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Effect of Temperature on Survival of Airborne *Mycoplasma pneumoniae*

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Aerosols of *Mycoplasma pneumoniae* were prepared at each of eight relative humidities between 0 and 85% and at five separate temperatures between 10 and 43 C. Survival of these organisms was found to be a function of both relative humidity and temperature. However, the temperature response was mediated by humidity in that the effects of temperature could be observed only if some water vapor was present. At all temperatures, survival of *M. pneumoniae* in aerosols was found to be best at the extremes of relative humidity. The effects of temperature were such that irrespective of relative humidity an increase in temperature resulted in a decreased airborne survival time.

Although several hypotheses have been proposed to explain the loss in viability of airborne bacteria (2, 4, 6, 13), the general phenomenon remains unexplained. An earlier attempt (14) to demonstrate the mechanisms of airborne death by using mycoplasma was unsuccessful. However, this work did show that the response of mycoplasmas to stresses of aerosolization and to changes in relative humidity (RH) was similar to those of bacteria. These results demonstrated that airborne survival did not depend on the structural dissimilarities between bacteria and mycoplasmas, but they raised the question as to whether other controllable environmental parameters would have an effect on the survival of these organisms in the airborne state.

The most frequent environmental change to which an airborne particle is exposed, with the exception of humidity, is temperature. Thus, whether RH acts independently or in conjunction with changes in temperature to enhance the death of airborne particles should be determined if the true nature of the airborne mycoplasma, its survival, transmissibility, and infectivity are to be understood.

Previous studies (7, 8, 11) with airborne bacteria revealed an inverse relationship between survival and temperature, and the question was raised as to whether this is a general phenomenon extending to other organisms such as the mycoplasmas. These studies of temperature on airborne bacteria were conducted over relatively short time spans either in static or in stirred settling chambers, and it seemed worthwhile to extend our studies to include a long-term aerosol storage.

MATERIALS AND METHODS

Organism and growth conditions. *Mycoplasma pneumoniae* M52 was grown in PPLO Broth (Difco), 20% horse serum, and 2% yeast extract under conditions previously reported (15).

Aerosolization. Several large, batch cultures of *M. pneumoniae* were pooled and concentrated 10-fold by centrifugation. This material, sufficient to complete all experiments, was separated into 70-ml amounts and was stored at −70 C until used. Aerosols containing *M. pneumoniae* were prepared from a thawed culture (slurry) by using a Wells refluxing nebulizer and aerosolizing the slurry into eight, 1,200-liter, stainless-steel, rotating drums (9) which were in a temperature controlled room. After the 10-min preaerosolization period necessary to produce a uniform dispersal of single cells throughout the slurry (15), the drums were filled in a sequential but random order. Each drum was filled for 1 min, giving a total reflux time of approximately 20 min. The number of colony-forming units per milliliter in the slurry remained constant during the period of drum fill, assuring an equal concentration of viable particles in each drum. Using an Andersen sampler (1), samples were taken from the drums at various times throughout the experiments. These samples showed that the aerosols were composed of particles which were fairly uniform in size and relatively monodispersed, and that 99% of the particles were smaller than 3 μm in diameter (15). At 5 min before 1-min aerosol fill, and for 5 min after the fill, drum rotation was cycled by using a 30-sec rotation period followed by a 30-sec stationary period. This procedure facilitated the establishment of a uniform aerosol distribution within the drum. Samples were taken 5 min after drum fill and at selected intervals for up to 12 hr. The observed physical half-life of a test aerosol within the slowly rotating drum (approximately 2 rev/min) was in excess of 2 days with normal sampling. With minimal sampling, the observed half-life was in excess of 200 hr.

Samples of aerosol were taken directly from the drums with either 1-, 10- or 100-ml syringes. The aerosol was then impacted directly onto an agar surface by means of a standard slit aerosol sampler having an air sampling rate of 10 liters per min. All data shown have been corrected for physical aerosol loss as determined by measuring the relative light scatter of the aerosol with a forward angle light scatter photometer.

The effect of eight different RH values (0, 13, 25, 38, 51, 67, 75, and 85%) on the viability of *M. pneumoniae* in aerosols held at five different temperatures (10, 15, 27, 38, and 43 C) was determined during these studies. Temperatures were determined by means of a thermocouple, and the room containing the rotating drums was maintained within 0.5 C of the desired temperatures. A Cambridge dewpoint hygrometer was used to measure the RH in the drums. The desired RH was achieved by mixing wet air with dry air as it entered the drum.

RESULTS

One of the principal causes of the apparent variation in survival of airborne microbes is the difference in the preparation and handling of slurry materials (5, 10). To eliminate as many variables from our procedures as possible and to reduce variation in aerosol response resulting from culture procedures, sufficient slurry for all experiments was produced at one time. However, the third and fourth replicates of each experiment were performed by using a different frozen slurry prepared in the same manner as was the first culture.

The results obtained with the concentrated frozen slurry compared favorably with results obtained from a fresh slurry. Figure 1 shows results representative of the stability of airborne *M. pneumoniae* atomized at *0*, *38*, and *67%* RH. Effects of other RH values used in these experiments on both types of slurry were determined, and they were similar to those shown in the Fig. 1. It is apparent that the frozen preparations produced a uniform and reproducible aerosol survival pattern that was similar to that of the fresh slurry. However, aerosol survival of the organisms from the frozen slurry was greater than that from the fresh material, a difference which was not attributable to a higher initial number of organisms in the frozen slurry. All subsequent experiments were performed with slurries from frozen culture material.

Figures 2 and 3 show the effect of temperature and humidity on the survival of *M. pneumoniae* when held in the airborne state for up to 6 hr. All experiments were continued for a total of 12 hr, although each decay rate was well established by the 6th hr and no changes occurred between aerosol ages 6 and 12 hr. Each point represents the mean of four separate experiments. Although

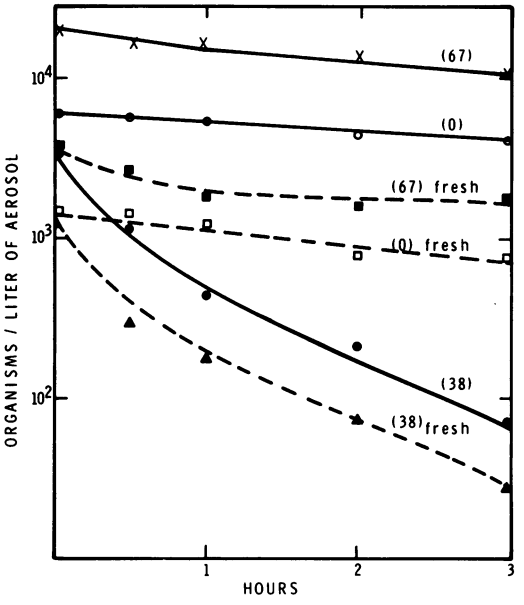

FIG. 1. *Comparison of survival of airborne M. pneumoniae from frozen and freshly prepared slurries. The temperature was 21 C, with the relative humidities indicated on the figure.*

survival of *M. pneumoniae* was determined at each temperature for all RH values, the data are also representative of the other RH and temperature experiments, in which the results were similar to the pattern shown.

Figures 1–3 show the rate of change in colony-forming units obtained by our techniques and they represent the actual number of organisms recovered during the experiments. The number of surviving cells indicated at the ordinate axis represents the number found in the first sample, taken 5 min after filling the drums. Differences in the number of surviving organisms at different RH values at this time were apparently related to initial decay rates, which Webb (13) has characterized as differing significantly from those shown. The reason for this initial loss of viable *M. pneumoniae* is not known; however, each rotating drum was filled uniformly and the early loss of viable cells prior to the first sample is a reproducible observation.

The effect of temperature is minimized when humidity is very low (Fig. 2A). In fact, considering the duration of each experiment, survival under these conditions is virtually independent of temperature. However, at 25% RH (Fig. 2B) it is obvious that higher temperatures have an immediate and sustained deleterious effect on the organisms. This effect of temperature was further accentuated as RH was increased up to 85%

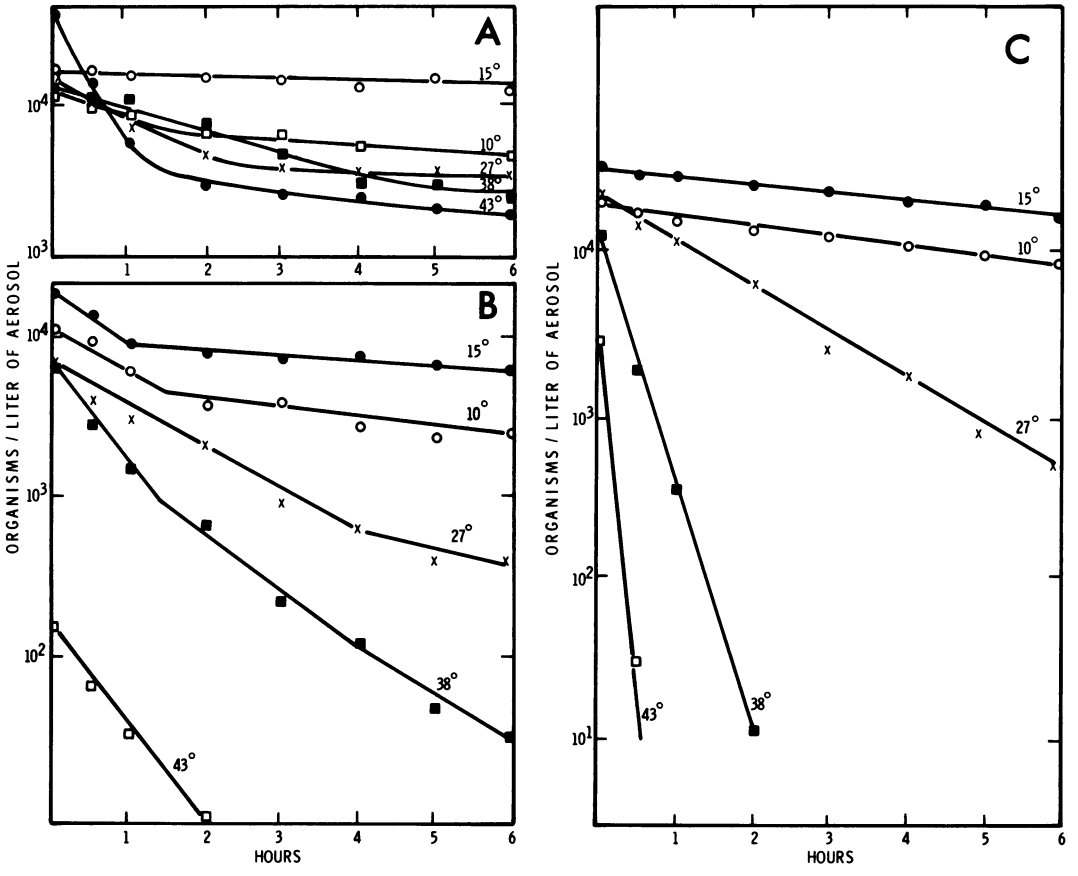

FIG. 2. *Effect of temperature on the survival of M. pneumoniae at (A) 13% RH, (B) 25% RH, and (C) 85% RH. Data shown represent the mean of four experiments at each RH and temperature. The extremes were within 10% of the values shown. Temperature is expressed in degrees centigrade.*

(Fig. 2C), at which point greater than 99% of the organisms lost viability within 30 min at 43 C.

Additional effects of humidity on survival at various temperatures are summarized in Fig. 3. At 43 C (Fig. 3A), *M. pneumoniae* survived only at low RH levels, and death was most rapid at RH values generally considered to be most lethal. At 43 C and 52% RH, no cells were recovered in the first sample, whereas other mid and high RH levels led to rapid but demonstrable loss of all airborne organisms.

The work of Kethley, Fincher, and Cown (11) demonstrated that the greatest stability for *Serratia marcescens* was near 18 C if specific methods were used to recover this organism. Figure 4 suggests that this may also be true for *M. pneumoniae* collected by procedures used in our study. This figure also shows the effect of humidity and temperature on aerosols of *M. pneumoniae* after 1 hr and it clearly illustrates the sustained survival of these organisms at low RH despite an increase in temperature.

DISCUSSION

The survival of *M. pneumoniae* in aerosols at a single temperature (15) indicated that the survival of these organisms at 27 C was dependent upon the RH of the aerosol. However, the data shown in Fig. 2–4 indicate that whereas humidity may play the major role in the survival of airborne microorganisms, temperature is also related in a complex way to survival. For example, at low temperatures the survival of airborne *M. pneumoniae* appears to be solely a function of the RH with a survival pattern unique to the organism involved (Fig. 3B), but when temperature is raised to between 27 and 43 C, a much greater sensitivity with respect to temperature and humidity is observed.

The effect of RH on the survival of *M. pneumoniae* is similar at all temperatures in that those RH values which are most deleterious at one temperature are also most deleterious at the other temperatures. It is of interest that survival is bet-

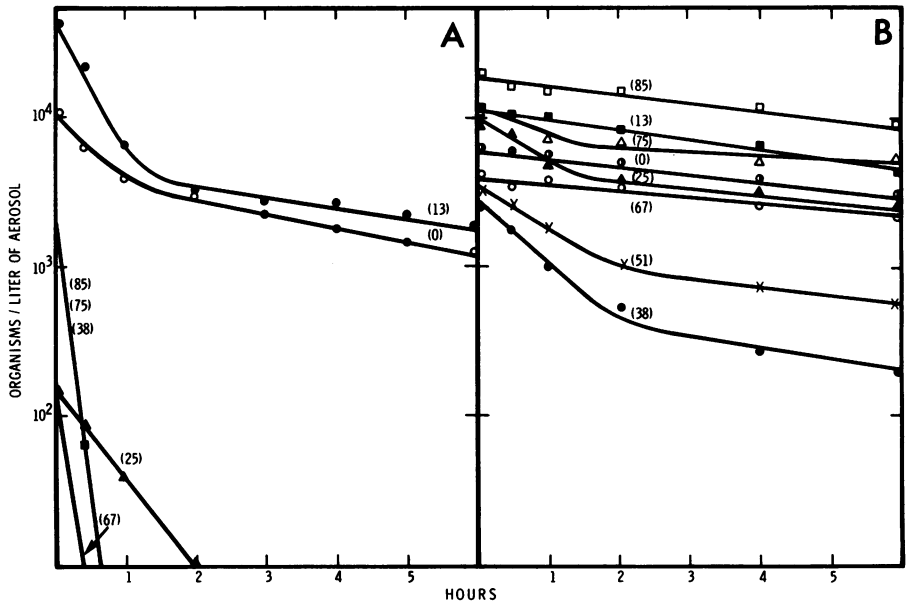

FIG. 3. *Effect of humidity on the survival of M. pneumoniae at (A) 43 and (B) 10 C. Curves represent the mean of four experiments at each RH and temperature, and all experiments were reproducible to within 10% of the values shown. Values in parentheses represent per cent relative humidity.*

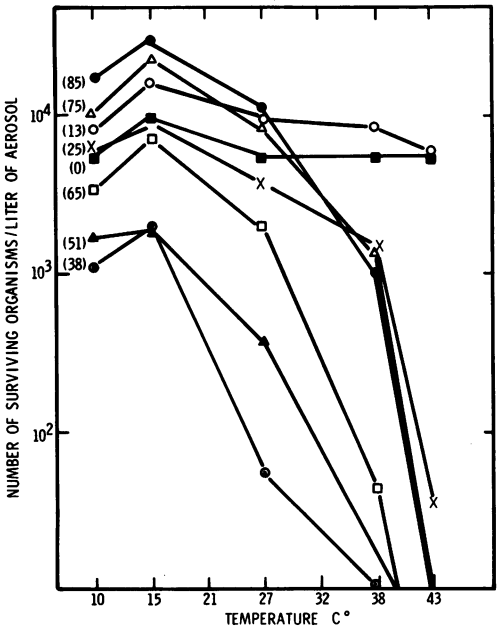

FIG. 4. *Effect of temperature and relative humidity on airborne M. pneumoniae, expressed as the number of surviving cells determined after 1 hr. Values in parentheses represent per cent relative humidity.*

ter at 15 than at 10 C. Although this difference is small, it is consistent and occurs at all RH levels studied. Such a survival pattern may reflect a need by the cell for metabolic functions which are limited by lower temperatures. The reasons for death of airborne mycoplasmas, whether due to failure of required anabolic processes, energy production, structural failure, or possibly to a resultant number of these forces which overlap to produce a condition lethal for the organism, are not known. However, it is apparent that the temperature of the environment surrounding the aerosol can and does play a major role in the stability of airborne organisms.

The effect of temperature on airborne microorganisms has received only limited investigation. In these studies (7, 8, 11, 13), there appears to be general phenomenon which results in decreased survival of the airborne cells as the temperature increases. As previous studies with airborne mycoplasmas (3, 12, 14, 15) have shown, the great physiological and structural differences between the true bacteria and mycoplasma do not cause significantly different response between these species when placed in the airborne state. The pattern of survival (Fig. 4) of *M. pneumoniae* in response to different temperature and humidity conditions is similar to those of the bacteria thus far studied, although we note that the bacteria have not been examined over the extended periods of airborne stress reported here. Loss of viability of bacteria appears to reach a logarithmic rate fairly early in the stress period, such that little change in the decay rate with time would be anticipated for those bacteria thus far studied.

Similarities between *M. pneumoniae* and bac-

teria in response to airborne stresses suggest that the phenomenon shown here is general in nature and that all organisms may respond similarly to temperature and RH changes when aerosolized.

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