Chemical Breakdown of Technetium-99m DTPA During Nebulization

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Aerosols of ^{99m}Tc diethylenetriaminepentaacetic acid ([^{99m}Tc]DTPA) used for measuring lung permeability and lung ventilation require a radioaerosol delivery system to produce an aerosol with reproducible size and radiochemical purity. To test how well nebulizers meet this requirement, radiochemical purity of aerosols produced with a jet and an ultrasonic nebulizer was evaluated. The activity median aerodynamic diameter (AMAD) and geometric standard deviation (σ_g) of radioaerosols were 0.46 μm (σ_g = 1.6) for the jet nebulizer and 0.70 μm (σ_g = 1.7) for the ultrasonic nebulizer. Paper and liquid chromatographic assays were obtained on the [^{99m}Tc]DTPA aerosol solute produced with each nebulizer. The results of these tests showed major differences in radiochemical purity. Aerosols produced in the jet nebulizer consistently showed greater than 90% of the radioactivity bound to the DTPA ligand whereas aerosols produced in the ultrasonic nebulizer showed <10% of the radioactivity bound to DTPA. The results support the need to test radiochemical purity of aerosols before using an aerosol nebulizer for pulmonary imaging and clearance studies.

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Inhaled radioaerosols are commonly used to determine the distribution of ventilation in the lungs and to obtain an index of lung permeability (*1–3*). The diagnostic utility of these radioaerosols requires adequate alveolar deposition and a stable bond between the radioisotope and ligand. Aerosol deposition is influenced by particle size and geometric standard deviation (*4,5*). Increasing particle size and geometric standard deviation increases large airway deposition (*6*). Particles >3 μm deposit in large ciliated airways. If an aerosol solute disassociates during aerosol generation or after deposition in the lung, the rate of clearance from the lungs and the calculated index of permeability would be altered. For example, technetium-99m diethylenetriaminepentaacetic acid ([^{99m}Tc]DTPA) is cleared from normal human lungs with a half-time ($t_{½}$) of between 60 to 80 min (*4,5*). If the compound disassociates to ^{99m}Tc 0_4^- and DTPA, the ^{99m}Tc 0_4^- fraction will leave the lung with a $t_{½}$ on the order of a few minutes (*6*).

Radioaerosols are commonly produced by jet or ultrasonic nebulizers. The majority of nuclear medicine departments use jet nebulizers because of their lower cost and generally simpler operation. Aerosol production, however, is significantly more efficient with the ultrasonic nebulizer. This increased efficiency increases aerosol concentration and reduces aerosol administration time. Furthermore, aerosol production from the ultrasonic nebulizer is independent of airflow. Control over aerosol delivery is achieved by changing the flow through the ultrasonic nebulizer or by adding diluting air. The advantages of ultrasonic nebulizers over jet nebulizers led to this study. Aerosol particle size and radiochemical purity from each nebulizer was studied.

To examine the effect of aerosolization on the stability of [^{99m}Tc]DTPA, we compared the radiochemical purity of [^{99m}Tc]DTPA solute before and after aerosolization using paper and liquid chromatographic assays. To determine s.e.e. if aerosolized [^{99m}Tc]DTPA disassociates after deposition in the lungs or clearance from the lungs, blood, and urine samples from five female beagle dogs that inhaled aerosols from both types of nebulizers were tested.

MATERIALS AND METHODS

Radiopharmaceutical

The [^{99m}Tc]DTPA used for this investigation was prepared from a commercially supplied kit.* Each vial contained 10 mg

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$CaNa_3$ DTPA and 0.50 mg $SnCl_2 \cdot 2H_2O$. The solute for the nebulizers was prepared by adding 50 mCi (1.85 GBq) of pertechnetate in 5.0 ml of physiological saline to the vial.

Aerosol Production

Aerosol was generated using an ultrasonic[†] and a jet nebulizer.[‡] The ultrasonic nebulizer was tested with the ultrasonic coupling fluid at room temperature and cooled with an ice bath. A Mercer seven-stage cascade impactor attached directly to the output of each nebulizer determined the AMAD and σ_g of the aerosol (*7*). The size distribution of the aerosol was determined by passing the aerosol through a series of seven consecutive stages in the impactor designed to deposit progressively smaller particles. Using logarithmic probability graph paper, the cumulative activity distribution is plotted against the effective cutoff diameter of each stage. The AMAD is the particle size at 50% of the total cumulative activity. The σ_g measures the polydispersity of the aerosol and is the particle size at 50% of the cumulative activity divided by the particle size at 15.9% of the cumulative activity or the particle size at 84.1% of the total cumulative activity divided by the particle size at 50% of the total cumulative activity.

Chromatography

Radiochemical purity was assayed using paper and liquid column chromatographic techniques (*8,9*). Strips of Whatman No. 3 chromatographic paper, 10 cm in length, were spotted with the radiopharmaceutical and developed in acetone until the solvent front reached a height of 10 cm. Each strip was cut into 1-cm pieces and counted in a NaI(Tl) well-type scintillation counter to determine the R_f profile of the material being tested. R_f equals the distance the activity peak has moved from the origin, divided by the distance the solvent front has moved from the origin. For liquid chromatography, aliquots of the radiopharmaceutical were loaded on a 25 cm G-25 Sephadex column and eluted with physiologic saline. Consecutive 1-ml samples of eluate were collected for NaI(Tl) scintillation detector counting. Standards were run to identify the eluate fraction containing [^{99m}Tc] 0_4^- and [^{99m}Tc]DTPA.

Radiochemical purity was determined at three different times during the aerosol study; as stock solution prior to nebulization; as an aerosol at the inhalation valve prior to administration; at the time of the appearance in blood and urine following aerosol administration. The aerosol solute was assayed by collecting the aerosol prior to inhalation on a Mercer seven stage cascade impacter. The coverslip of each impactor stage was rinsed in physiologic saline and assayed for radiochemical purity.

Animal Studies

Five female beagle dogs weighing 8.5 to 12.3 kg were studied twice with each nebulizer. Two additional studies were made

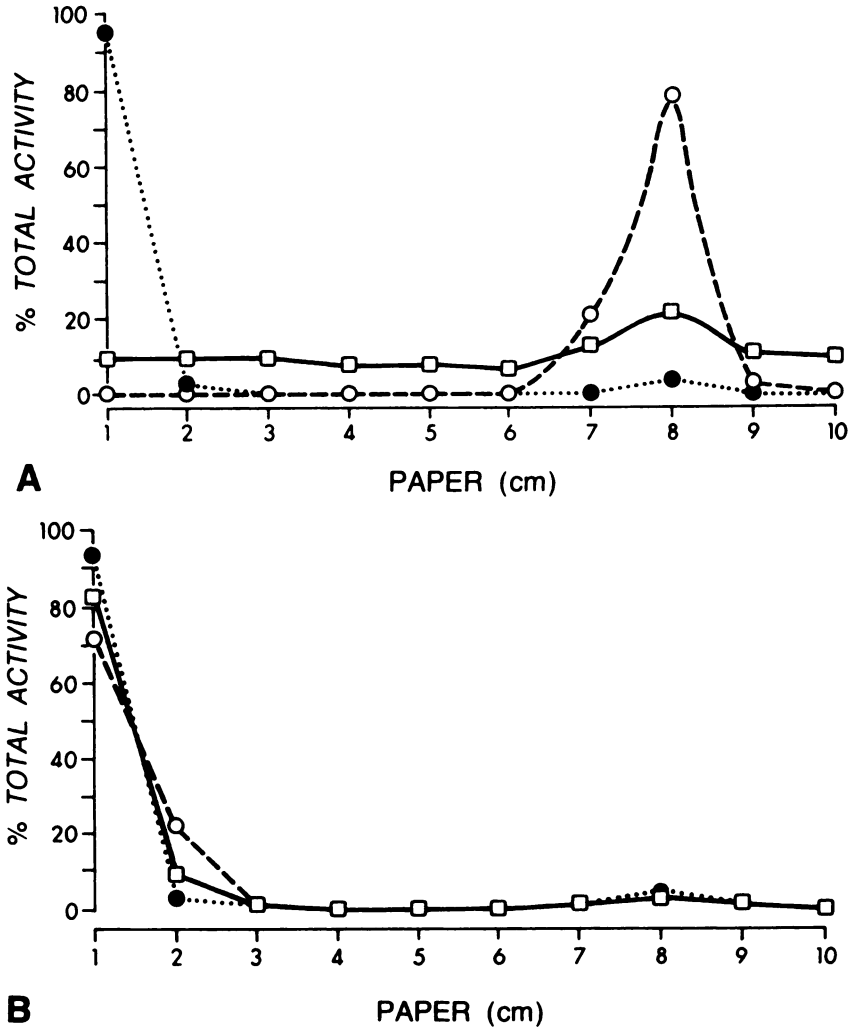


FIGURE 1
A: [^{99m}Tc]DTPA after ultrasonic nebulization. Paper chromatographic profiles of [^{99m}Tc]DTPA stock solution (control) (●), a $^{99m}TcO_4^-$ standard (○), and the ^{99m}Tc aerosol solute (□). The aerosol solute produced with the ultrasonic nebulizer shows no R_f peak at values corresponding to either [^{99m}Tc]DTPA or $^{99m}TcO_4^-$. B: Paper chromatographic profiles of [^{99m}Tc]DTPA stock solution (control) (●), aerosol particles <1.5 μ (□), and aerosol particles >1.5 μ (○). Aerosols produced with a jet nebulizer showed >90% of the total activity at the R_f corresponding to [^{99m}Tc] DTPA.

TABLE 1
Percent Activity at R_f of [^{99m}Tc]DTPA*

Chromatography	Stock solution	Jet nebulizer aerosol solute	Ultrasonic nebulizer aerosol solute	Ultrasonic nebulizer aerosol solute cooled coupling fluid
Paper	97 ± 1.2	96 ± 1.4	5 ± 4.7	93 ± 2.3
Liquid	98 ± 1.6	98 ± 1.8	4 ± 6.2	94 ± 3.8

* Mean based on ten measurements ± s.d.

on each animal using an ultrasonic nebulizer with the coupling fluid cooled to 0°C. Animals were anesthetized with 25 mg/kg of sodium pentobarbitol, and aerosol was administered through a cuffed endotracheal tube 1.0 cm in diameter which had been introduced into the trachea. A small catheter was placed into the bladder to obtain urine samples following radioaerosol administration. The animals inhaled radioaerosols for two minutes through the endotracheal tube. The animals were ventilated by respirator pump at a tidal volume of 350 ml and a frequency of seven breaths per minute during inhalation. Blood and urine samples were collected at 10 min following aerosol inhalation.

RESULTS

Aerosol Characteristics

The AMAD and σ_g of the radioaerosols were 0.46 μm ($\overline{0}_g$ = 1.6) for the jet nebulizer and 0.70 μm (σ_g = 1.7) for the ultrasonic nebulizer.

Chromatography

Stock solution and aerosol. Using paper chromatography, [^{99m}Tc] 0_4^- moved with the solvent front (R_f = 1.0) and [^{99m}Tc]DTPA remained at the origin (R_f = 0.0). With liquid column chromatography, [^{99m}Tc] DTPA eluted first; the smaller [^{99m}Tc] 0_4^- ions eluted second; and hydrolyzed ^{99m}Tc remained bound to the column. The chemical purity of all stock solutions measured prior to nebulization showed >95% of ^{99m}Tc bound to DTPA. Ultrasonically generated aerosols showed <10% of the total activity at the R_f value of [^{99m}Tc]DTPA (Fig. 1A) with both paper and liquid chromatography (Table 1). Aerosols generated from stock solution by jet nebulization measured >90% of the total activity at the R_f value of [^{99m}Tc]DTPA (Fig. 1B). The results were consistent for solution obtained from each stage of the impactors.

The use of an ice water bath as ultrasonic coupling fluid decreased the chemical breakdown of [^{99m}Tc] DTPA to <10% (Fig. 2). The influence of temperature on the chemical stability of [^{99m}Tc]DTPA was confirmed by heating a solution of [^{99m}Tc]DTPA to 80°C for 2 min (Fig. 3). The chromatographic profile seen from heating the solution is similar to the pattern seen by ultrasonic destruction of [^{99m}Tc]DTPA.

Urine and plasma analyses. Urine samples from animals that inhaled aerosol produced in the jet nebulizer and those produced in the ultrasonic nebulizer when the ultrasonic coupling fluid was cooled to 0°C showed >90% of the activity at the R_f of [^{99m}Tc]DTPA (Table 2). Chromatographic analysis of urine samples from animals that inhaled aerosol from the ultrasonic nebulizer showed <10% of the activity at the R_f of [^{99m}Tc]DTPA. Plasma samples from each animal were assayed for [^{99m}Tc]DTPA. Although activity levels were too low in the plasma to obtain precise R_f values, qualitatively the chromatographic profile was similar to the one obtained from urine analyses.

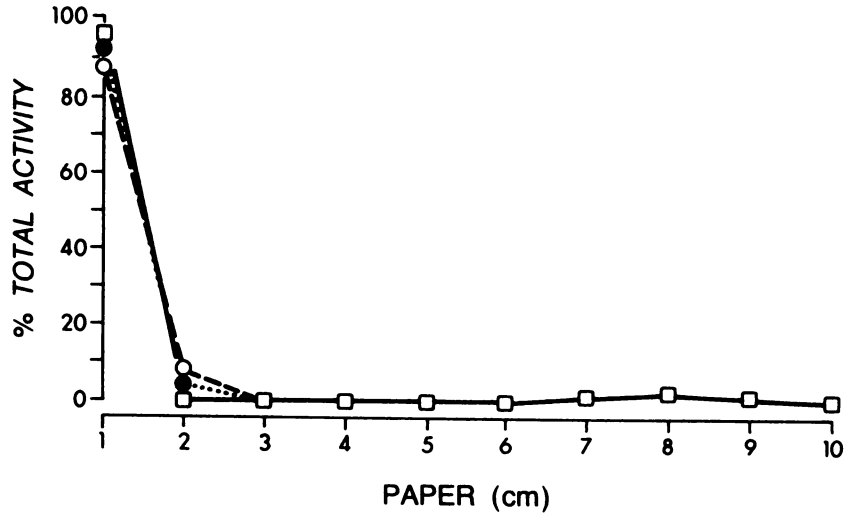


FIGURE 2
Paper chromatographic profile of an ultrasonically generated aerosol with a coupling fluid cooled to 0°C. [^{99m}Tc] DTPA stock solution (□), [^{99m}Tc] DTPA following nebulization (●), ^{99m}Tc activity in urine after [^{99m}Tc] DTPA aerosol inhalation (○).

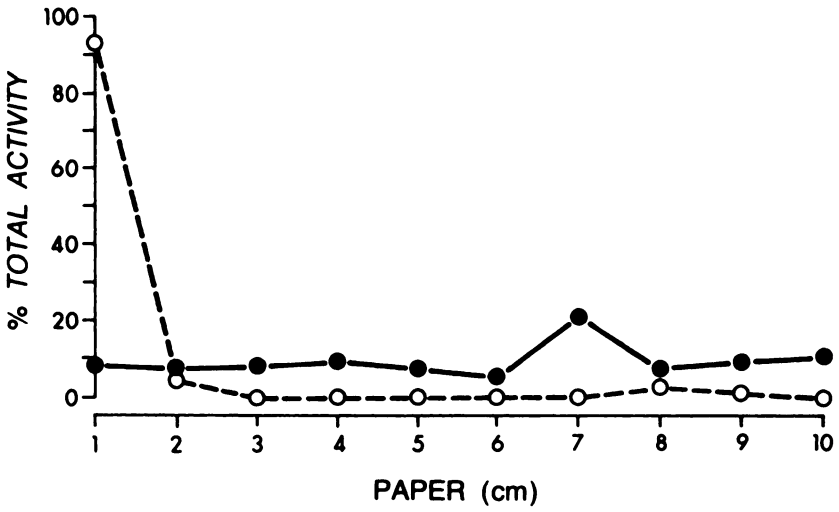


FIGURE 3
Paper chromatographic profile of solution of [^{99m}Tc]DTPA before heating (○), and following heating for 2 min at 80°C (●). Heating eliminates the R_f peak for [^{99m}Tc]DTPA and a small peak appears at the R_f position of $^{99m}TcO_4^-$.

DISCUSSION

Our results indicate that an ultrasonic nebulizer used to generate a [^{99m}Tc]DTPA aerosol may destroy the [^{99m}Tc]DTPA molecule. Stock solutions of [^{99m}Tc]DTPA and aerosolized [^{99m}Tc]DTPA were tested to determine the presence of common chemical impurities (*10*). Neither free $^{99m}TcO_4^-$ resulting from the oxidation of stannous ion or the reoxidation of ^{99m}Tc nor [^{99m}Tc] dioxide formed from hydrolyzed stannous ion were detected in chromatographic tests. Since these were not seen in the stock solution or following ultrasonic nebulization, another mechanism of chemical breakdown must be occurring in the aerosols prepared with the ultrasonic nebulizers.

The chromatographic profile of molecular destruction seen in ultrasonically produced aerosol led to the examination of the methods of aerosol generation. The jet nebulizer provides a simple method of aerosol generation (Fig. 4A). Air is forced through a small orifice at high velocity, causing a low pressure area to exist at the tip of an air inlet tube. The low pressure forces liquid up the feed tube. As the liquid exits the tube the high velocity air stream breaks the drop into aerosol particles; evaporation is believed to cool the liquid in the nebulizer reservoir. This type of aerosol generation does not appear to affect the molecular structure of [^{99m}Tc]DTPA.

TABLE 2
Percent Activity in Urine at R_f of [^{99m}Tc]DTPA*

Animal number	Jet nebulizer		Ultrasonic nebulizer		Ultrasonic with cooled coupling fluid	
	A	B	A	B	A	B
1	91	90	6	10	92	94
2	94	94	6	8	94	91
3	92	98	9	9	95	92
4	96	92	6	9	97	94
5	98	92	9	7	91	93
	94 ± 2.8†		8 ± 1.5		93 ± 1.9	

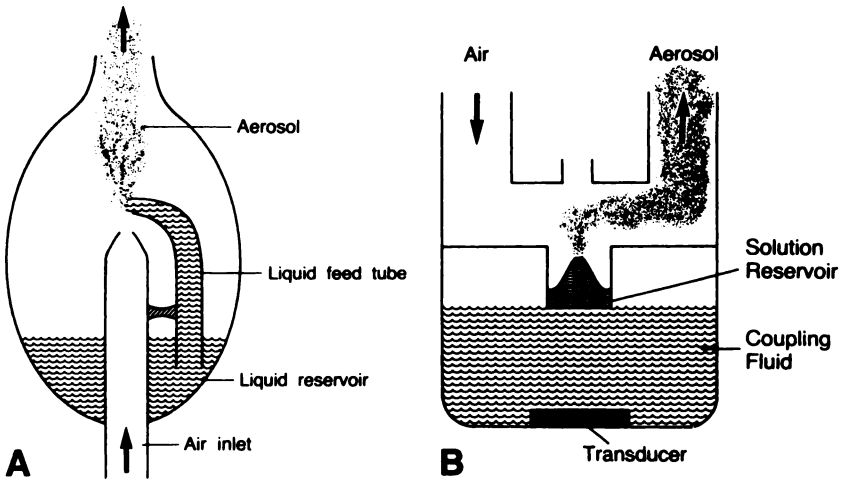
* A and B are separate experiments on same animal.
† Mean ± s.d. for A & B.

The ultrasonic nebulizer, (Fig. 4B), operates on different principles. Line current is converted into high frequency vibrations, on the order of 1–2 MHz range, in the transducer (*11*). The transducer focuses the mechanical vibrations to create an intense acoustic field in the coupling fluid. The energy is transmitted through the fluid via molecular motion. These intense adiabatic compressions occur with localized density and temperature changes. The massive force of energy causes a pressure gradient along the cylindrical axis of the transducer. The gradiant produces a geyser which ultimately generates an aerosol.

It has been postulated by Prudhomme and Graber (*12*) that ultrasonic molecular destruction of polypeptides is a result of cavitation. Cavitation is the formation of cavities caused by negative pressure in an ultrasonic geyser. Their collapse creates intense destructive shock waves. An increase in temperature creates a proportional increase in vapor pressure that cushions the collapse of the cavity. If this were the mode of [^{99m}Tc]DTPA destruction, lowering the temperature would increase rather than decrease chemical degradation. In various aqueous solutions, H_2O in collapsing cavitation bubbles leads to the formation of ·OH and ·H radicals (*13*). It is postulated that these highly reactive free radicals might attack the [^{99m}Tc]DTPA chelate bond.

Temperature elevation (*14–16*) and cavitation (*17*) are well-established mechanisms for chemical degradation in an ultrasonic beam. Neither theory adequately explains why lowering the temperature of the ultrasonic coupling fluid dramatically reduces chemical decomposition. A massive focused temperature increase at the

FIGURE 4
A: A schematic representation of a jet nebulizer. A high velocity air stream is forced through the air inlet tube. An area of low pressure at the top of the air inlet tube causes liquid to move up the liquid feed tube. As the liquid exists, the high velocity air stream breaks it into aerosol particles. B: A schematic representation of an ultrasonic nebulizer. High frequency vibrations are formed in the transducer and are transferred to the coupling fluid. Energy is transmitted from the coupling fluid to the nebulizer reservoir by molecular motions which cause localized temperature and density changes. A large pressure gradient is formed along the cylindrical axis of the transducer producing a geyser which generates the aerosol.



tip of an ultrasonic geyser is not expected to respond to a bulk temperature reduction in the connecting fluid. It is our feeling that a local temperature increase is a major factor influencing [^{99m}Tc]DTPA degradation, however, a combination of several processes is probably necessary to achieve the observed chemical breakdown.

CONCLUSION

The radiochemical purity of [^{99m}Tc]DTPA aerosols generated by a Dautrebande, Model D-31 jet nebulizer was >90%. Technetium-99m DTPA aerosol produced by a Heyer, Model USE77 ultrasonic nebulizer showed radiochemical purity <10%. Cooling the ultrasonic coupling fluid to 0°C increased the radiochemical purity to >90%. These observations indicate that radiochemical purity of ultrasonically generated aerosols should be evaluated prior to their use in diagnostic tests.

NOTES

* E.R. Squibb and Sons, Princeton, NJ.
† Heyer Company, Model USE77, Bad Ems, Germany.
‡ Dautrebande Jet Nebulizer, Model D-31.

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