

## Distribution of Nebulized Aerosols in Spontaneously-breathing Puppies

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The pulmonary distribution of nebulized fluorescent dyes was studied in 22 beagle puppies that breathed aerosols for either one or four hours. Brilliant fluorescence was seen in the nasopharynx, trachea, mainstem bronchi and esophagus of all dogs whether they breathed aerosols from a pneumatic or an ultrasonic nebulizer. Aerosols from both nebulizers were deposited throughout the airway and to the alveolar ductules within one hour of exposure. There was more deposition of dye in the small airways and alveoli as the nebulizer output and/or duration of exposure was increased. Mean particle diameter of aerosols decreased as the particles moved from the nebulizer output to the mid-point of the mist tent. It is concluded that water aerosols reach the gas exchange area of the lung in grossly detectable amounts. The quantity of aerosol generated, particle size, and length of exposure all influence the quantity of aerosol deposited. (Key words: Lung; aerosol deposition; Aerosol; lung deposition; Humidification.)

MANY STUDIES report the uptake of water particles in the lung as the difference between the amount inhaled and the amount exhaled.<sup>1-3</sup> These reports assume all retained water is distributed within the lung. Recent human studies with technetium-labeled water

have questioned whether ultrasonic aerosols reach the gas exchange areas of human lungs in significant quantities.<sup>4</sup> Reports dealing with radioactive labeling of technetium more accurately identify the intrapulmonary retention of water aerosols. These reports express the retention as a fraction of the total volume of water aerosol generated and imply that the greater the retention, the greater the possible benefit to patients with pulmonary disease. Wolfsdorf *et al.*<sup>4</sup> found that the amount of water retained was very small in spontaneously breathing man and questioned whether significant water is retained in the lung. This observation is contrary to findings in animal studies in which spontaneously breathing puppies retained sufficient ultrasonic saline aerosol to develop severe pulmonary congestion and bronchial pneumonia.<sup>2</sup> Matthews and Doershuk<sup>5</sup> have questioned how the effects of evaporation in a mist tent would effect the final size of the particles inhaled, and whether, in turn, this would influence retention.

These reports prompted us to study the distribution of water aerosols in the lung. We have evaluated how the type of nebulizer (pneumatic-surface tension or ultrasonic), output volume of the nebulizer, and length of exposure affect the distribution of aerosolized water in the lung. Finally, we wanted to know whether the spectrum of water particle sizes changed in a mist tent as used clinically.

### Materials and Methods

Twenty-two beagle dogs, 2 months of age, were studied in three groups. The 12 beagles in Group I were divided into three experimental subgroups (table 1). Each dog was placed in a 3 × 3 × 3-foot cage enclosed in a plastic oxygen tent cover (fig. 1). The four dogs in subgroup A breathed for one hour an

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The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

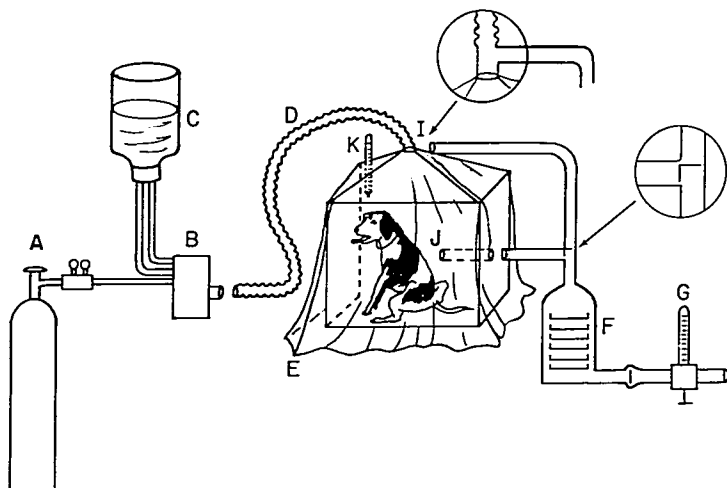


FIG. 1. Schematic drawing showing A, compressed air and valves; B, nebulizer; C, reservoir of dye; D, corrugated delivery hose; E, plastic enclosed cage; F, Andersen 6 stage impactor column; G, flowmeter; H, source of vacuum; I, sampling point for particles directly from nebulizer; J, sampling point from mid-tent; K, thermometer.

TABLE 1. Studies of the Intrapulmonary Distribution of Nebulized Dyes in 22 Beagle Puppies

	Number of Animals	Exposure (Hours)	Dye	Nebulizer
Group I				
A	4	1	Fluorescein	Pneumatic
B	4	1	Fluorescein	Ultrasonic #
C	4	1	Fluorescein	Ultrasonic #
Group II				
A	3	4	Fluorescein	Pneumatic
B	3	4	Fluorescein	Ultrasonic #
Group III				
A	2	1	Rhodamine-6 G	Pneumatic
B	2	1	Rhodamine-6 G	Ultrasonic #

aerosol of distilled water containing fluorescein dye (1.5 per cent) produced by a pneumatic nebulizer.\*\* The aerosol was delivered through the top of the tent via a

corrugated tube 60 cm long, I.D. 4 cm. The four dogs in subgroup B breathed for one hour fluorescein dye (1.5 per cent) aerosol produced by an ultrasonic nebulizer†† at an

\*\* Hydrosphere, Owens-Illinois, Inc., Toledo, Ohio.

†† Ultrasonic Model 35, Devilbiss, Co., Somerset, Pennsylvania.

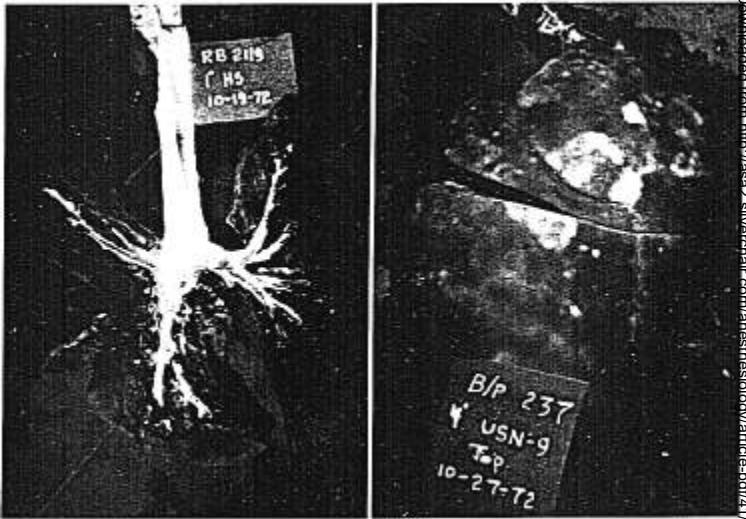


FIG. 2. Photographs of dog lung after spontaneous breathing of fluorescent dyes, taken under ultraviolet (A and B) and mercury (C and D) light. A (above, left), cut surface after one-hour exposure to 1.5 per cent fluorescein aerosol produced by a pneumatic nebulizer; B (above, right), pleural surface after four-hour exposure to 1.5 per cent fluorescein mist produced by an ultrasonic nebulizer at a power setting of 9. Facing page: C (above), and D (below), terminal bronchiole and alveolar ductules after one-hour exposure to rhodamine-6 G (1.5 per cent) aerosol produced by an ultrasonic nebulizer at a power setting of 9 (330 $\times$ ).

output power setting of 3, and delivered through a 60-cm long corrugated tube, 2.5 cm I.D. A power setting of 3 was selected because at this setting the ultrasonic and pneumatic nebulizers have roughly equivalent water density-per-volume outputs (44 mg/m<sup>3</sup>).<sup>7</sup> The dogs in subgroup C breathed for one hour an aerosol produced by the ultrasonic nebulizer at a power setting of 9 (maximum output).

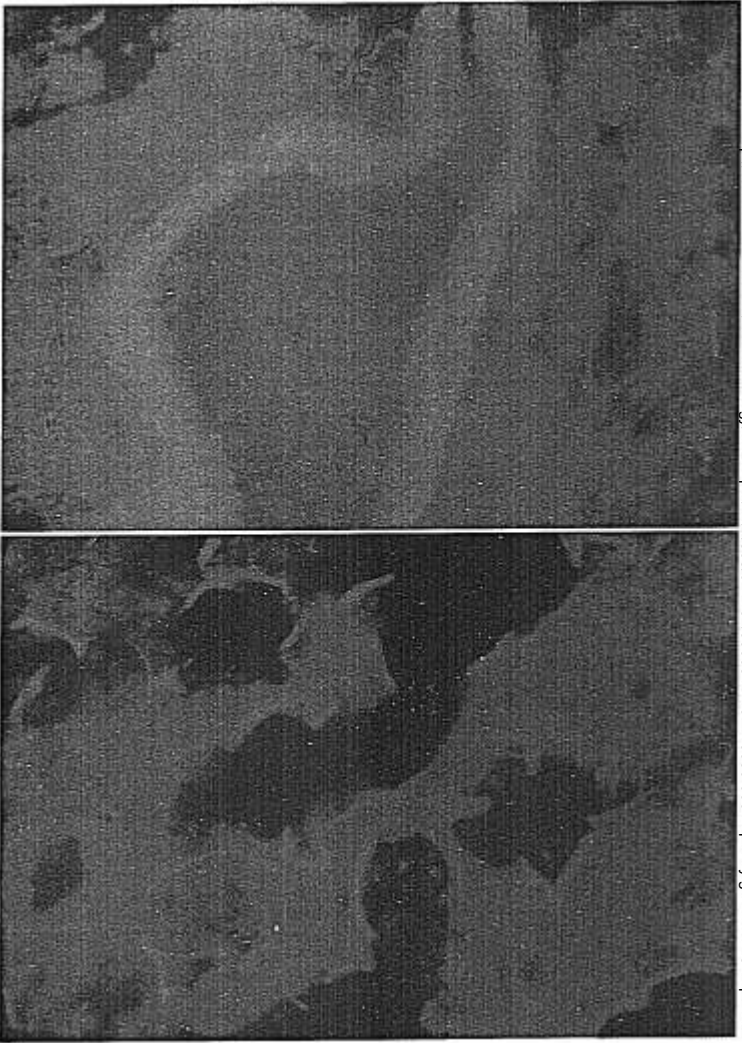
In Group II, six beagles were exposed to fluorescein dye (1.5 per cent) aerosol for four hours, three via the pneumatic nebulizer and three via the ultrasonic nebulizer at an output setting of 9.

In Group III, four beagles breathed for one hour an aerosol of rhodamine-6 G dye (1.5 per cent), rather than fluorescein dye. The aerosol was generated by the pneumatic nebulizer for

two of these dogs, and by the ultrasonic nebulizer at a power setting of 9 for the other two. The temperature within the tent was recorded throughout every experiment.

At the conclusion of each exposure, puppies were sacrificed with intravenous injection of sodium thiopental and potassium chloride, and the skin was removed to eliminate the major portion of the "rained out" fluorescent dye. Each animal's airway, from the external nares and mouth to the small bronchial branches, was dissected carefully with separate instruments to minimize the possibility of spreading fluorescent dye from one area to another. The lung was also cut in serial sections to study the more distal areas. The deposition pattern of fluorescein dye was documented by photography under ultraviolet

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light. The heart, esophagus, and all intra-abdominal organs were similarly examined under ultraviolet light for fluorescence.

Frozen sections were taken from the periphery of the lungs of the beagles exposed to rhodamine-6 G dye. These were fixed in acetic acid and mounted with glycerol. The sections were examined with a Leitz microscope with Phlome illumination, using a mercury light and appropriate filtration.

Samples of the rhodamine-6 G aerosols were collected from the end of the corrugated tubing and from the mid-point in the tent with each nebulizer. These samples were run through an Andersen six-stage impactor column at a flow of 1 cubic foot/min (fig. 1).<sup>11</sup> The aerosols were impacted onto 100-mm plastic petri dishes, from which they were eluted with 100 ml of distilled water. The dye from each stage was analyzed in a fluorescent spectrofluorometer.<sup>11</sup> The results from each stage were expressed as a percentage of the total of all stages and were plotted as accumulative percentage on a probability scale such that the 50 per cent line represents the mass median.

## Results

At necropsy, brilliant fluorescence was seen in the nasopharynx, trachea, mainstem bronchi, and esophagus of every dog, and in the stomach of all but one dog. No other intra-abdominal organ examined showed fluorescence except the gallbladder. Fluorescent spots, 1-3 mm in diameter, were visible on the pleural surface of the lungs in all but one dog exposed to fluorescein dye via the pneumatic nebulizer for one hour (Group IA). On the cut surface of the lung small airways fluoresced brilliantly (fig. 2A). After four hours of exposure there was more deposition of fluorescein dye visible in the mainstem bronchus and on the pleural surface, but the amount visible in the smaller airways was similar to that which was present after the 1-hour exposure.

The dogs exposed for 1 hour to the fluorescein aerosol from the ultrasonic nebulizer had diffuse areas of dull fluorescence on the pleural surfaces of the lungs, especially at the

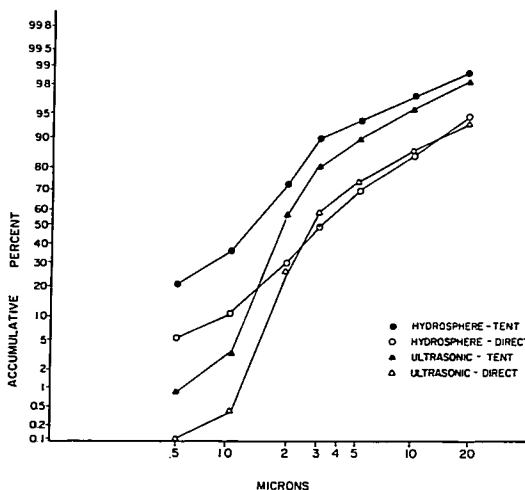
edges of the lobe. There were no prominent discrete fluorescent spots on the pleural surface as was seen in Group IA, but the cut surfaces of the lungs were similar in the two groups. The lungs of the dogs exposed to aerosol from the ultrasonic nebulizer at a power setting of 9 for one hour (Group IC) were indistinguishable from those of the one-hour pneumatic-nebulizer group. After breathing of fluorescein aerosol from the ultrasonic nebulizer set at 9 for four hours, there were increased amounts of dye in the mainstem bronchus and the small airways (fig. 2B). Again, there were large areas of fluorescence on the pleural surfaces, as well as an increase in the number of discrete brilliant fluorescent spots. Grossly, we interpreted the quantity of fluorescent material to be slightly greater with the ultrasonic nebulizer than with the pneumatic nebulizer after four hours, although the distribution patterns were similar.

The gross fluorescent pattern of the lungs of the dogs that breathed rhodamine-6 G dye aerosol was similar to those of the fluorescein Groups IA and IC. On frozen section fluorescence indicative of rhodamine-6 G dye was present in the terminal bronchioles and alveolar ductules of the lungs in both groups (fig. 2, C and D).

The temperature in the tents remained constant (22 C) throughout the experiments regardless of the nebulizer used. The mass median diameter of the aerosol containing rhodamine-6 G dye decreased in size as it moved from the nebulizer output hose to mid-tent. The mean diameter of the particles changed from 2.8 $\mu$  at the exit hose of the ultrasonic nebulizer to 1.8 $\mu$  in mid-tent (fig. 3), whereas the mass median particle size from the pneumatic nebulizer decreased from 3 $\mu$  at the exit hose to 1.3 $\mu$  in the tent. The percentage of particles 0.5 $\mu$  or less in size increased from 5.5 per cent at the exit of the pneumatic nebulizer to 20 per cent when sampled at mid-tent, versus a change from 0.1 per cent to 0.8 per cent with the ultrasonic nebulizer. Approximately 14 per cent of the particles directly from both nebulizers were 10 $\mu$  or larger in diameter, whereas only 4 per cent were that large in the tent (fig. 3). The shift in particle sizes from the output hose to mid-tent for each nebulizer was significant at all points ( $P < 0.001$ ).

11 Spectrofluorometer Model MTF-2A, Perkin Elmer, Norwalk, Connecticut.

FIG. 3. Distribution of particle sizes produced by the pneumatic and ultrasonic nebulizer directly and at mid-tent position. Particle size is expressed as the accumulative per cent (probability scale) of the total distribution plotted against log particle diameter (microns).



### Discussion

In the present study we have demonstrated in spontaneously-breathing dogs that liquid aerosols are distributed throughout the conducting airways and into the gas exchange area at the level of the alveolar ductules. We also have documented that there is a shift from larger to smaller aerosol particles within a mist tent. The intensity of fluorescence and, therefore, the quantity of water delivered to the lung increased with increased exposure time. The greatest deposition in the periphery of the lung was seen in the dogs exposed to ultrasonic aerosol at high output for four hours. When the two nebulizers were studied at comparable water outputs, however, more peripheral fluorescence was visible with the pneumatic nebulizer than with the ultrasonic nebulizer at a setting of 3. Since the mass median diameter of particles from the pneumatic nebulizer was smaller ( $1.3\mu$ ) than that of particles from the ultrasonic nebulizer ( $1.8\mu$ ), deeper penetration and greater retention may have resulted. The ultrasonic nebulizer at a setting of 9 produces more than twice

the water density per volume of gas than either the pneumatic nebulizer or the ultrasonic nebulizer at a power setting of 3 at 37 C.<sup>7</sup> This greater volume explains the increased deposition at this power setting over that produced by the pneumatic nebulizer at four hours. These findings suggest that particle size, quantity of nebulizer water output, and duration of exposure influence the distribution and retention of aerosolized liquids.

We believe that the decrease in particle sizes within the mist tent is due to evaporation of the particles as they humidify entrained air and not due to the larger particles raining out, since the method of sampling would catch all particles passing the tube in any given period. The fact that supplemental air is delivered into the mist tent during clinical use is important to consider in any aerosol study. Whereas our study permitted entrainment of ambient air, techniques using radioactive substances frequently require a closed system to prevent atmospheric contamination and, therefore, the particles studied may actually be larger than would occur with the same nebulizer in clinical use.

The dogs used in this study were all considered normal on physical examination. We did not find any preponderance of distribution of liquids to dependent parts of the lungs. Since our dogs were not anesthetized, they were free to move within the tent during the exposure period. Constant observation of the dogs within the mist tent was attempted, but good visualization was not always possible due to the opacity of the dye aerosol. Since the temperature within the tent remained constant at room temperature (22 C), we did not observe panting during the exposure period. Had panting occurred, it would have resulted in a more proximal distribution of aerosols than we found. We would also expect that distribution of aerosol in diseased lungs would be different, in that the aerosol could not penetrate distal to areas of small-airway occlusion.

Fluorescein dye was chosen for this experiment as a continuation of previous work done by Modell *et al.*<sup>9</sup> Once we documented that fluorescent material reached the smaller airways, we elected to see whether it could be found in the alveolar ductules. Background fluorescence of tissue in histologic sections negated the possibility of using fluorescein in that part of the experiment. Rhodamine-6 G dye was chosen since there is no tissue fluorescence in its light range. By studying the distribution of particles within the mist tent with rhodamine-6 G aerosols we could correlate the results with our findings on histologic section. The dog lung terminal bronchiolus does not terminate in an acinus but continues as a long alveolated ductule which is a gas exchange area.<sup>10</sup> Since fluorescence was seen in the ductule, we confirmed that aerosols do reach this level.

It was not the purpose of this study to quantitate the retention-to-delivery ratio of aerosols or the pathologic changes that might occur due to prolonged exposure to aerosols. The studies which found a small percentage of labeled aerosol retained in the lungs compared with that delivered imply an ineffective delivery system, whereas the water retained, even though small in amount compared with that delivered, may produce the desired result. Previous studies in our laboratory in unanesthetized animals have shown that chronic exposure to ultrasonic

aerosols can have deleterious effects.<sup>5</sup> Furthermore, in intubated subjects, both human and animal, these effects can be accelerated.<sup>11,12</sup>

We have shown that aerosols are deposited throughout the airway and into the alveolar ductules within one hour in sufficient volume to cause brilliant visible fluorescence. The quantity of aerosol, particle size, and length of exposure all influence the quantity of aerosol deposited. All these factors should be taken into consideration when mist-tent therapy is considered in the treatment of patients with pulmonary disease.

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