Aerosol Survival of *Pasteurella tularensis* and the Influence of Relative Humidity

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The aerosol survival in air was determined for *Pasteurella tularensis* live vaccine strain (LVS) as a function of relative humidity (RH). Three different preparations of bacteria were used: (i) liquid suspension of *P. tularensis* LVS in spent culture medium; (ii) powders of *P. tularensis* LVS freeze-dried in spent culture fluid; (iii) *P. tularensis* LVS freeze-dried in spent culture fluid and then reconstituted with distilled water and disseminated as a liquid suspension. Preparation (i) gave greatest survival at high RH and lowest survival at intermediate RH. Preparation (ii), in contrast, gave greatest survival at low RH and minimum survival at 81% RH. Preparation (iii) was the same as preparation (i), i.e., the process of freeze-drying and reconstituting with distilled water before aerosol formation had little or no effect upon aerosol survival as a function of RH. Hence, control of aerosol survival appears to be through the water content of *P. tularensis* LVS at the moment of aerosol generation rather than the water content of the bacteria in the aerosol phase.

Of the many factors that influence aerosol survival of microorganisms (2), the role of relative humidity (RH) has been widely studied. The mechanism of its influence is not clearly understood (2); however, the manner of dehydration of the aerosol seems to be involved (3-12, 16-20). Webb (22, 23), on the other hand, considers that it is dehydration that influences aerosol survival. For bacteria aerosolized from a suspension in distilled water, at constant temperature and pressure, it is possible that RH exerts its influence by controlling the rate of evaporation of water from the aerosol droplet (2, 21). However, this possibility can be ruled out for *Escherichia coli* B in aerosols in nitrogen, argon, and helium (7).

Recently, strict comparisons have been made between aerosol survival of bacteria disseminated from the wet and dry states (10, 11). The results of these comparisons again indicated that rehydration, rather than dehydration, markedly influenced aerosol survival. Furthermore, aerosol survival as a function of RH was different for *E. coli* B [and for *Pasteurella tularensis* live vaccine strain (LVS)] disseminated from the wet and dry states, either into air or into purified nitrogen atmospheres (10, 11); i.e., the RH of the stored aerosol did not control survival. Possibly the freeze-drying, by means of which bacterial powders were made, changed the bacteria and suspending medium such that the pattern of aerosol survival of the bacteria as a function of RH was different for wet and dry dissemination (10, 11). The purpose of the work described in the present paper is to investigate this possibility. The technique employed was to compare aerosol survival as a function of RH for the following conditions: aerosols produced from (i) liquid suspensions of *P. tularensis* LVS in spent culture fluid; (ii) powders of *P. tularensis* LVS freeze-dried in spent culture fluid; (iii) *P. tularensis* LVS freeze-dried in spent culture fluid and then reconstituted with distilled water and disseminated as a liquid suspension.

**MATERIALS AND METHODS**

**Growth of *P. tularensis* and preparation for dissemination.** Details of conditions of growth have been given previously (11). After growth, three types of bacterial preparations were made. (i) The bacteria were collected by centrifugation and resuspended in spent culture fluid to give $10^8$ bacteria/ml. (ii) Samples (1 ml) of the resting-phase culture ($2 \times 10^{10}$ bacteria/ml) were freeze-dried in serum bottles for 5 hr to produce the powders for dry dissemination. Survival after freeze-drying was 20%. (iii) Samples (5 ml) of the resting-phase culture were freeze-dried in serum bottles for 5 hr. After freeze-drying, each serum bottle was reconstituted with 5 ml of double glass-distilled water.

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**Aerosol apparatus.** Liquid cultures were disseminated with a Wells atomizer (24). Freeze-dried powders were disseminated pneumatically as previously described (10, 11, 13). Aerosols were stored in air in a facility incorporating eight rotating drums (15) maintained at 26.8 C. Aerosols were usually sampled directly onto agar with the syringe-slit sampler technique (15).

**Assay.** The suspensions used for generating the aerosols were diluted as required and plated on blood-agar (14) for *P. tularensis* LVS and on tryptone agar (4) for *Bacillus subtilis* var. *niger* spores. The blood-agar contained penicillin to inhibit spore growth. After collection from the aerosol, blood-agar plates were incubated for 72 hr at 37 C and tryptone plates were incubated overnight at 37 C. After incubation, the colonies were counted. Percent viability was calculated from the ratio of *P. tularensis* LVS to *B. subtilis* var. *niger*, normalized to the ratio in the preparations used for generating aerosols as being 100% viability.

Only survival in air was measured because the toxic effects of oxygen on aerosol survival of *P. tularensis* LVS were small (11). All experiments were performed four times. For wet dissemination, the experimental error was ±20% of the survival value for good survival and ±70% of the survival value for poor survival. For dry dissemination, the errors were ±10 and ±50%, respectively.

**RESULTS**

**Survival of *P. tularensis* LVS disseminated from the wet state.** The 15-min survival values in aerosols generated from a type 1 suspension are given in Fig. 1. Survival was greatest at high RH and lowest in the mid-range of RH. At low RH, survival increased.

**Survival of *P. tularensis* LVS disseminated from the dry state.** The 15-min survival in aerosols generated from a type 2 suspension are given in Fig. 1. Unlike wet dissemination, survival was greatest at low RH, and minimum survival occurred at 81% RH. The RH for minimum survival was different from that for wet dissemination.

**Survival of freeze-dried *P. tularensis* LVS reconstituted with distilled water and disseminated from the wet state.** The aerosol survival of *P. tularensis* LVS (type 3 preparation) is given in Fig. 1. These results were very similar to those found for aerosols produced from a type 1 suspension, i.e., the processes of freeze-drying and reconstituting with distilled water before aerosol formation had little or no effect upon aerosol survival as a function of RH.

**DISCUSSION**

The aerosol survival of *P. tularensis* LVS disseminated from the wet and dry states has been determined previously (11). The present results are similar but differ in two ways. For wet dissemination, minimum survival occurred at 50 to 55% RH in the previous study (11), whereas in this study minimum survival occurred between 30 and 60% RH. For dry dissemination, minimum survival occurred at 81% RH, compared to 75% RH in the previous study (11). Differences of this nature have been observed previously, even within the same laboratory; e.g., compare the results of Cox (4) with those of Anderson (1). The reasons for such differences are not clear and indicate that unknown factors influence aerosol survival.

It has been suggested that the differences in aerosol survival of wet- and dry-disseminated *P. tularensis* LVS could be because the process of freeze-drying (used to prepare the dry powders) changes the composition of the bacterial suspending medium. Such a change then causes minimum survival to occur at different RH values for wet and dry dissemination. This possibility is eliminated by data given in Fig. 1, because *P. tularensis* LVS freeze-dried and then reconstituted with distilled water and disseminated from the wet state behaves in the same way as a wet-disseminated aerosol in which the freeze-drying step is omitted; i.e., a component in the bacterial suspending fluid toxic in the aerosol at 30 to 60% RH for wet
dissemination is not modified by freeze-drying to become another component toxic at 81% RH for dry dissemination. Hence, the present results indicate that RH does not control aerosol survival of P. tularensis LVS, as was also suggested by Cox (11). [The effects of hysteresis in sorption isotherms and also equilibration of the aerosol particle with its environment have been previously dismissed as the causes of the differences between survival of wet- and dry-disseminated aerosols (10, 11)]. A possible explanation of the present results is that the direction of water flow is important with regard to aerosol survival. For wet dissemination, water leaves the bacteria as they come to equilibrium with the RH of their environment. For dry dissemination, water is gained by the bacteria as they come to equilibrium with the RH of their environment. It is possible that a biologically essential component in the bacteria can exist as different hydrates (as can copper sulfate) with a particular hydrate rapidly becoming biologically inactive. Such a scheme would give the result that a minimum occurs in survival versus RH plots, as found here. Application of the law of mass action indicates that the RH for minimum survival could be different for wet and dry dissemination. This model is to be discussed further in a separate communication.

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