Relationship Between Atmospheric Temperature and Survival of Airborne Bacteria

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Effects of temperatures ranging from −40 to 49 C on the behavior of airborne Serratia marcescens, Escherichia coli, and Bacillus subtilis var. niger were investigated. Aerosol decay rates of B. subtilis spores were not significantly affected by the temperature and remained approximately constant within the temperature range studied. The survival of airborne S. marcescens and E. coli was closely related to the temperature. An increase in temperature from −18 to 49 C resulted in a progressive increase of the biological death rate, and the relationship between the biological death rate and the temperature appeared to be linear. An increase in temperature from 24 to 49 C resulted in significantly reduced aerosol recoveries of the two vegetative organisms. At −40 C, the aerosol recovery of all three agents was consistently lower than at −18 to 24 C.

The major atmospheric factors affecting the survival of airborne microorganisms are humidity and temperature. Although numerous investigators have reported studies of the effects of humidity on survival of airborne bacteria and viruses, literature pertaining to the influence of temperature is much more limited. The experimental studies described in this paper examined the effects of various temperatures ranging from −40 to 49 C on the survival of airborne Serratia marcescens, Escherichia coli, and spores of Bacillus subtilis var. niger.

MATERIALS AND METHODS

Concentrated stock cultures of S. marcescens strain 8 UK (ATCC 14041), E. coli strain 162 (Porton), and B. subtilis (batch no. 91) were kindly provided by the U.S. Army, Fort Detrick. The microorganisms were deep-fermented, concentrated by centrifugation, freeze-pelleted, and stored in individual vials at dry ice temperature until used. Aerosol experiments conducted with these organisms at various storage intervals indicated no changes in aerosol characteristics at 24 C and 85% relative humidity (RH) over long periods of storage. The microorganisms were thawed and suspended in gelatin phosphate diluent, and 1 ml of the suspension was disseminated by means of a simple two-fluid atomizer (FK-8 aerosol gun). The atomizer, operated at 50 psig of nitrogen, disseminated 1 ml of fill volume in less than 5 sec and produced an aerosol cloud having a majority of the airborne particles in the 1- to 5-μm size range. The microorganisms were disseminated into a 2,500-liter, Freon-tight stainless-steel aerosol chamber with an attached bacteriological safety cabinet system. To maintain a homogenous distribution of the cloud, a small fan was operated in the aerosol chamber throughout an experimental trial.

The desired temperature and humidity conditions in the aerosol chamber were established before the dissemination and were maintained and monitored at the selected condition after the microorganisms were introduced into the chamber. The air temperature in the chamber was continuously measured and recorded with a resistance bulb connected with a resistance-to-current converter (model 694, Foxboro Co.). The dew point was determined by means of an electronic-type, humidity-sensitive dew cell element (model 2711 AG, Foxboro Co.). The dew point measurements were converted to per cent RH by using standard conversion tables. The temperatures used in the studies ranged from −40 to 49 C with 2 to 3 C variation in the specific temperatures examined from experiment to experiment. At −2 C and above, the humidity in the aerosol chamber was maintained at 85 ± 5% RH, whereas at −18 C and below essentially saturated atmosphere was used.

To estimate the biological decay rate of the airborne microorganisms, suitable cloud tracers were used. For B. subtilis spores, sodium fluorescein served as the tracer and the dissemination mixture consisted of B. subtilis spores suspended in gelatin phosphate diluent containing 0.4 g of sodium fluorescin per 100 ml of the diluent. To determine the death rate of airborne S. marcescens or E. coli, spores of B. subtilis were used as the physical decay tracer. The ratio of the vegetative microorganisms to the spore tracer in the dissemination mixture was approximately 10:1.

For the quantitative assay of the airborne bacteria, duplicate aerosol samples were collected for 1 min at
4-, 16-, 32-, and 64-min cloud ages. The samples were obtained with all-glass impingers (AGI-30 (1)) operated at a sampling rate of 12.5 liters per min. A single-stage impactor (4), designed to remove particles larger than 5 µm, replaced the usual curved stem of the AGI-30. The AGI-30 contained 20 mL of tryptose saline, gelatin saline, or distilled water as collecting fluids for S. marcescens, E. coli, and B. subtilis, respectively. To prevent foaming during aspiration, the collecting fluids contained 0.15% Dow-Corning Antifoam A emulsion. During experiments involving subfreezing temperatures, the aerosol samplers were placed in a water bath maintained at approximately 21°C to prevent freezing of the collecting fluid.

The contents of the duplicate AGI-30 samplers were pooled, and standard bacteriological dilution and plating techniques were used to assay the collected microorganisms. The number of viable S. marcescens and E. coli was determined on Difco Castone-agar containing Brilliant Green and cycloheximide as inhibitors of the spore tracer and fungal contaminants. B. subtilis was assayed on Tryptose Agar (Difco) with potassium tellurite and cycloheximide added as inhibitors. The plates were incubated at 37°C for 24 hr and counted. Sodium fluorescein was assayed fluorometrically in a Coleman photofluorometer by comparison with freshly prepared standard solutions.

Within an experiment, a minimum of six replicate aerosol trials were conducted at each temperature condition. The estimates of the aerosol parameters studied in these experiments were compared by standard analysis of variance techniques. The 5% level of probability was selected to determine the significance of the differences observed among treatment means.

RESULTS AND DISCUSSION

The decay of a microbial aerosol can be estimated by quantitating the numbers of airborne organisms per unit of air volume at various cloud ages. The slope of the resulting curve describes the rate of decay of the aerosol cloud and can be expressed as per cent per minute (%/min). This total decay rate of a biological cloud includes losses caused by physical factors, such as fallout or deposition on the walls of the chamber (physical decay), and also those caused by biological inactivation of the microorganisms (biological decay). Estimates of physical decay of aerosols can be made on the basis of the recovery of a suitable tracer, such as sodium fluorescein or spores of B. subtilis, disseminated as an intimate mixture with the more labile vegetative microorganisms. The difference between the total decay rate and the physical decay rate provides an estimate of the biological decay or the death rate of the microorganisms.

Spores of B. subtilis are known to exhibit high biological stability within a wide range of temperatures, and it has been assumed that the spores will also remain viable when airborne under such conditions. Accordingly, spores of B. subtilis are frequently used as a physical tracer in aerosol studies involving less stable bacteria and viruses. Results of our investigations confirmed the high aerosol stability of this microorganism within the temperature range of −29 to 49°C. Although the differences among estimates of biological decay rate in this temperature range were not supported statistically (Table 1), at 49°C, the death rate appeared to be higher than at the other temperatures. It was also important to note that, except at 49°C, the 95% confidence limits encompassed zero decay, suggesting that the biological losses were undetectable. Aerosol recoveries of B. subtilis at 4-min cloud age were similar within the temperature range of 4 to 49°C. The mean recovery calculated for the five temperature conditions was 55.9 ± 8.0%. At −29°C, however, aerosol recovery estimates were significantly reduced (28.0 ± 3.7%). This lower aerosol recovery of B. subtilis at −29°C was paralleled by the recovery values obtained for the sodium fluorescein physical tracer and probably reflects, in part, a reduction in atomizer output and efficiency.

Table 1. Effect of temperature on airborne B. subtilis var. niger spores

| Temp | Biological decay rate (%/min) | Per cent recovery at 4-min cloud age |
|------|-------------------------------|------------------------------------|
| C    |                               |                                    |
| −29  | 0.19                          | 28.0                               |
| 4    | 0.10                          | 55.1                               |
| 10   | 0.04                          | 65.4                               |
| 16   | 0.05                          | 62.8                               |
| 24   | −0.09                         | 50.3                               |
| 49   | 0.49                          | 46.0                               |

* At the temperatures tested, differences among estimates of biological decay rate were not supported statistically.

Aerosol recoveries were similar within the temperature range of 4 to 49°C. At −29°C, however, aerosol recovery estimates were significantly reduced.
A similar relationship between temperature and aerosol decay was seen for airborne *E. coli* (Fig. 1); however, the slope of the curve was not as steep as that observed for *S. marcescens*. Differences in biological decay rate estimates within the temperature range of −40 to 24°C could not be discriminated statistically, although numerical values indicated a rate of decay almost three times as rapid at −2 and 24°C (2.36%/min) as at −40 or −18°C (0.90%/min). At 35 and 49°C, the death rate increased significantly to a mean value of 8.65%/min.

The influence of temperature is further illustrated in Fig. 2, which shows the aerosol recovery of *S. marcescens* at various cloud ages. The family of curves based on observations obtained at intervals during the 1-hr cloud age indicated that a progressive increase in temperature usually resulted in a decrease in initial aerosol recovery. Moreover, the slope of the curves made it apparent that a concomitant increase in biological decay rate was present. The most evident exception in this relationship appeared to be the behavior of airborne *S. marcescens* at the lowest temperature. The recovery at −40°C resembled more closely that observed at 24°C rather than those at −18 or −2°C.

Aerosol recoveries of *S. marcescens*, *E. coli*, and *B. subtilis* spores obtained at an early cloud age (4 min) are shown in Fig. 3. Aerosol recoveries of all three microorganisms were low at −40°C. Recoveries of *B. subtilis* within the temperature range of −18 to 49°C were remarkably uniform, confirming the high aerosol stability of this agent. The differences among aerosol recoveries of *S. marcescens* and *E. coli* were relatively uniform at temperatures ranging from −18 to 24°C. However, between 29 and 49°C, progressively lower aerosol concentrations were observed.

Studies concerned with the effects of temperature on airborne bacterial agents have been reported by only a few investigators. Ferry et al. (2) studied the death rate of airborne *Micrococcus candidus* at 15, 25, and 35°C in a nitrogen atmosphere. The authors concluded that the rate of the decay appeared to increase two- to three-fold for each 10°C increment of temperature.
Agreement with those reported by the other investigators. The data suggested that a progressive increase in temperature from $-18$ to $49\,^\circ C$ resulted in an increase in the biological death rate of *S. marcescens*. An increment of $10\,^\circ C$ resulted in approximately a threefold increase in the rate of biological decay. However, this linearity of response did not extend to the lowest temperature studied, namely $-40\,^\circ C$. At this point, the biological decay rate resembled more closely that at $24\,^\circ C$ rather than that at $-18$ or $-2\,^\circ C$.

The significantly lower aerosol recoveries of all three agents at $-40\,^\circ C$ were probably due, in part, to changes in physical characteristics of the slurry and to the configuration of the particles upon dissemination at this low temperature. The extremely low temperature and the concomitant saturated atmosphere could retard or totally prevent drying of the airborne particles and thus influence the initial particle size distribution of the aerosol cloud. Such alteration of particle size distribution would in turn be reflected in estimates of aerosol recovery, especially when a particle size discriminating device is used in aerosol sampling.

Calculation of aerosol survival based on the ratio of *S. marcescens* to *B. subtilis* in suspension and cloud samples suggested that the low recoveries of *S. marcescens* at $-40\,^\circ C$ were not limited to changes in physical characteristics of the aerosol, but were in part due to the initial lower survival of the vegetative microorganism. The estimate of the per cent survival of this vegetative microorganism in particles of $5\,\mu m$ or less in diameter at $4$-min cloud age was approximately $60\%$ at $-40\,^\circ C$ as compared to essentially $100\%$ at $-18$, $-2$, and $24\,^\circ C$. The results obtained with *E. coli* likewise indicated a lower initial survival at $-40\,^\circ C$.

The rapid increase in biological death rate and the decrease in aerosol recovery of *S. marcescens* and *E. coli* during early cloud ages in the temperature range of $24$ to $29\,^\circ C$ are of importance. For example, very frequently investigators interested in the effects of RH on airborne bacteria report the temperature as "ambient," which indeed could fall within this critical range and thus could have a significant effect on the resulting data. The behavior of airborne organisms at various temperatures cannot be predicted on the basis of a single model system or for a given organism on the basis of studies at only one or two temperature conditions. For a thorough characterization of behavior of airborne microorganisms, a broad spectrum of temperatures must be used and extrapolation of the results

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**Fig. 3.** Effect of temperature on aerosol recovery of *S. marcescens*, *E. coli*, and *B. subtilis* spores at 4-min cloud age.
into lower or higher temperatures should be avoided. When effects at extreme temperatures are investigated, a condition at which the airborne behavior of an organism is well established should serve as a basis of comparison.

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LITERATURE CITED

1. Brachman, P. S., R. Ehrlich, H. F. Eichenwald, V. J. Cabelli, T. W. Kethley, S. H. Madin, J. R. Maltman, G. Middlebrook, J. D. Morton, J. H. Silver, and E. K. Wolfe. 1964. Standard sampler for assay of airborne microorganisms. Science (Washington) 144:1295.

2. Ferry, R. M., W. F. Brown, and E. B. Damon. 1958. Studies of the loss of viability of bacterial aerosols. III. Factors affecting death rates of certain nonpathogens. J. Hyg. 54:389–403.

3. Kethley, T. W., E. L. Fincher, and W. B. Cown. 1957. The effect of sampling method upon the apparent response of airborne bacteria to temperature and relative humidity. J. Infec. Dis. 100:97–102.

4. Malligo, J. E., and L. S. Idoine. 1964. Single-stage impaction device for particle sizing biological aerosols. Appl. Microbiol. 12:32–36.

5. Webb, S. J. 1959. Factors affecting the viability of airborne bacteria. I. Bacteria aerosolized from distilled water. Can. J. Microbiol. 5:649–669.

6. Won, W. D., and H. Ross. 1968. Behavior of microbial aerosols in a −30°C environment. Cryobiology 4:337–340.