CHARACTERISTICS OF DRIED SERRATIA MARCESCENS IN THE AIRBORNE STATE

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Although there have been many studies of the endurance and infectivity of bacteria atomized from liquid menstrua to simulate, for one purpose, the behavior of bacteria spread by coughs and sneezes (Wells, 1955), few studies of powdered bacterial aerosols have been reported. The latter appears to simulate airborne dusts from roads, floors, bedding, and fomites in general, and is, therefore, of possible public health importance.

Druett et al. (1953) states that as early as 1888 Buchner dispersed dried anthrax spores to infect small animals. Goss (1905) infected mice with powdered aerosols of both the plague organism and pneumococci. Lidwell and Lowbury (1950) investigated the survival of organisms in natural dusts from floor sweepings. Recently Beebe (1959) investigated the airborne behavior of dry Pasteurella pestis as influenced by relative humidity and exposure to ultraviolet irradiation. However, in the reports cited, the loss of viability was not studied in a manner which might elucidate death mechanisms or the infectivity of measured dosages. The lack of suitable laboratory dispersion methods for powdered bacterial preparations has been a great deterrent to such studies, especially when compared with the ease of atomization of liquidborne organisms.

The present paper is concerned with the generalized physical and biological behavior of aerosols of dried Serratia marcescens as studied under the condition of stirred settling. In general, the findings demonstrate that powdered aerosols can be dispersed in a form suitable for the study of survival, infectivity, and certain physical properties which influence the cloud of dry bacteria.

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MATERIALS AND METHODS

The aerosol chambers (cubic-meter boxes with fans to stir air), the particle sizing method (slope analysis of the decay of light scattered at right angles by the particles), the multiple-jet (Naval Biological Laboratory, NBL) disperser, the Stanford jet, and the CO$_2$ pistol disperser have been described earlier (Dimmick, Hatch, and Ng, 1958; Dimmick, 1959; Perkins et al., 1952; Gordieyeff, 1957).

S. marcescens strain 8UK, from two sources, was employed in the study. One was the laboratory stock culture grown on heart infusion broth (Difco) for 24 hr at 34°C and freeze-dried directly from the growth medium after the addition of 2 to 4 per cent sucrose. The other was a gel-dried preparation of S. marcescens furnished by the Western Utilization Branch of the Department of Agriculture (Matchett, 1956). Counts of viable bacilli were determined by appropriate dilution of a weighed powdered sample in 1 per cent peptone and subsequent surface plate count on blood agar base (Difco) medium. The number of viable cells in the dried solids varied from $10^6$ to $10^8$ cells per mg.

Powders (heart infusion broth cultures only) to be dispensed from the CO$_2$ pistol were ball-ground for periods of from 10 to 20 min in a glass mill 3 inches in diameter, 120 rpm, with 1/4-inch steel ball bearings; the other dispersers provided grinding during aerosolization.

Different weights of the ground powders were placed into tared no. 4 gelatin capsules, and the capsules were fired from within the chamber by means of the pistol. By this procedure dispersal was instantaneous. Unground material was prepared as previously described (Dimmick, 1959) and, using either the Stanford or NBL jet, dispersion was continued until a light scatter monitor read between 95 and 100 per cent.

After dispersion (zero aerosol time), 10 to 20
samples of the organisms remaining airborne were obtained at appropriate intervals by either an impinger method employing 1 per cent peptone as the diluent, the slit sampler method using BAB plates (Bourdillion, Lidwell, and Thomas, 1941), the sieve method (Andersen, 1958) using BAB plates or, rarely, by settling on BAB plates. The temperature and relative humidity within the chambers were noted at zero time and at regular intervals thereafter. Because the sample volumes were small (2 to 6 L) the simple expedient of replacing the sampled air with filtered room air was employed.

Colony counts were converted to the number of particles containing viable organisms per liter of air, and the log of this number was plotted versus time to obtain the total biological decay curve (physical plus biological). The loss of particles from the aerosol by sedimentation (physical decay) was obtained from the light scatter decay curve (Dimmick et al., 1958). Exponential portions of these slopes, usually after 60 min aerosol time, were employed to calculate the corrected biological half-life ($T_2$) by use of the formula $T_2 = (T_1 \times T_2)/(T_1 - T_2)$ where $T_1$ is the physical half-life and $T_2$ the total biological half-life. In instances wherein the physical decay rate was changing, as in the early stage of aerosol life, the total biological half-life could not be corrected for physical decay.

Some aerosols were atomized directly from the 24-hr growth culture (heart infusion broth) by a Wells-type atomizer, for comparative purposes. Aerosol containment, particle sizing, and sampling were performed as described above.

**RESULTS**

The mass median diameters of aerosols dispersed from the CO$_2$ pistol varied from 6 to 15 $\mu$, and only about 1 per cent of the material was in a size range (1 to 3 $\mu$) considered suitable for respiratory studies. Twenty to 40 mg of material were required to produce aerosols of sufficient viable airborne content (at least $10^5$ organisms per liter at 1 min aerosol time) to sample accurately. The averages of the mass median diameters varied with relative humidity as shown in figure 1.

Crinding dried cells in the ball mill caused a decrease of viability of 10 to 20 per cent during the first 15 min and 40 to 50 per cent during a 2-hr period. In an exploratory test, powder col-

![Figure 1](http://jb.asm.org/)  
*Figure 1.* The relationship between particle size and relative humidity of powdered Serratia marcescens dispersed by the CO$_2$ pistol and the Stanford jet.

Total variation is shown by the vertical lines. A change in density could be partially responsible for the changes shown here.
lected from the walls of a 1-L flask into which capsules had been fired (5 tests) contained an average of 80 per cent of the original concentration of living bacilli and varied between the limits of 70 and 100 per cent. Thus, low initial recoveries which had been observed in aerosols (0.6 per cent or less based on the theoretical concentration dispersed) could not be attributed to death by reason of explosive release of aerosol.

Aerosols from the Stanford jet consisted of particles about 6 μ mass median diameter, and about 4 mg were necessary to produce aerosols of the required bacterial content. As much as 30 per cent was in the 1 to 3 μ range. The size of these particles varied with relative humidity as shown in figure 1.

The NBL jet dispersed particles in the range of 2 to 4 μ mass median diameter, required 1.2 to 4 mg of material per aerosol, and frequently more than 60 per cent of the particles in an individual aerosol were in the 1 to 3 μ range, whereas atomized particles were in sizes below 2 μ mass median diameter and required 1 to 2 ml of liquid per aerosol. With the latter two methods of dispersion there was no certain indication of a change of particle size with relative humidity; however, the largest sized particles showed the greatest variation. A typical light scatter monitor record of an aerosol dispersed by the NBL jet is shown in figure 2. Initial viable bacilli recoveries have been reported elsewhere (Dimmick, 1959).

Typical total biological decay patterns obtained after dispersion from the NBL jet are shown in figures 3 and 4. A period of rapid loss of viability occurred initially, followed by a period of slower decay which usually continued to the limits of sampling ability; infrequently a state of increased decay rate was noted after 200 min. This behavior is common with atomized aerosols. Deviations between slit sampler data and impinger sampler data during the initial decay period result from the presence of large particles which contained more than one viable cell. These differences, at zero time, were between 500 and 1,000 times for aerosols dispersed from the CO₂ pistol, 5 to 100 times for the jets, and 0 to 5 times for atomized aerosols.

The effect is more clearly shown in figure 4, the data of which were obtained when the

Figure 2. Record of the behavior of a powdered aerosol of Serratia marcescens as indicated by a right-angle light scatter monitor.

Dispersion (NBL jet) started in upper right and ceased at "zero" aerosol time. In lower left the time scale was changed from minutes to hours. Total observed time was 3½ hr. Steps in jet adjustment are indicated for the dispersion period.
Figure 3. Example of the decay of viability of aerosols of Serratia marcescens dispersed from the NBL jet.

It might appear that the transition point, at about 75 min, was associated with the appearance of single cell particles as indicated by agreement between slit sampler and impinger counts, but this agreement did not always occur.

Figure 4. The decay of the viability of an aerosol of powdered Serratia marcescens as indicated by an Andersen sampler. Values plotted are the sums of the stages. The effect of the change of particle size is shown in the table.

Andersen sampler had been utilized to determine loss of viability. Data from each stage of the sampler indicated a different half-life, and the distribution of colonies per stage was in reasonable agreement with the particle size distribution as estimated from the physical decay pattern. However, the half-lives per sampler stage did not relate to particle size; in some instances the
Figure 5. The relationship between biological half-life and relative humidity of powdered aerosols of *Serratia marcescens* dispersed from the CO₂ pistol. Total variation is shown by the vertical lines. Some aerosols, atomized from the growth medium, are included for comparison.

Figure 6. The relationship between biological half-life and relative humidity of powdered aerosols of *Serratia marcescens* dispersed from the NBL jet. Total variation is shown by vertical lines. Also shown is the relationship between relative humidity and the initial half-lives of aerosols dispersed from the pistol.
half-life of the first stage exceeded the expected half-life, based on a 10 μm size estimate, by fourfold.

When values of corrected biological half-lives were plotted versus the initial relative humidity, the relationships indicated in figures 5 and 6 were obtained. For comparison the figures show results from pistol-dispersed aerosols, jet-dispersed aerosols, and aerosols atomized directly from the growth medium. Loss of viability was rapid at humidities above 50 per cent, and there appeared to be another area of rapid loss at about 15 per cent relative humidity. Highest survival levels were apparent at humidities in the range of 30 per cent and near zero relative humidity. Aerosols dispersed by the pistol or by the jets manifested the same relative humidity relationships, although correcting the former for physical fallout resulted in what appeared to be increased stability. Conversely, atomized bacilli died at greater rates at the lower humidities and were most stable at humidities above 50 per cent.

No significant differences were noted in the behavior of the gel-dried preparation when compared with the freeze-dried material, either in physical fallout or biological response to differences in humidity. Unless noted otherwise, the figures include data from both types of preparations.

DISCUSSION

The results of these studies show that standardized preparations of dried organisms can be employed in aerosol tests in much the same manner as those atomized from liquid menstrua.

One exception, which may apply only to the organism tested, was the difference in aerosol stability between “wet” and “dry” aerosols in relationship to relative humidity. In each instance, highest stability appeared to be associated with those preparations which encountered the least change in their environment, that is, the dry cells remained dry at low relative humidities, and the atomized cells did not dry completely at high relative humidities. (Tests have shown that the dried menstruum takes up water from the air at relative humidity conditions as low as 5 per cent.) Presumably the water content of both types of menstruum would be identical when in equilibrium with a given vapor pressure condition. However, since complex media were employed, there remains the possibility that hydrated salts or different “bound water” energy states formed after dispersion of the aerosol and that the adsorption-desorption isotherms were not identical. This relationship has been suggested by Orr, Hurd, and Corbett, (1958) for small particles of salts. That the cells survival is critically dependent on water content has been amply documented (Kethley et al., 1957a, b).

The similarity between aerosols atomized from liquids and the powdered type is further shown by the existence in both types of an initial rapid decay followed by the period of slower, secondary decay. The transition point between these events seemed particularly marked, however, in powdered aerosols. The parallel variation in initial and secondary rates with changing relative humidity, as shown in figures 5 and 6, is suggestive of similar death mechanisms influencing both rates. The presence of two rates might have been influenced in the pistol-dispersed aerosols by the presence of large particles, but with jet-dispersed aerosols there was no indication of a sufficiently high number of large particles to have accounted for the initial rapid loss of viability. The errors in the assignment of particular significance to either the exponential decay or the apparently sharp transition point have been discussed by Monk and Mattuck (1956).

As noted in figures 5 and 6, there was considerable variation of the corrected biological half-lives especially in the 20 to 60 per cent relative humidity range. In some instances the aerosol was divided during dispersion into the two chambers where conditions were apparently identical. Individual determinations of viable cells remaining within the aerosol, as indicated by all the samplers employed, deviated from a line of best fit no more than 5 per cent, yet the biological decays were frequently quite different.

The cause of this variability is not clear, but may be due to a lack of real identity of conditions within the two test chambers. The most suspect measurement is that of relative humidity. Recent tests of a preliminary nature have shown that the protein membrane type hygrometers change in sensitivity and “zero” setting as dust collects on the element during use and also that atomized suspensions of washed cells exhibit an extreme sensitivity to slight changes in moisture content of the air. These observations point out the need for further experiments utilizing equipment
characteristics of Dried *S. marcescens* capable of a high degree of control and accuracy. Of course, it may not be relative humidity alone which influences the death rate. The absolute moisture content of the droplet as a function of environmental vapor pressure, which is in turn temperature dependent, is probably the fundamental variable. Presumably the airborne cell reacts to a microenvironment which equilibrates rapidly with changes in the external environment.

Another problem is that of assuring a high content of viable bacteria in the dried powder if, for example, the influence of a defined medium on the aerosol survival level is to be tested. The medium, or supporting menstruum, must provide a high level of survival during the freeze-drying step and must have physical characteristics suitable for dispersion. These problems are unique to each instance.

The jet-dispersed aerosols were superior in most respects to those dispersed by the pistol; fewer cells were required, higher particulate content in the aerosols was obtained, the aerosols existed for longer periods of time, and the content of particles in the 1 to 3 μ range was greatly increased. An additional advantage was the ability to disperse continuously, as shown in figure 2, which provided a more adequate degree of control than explosive methods of dispersing aerosols as well as presenting the possibility of utilizing the jets in conjunction with dynamic-type chambers as atomizers have been employed. In contrast to pistol-dispersed aerosols, the physical decay of atomized aerosols, and of most of the jet-dispersed aerosols, was so slight compared to the total biological decay that correction for physical decay was negligible.

Pistol-dispersed aerosols are easy to create and should be useful, however, for studies of non-pathogens if suitable correction for physical decay is employed. In this regard it may be noted that the uncorrected biological half-lives of the pistol-dispersed cells were similar to the corrected half-lives of cells dispersed from the jets. Apparently the physical behavior of the former, as measured by light scatter decay, was not that of the particles containing viable bacilli, or erosive forces within the jet disperser had in some way sensitized the cells. The latter possibility has not been tested.

**SUMMARY**

Aerosols of freeze-dried cultures of *Serratia marcescens* were dispersed from the dried state into stirred settling chambers by an explosive method and an erosive method. The decrease of the numbers of viable airborne bacilli was obtained by sampling with conventional instruments. Two decay rates were apparent. An initial rapid loss of viability was followed by a slower decay which lasted for periods as long as 5 hr. The relative humidity within the chamber was shown to influence the rate of decay; at high humidities the rates were rapid, whereas decreased rates of loss were noted at low humidities. These relationships are almost the reverse of those found in aerosols atomized from liquid menstrua. In the midranges of humidity, survival was difficult to predict, and the variations are probably referable to unmeasured changes in humidity and temperature. Both the initial and secondary biological decay rates varied with relative humidity in the same manner, suggesting that similar mechanisms of death were involved.

**REFERENCES**


