
Before aerosolization	93 ± 6	96 ± 7	37 ± 16
After aerosolization	89 ± 6	67 ± 22*	37 ± 23

*$p < 0.05$.

TABLE 5
Oil/Water Partition Coefficients (P) of Various Solutes* (n = 4)

Solute	P, $\bar{x}$ ± s.d.	log P	% in water
$^{99m}TcO_4$	0.052 ± 0.021	−1.28	95.1
[^{99m}Tc]GH	0.0041 ± 0.003	−2.38	99.6
[^{51}Cr]EDTA	0.00438 ± 0.0018	−2.36	99.6
[^{99m}Tc]DTPA	0.014 ± 0.0005	−1.85	98.6
[^{111}In]DTPA	0.0135 ± 0.0078	−1.87	98.7
[^{67}Ga]DFOM	0.023 ± 0.0017	−1.64	99.8
[^{99m}Tc]DX	0.0029 ± 0.002	−2.54	99.7
[^{111}In]TF	0.013 ± 0.0012	−1.88	98.7

*Abbreviations are defined in Table 1.

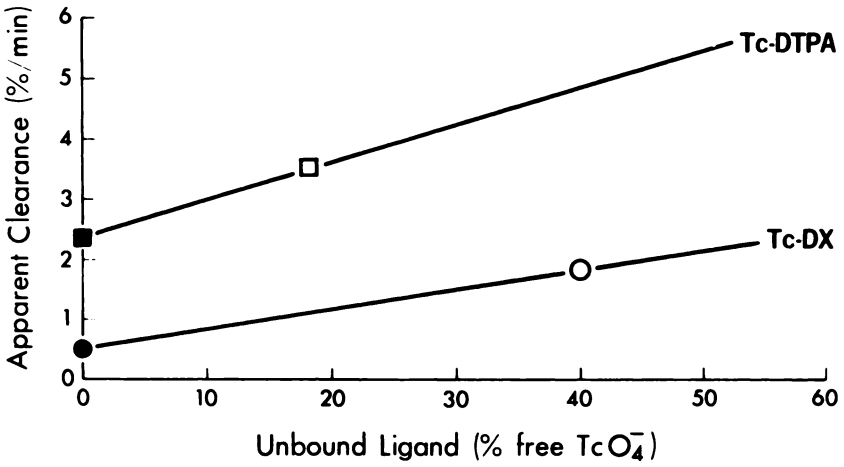

FIGURE 4
Plot of calculated apparent respiratory epithelium clearance of [^{99m}Tc]DTPA (top line) and of [^{99m}Tc]DX (bottom line) against the percentage of free TcO_4^-. Apparent clearances were calculated using the sum of decreasing activities of free TcO_4^- and [^{99m}Tc]DTPA or [^{99m}Tc]DX, for various ratios of free TcO_4^-. Observed clearances (○, □) and "real" clearances (●, ■ = no free [^{99m}Tc]O_4) are shown.

eight solutes, the adequacy of binding seemed to be the most likely problem, particularly of those labeled with ^{99m}Tc. Accordingly, we designed our study to include experiments on the completeness of the bond between ^{99m}Tc and the three ligands with which it was combined.

The reason for suspecting ^{99m}Tc is that its binding is susceptible to oxidation. This is seldom a problem when [^{99m}Tc]DTPA or other ^{99m}Tc-labeled compounds are prepared in the usual way in the presence of a reducing agent (tin) and a nitrogen environment, and then injected intravenously or into a body cavity. But oxidation seems especially likely during aerosolization, and might occur after deposition of a ^{99m}Tc-compound in the oxygen-rich environment of the lungs. Unfortunately, following aerosolization, aliquots of serum small enough to perform thin layer chromatography or electrophoresis had inadequate counts to distinguish breakdown products from background. Urine specimens would have given misleading data because TcO_4 has a different volume of distribution than [^{99m}Tc]DX, [^{99m}Tc]GH and [^{99m}Tc]DTPA, and is slowly cleared by the kidneys. The one place that we could obtain adequate samples for testing of ^{99m}Tc-binding was the nebulizer before and after aerosolization. This approach does not assay the effects of oxidation, which takes place during transit from the nebulizer to the moment of entry into the bloodstream, changes which might affect the rate of clearance.

We found that ^{99m}Tc was not completely bound with ligand before aerosolization (93% for [^{99m}Tc]GH, 96% for [^{99m}Tc]DTPA and 37% for [^{99m}Tc]DX) and this became even worse after aerosolization (89% for [^{99m}Tc]GH, 67% for [^{99m}Tc]DTPA and 37% for [^{99m}Tc]DX), although only the change in [^{99m}Tc]DTPA was significant. The more striking observation was that binding was quite unpredictable. Waldman et al. (*28*) reported extensive unbinding of [^{99m}Tc]DTPA after aerosolization in an ultrasonic nebulizer but not in a jet nebulizer. In the studies of Saha and Boyd (*29*) the binding of [^{99m}Tc]GH (85.5%) and [^{99m}Tc]DTPA (95.2%) was similar to our preaerosolization values.

The extent of unbinding, as shown in Figure 4, can explain the increased clearances of [^{99m}Tc]DTPA and [^{99m}Tc]DX relative to the values predicted from their molecular weights. To construct this graph, which shows the effects on calculated clearance of increasing percentages of TcO_4 in a mixture of [^{99m}Tc]DTPA or [^{99m}Tc]DX, we have assumed that the "real" clearance of TcO_4 is 6.32%/min (measured value), that the "real" clearance of [^{99m}Tc]DTPA is 2.35%/min (the same as [^{111}In]DTPA), and that the "real" clearance of [^{99m}Tc]DX is 0.5%/min (interpolated value from Figure 2). To account for the *observed* clearances of [^{99m}Tc]DTPA (3.51%/min) and [^{99m}Tc]DX (1.81%/min), the graph predicts the presence of 18% and 40% free TcO_4, respectively; these percentages are in the ranges of those actually found in the nebulizer after aerosolization (Table 4).

We have based this analysis on the results of the electrophoretic studies, which suggest that TcO_4 and the intact radiopharmaceutical are the principal two compounds involved. However, because technetium has eight different states of oxidation and may form numerous compounds, there may be many different products involved, each with its own rate of clearance. The composite clearance of all these substances does not necessarily produce a multiphase curve, but can result in a straight line such as we regularly observed (after correction).

Another apparent outlier in the continuum of values of respiratory clearance versus molecular weight was [^{99m}Tc]GH. Because this compound was admixed with substantial amount of TcO_4, a higher than predicted clearance might have been anticipated, as just discussed. Remarkably, respiratory clearance of [^{99m}Tc]GH (1.50 %/min) was slower than that of [^{51}Cr]EDTA, a substance of similar molecular weight. We believe that this phenomenon is best explained by retention of [^{99m}Tc]GH within the lungs, as described below.

Significant unbinding is not likely to have occurred with the other radiopharmaceuticals we tested. This conclusion is based on the intensity of binding, which

is reflected in their log stability constants: [^{51}Cr]EDTA = 10^{23} (*30*); [^{111}In]DTPA = 10^{29} (*31*); [^{111}In]TF = 10^{30} (*31*); and [^{67}Ga]DFOM = 10^{20} (*31*). For comparison, the log stability constant of [^{99m}Tc]DTPA is 10^{17} and that of [^{99m}Tc]GH is 10^{16} (*32*).

Uptake Within the Lungs

Uptake of the radiopharmaceutical or any radioactive part of it, either by proteins or by cells, will cause retention of the solute within the lungs that, in turn, will decrease the measured clearance. As stated, we believe this is the explanation for the slow (relative to [^{51}Cr]EDTA) clearance of [^{99m}Tc]GH. If TcO_4 were the component retained in the lungs, the same phenomenon should have occurred with [^{99m}Tc]DTPA and [^{99m}Tc]DX, which were also admixed with considerable TcO_4. However, respiratory clearance of [^{99m}Tc]GH appeared relatively slow whereas clearances of [^{99m}Tc]DTPA and [^{99m}Tc]DX appeared relatively fast. Because unbinding and accumulation within the lungs of TcO_4 is not a uniformly satisfactory explanation, we postulate that all or part of [^{99m}Tc]GH itself is retained within the lungs. Technetium-99m GH is known to be taken up by the kidneys (*33*) and by brain tumors (*34*). Recently, it was reported that not only is [^{99m}Tc]GH also concentrated in lung cancers, but in a variety of pulmonary inflammatory diseases as well (*35*); whether this is a unique property of [^{99m}Tc]GH is unknown. The mechanism of [^{99m}Tc]GH uptake is not well established, but transcellular transport as an analog of glucose has been suggested; this would provide a straightforward explanation for our findings.

It should also be noted that indium and gallium radiopharmaceuticals will exchange their metals to transferrin unless the metal is bound with a log stability $>10^{30}$ (*31*). Because transferrin may be found in macrophages in both the interstitium and alveolar lumen (*36*), the possibility exists that some exchange between transferrin and [^{111}In]DTPA, [^{67}Ga]DFOM or [^{111}In]TF might have occurred with retention of radioactivity within the dogs' lungs. We do not know to what extent intrapulmonary retention might have retarded the clearances of these three radiopharmaceuticals. However, there are three reasons to believe this effect was probably negligible: (a) because of the high binding constants of the parent compounds; (b) because the clearances of [^{111}In]DTPA and [^{67}Ga]DFOM were consistent with their molecular weights relative to each other and to [^{51}Cr]EDTA; and (c) because previous studies of pulmonary clearance of aerosolized ^{131}I-labeled human serum albumin, whose molecular weight approaches that of [^{111}In]TF, showed extremely slow removal after deposition on nonciliated portions of the respiratory epithelium (*37*).

Liposolubility

We studied the liposolubility of all eight solutes because the results of previous aerosol studies had shown much more rapid transfer into the bloodstream of a lipophilic solute, [^{125}I]antipyrine, compared to a hydrophilic solute, [^{99m}Tc]DTPA (*4*). Comparable results were obtained after instillation of a liquid bolus containing lipophilic and hydrophilic solutes into the lungs (*38*). In contrast, Waldeman and Weber (*39*) noted slower respiratory clearances of three lipophilic HIDA derivatives compared to TcO_4 and [^{99m}Tc]DTPA. However, given the small differences in oil/water partition among the eight solutes that we observed, it is unlikely that liposolubility had an important effect on respiratory clearance. The increased oil/water partition coefficient of TcO_4, relative to the other solutes, may have had a slight accelerating effect on the transfer of this already rapidly moving radionuclide.

In conclusion, our study demonstrates that several factors influence the rate of respiratory clearance of aerosolized radiopharmaceuticals. Low molecular weight solutes cross the respiratory membrane faster than high molecular weight solutes. However, within a relatively narrow range of molecular weights, the physicochemical properties of a particular radiolabeled solute affect its rate of clearance. Among these factors, binding is critical, especially with ^{99m}Tc-labeled radiopharmaceuticals such as [^{99m}Tc]DTPA and [^{99m}Tc]DX. Uptake of radioactivity by cells with retention in the lungs appears likely for solutes such as [^{99m}Tc]GH, but this has not been conclusively established. Of the eight solutes that we studied, liposolubility probably did not have a major effect on their rates of respiratory clearance.

NOTES

* Harvard Manufacturing, Dover, MA.
† Harshaw, Solon, OH.
‡ DuPont Company, No. Billerica, MA.
§ ICN, Irvine, CA.
¶ Medi-Physics, Richmond, CA.
** CIBA, Summit, NJ.
†† Mallinckrodt, St. Louis, MO.
‡‡ Sigma Chemical Corp., St. Louis, MO.
§§ Venticis, Syncor Intl., Sylmar, CA.
¶¶ Main-Flow Filter, Bourns, Riverside, CA.
*** Intox, Albuquerque, NM.
††† Berkeley Nucleonics Corp., Berkeley, CA.
‡‡‡ Digital Equipment Corporation, Maynard, MA.
§§§ Helana 1283, Helana, Beaumont, TX.
¶¶¶ Beckman 5500, Beckman Co., Irvine, CA.

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