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Aerosol Survival of *Escherichia coli* B Disseminated from the Dry State

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Survival was determined for *Escherichia coli* B disseminated as an aerosol from the dry state. Survival in nitrogen, like that for wet dissemination, was better at low than at high relative humidity (RH). At high RH, survival was characterized by critical zones of instability in survival as a function of RH, instability occurring at 100, 95, 78, 70, and 60% RH. In air, survival was inferior to that in nitrogen at low RH, whereas the converse was found at high RH. The effect was attributed to oxygen. In general, results support the conclusion that to the first approximation survival is related to bacterial water content, the latter increasing with RH. However, a more detailed analysis of results indicates that survival might not be exactly related to bacterial water content. It is shown that death occurred because of rehydration and that the pretreatment of *E. coli* B affected its aerosol stability characteristics; i.e., wet and dry disseminated aerosols are not equivalent.

The aerosol survival of microorganisms has been reviewed in general terms by Anderson and Cox (1). This account showed that much work had been done using wet disseminated aerosols but that little work had been done using dry disseminated aerosols. The work reported in this paper was performed to help redress this imbalance and because it was suggested by Cox (8) that aerosol survival was related to water content of bacteria. Hence, phenomena such as critical minima in survival as a function of relative humidity (RH) (5–10) and toxicity of oxygen (3, 5, 9, 11, 13, 14) should occur also for aerosols generated from the dry state.

MATERIALS AND METHODS

Aerosols were generated with a pneumatic disseminator designed by E. G. Flurie (C. S. Cox et al., *unpublished data*; E. G. Flurie and C. Rodderick *unpublished data*), and were stored in a rotating drum (5). Freeze-dried powders were prepared from distilled water-washed 16-hr cultures of *Escherichia coli* B by using a "waffle iron" freeze-drier (15). After preparation, the powders were stored in vacuo at −70 C.

RH was controlled by mixing wet and dry streams of gas. RH was measured by means of wet and dry bulb thermometers (matched from 0 to 50 C, calibrated to 0.1 C). Measurement of RH at very high RH is not particularly easy but can be accomplished because, at 26.5 C (the temperature of the experiments), 97% RH, for example, is equivalent to a wet bulb depression of 0.3 C (8), which is very readily measured.

In initial experiments, spores of *Bacillus subtilis* var. *niger* were used as a tracer for physical decay (1). However, it soon became evident that *Escherichia coli* B and *B. subtilis* var. *niger* spores did not show the same physical decay (C. S. Cox et al., *unpublished data*). Therefore, the freeze-dried *E. coli* B were tagged with ^{14}C (by incorporating ^{14}C-labeled glucose into the growth medium) to determine physical decay. The disadvantages of the ^{14}C method (8), namely an increased radioactive count in the spray suspension between initiation and completion (1 min) of dissemination, did not occur for the present studies, although the effect of collecting fluid was still present (8). The latter was caused by the addition of sucrose to the collecting fluid which resulted in quenching during the scintillation assay of the ^{14}C.

Variables considered in this study of aerosol survival at various relative humidities were survival in nitrogen versus air, storage of powder versus no storage, use of sucrose during freeze-drying versus no sucrose, and use of sucrose in the collecting fluid versus no sucrose.

By means of six replicate experiments in each of the two cases below, it was established that the following errors occurred in the data; for good survival, the error was ± 10% of the survival value, whereas, for poor survival, the error was ± 50% of the survival value. Furthermore, the more critical data, such as the positions of minima in survival versus RH curves, were confirmed by duplicate experiments.

Other details were as previously described (5).

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RESULTS

Aerosol survival of E. coli B in nitrogen. Survivals at aerosol ages of 2, 15 and 30 min are given in Fig. 1. It is apparent that survival was better at low than at high RH. At high RH, the survival was characterized by critical zones of instability in survival as a function of RH, instability occurring at 100, 95, 78, 70, and 60% RH (Fig. 1C).

Figure 2 shows the aerosol survival after storing the freeze-dried powder of *E. coli* B in vacuo at −70 C for 7 days. (The data in Fig. 1 were obtained within 24 hr of preparing the powder.) The aerosol survival was again higher at low than at high RH. Comparison of Fig. 2 with Fig. 1 indicated that storage of *E. coli* B in vacuo for 7 days at −70 C caused a decrease in aerosol stability especially at low RH. Figure 3 gives the aerosol survival of *E. coli* B freeze-dried in the presence of 0.3 M sucrose. Although the presence of sucrose enhanced survival during freeze-drying (21% survival freeze-dried from distilled water and 39% survival freeze-dried from sucrose), it

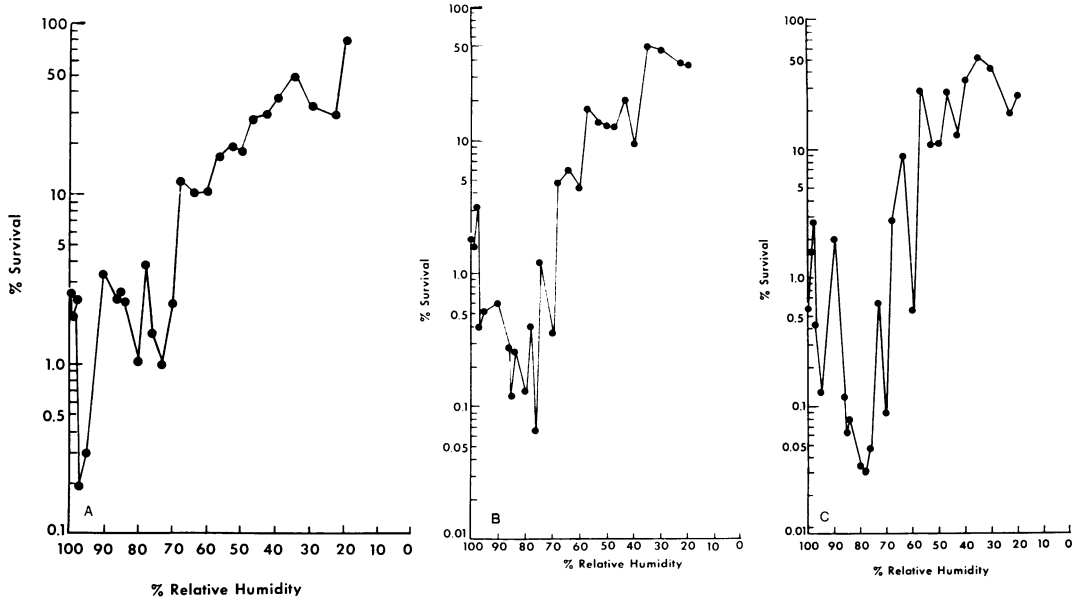

FIG. 1. *Aerosol survival in nitrogen of Escherichia coli B, powder age 1 day, as a function of relative humidity. (A) At an aerosol age of 2 min, (B) at an aerosol age of 15 min, and (C) at an aerosol age of 30 min.*

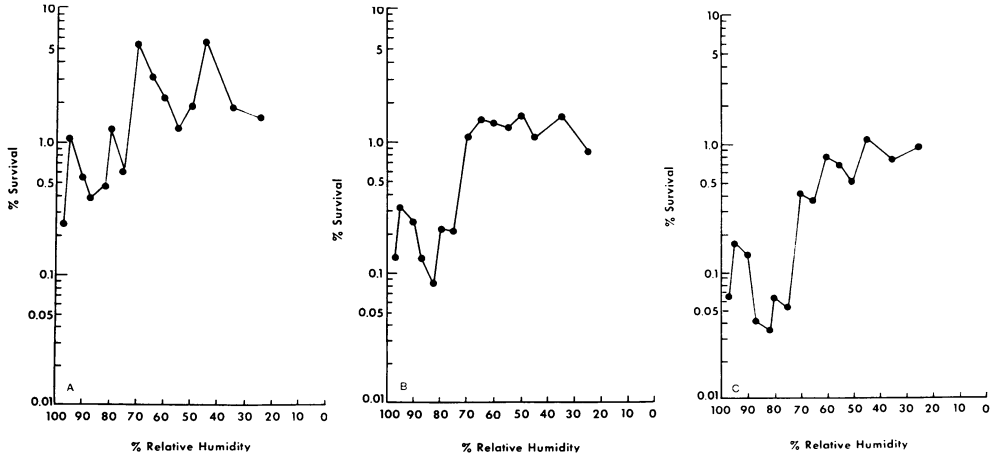

FIG. 2. *Aerosol survival in nitrogen of Escherichia coli B, powder age 7 days, as a function of relative humidity. (A) At an aerosol age of 2 min, (B) at an aerosol age of 15 min, and (C) at an aerosol age of 30 min.*

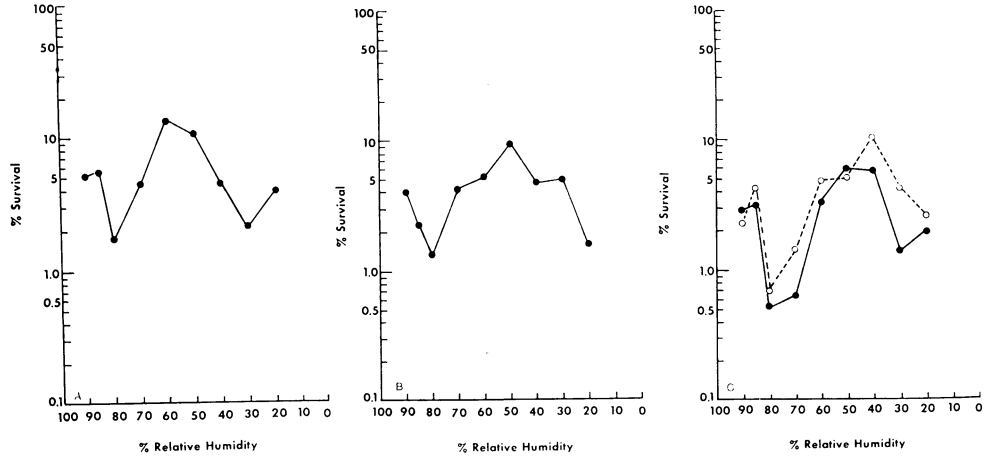

FIG. 3. *Aerosol survival in nitrogen of Escherichia coli B, powder age 7 days, as a function of relative humidity. (A) At an aerosol age of 2 min, protecting agent sucrose; (B) at an aerosol age of 15 min, protecting agent sucrose; and (C) at an aerosol age of 30 min, protecting agent sucrose. Collection in phosphate buffer (●); collection in phosphate buffer + 1 M sucrose (○).*

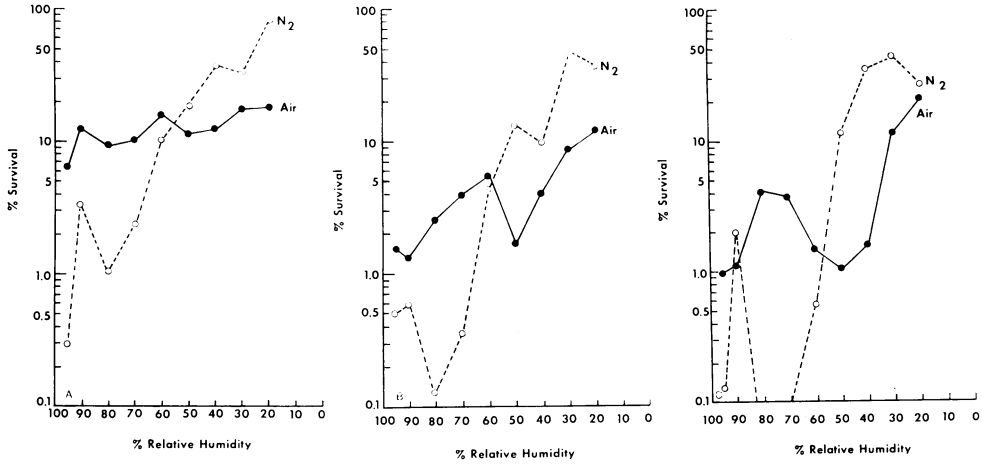

FIG. 4. *Aerosol survival of Escherichia coli B as a function of relative humidity, powder age 1 day. Air (●); nitrogen (○). (A) At an aerosol age of 2 min; (B) at an aerosol age of 15 min; (C) at an aerosol age of 30 min.*

was not generally beneficial in enhancing aerosol survival. Including 1 M sucrose in the collecting fluid caused little effect (Fig. 3C). This was the case also for data in Fig. 1, except at very high RH where the effect was to enhance survival.

Aerosol survival of E. coli B in air. Figure 4 shows data which compare the aerosol survival of *E. coli* B in air and in nitrogen. Survival in air was inferior to that in nitrogen at low RH, whereas the converse was found at high RH. At high RH, air in place of nitrogen tended to eliminate the critical regions in survival as a function of RH.

DISCUSSION

Cox (8), after showing that evaporation rate did not influence aerosol survival, suggested that RH was important with regard to survival through its influence on bacterial water content. If this were so, then many of the phenomena shown for wet disseminated aerosols should be observable with dry disseminated aerosols. Of these phenomena, the most important were the extremely critical nature of the response of survival to RH at high RH (5–10) and the toxic nature of air due to oxygen below 70% RH (3,

5, 9, 11, 13, 14). In general, the experiments reported in this paper support the above observations, in that critical minima appeared at high RH and that air was toxic below 57% RH. Also, these data, for dry dissemination where evaporation in aerosols does not occur, support the conclusion that rehydration rather than evaporation is the important process with regard to survival in aerosols (4–10). However, more exacting comparisons of the present data with those for wet dissemination enable more information to be obtained. For dry dissemination into nitrogen, minima occurred at 100, 95, 78, 70, and 60% RH, whereas for comparable wet dissemination, minima occurred at 100, 97, 86.6, and 85.7% RH (5, 8). If survival were truly related to water content, then the two sets of data should nearly coincide. Exact numerical equivalence would not be expected owing to hysteresis in the sorption isotherm (2). Such hysteresis would result in the minima for dry dissemination, occurring at higher RH than the minima for wet dissemination. This would also be the situation for bacteria that had not reached equilibrium with regard to water content and RH. Clearly the data show that the reverse situation is the case. At this stage, it is not known why the positions of the minima for wet and dry dissemination do not agree more closely. One explanation is that aerosol survival of *E. coli* B does not truly depend upon bacterial water content. If so, the effect of RH must operate through some other mechanism, which at this time is obscure. Also, the effect of air at high RH was different for wet and dry dissemination. For wet dissemination, the responses in air and nitrogen at high RH were very similar (5, 9, 11), whereas for dry dissemination, air prevented the appearance of the minima. In addition, the results with sucrose do not agree with those for wet dissemination (4). Hence, the conclusion is that wet and dry disseminated aerosols are not equivalent, i.e., the pretreatment of *E. coli* B before aerosolization must affect its aerosol stability characteristics.

As stated earlier, the evaporation of water from the aerosol particle does not influence aerosol survival, as was also shown by an entirely different technique (8). Hence, in nitrogen, death must occur through rehydration, as previously suggested (4–10). In the region of the critical minima in survival as a function of RH, it is rehydration during collection in the impinger that causes loss of viability (5–10).

The protecting effect of air at high RH for dry dissemination suggests that oxygen can inhibit the occurrence of critical minima in survival as a function of RH. Cox (10) found that for wet dissemination, the occurrence of critical minima was associated with failure of aerobic metabolism. Anaerobic metabolism was also inhibited (Cox, *unpublished data*). Since the same cause of loss of viability probably occurred for dry dissemination, then oxygen must be able to, at least partially, inhibit the mechanism causing loss of aerobic and anaerobic metabolism. This inhibition may be a free radical-induced phenomenon (11). However, oxygen-induced free radicals do not exist in the presence of appreciable amounts of water (12). Therefore, some other phenomenon probably occurred; further work is thus required to elucidate this preliminary finding.

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LITERATURE CITED

1. Anderson, J. D., and C. S. Cox. 1967. Microbial survival. Symp. Soc. Gen. Microbiol. 17:203–226.
2. Bateman, J. B., C. L. Stevens, W. B. Mercer, and E. L. Carstensen. 1962. Relative humidity and the killing of bacteria: the variation of cellular water content with external relative humidity or osmolality. J. Gen. Microbiol. 29:207–219.
3. Benbough, J. E. 1969. Factors affecting the toxicity of oxygen towards airborne coliform bacteria. J. Gen. Microbiol. 56:241–250.
4. Cox, C. S. 1965. The mode of action of protecting agents. 1st Int. Symp. Aerobiol., p. 345–368.
5. Cox, C. S. 1966. The survival of *Escherichia coli* atomized into air and into nitrogen from distilled water and from solutions of protecting agents, as a function of relative humidity. J. Gen. Microbiol. 43:383–399.
6. Cox, C. S. 1966. The survival of *Escherichia coli* in nitrogen under changing conditions of relative humidity. J. Gen. Microbiol. 45:283–288.
7. Cox, C. S. 1967. The aerosol survival of *Escherichia coli* Jepp sprayed from protecting agents into nitrogen atmospheres under shifting conditions of relative humidity. J. Gen. Microbiol. 49:109–114.
8. Cox, C. S. 1968. The aerosol survival of *Escherichia coli* B in nitrogen, argon and helium atmospheres and the influence of relative humidity. J. Gen. Microbiol. 50:139–147.
9. Cox, C. S. 1968. The aerosol survival and cause of death of *Escherichia coli* K12. J. Gen. Microbiol. 54:169–175.
10. Cox, C. S. 1969. The cause of loss of viability of airborne *Escherichia coli* K12. J. Gen. Microbiol. 57:77–80.
11. Cox, C. S. and F. Baldwin. 1967. The toxic effect of oxygen upon the aerosol survival of *Escherichia coli* B. J. Gen. Microbiol. 49:115–117.
12. Heckly, R. J., R. L. Dimmick, and J. J. Windle. 1963. Free radical formation and survival of lyophilized microorganisms. J. Bacteriol. 85:961–966.
13. Hess, G. E. 1965. Effects of oxygen on aerosolized *Serratia marcescens*. Appl. Microbiol. 13:781–787.
14. Webb, S. J. 1967. The influence of oxygen and inositol on the survival of semidried microorganisms. Can. J. Microbiol. 13:733–742.
15. Zimmerman, L., 1962. Survival of *Serratia marcescens* after freeze-drying or aerosolization at unfavorable humidity. I. Effect of sugars. J. Bacteriol. 84:1297–1302.