Modified Spinning Top Homogeneous Spray Apparatus for Use in Experimental Respiratory Disease Studies

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The May spinning top generator was adapted to a modified Henderson tube for producing large aerosol particles (> 4 μm) to obtain almost exclusive upper respiratory tract deposition of infectious aerosols in exposed mice. The system was installed in a biological safety cabinet to permit experimentation with pathogens. A novel mechanism utilizing parts from a machinists micrometer and the mechanical stage from a light microscope was developed for the spinning top generator as a means for precisely positioning the liquid feed needle. Aerosol light-scatter properties were continuously analyzed to provide relative measures of particle size distribution and aerosol concentration. When mice were exposed to influenza virus aerosols in which none of the virus was contained in particles with aerodynamic diameters < 4 μm, essentially all of the virus was deposited in the upper respiratory tract tissues.

The settling diameter, i.e., the diameter of aerodynamically equivalent spheres of unit density, of airborne particles markedly affects their regional deposition properties in the respiratory tract when inhaled by man or experimental animals (2, 4, 11, 17). Also, the respiratory dose response characteristics and organism survival properties of infectious microbial aerosols are highly dependent upon the aerodynamic size (3, 5, 6, 8, 16, 18). Therefore, quantitative studies of infectious diseases transmitted by the aerosol route demand consideration and control of aerosol particle size.

We have recently initiated research to investigate the pathogenesis, prophylaxis, and therapy of infectious respiratory diseases in experimental animals. Implicit in this program is the need to control the size of aerosol particles containing the infectious microorganisms, to produce aerosols of small particles that would be expected to deposit largely in the lungs of experimental animals, and to produce aerosols of large particles that would be expected to deposit in the upper respiratory tract. Hatch and Gross (11) have reviewed the extensive work on particle deposition as a function of size in the respiratory tract of man, the monkey, and the guinea pig. They conclude that particles smaller than 2.0 μm will deposit almost exclusively in the lung alveoli whereas particles larger than 4.0 μm will deposit largely in the upper respiratory tract structures (nasal chamber, trachea, bronchi, and finer airways).

We used the Collison spray device in a Henderson apparatus (12), modified by the incorporation of an animal exposure box, for creating small-particle aerosols to achieve mainly alveolar deposition in mice and have adapted the spinning top disseminator after the design of K. R. May (14, 15) to a similarly modified Henderson apparatus as a means for producing large-particle aerosols. This report describes the aerosol system with spinning top disseminator as we have developed it for use in our studies and presents results showing dissemination performance characteristics including, particularly, particle size distribution properties. Also presented are results of a study on influenza virus deposition in the respiratory tract of mice after exposure to aerosols produced with the spinning top.

MATERIALS AND METHODS

Aerosol generation. A May spinning top homogeneous spray apparatus (14, 15) (manufactured by Research Engineers Limited, London, and marketed in the U.S. by Billings and Gussman, Inc., Waltham, Mass.) was used. We modified the device, however, to provide for precise lateral and vertical adjustments of the liquid feed tube. The modification as depicted in Fig. 1 incorporates the mechanical stage from a light microscope to achieve the lateral adjustment and parts from a machinists micrometer for adjustment of the clearance between the liquid feed needle and the spinning top. We also made several pairs of annulus extension rings which were employed to ensure maximal efficiency of the satellite extraction feature of the spinning top generator (see ref. 15). The rings were sized so that by selecting the proper pair the "halo"
of primary droplets was always formed from 0.5 to 1 inch beyond the outer edge of the annulus.

**Aerosol system.** The main features of the aerosol system including air-flow patterns are depicted schematically in Fig. 2 and 3. All of the components except the mainframe of the light-scatter particle counting instrument were contained within a biological safety cabinet. The total volume of filtered secondary air introduced into the system varied as a function of working air pressure applied to the spinning top generator, since the flow inward between the annulus rings is directly related to the pressure used to drive the spinning top. However, the air that entrained the primary droplets, specifically that which flowed downward through the mixing cylinder and thence through the transit tube and the exposure box, was controlled at 56 liters per min.

**Test suspensions.** Heart infusion broth (HIB) and uninfected allantoic fluid (UAF) harvested from embryonated eggs after 12 days of incubation were used in experiments designed to calibrate the system and evaluate its performance. A physical tracer, uranine dye, was added to a final concentration of 0.2% (wt/vol) just prior to use in aerosol trials.

**Aerodynamic aerosol particle size measurements.** Aerodynamic particle size distributions were measured only in the calibration and evaluation experiments performed with HIB and UAF. The aerosols were sampled with a series of single stage impactor devices (SSI) for which the respective aerodynamic particle diameters associated with 50% collection efficiency were 3, 5, 7, 9, 11, and 13 μm (13). Both the glycerin-coated glass slides used in the impactor sections and the impinger collection media were assayed; slides were rinsed with 20 ml of distilled water. Fluorescence was measured by a Photovolt model 54 fluorophotometer (Photovolt Corp., New York, N.Y.).

**Light-scattering particle size measurements.** Light-scatter measurements were obtained with a programmable particle counting system with gas dilution unit (model 202, Royco Instruments, Inc., Menlo Park, Calif.). The optical bench, consisting of the sampling tube, diluter, light source, optics, photomultiplier, and preamplifier, was removed from the instrument and relocated inside the biological safety cabinet directly beneath the aerosol transit tube. The sampling tube was mated to a transition-flow diverting section which extended through the wall of the transit tube and was terminated with an axially mounted aerosol sampling probe. Signal and power cable extensions connected the optical bench to the mainframe.

To relate light-scatter properties to particle size, the instrument was first calibrated with uniformly sized latex spheres in accordance with the manufacturer's instructions. Thereafter, the particle number and size data generated by the instrument in the aerosol trials were converted to measures of particle volume (approximating mass) to provide a basis for developing meaningful relationships between the optical and aerodynamic particle diameters.

**Virus suspension.** Mouse virulent preparations of strain Aichi/2/68 (H3N2) influenza virus were used in the respiratory tract deposition and recovery studies in mice. Increased virulence was achieved in nine passages by intranasal instillation of fluid supernatants from homogenates of infected mouse lungs. The final spray product was produced in embryonated eggs. Virus-infected allantoic fluids were harvested, pooled, and frozen as small portions for storage at −70 C. The thawed product contained approximately 10^6 egg median infectious doses (EID₅₀) per ml.

**Mouse exposures and necropsies.** Six- to seven-week-old randomly bred, white Swiss-ICR mice, weighing 20 to 25 g each, procured from Microbiological Associates, Walkersville, Md., were employed in the respiratory tract deposition and recovery study. Twelve mice were exposed to the aerosol for a period of 10 min in each trial. Immediately after exposure, the mice were killed and groups of three were allocated randomly to each of four technicians who independently collected respiratory tissue specimens. Respective tissues from the three mice comprising each group were pooled and homogenized in tissue grinders containing measured volumes of HIB with 250 U of penicillin and 250 μg of streptomycin added per ml. The respiratory tissues were removed as three separate specimens: (i) the lungs were taken as individual lobes and pooled; (ii) trachea samples were collected which included the mainstem bronchi, portions of the secondary bronchi, and the trachea up to the epiglottis; and (iii) nasopharyngeal tissues were taken by trimming the head to exclude the mandible.

**FIG. 1. Liquid feed tube adjustment mechanism for May spinning top aerosol generator consisting of: (A) mechanical stage from American Optical Co. light microscope, (B) liquid feed tube terminating with a 23-gauge hypodermic needle entering (C) the barrel and thimble from a machinists micrometer mounted on (D) an upper plastic (plexiglass) plate attached to the X-X lateral adjustment rack of the mechanical stage, and (E) a strut mounted, fixed, plastic plate to which is affixed the Y-Y lateral adjustment rack.
tongue, and cranial leaving the nasal turbinates, soft palate, pharynx, and epiglottis.

**Virus aerosol sampling.** All-glass impingers (1) containing 20 ml of HIB with 100 U of penicillin and 100 μg of streptomycin per ml were employed to collect 1-min samples of the influenza virus aerosols from 1 to 2 and from 9 to 10 min during dissemination in each trial. The contents of the two samples were pooled and assayed for virus to provide measures of aerosol concentrations.

**Virus assays.** All assays of virus spray suspensions, aerosol samples, and mouse respiratory tissue homogenates were performed in 9- to 11-day-old embryonated eggs. Eggs were inoculated with 0.1-ml portions of appropriate sample dilutions, incubated 48 h, and tested for viral hemagglutinin activity using guinea pig erythrocytes.

**RESULTS**

**Calibration and performance study.** Four replicate aerosols were created at each of three rotational velocities as determined by the working air pressure applied to the spinning top generator. The pressures employed were 20, 26, and 30 lb/in². Both HIB and UAF, each with uranine dye added as a physical tracer, were disseminated. After a 1-min equilibrium period in each trial, the light-scatter properties of the aerosols were measured, and the SSI devices were employed in random sequence to measure the aerodynamic size properties and total dye recoveries. Computationally, the particle size data were reduced to the mass median particle diameter (MMD) and the geometric standard deviation (GSD) of a log-normal distribution. Analyses of variance were computed on the logarithms of MMDs, GSDs, and total aerosol recoveries. The comparative results are summarized in Table 1.

The effect of generator operating pressure on aerosol MMD values tested highly significant \( P < 0.01 \) in the analysis of both the SSI and light-scatter results. With both HIB and UAF, MMD values decreased with increasing operating pressure. The MMD values with HIB and allantoic fluid were generally similar within operating pressure and sampling system.

GSD values were generally not affected by operating pressure or spray fluid except that on the basis of light-scatter measurements somewhat higher GSDs, suggesting wider particle size ranges, were obtained with allantoic fluid than with HIB. Otherwise, GSD levels approached the value of 1.25. This observation was especially encouraging since the particle size discriminating properties of the SSI device are such that a GSD value of 1.25 would be predicted when sampling strictly monodisperse aerosols.

On the basis of aerodynamic particle size (SSI measurements), none of the aerosol mass with either HIB or allantoic fluid was in particles <4 μm in diameter when the generator was operated at 20 lb/in². Presumptively, therefore, the aerosols with these treatments met the criteria for upper respiratory exposure of experimental animals. Depending upon spray fluid...
and operating pressure, from 0.5 to 3.0% of the mass was in particles <4 μm in diameter with the remaining treatments. The fact that the GSDs associated with the optical size distributions did not approach the theoretical minimal value of 1.0 was attributed primarily to coincidence, that is, the random occurrence of two or more particles in the sensing volume at the same time.

The interaction of generator operating pressure and spray fluid tested highly significant (P < 0.01) when comparing total aerosol recoveries, both with SSI and light scatter. With HIB, the mean aerosol concentrations decreased markedly as generator operating pressure was increased. With UAF, the aerosol concentration pattern was curvilinear; the highest concentrations were measured at 26 lb/in². These results suggested that some particles of the main aerosols might have been removed with the satellite particles, and the proportions removed were dependent upon both generator operating pressure and spray fluid.

**Mouse respiratory tract deposition.** An influenza virus deposition study in mice was conducted with the spinning top generator aerosolizing a 1:10 dilution of virus in HIB at the rate of 1 ml/min. The generator was operated at a working air pressure of 20 lb/in². Estimates of the total doses inhaled by the mice were computed from the measured aerosol concentrations (all-glass impinger results) and Guyton's formula (9) for mouse inhalation rates.

Among the 20 groups of three mice which were exposed in five aerosol trials, essentially all of the virus recovered immediately after challenge was found in the nasopharynx. Virus was recovered from the nasopharynx sample of all groups; the mean recovery per mouse was 10⁻⁹ EID₅₀ (standard error ± 0.1). Nonquantifiable traces of virus were found in 12 of the 20 samples of trachea homogenates and three of the 20 samples of lung homogenates. Hence, the aim of achieving almost exclusive upper respiratory exposure in mice was reached in these studies.

Aerosol particle size distribution properties based on light-scatter measurements, and virus aerosol concentrations as determined from impinger sample collections, are summarized in Table 2. In the absence of dye tracer and with a mixture of HIB and allantoic fluid (virus suspension), light-scatter MMDs as well as GSDs were somewhat higher than those observed at the 20-lb/in² operating pressure in the calibration trials. Between 4 and 7% of the airborne mass, depending upon which trial was considered, was in particles <4 μm (but generally >3 μm). However, on the basis of the relationships between light-scatter and aerodynamic size

### Table 1. Particle size distribution and recovery properties of dye traced HIB and UAF aerosols produced with spinning top generator

| Determinants                  | Operating pressure (lb/in²) | 20          | 26          | 30          |
|-------------------------------|----------------------------|-------------|-------------|-------------|
|                               |                            | HIB         | UAF         | HIB         | UAF         |
| SSI                           |                            | HIB         | UAF         | HIB         | UAF         |
| MMD (μm)                      | 8.60                       | 8.09        | 6.73        | 6.51        | 6.18        | 5.95        |
| GSD                           | 1.23                       | 1.22        | 1.25        | 1.27        | 1.29        | 1.25        |
| Total recovery (μg of dye     | 4.23                       | 2.86        | 1.42        | 4.18        | 0.64        | 3.37        |
| per liter)                    |                            |             |             |             |             |             |
| Light scatter                 |                            | HIB         | UAF         | HIB         | UAF         |
| MMD (μm)                      | 6.09                       | 6.15        | 5.78        | 5.63        | 3.15        | 4.69        |
| GSD                           | 1.31                       | 1.36        | 1.29        | 1.37        | 1.25        | 1.33        |
| Total recovery (cc × 10⁻¹      | 2.07                       | 1.24        | 1.54        | 1.43        | 0.16        | 0.82        |
| per liter)                    |                            |             |             |             |             |             |

* Volume of airborne spray suspension, computed from summation of particle volumes.
DISCUSSION

An aerosol system which incorporates a May spinning top aerosol generator for producing large-particle microbial aerosols to achieve upper respiratory tract exposures of mice has been described. The system design represents what we consider a compromise among various interacting factors. Druett and May (7) incorporated the generator in an inverted U-tube type of system and apparently succeeded in producing aerosols of uniformly large particles. Their system was several feet high and operated at a relatively high volume flow rate (150 liters per min.). We were interested in adapting the system to a more conventional horizontal safety cabinet which in our case already existed (30 inches wide by 30 inches high by 96 inches long). With this restriction, a horizontal system was indicated and a primary concern became the sizing of the aerosol transit tube. We selected a 1.5-inch diameter as a practical size in which the aerosol losses due to gravitational settling and inertial deposition could be held to acceptable levels at volume flow rates that were consistent with the achievement of reasonable aerosol concentrations from our virus suspensions, given a maximum liquid feed rate of about 1 ml/min.

With our system contained in a biological safety hood, having attached arm-length rubber gloves for experimentation with pathogens, the modification for precisely adjusting the liquid feed tube proved essential. Without it, even the most painstaking attempts to center properly the feed needle-to-top clearance were unsuccessful. Similarly, we consider the instrumentation for monitoring light-scattering properties on a continuous basis as almost essential. Investigations of pathogenesis necessarily involve comprehensive, tedious, and time-consuming studies in the infected host for periods as long as several weeks. Accordingly, it is important that the investigator be alerted to any equipment malfunctions as soon as they occur so that costly biological assays can be avoided. Light scattering can only provide relative measures of aerodynamic aerosol properties but has the advantages of detecting malfunctions immediately, requires minimal effort, and can be measured without interfering with the primary animal exposures.

Although the results of our limited effort to establish relationships between optical and aerodynamic particle size distributions satisfied our immediate requirements, we plan to continue our investigations to obtain a broader base of data, including other spray suspensions and a wider range of mean particle diameters. We also plan to modify the liquid feed needle adjustment slightly by replacing the machinists' micrometer head presently used with one which incorporates a nonrotating spindle. This change should eliminate the need to readjust the needle laterally each time a clearance adjustment is made.

An average total recovery of only 0.63% of the presented doses from the respiratory tract of mice seemed remarkably low. The scientific literature, however, does not contain information that can be used as a basis for judgment in this regard. Harper and Morton (10) have reported on the exposure of guinea pigs primarily, and only a few monkeys and mice, to isotopically labeled bacteria. Their mice were exposed only to 1-μm particles (single bacterial spores). By comparison with predicted doses, based on Guyton's estimates of minute-volumes (9), 34% of the radiolabel was recovered from the respiratory tract in one group of eight mice; 19% was recovered from another group of 10 mice. Although material balance was not an objective of our work and we did not attempt to measure physical deposition efficiencies, it seems unlikely that the disparity between our recoveries and those of Harper and Morton could have resulted from physical factors alone, i.e., particle size differences. We have concluded, therefore, that considerable in vivo inactivation of virus must have occurred during and immediately after exposures.

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