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Continuous Aerosol Therapy System Using a Modified Collison Nebulizer

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A Collison nebulizer was incorporated into an exposure system for administering antiviral compounds as continuous aerosols to mice infected with influenza virus. The nebulizer was modified to control aerosol output by varying the liquid feed rate. A multiple regression equation was developed from data obtained with uranine dye to define the aerosol concentration of the dye in the system as a function of the concentration of the dye in the spray fluid and the rate at which it was aerosolized. The rate of change of the concentration of the test solution due to evaporative losses was also ascertained for a 1-ml/min feed rate over a 23.5-h period of operation. Procedures are outlined for using these relationships to determine the concentration of a given drug that will result in a given dose. Performance data for the drug ribavirin are presented.

The efficacy of aerosol vaccination of experimental animals and man against respiratory infection is well documented (5, 6, 8, 9, 12, 15). For the most part these studies have been undertaken to test the hypothesis that the antigen would be more effective in inducing host immunity if the route of vaccine administration was the same as the route of disease acquisition. The same rationale has been applied to studies on therapeutic management of respiratory infections (1, 2, 7, 13, 14, 16, 18). We have recently initiated a program to investigate the effectiveness of aerosols of potential antiviral compounds in the treatment of respiratory infection induced by influenza virus in laboratory animals. One of our objectives has been to compare the efficacy of a drug administered as a continuous aerosol with that resulting from the same dose of drug given either by short-term intermittent aerosols or by intraperitoneal injection (17, 18). This report describes the system we have developed for continuous aerosol therapy of laboratory mice and discusses the procedures followed to calibrate and characterize the system. We also present performance data for a modified Collison nebulizer which is basic to the system and describe the procedures for using the data to determine the concentration of drug required to achieve a specified dose, or to calculate the dose administered when the concentration of the drug is known.

MATERIALS AND METHODS

Aerosol system. The general features of the aerosol system are depicted schematically in Fig. 1. Plastic animal holding cages (22.9 cm wide by 45.7 cm long by 15.2 cm deep) were converted to therapy chambers by outfitting them with gasketed covers that were clamped on to achieve an air-tight seal. The output of the Collison nebulizer and secondary air were introduced into the first cage through a tube extending through the cover. Aerosol was forced from the cage through a second tube in the cover and then through a 2-cm rubber tube to the inlet of the second cage. The system consisted of four cages interconnected in the manner described. Watering tubes extended into the cages through tight-fitting rubber bushings in the covers. The ventilation rate, including the input from the nebulizer, was 15 liters/min. The entire system was housed in a biological safety cabinet.

Aerosol generation. Aerosols were generated by a Collison nebulizer (11) which we modified in order to vary the concentration of the aerosol output by controlling the liquid feed rate. Details of the modified nebulizer are shown in Fig. 2. Two of the liquid intakes were closed with machine-screw plugs. The third intake was fitted with a plastic tube through which the solution to be aerosolized was pumped at the desired rate. The liquid-feed tube adaptation was effected by cutting an infant feeding tube (size 5, French) to an appropriate length, inserting a 2-cm length of 19-gauge hypodermic needle stock about 1 cm into the end of the feeding tube to prevent it from collapsing, and then "screwing" it into the threaded liquid intake of the nebulizer. A hole in the side of the jar accepted a two-hole rubber stopper with feed-through tubes to connect the nebulizer to the liquid feed pump, and to connect a liquid return pick-up tube to the reservoir. The reservoir was maintained air-tight; therefore, as liquid was withdrawn by the pump, a suction was created to return the run-off from the jar to the reservoir. By extending the pick-up tube to the bottom of the jar and tilting the jar slightly, it was possible to prevent an accumulation of spray solution.

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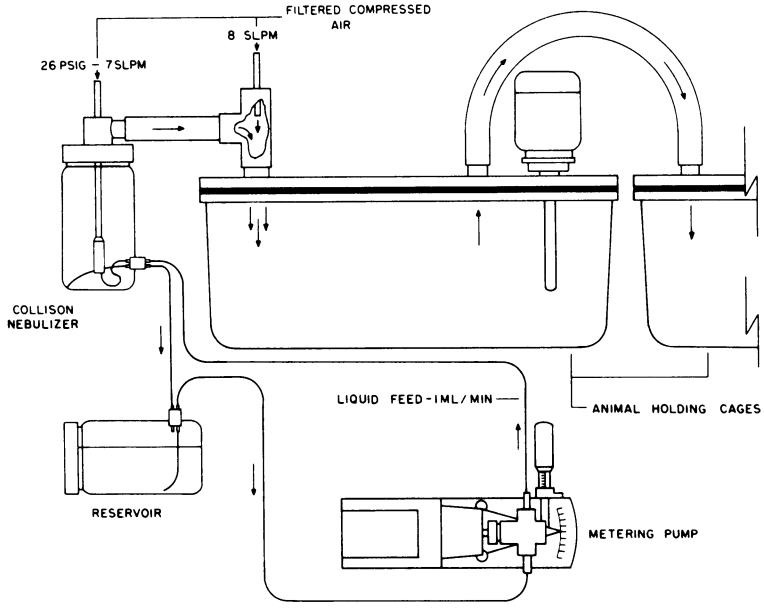

FIG. 1. *Schematic representation of continuous aerosol therapy system.*

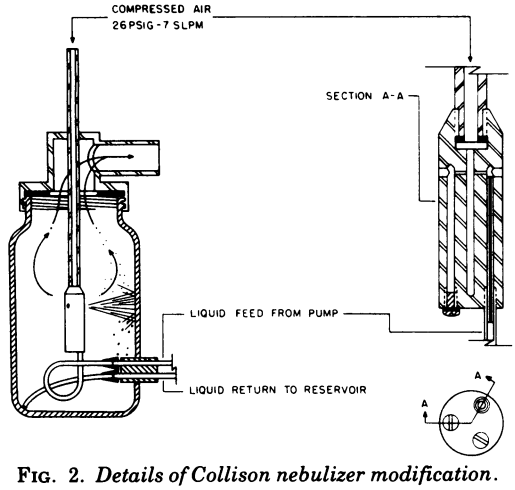

FIG. 2. *Details of Collison nebulizer modification.*

The performance characteristics of the nebulizer were determined at four different liquid feed rates: 0.1, 0.4, 0.7, and 1.0 ml/min. However, all of the studies to characterize the system were conducted with a 1.0-ml/min feed rate. A variable speed syringe pump (model 355, Sage Instruments, Cambridge, Mass.) supplied the nebulizer with fresh test solutions in all of the studies except one, in which aerosols were generated continuously over a 23.5-h period. In that case, a metering pump (model RRP 1 G 20, Fluid Metering, Inc., Oyster Bay, N. Y.) was used and the solutions were recycled through the reservoir.

Test solutions. Uranine dye (Fisher Scientific Co., Fair Lawn, N. J.) dissolved in distilled water was used at concentrations of 10, 30, and 50 mg/ml to calibrate the nebulizer, and at 30 mg/ml to characterize the system. Base-line data were also obtained for 30 mg of aqueous solutions of ribavirin per ml (kindly supplied by ICN Pharmaceuticals, Inc., Irvine, Calif.).

Aerosol sampling. The aerosol parameters pertaining to a specified cage in the system were determined from samples collected from the tube that connected it to the following cage, at a point adjacent to the exhaust tube of the cage. All-glass impingers (AGI) were used to determine total aerosol concentrations, expressed as micrograms of dye (or ribavirin) in particles of all sizes per liter of air in the aerosol system (4). Particle size data were obtained with a series of single-stage impactor devices for which the respective aerodynamic particle diameters associated with 50% collection efficiency were 1, 3, and 5 μm (10). The collection medium in both types of samplers was distilled water. Samples from the dye aerosols were assayed for fluorescence using a model 54 fluorophotometer (Photovolt Corp., New York, N. Y.); a spectrophotometric procedure was used to evaluate the ribavirin samples (17).

The data obtained from the impingers of the single-stage impactor devices were used in conjunction with those obtained with the AGI to characterize the aerosols in terms of two parameters: the mass median diameter (MMD) and the geometric standard deviation (GSD) of a log-normal distribution. Analyses of variances were computed on the logarithms of MMD, GSD, and total aerosol concentrations.

Mice. In all of the studies described, except the nebulizer calibrations, each of the cages in the system housed fifteen 5- to 6-week-old random-bred, white Swiss mice weighing 20 to 25 g each.

RESULTS

Nebulizer calibration. Four replicate aerosols were generated with each of 12 combinations of solution concentration and liquid feed rate. The system was allowed to operate for 5 min to establish steady-state conditions before sampling was initiated. Samples were collected from the first cage.

Total aerosol concentrations increased significantly ($P < 0.001$) in response to increases in either solution concentration or liquid feed-rate (Table 1). The relationship between aerosol concentration and the two variables may be described by the following equation:

$$\hat{Y} = 0.5728 + 2.6045X_1 - 0.5758X_1^2 + 0.2925X_2 - 0.3162_2{}^2 \quad 1$$

where, in logarithms, $\hat{Y}$ is the estimated aerosol concentration in micrograms per liter, X_1 is the concentration of the dye solution in milligrams per milliliter and X_2 is the liquid feed rate in milliliters per minute.

Particle size distributions were essentially the same regardless of solution concentration or feed rate. Although MMD increased ($P < 0.001$) with increasing solution concentration, the differences were too small to be of practical importance in regard to respiratory tract deposition and retention. There was no demonstrable effect of feed rate on MMD or of either of the variables on GSD. The overall geometric means or MMD and GSD were 1.38 μm and 1.79, respectively.

Cage-to-cage comparisons. Six replicate aerosols were generated to compare the aerosol parameters among cages. Samples were collected at the effluent site of each cage after a 20-min equilibration period.

The mean total aerosol concentration in the first cage (Table 2) was lower ($P < 0.01$) than that obtained at the same conditions in the nebulizer calibration study. This difference was associated entirely with particles having aerodynamic diameters in the range of 3 to 5 μm; recoveries in the ≤1- and ≤3-μm size classifications were essentially the same as those obtained previously. Since droplets produced by air-blast atomization characteristically carry high electrostatic charges, the lower aerosol concentration may have been the result of particle precipitation associated with the introduction of animal fur into the system; in this study each cage contained 15 mice.

The dye concentration of the aerosols decreased in each successive cage ($P < 0.01$). The loss associated with each cage and the tube that connected it to the preceding cage amounted to about 8.8%. There was no evidence that the particle size distribution changed materially as a result of the aerosol passing through successive cages.

TABLE 1. *Mean total concentrations and particle size distribution parameters of uranine dye aerosols produced with a modified Collison nebulizer*

Variables		Parameter		
Dye concn (mg/ml)	Liquid feed rate (ml/min)	Total aerosol concn (μg/liter)	MMD (μm)	GSD
10	0.1	8.08	1.06	1.99
	0.4	23.3	1.10	1.97
	0.7	28.2	1.00	1.93
	1.0	35.9	1.17	1.54
30	0.1	25.5	1.40	1.57
	0.4	73.3	1.53	1.71
	0.7	100.0	1.46	2.02
	1.0	105.2	1.43	1.99
50	0.1	41.7	1.79	1.67
	0.4	108.2	1.71	1.76
	0.7	144.4	1.50	1.70
	1.0	146.9	1.42	1.67

TABLE 2. *Mean concentrations of uranine aerosols (micrograms per liter) as a function of aerodynamic particle diameters*

Sample source	Particle diameter classification			
	Total	≤5 μm	≤3 μm	≤1 μm
Cage 1	83.7	ND[a]	88.7	29.7
Cage 2	81.9	ND	81.3	25.7
Cage 3	73.6	ND	73.3	22.3
Cage 4	64.5	ND	60.0	16.0
Cage 1[b]	105.2	100.2	92.7	30.2

[a] ND, Not determined.
[b] From nebulizer calibrations.

Continuous aerosol generation. Aerosols were generated continuously for 23.5 h on each of 5 days. At the beginning of each day, the reservoir was charged with 275 ml of dye solution (32.3 mg/ml). At 1, 3, 5, 7, and 23.5 h, AGI samples were collected from the first cage to determine aerosol dye concentrations, and the liquid in the reservoir was sampled to measure solute concentration. The volume of solution in the reservoir was measured at the end of the 23.5-h period.

In the Collison nebulizer, over 99% of the droplet mass is associated with droplets that either impinge on the wall or are too large to escape the jar because their settling velocities exceed the velocity of the upward airflow to the outlet port (11). The evaporation of solvent from these droplets results in an increase in the concentration of the spray solution which is contin-

ually recycled. This concentrating effect is described by the following equation:

$$C_t = C_0 \left(\frac{V_0}{V_0 - k_1 t} \right)^{k_2} \quad 2$$

where, C_t is the solute concentration after t minutes of operation; C_0 and V_0 are the initial solute concentration and solution volume, respectively; k_1 is the total time-rate expenditure of solution due to evaporation and aerosol production; and k_2 is the loss of solvent due to evaporation expressed as a dimensionless fraction of the total liquid loss. After 23.5 h, the mean volume of residual solution was 106 ml with a mean concentration of 47.8 mg/ml. These values were used in conjunction with the initial measurements of volume and concentration to establish k_1 and k_2 in equation 2 at 0.12 ml/min and 0.41, respectively. In practical terms, therefore, the system expended solution at an average rate of 0.12 ml/min; 0.07 ml/min was converted to effective aerosol while 0.05 ml/min of solvent was evaporated. The mean solution concentrations measured at 1, 3, 5, and 7 h were slightly lower than the values predicted by equation 2 but, at most, differed by less than 5% (Table 3).

Measured aerosol concentrations at 3, 5, 7, and 23.5 h were lower than the values predicted by equation 1 by an average of 6.5% when the measured values of solution concentration were used in the computation and by about 8% when the concentrations predicted by equation 2 were used. The lower-than-predicted concentrations observed in the cage-to-cage comparisons (Table 2) where mice were introduced into the system were still evident in this study at the end of 1 h of operation. However, by the 3rd h, a state of equilibrium appeared to have been reached.

Ribavirin. Base-line data were obtained with ribavirin by creating five replicate aerosols which were sampled at cages 1 and 3 beginning 20 min after the nebulizer was activated. The aerosol concentrations and particle size distributions from cage 1 were similar to those obtained with dye calibration (Table 4). In addition, the concentration of ribavirin in cage 3 was consistent with the 8.8% loss per cage determined in the cage-to-cage comparisons, and as before the particle size distribution in cage 3 was essentially the same as that in cage 1.

TABLE 3. *Effects of continuous operation of a modified Collison nebulizer on solution and aerosol concentration of uranine dye*

Hours after activation of nebulizer	Item			
	Solution (mg/ml)		Aerosol (μg/liter)	
	Measured	Predicted	Measured	Predicted
0	32.3	32.3	83.7[a]	111.6
1	31.8	32.6	88.4	112.5
3	31.9	33.4	104.8	114.9
5	34.0	34.4	113.2	117.8
7	34.6	35.1	110.7	119.7
23.5	47.8	47.8	136.8	150.8

[a] From cage-to-cage comparisons (t = ~30 min).

TABLE 4. *Aerosol parameters of aerosols of ribavirin*

Parameter	Sample source	
	Cage 1	Cage 3
Total aerosol concentration (μg/liter)	101.4	88.3
MMD (μm)	1.37	1.37
GSD	1.69	1.93

DISCUSSION

We have described a system which has proved successful for administering aerosols of antiviral drugs to mice with experimentally induced viral respiratory infections (17, 18) and have presented the results of system characterization studies. Our purpose for this was to delineate the operating principles involved and to identify the variables that affect performance in the continuous generation of small-particle aerosols.

It was essential to quantitate the effects of solute concentration and liquid feed rate on aerosol concentrations, because, at a given ventilation rate and with continuous aerosolization, control of aerosol concentration is the only means for controlling dose. We have shown that these variables were mutually independent and that, as would have been predicted, their independent effects were direct and curvilinear. The curvilinear relationships suggest, of course, that a maximum exists for each variable beyond which there will be no further increase in aerosol concentration. Having determined with uranine both the form of, and the coefficients for, equation 1, that relationship becomes the basis for extrapolating the aerosol concentration of drug that will result for any combination of solute (drug) concentration and liquid feed rate.

Although our experience has shown that the calibration data obtained with uranine can be used to predict the performance of the system with aerosols of ribavirin, it does not necessarily follow that this will hold for all other drugs. Since the output of the Collison nebulizer will vary as a function of the physical properties of the solution being aerosolized, and the electro-

static charge carried by the particles will vary with the ionic strength or conductivity of the solution, sufficient base-line data should be obtained with each new compound to verify the predictive equations or determine the correction factors that should be applied.

The average aerosol concentration ($\bar{Y}$) that will result in a specified dose for an exposed animal can be determined from the following equation:

$$\bar{Y} = \frac{d}{mtr} \qquad 3$$

where d is the dose, m is the respiratory minute volume (liters/minute) of the animal, t is the period of time (minutes) during which the dose is administered, and r is the respiratory retention rate. Now from equation 1:

$$\bar{Y} = \frac{1}{b-a}\int_a^b (-0.5728 + 2.6054X_1 - 0.5758X_1^2 + 0.2925X_2 - 0.3162X_2^2)dX_1 \qquad 4$$

where the limits of integration a and b are the initial and final solution concentrations, respectively. It follows that the initial solution concentration required to achieve a given dose can be found by substituting the liquid feed rate to be used for X_2 in equation 4; integrating between the limits $a = X_1$ and $b = X_1 + \log (C_t/C_0)$, where (C_t/C_0) is defined for the period of therapy by equation 2; and solving the resultant quadratic equation for X_1 after substituting the value for $\bar{Y}$ which will, by equation 3, yield the desired dose.

Two inherent shortcomings of our system are the cage-to-cage differences in aerosol concentrations and the increase of aerosol concentration as a function of time due to increasing solute concentration in the spray solution. Fortunately, these differences followed predictable patterns and could, therefore, be quantitated. Aerosol concentrations in successive cages closely approximated a geometrical progression described by the following general equation:

$$\hat{Y}_n = ae^{k(n-1)} \qquad 5$$

where $\hat{Y}_n$ is the estimated aerosol concentration in the nth cage, a and k are constants determined by the geometry of the system and the concentration of the aerosol entering the first cage, and e is the base of the natural system of logarithms. For our system, the values of the constants a and k pertaining to a 30-mg/ml solution and a 1-ml/min feed rate were 86.2 μg/liter and -0.092, respectively. The fractional loss per cage, L, was given by the following equation:

$$L = 1 - e^{-0.092} \qquad 6$$

We did not define fully the aerosol concentration-time relationship which in general is obtained by substituting the logarithmic form of the expression for solution concentration (equation 2) for X_1 in equation 1. Since the range of aerosol concentrations obtained with the 1-ml/min feed rate was consistent with the dose requirements of our initial therapy studies, that was the only feed rate for which we determined k_1 and k_2 of equation 2. Therefore, our predictive capabilities with respect to either increase of aerosol concentration with time or the concentration of solution required to yield a given dose are presently limited to the 1-ml/min feed rate.

The cage-to-cage effects could have been avoided by adopting alternative designs. One alternative would have been to use a cage of sufficient size to hold as many mice as our four-cage system holds. However, such a design would not have provided for isolation among groups of mice that had received different experimental treatments prior to therapy. A second alternative would have been a four-cage parallel system with one-fourth of the aerosol going to each, that is, dividing the aerosol-laden ventilation air equally among the cages. We were prevented from using such a design by a requirement to house the system in an existing biological safety cabinet which imposed dimensional limitations. Our series arrangement of cages represented a compromise that permitted selective isolation among groups of mice.

There were two alternative approaches to the problem of increasing solute concentrations in the spray solution with time. One would have been to avoid recycling of the spray solution. However, such a system would be unacceptably wasteful of drug solutions. The other approach would have been to continously replace solvent at a rate equal to the evaporation rate, but that would have further complicated the system with the need for additional equipment and space.

The total ventilation rate of the system was established with the objective of achieving the highest aerosol concentrations consistent with a maximum relative humidity of 70% in the fourth cage. An input of 8 liter of dry secondary air per min was determined on the basis of an estimated maximum input of 0.07 g of free water vapor per min from the nebulizer and about 0.003 g/min from each of 60 mice (3).

Preliminary data obtained at the exhaust of the fourth cage indicated that the system performed within the limit set for relative humidity and that the average increases in temperature and relative humidity were about 1°C and 10% per cage, respectively.

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