Homogeneous Bacterial Aerosols Produced with a Spinning-Disc Generator

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Aerosols composed of viable particles of a uniform size were produced with a commercial spinning-disc generator from aqueous suspensions of Bacillus subtilis var. niger spores containing various amounts of an inert material, dextran, to regulate aerosol particle size. Aerosols composed of single naked spores having an equivalent spherical diameter of 0.87 μm were produced from spore suspensions without dextran, whereas aerosols produced from suspensions containing 0.001, 0.01, 0.1, and 1% dextran had median diameters of 0.90, 1.04, 1.80, and 3.62 μm, respectively. Such aerosols, both homogeneous and viable, would be useful for calibrating air sampling devices, evaluating air filter systems, or for employment wherever aerosol behavior may be size-dependent.

Spinning-disc aerosol generators have been used for producing homogeneous nonbiological aerosols. Unlike most other dissemination devices (6), they put droplets of nearly uniform size. Walton and Prewett (7) and May (5) were among the first to develop spinning-disc-type aerosol generators. More recently, spinning-disc generators have been used by Whitby et al. (8) to study the effect of particle size on aerosol behavior and air filtration and by Flesch et al. (1) and Knuth (2) to produce aerosols of methylene blue dye for calibrating air samplers. Lippmann et al. (3, 4) designed one to study the effect of particle size on the deposition of inhaled iron oxide aerosols in the human respiratory tract. The vibrating reed also disseminates homogeneous droplets, but its droplet output is negligible compared with that of the spinning disc (9).

Recently, a spinning-disc aerosol generator (Environmental Research Corp., St. Paul, Minn.) has become commercially available. This paper reports on the utilization of this generator to produce aerosols both homogeneous and viable over a wide range of sizes by using bacterial spores together with a controlled amount of inert material accompanying each spore. Such aerosols would be useful for calibrating air sampling devices where particle collection efficiencies may be size-dependent and for evaluation of certain air filter systems.

MATERIALS AND METHODS

Spinning-disc aerosol generator. A diagram of the generator is shown in Fig. 1. The generator produces an aerosol by feeding a solution or suspension through a flowmeter onto the center of the disc [1 inch (2.54 cm) in diameter] rotating at a fixed speed of 60,000 rev/min. The liquid is spun off the disc in two groups of droplet sizes. The larger or primary droplets are used as the homogeneous test aerosol. The smaller or satellite droplets, which are about one-third the diameter of the primary droplets and several times more numerous, are heterogeneous in size. They are removed from the main air stream by a separate exhaust system.

The size of the primary droplet is a function of the size and speed of the disc and the density and surface tension of the liquid as described by the following formula used by Walton and Prewett (7):

\[ D_d = K/W (T/PD)^{1/2} \]  

where \( D_d \) is the droplet diameter, \( K \) is a dimensionless proportionality constant, \( W \) is the angular velocity of the disc, \( D \) is the disc diameter, \( P \) is the liquid density, and \( T \) is the liquid surface tension. With a fixed size and speed of the disc, droplet diameter becomes dependent on the density and surface tension of the liquid.

Aerosol particle size is directly proportional to the size of the primary droplets and the solute content of the liquid as described by the following formula, which assumes complete evaporation of the solvent in the droplet and the subsequent formation of a solid core sphere of the dissolved solute: (volume of particle/volume of droplet) = (volume of solute/volume of solution). Converting to units used in the tests the expression becomes:

\[ D_p/D_d = (\text{weight of solute/weight of solution})^{1/3} \]  

which assumes that the density of the solute is the same as that of the solution or, in the case of a dilute aqueous solution, not far from 1.0 g/ml. \( D_p \) is the
diameter of the evaporated particle, and $D_d$ is the liquid droplet diameter.

If the liquid droplet contains a spore, it can be assumed that the volume of the resultant evaporated particle equals the volume of an equivalent uninhabited particle plus the volume of the spore, because the spore detacts little volume from the liquid phase of the original droplet. By using diameter rather than volumes, the expression becomes

$$D_{sp} = (D_v^3 + D_s^3)^{1/3}$$

(3)

where $D_{sp}$ is the equivalent spherical diameter of a spore-bearing particle, $D_v$ is the diameter of an uninhabited particle, and $D_s$ is the equivalent spherical diameter of a spore, 0.87 µm. If, for example, the solute content of the suspension is such that the diameter of an uninhabited particle is 1 µm after the solvent has evaporated, then the diameter of a particle containing a single spore should be 1.18 µm: $D_{sp} = (1^3 + 0.87^3)^{1/3} = 1.659^{1/3} = 1.18$. With more concentrated suspensions that would result in larger evaporated particles, the difference between the size of the inhabited and uninhabited particles would be expected to be still smaller.

**Design of experiments.** Aerosols were produced with the spinning disc from aqueous suspensions of *Bacillus subtilis* var. *niger* spores containing various amounts of dissolved Dextran 2000 (Pharmacia, Uppsala, Sweden) to regulate aerosol particle size. These spores are nonpathogenic and highly resistant to biological decay (death); therefore, they make ideal viable tracers. The size of a spore was determined from aerosols generated from spore suspensions without dextran. Average dimensions, based on the measurement of 652 spores, were 0.68 by 1.17 µm which gives a volume of 0.34 µm³ and an equivalent spherical diameter of 0.87 µm, assuming spore shape to be that of a cylinder with hemispherical ends. Dextran 2000 is an anhydroglucose polymer with an average molecular weight of $2 \times 10^4$. It is stable, inert, and soluble in water.

Preliminary tests with dextran-spore aerosols were conducted by use of spore concentrations that theoretically would average about one spore in each of the primary droplets. However, examination of aerosol samples showed that the airborne particles were heterogeneous in size. The aerosols consisted mainly of particles containing a single spore, but there were also lesser numbers of uninhabited and multiply inhabited particles. Therefore, dilute spore suspensions were used so that the capabilities of the spinning-disc generator and the particle size parameters of the aerosols could be more accurately defined. This procedure naturally resulted in aerosols composed of dextran particles with and without spores, but almost all of the inhabited particles contained only a single spore. Aerosols were generated from spore suspensions in distilled water without dextran and from suspensions containing 0.001, 0.01, 0.1 and 1% dextran. The aerosols were sized with an electron microscope.

Spore concentration of the aerosols was determined from samples collected with cotton filters (10). Spores were dislodged from the cotton by shaking in water containing 0.1% Tween 80 (Atlas Chemical Industries, Inc., Wilmington, Del.) and assayed on pour plates of Tryptose Agar (Difco). Colonies were counted after 24 hr of incubation at 37 C.

**Aerosol particle sizing.** Aerosol particle size was determined with an electron microscope by use of aerosol samples collected on a collodion-coated specimen grid mounted in a Greenburg-Smith Impinger as described by Flesch et al. (1). The grids were then shadowed with uranium and examined in an electron microscope at a magnification of 1,870, and random areas of the grid were photographed. For sizing, the electron micrographs were projected on a screen of translucent paper, and the individual particles were measured perpendicular to the shadow. For each size interval, the number of particles, the percentage of the total number of particles, and the cumulative percentages were determined. The cumulative percentages, when plotted against particle diameter, gave a good straight-line fit on log probability paper. Therefore, the particle size distributions of the aerosols were log-normal. Populations of 25 to 150 particles were used to determine the following parameters: number median diameter (NMD), which is the 50% size, and geometric standard deviation (GSD), which indicates the degree of heterogeneity of the aerosol. A perfectly homogeneous aerosol has a GSD of 1.0, i.e., all of the particles of the same size. Aerosol GSD is

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**Fig. 1.** Flow diagram of ERC model 8320 spinning-disc aerosol generator.

**Fig. 2.** Size distribution of aerosol produced with the spinning-disc generator from a dilute suspension of *B. subtilis* var. *niger* spores containing 0.1% dextran.
TABLE 1. Particle size of aerosols produced with the spinning-disc generator from dilute B. subtilis var. niger spore suspensions containing various amounts of dextran.

| Dextran conen (by weight) | Aerosols sized | Mean NMD (μm)c | Mean GSD |
|---------------------------|----------------|----------------|---------|
| %                         | p              | sp             | d       |         |
| None                      | 6              | 0.87           | 1.09    |         |
| 0.001%                    | 2              | 0.90           | 17.9    | 1.09    |
| 0.01%                     | 4              | 0.78           | 16.7    | 1.13    |
| 0.1%                      | 10             | 1.73           | 17.3    | 1.11    |
| 1.0%                      | 20             | 3.60           | 16.7    | 1.10    |

* Dextran-spore suspensions titered $2 \times 10^7$ spores/ml; suspensions were fed onto disc at a rate of 2 to 5 ml/min.

* Abbreviations: p, solid dextran particle as observed from electron micrographs; sp, spore-bearing dextran particle as calculated from p, using equation 3; d, primary liquid droplet as calculated from p, using equation 2.

* Not applicable or no method of determining.

Results of 42 tests are shown in Table 1. The spinning disc routinely produced homogeneous aerosols as evidenced by the low GSD values. The GSD of the 42 aerosols sized ranged from 1.05 to 1.16 (not shown in Table 1). This compares favorably with reports in the literature. The mean NMD values of the dextran aerosols, as observed from electron micrographs, were 0.39, 0.78, 1.73, and 3.60, respectively, for dextran concentrations of 0.001, 0.01, 0.1, and 1.0%. Utilizing equation 2, the apparent diameters of the primary droplets were calculated, and, utilizing equation 3, the diameters of the spore-bearing particles were calculated from the dextran particle diameters. It should be noted that increasing the dextran concentration apparently did not alter the diameter of the primary droplets. The primary droplet NMD of the 42 aerosols sized varied from 16.1 to 18.4 μm (not shown in Table 1). This is considered good reproducibility. However, other aerosols not reported here, produced with the same generator but with a different disc, were also of good quality but had significantly larger droplet NMD values (19 μm). Therefore, aerosol particle size should
be determined upon any change in the generator or its operating conditions. The ability of a commercially available spinning-disc generator to produce homogeneous aerosols over a range of sizes by using bacteria as a viable tracer has been established. This type of aerosol is especially useful for calibrating air sampling devices, for evaluating air filters, or for use wherever aerosol behavior may be size-dependent.

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