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TECHNICAL MANUSCRIPT 565

AEROSOL SURVIVAL OF ESCHERICHIA COLI B DISSEMINATED FROM THE DRY STATE

Christopher S. Cox

DECEMBER 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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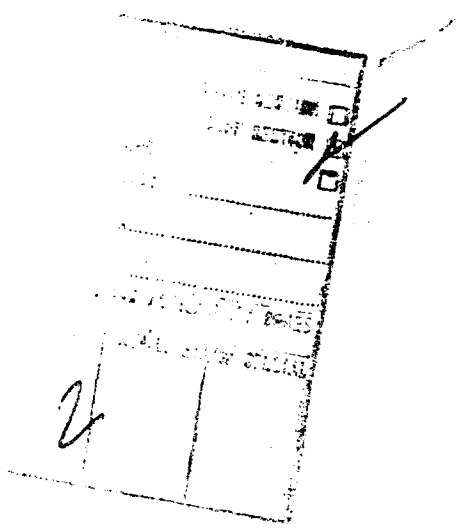

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AEROSOL SURVIVAL OF ESCHERICHIA COLI B DISSEMINATED FROM THE DRY STATE

Christopher S. Cox

Physical Science Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1T061101A91A

December 1969

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I thank Mr. Cameron Moffat for excellent technical assistance.

ABSTRACT

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 as a function of RH; instability occurred at 100, 95, 78, 70 and 60% RH. In air, percentage survival was less than that in nitrogen at low RH; the reverse was found at high RH. The effect was attributed to oxygen. In general, results support the conclusion that, at first glance, 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 as a result of rehydration and that treatment of E. coli B before aerosolization affected its aerosol stability characteristics.

I. INTRODUCTION*

The aerosol survival of microorganisms has been reviewed by Anderson and Cox.[1] Their account showed that although much work had been done using wet disseminated aerosols, little work had used dry disseminated aerosols. The work reported in the present paper was performed to help redress the balance and because Cox[2] suggested 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)[2-6] and toxicity of oxygen[3,7,8] should occur also for aerosols generated from the dry state. This study was performed to test this idea.

II. MATERIALS AND METHODS

Aerosols generated with a disseminator designed by E. Flurie** were stored in a rotating drum.[3] Freeze-dried powders were prepared with a Waffle Iron freeze dryer[9] from 16-hour cultures of Escherichia coli B washed in distilled water. Following preparation, the powders were stored in vacuo at -70 C.

In initial experiments, spores of Bacillus subtilis var. niger were used as a tracer for physical decay.[1] However, it soon became evident that E. coli B and B. subtilis var. niger spores did not show the same physical decay. Therefore, the freeze-dried E. coli B were tagged by C^{14} to determine physical decay. The disadvantages of the C^{14} method,[3] including an increased radioactive count in the spray suspension between initiation and completion (1 minute) of dissemination, did not occur for the present studies, although the effect of collecting fluid*** was still present.[2]

Variables considered in this study of aerosol survival at various relative humidities were: survival in air versus nitrogen; storage of powder versus no storage; use of sucrose during freeze-drying versus no sucrose; use of sucrose in the collecting fluid versus no sucrose. Data for critical minima were confirmed by duplicate experiments.

Details of preparation of materials and methods of testing have been described previously.[3]

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** Eugene G. Flurie, Special Operations Division, personal communication.

*** The addition of sucrose to the collecting fluid causes quenching during the scintillation assay of the radioactive isotope.

III. RESULTS

A. AEROSOL SURVIVAL OF ESCHERICHIA COLI B IN NITROGEN

Survival percentages at aerosol ages of 2, 15, and 30 minutes are shown in Figures 1, 2, and 3. 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 as a function of RH; instability occurred at 100, 95, 78, 70, and 60% RH (Fig. 3). The data in Figures 1 to 3 were obtained within 24 hours of preparing the powder.

Figures 4, 5, and 6 show the aerosol survival after storing the freeze-dried powder of E. coli B in vacuo at -70 C for 7 days. The aerosol survival was again higher at low RH than at high RH. Comparison of Figures 4 to 6 with Figures 1 to 3 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. Figures 7, 8, and 9 show the aerosol survival of E. coli B freeze-dried in the presence of 0.3 M sucrose. The presence of sucrose during freeze-drying enhanced survival (21% survival for freeze-dried from distilled water and 39% survival for freeze-dried from sucrose), but it was not generally beneficial in enhancing aerosol survival. Including 1 M sucrose in the collecting fluid caused little effect (Fig. 9), as was also the case for data in Figures 1 to 3, except at very high RH, where survival was enhanced.

B. AEROSOL SURVIVAL OF ESCHERICHIA COLI B IN AIR

Figures 10, 11, and 12 compare the aerosol survival of E. coli B in air and in nitrogen. Survival in air was less than that in nitrogen at low RH; the reverse 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.

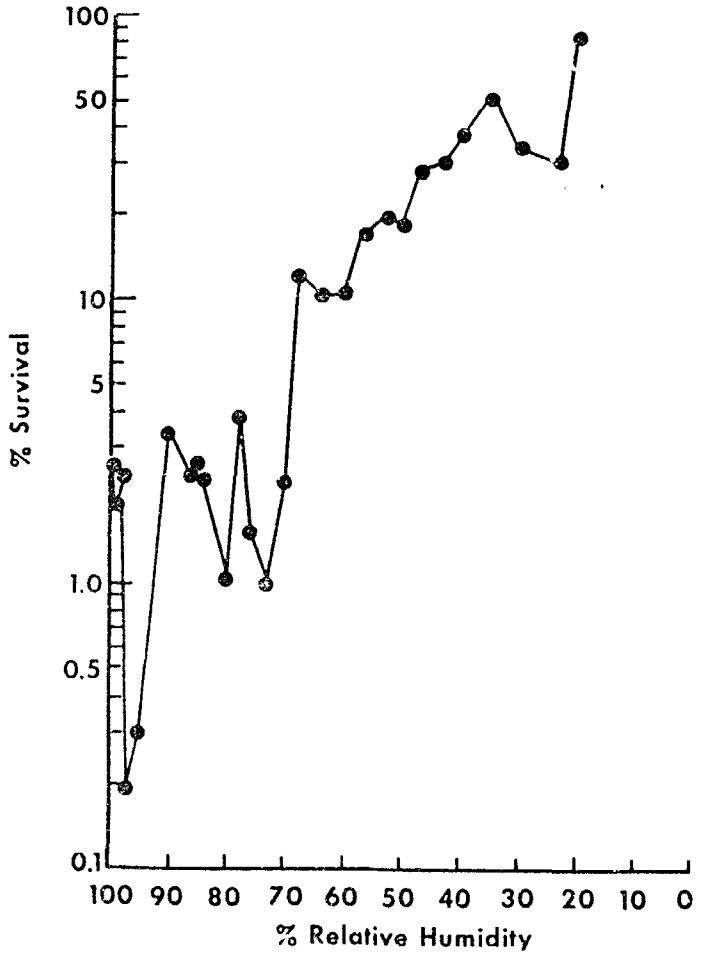

FIGURE 1. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 2 Minutes; Powder Age 1 Day.

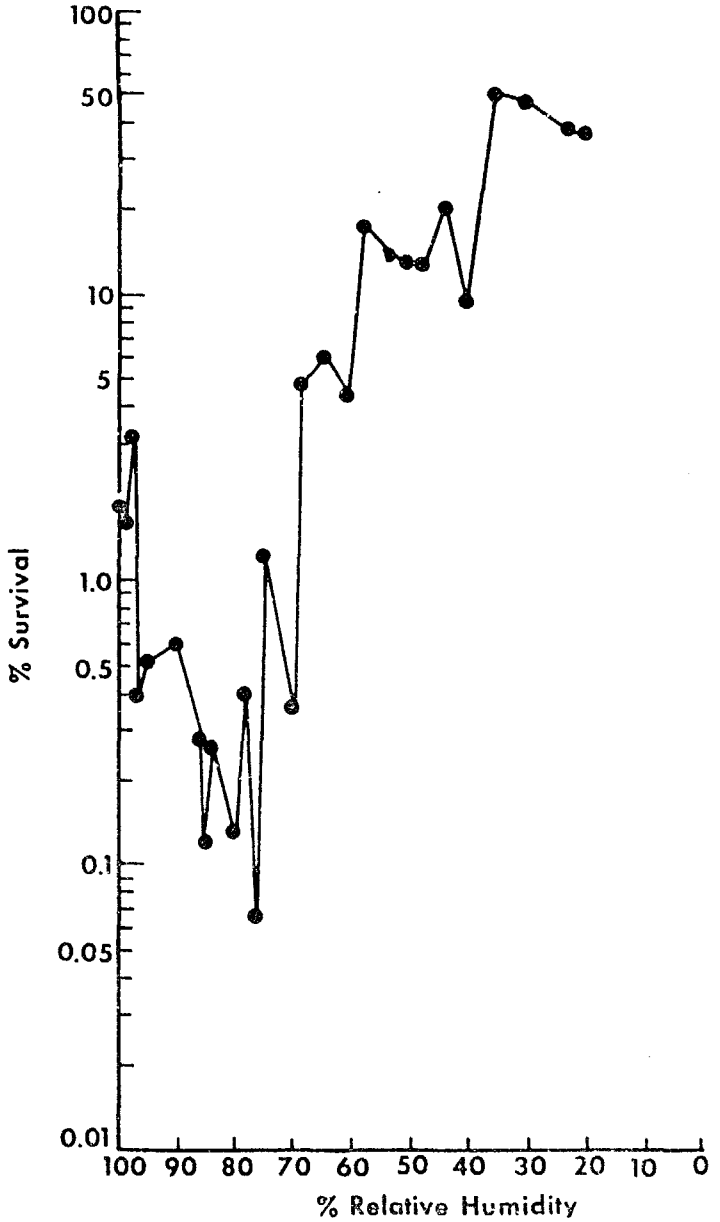

FIGURE 2. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 15 Minutes; Powder Age 1 Day.

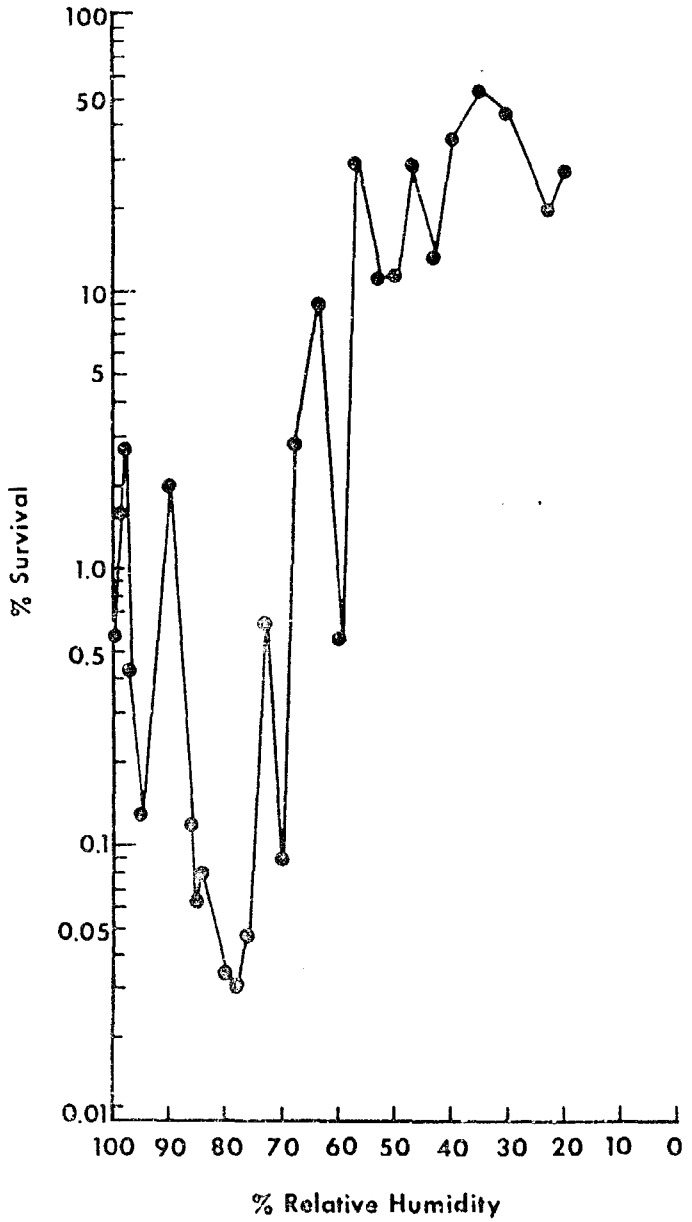

FIGURE 3. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 30 Minutes; Powder Age 1 Day.

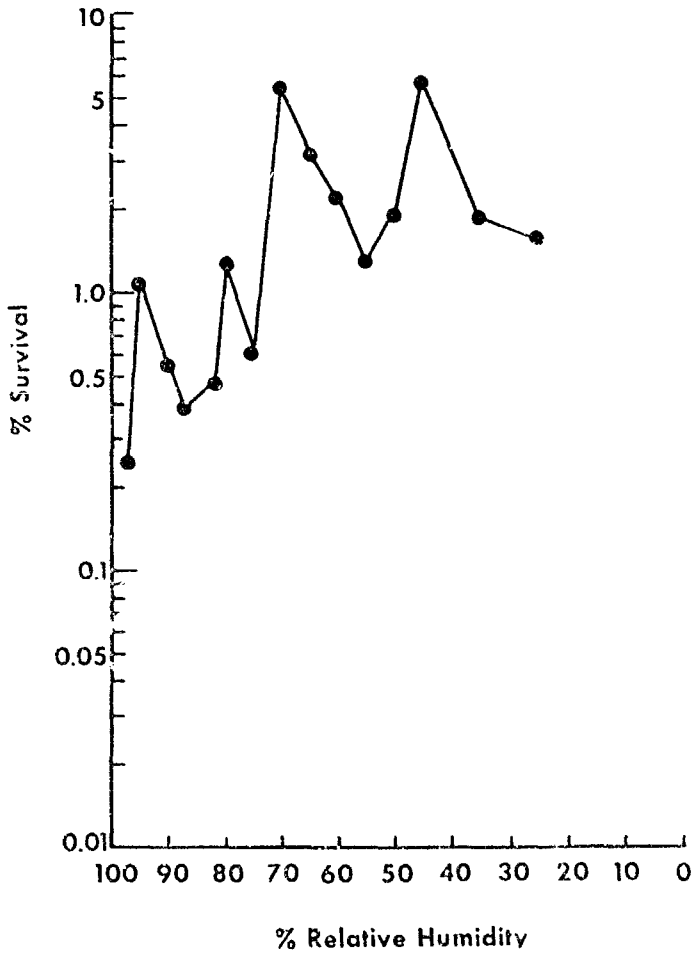

FIGURE 4. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 2 Minutes; Powder Age 7 Days.

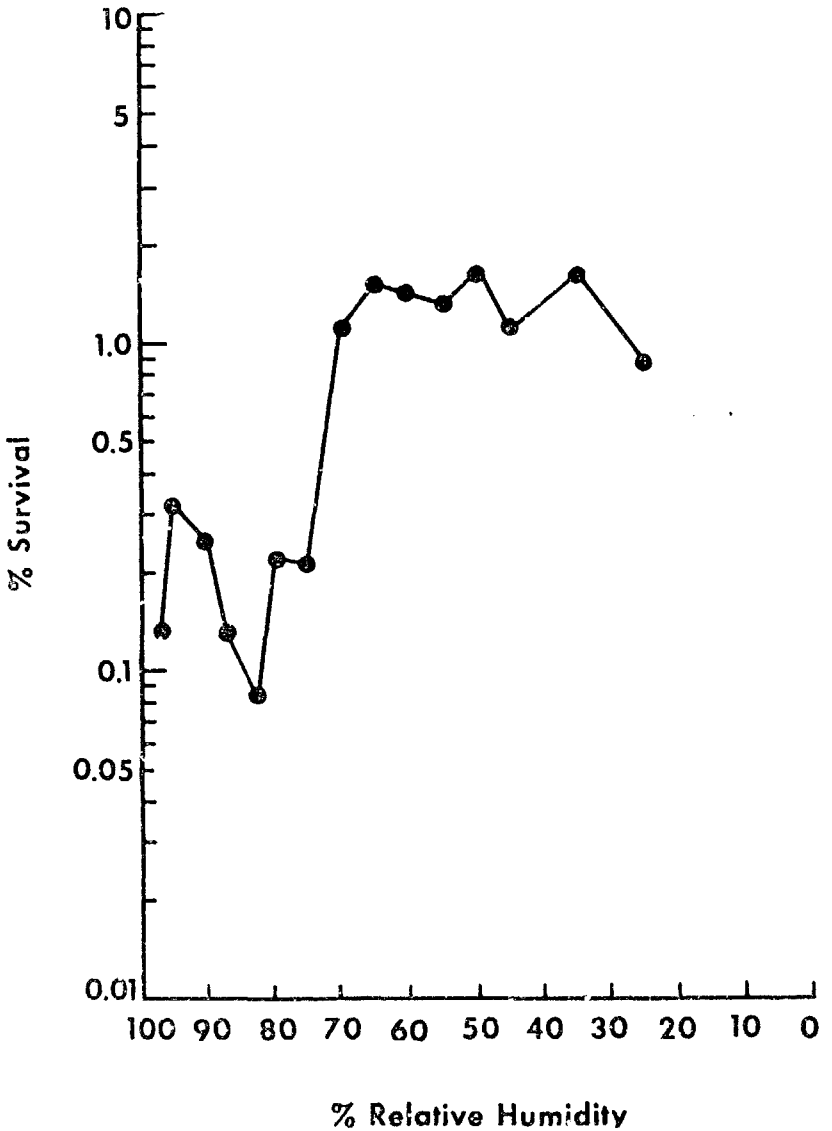

FIGURE 5. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 15 Minutes; Powder Age 7 Days.

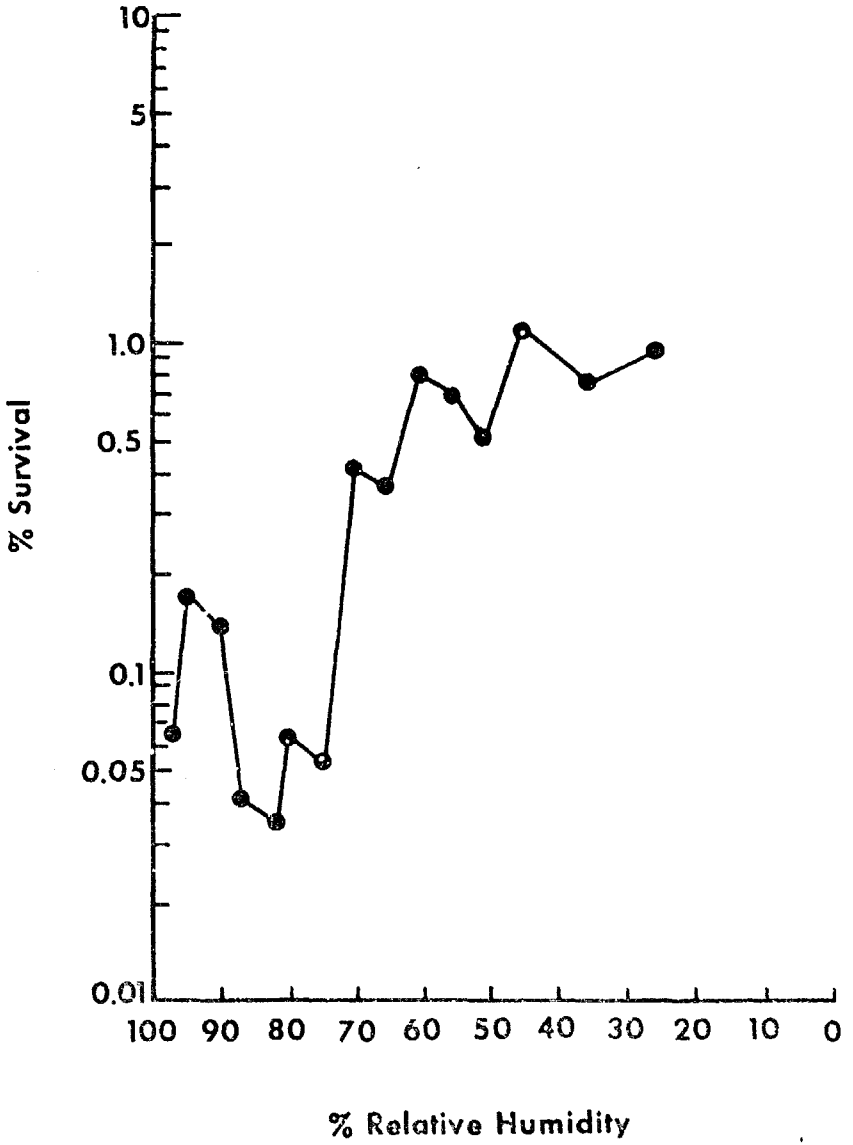

FIGURE 6. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 30 Minutes; Powder Age 7 Days.

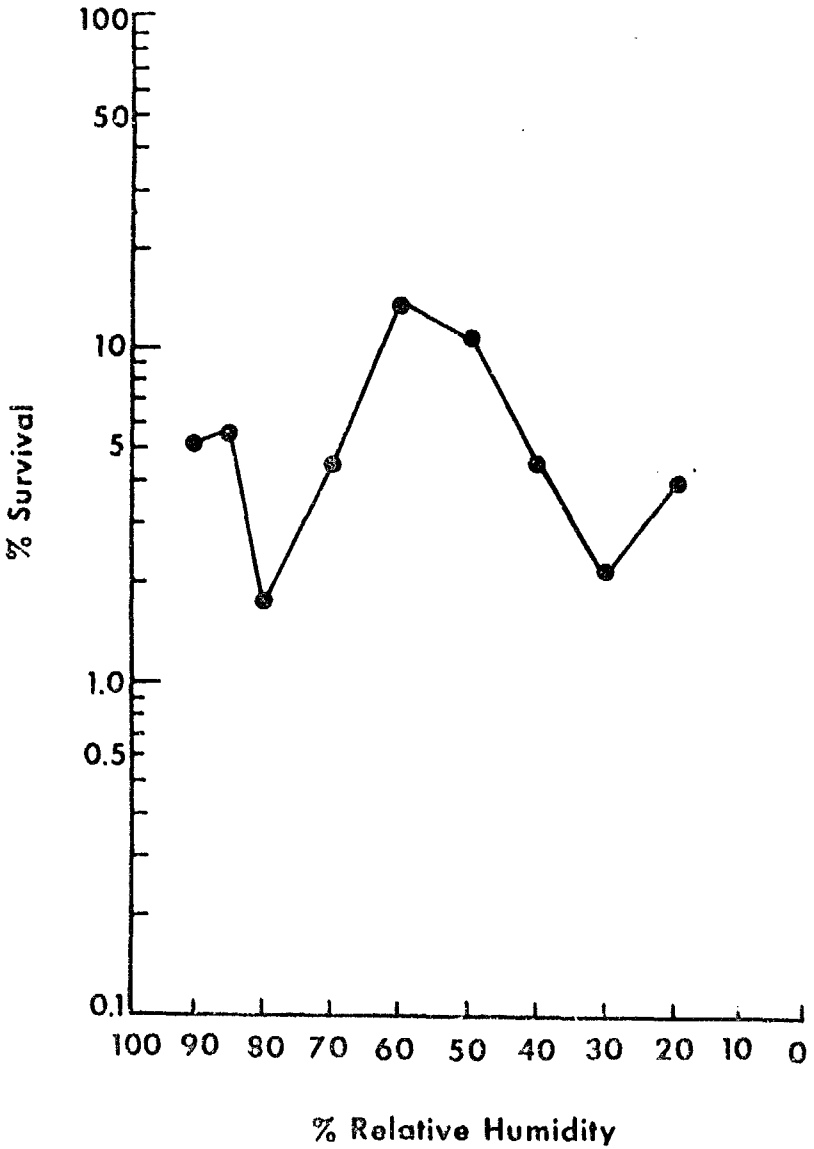

FIGURE 7. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 2 Minutes; Powder Age 7 Days; Protecting Agent Sucrose.

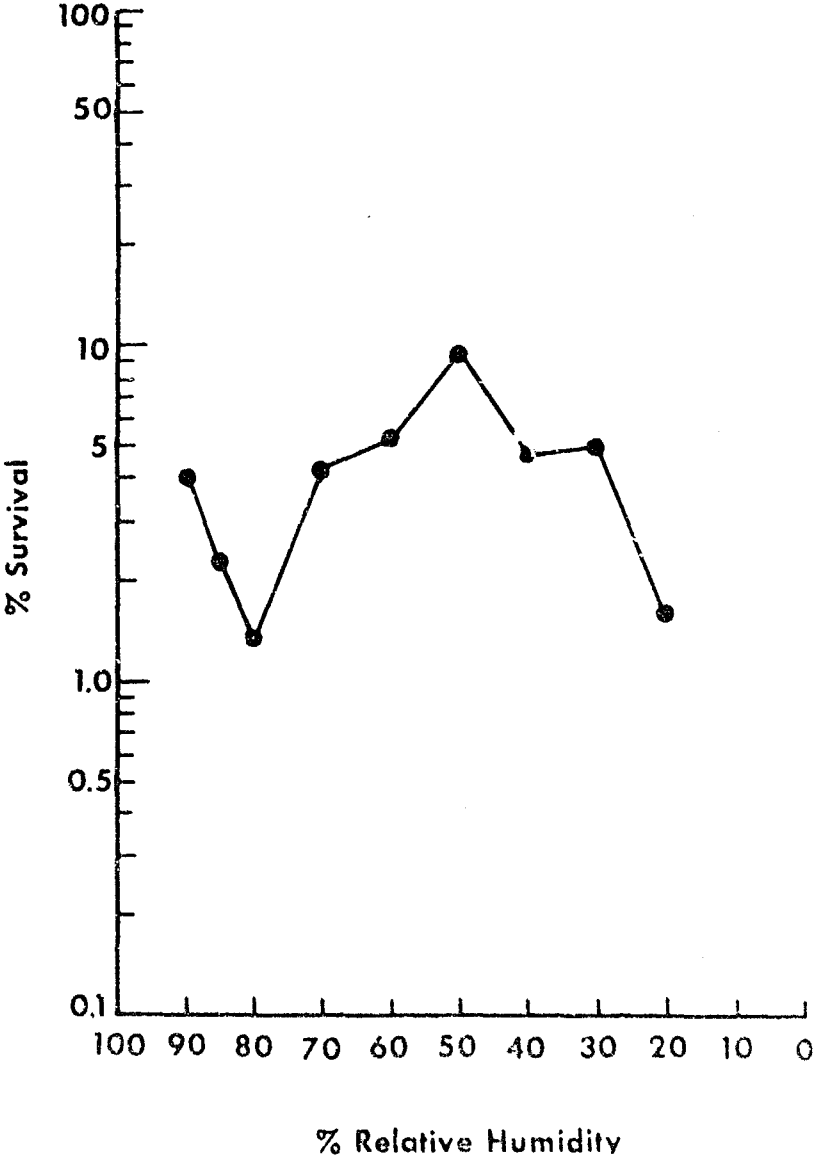

FIGURE 8. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 15 Minutes; Powder Age 7 Days; Protecting Agent Sucrose.

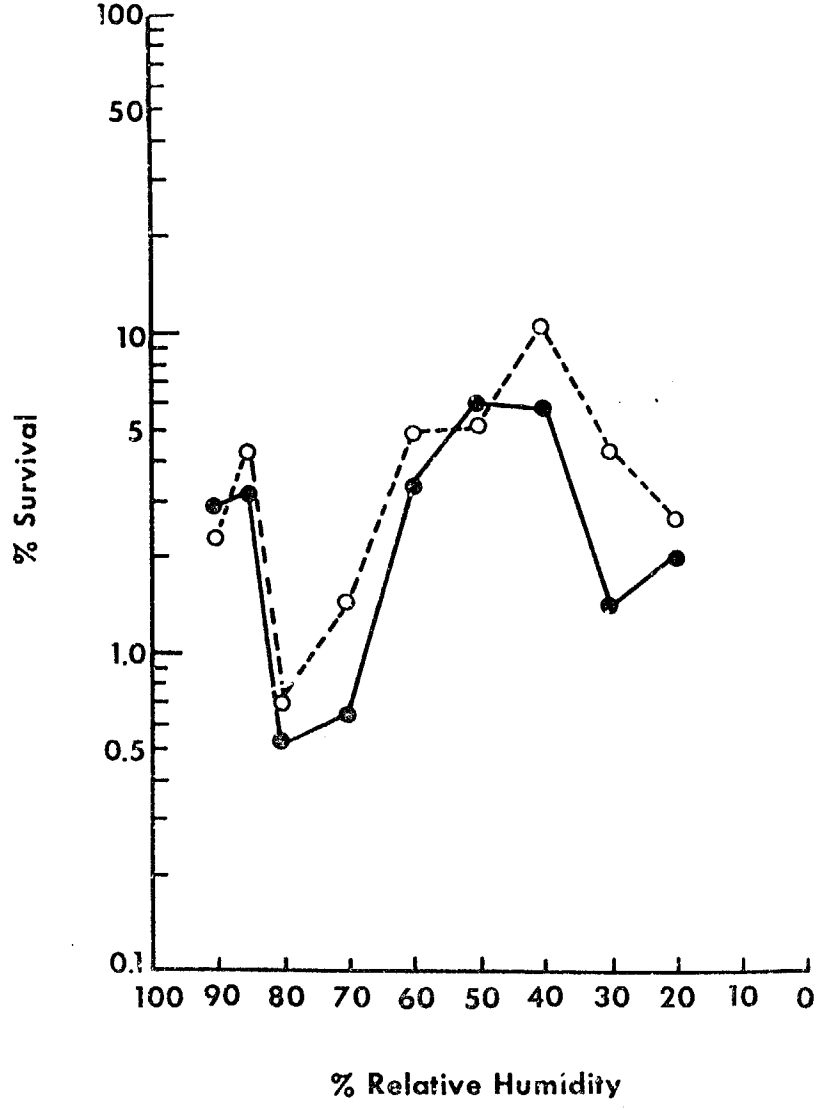

FIGURE 9. Aerosol Survival in Nitrogen of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 30 Minutes; Powder Age 7 Days; Protecting Agent Sucrose. ●—● Collection in phosphate buffer; O—O collection in phosphate buffer + sucrose.

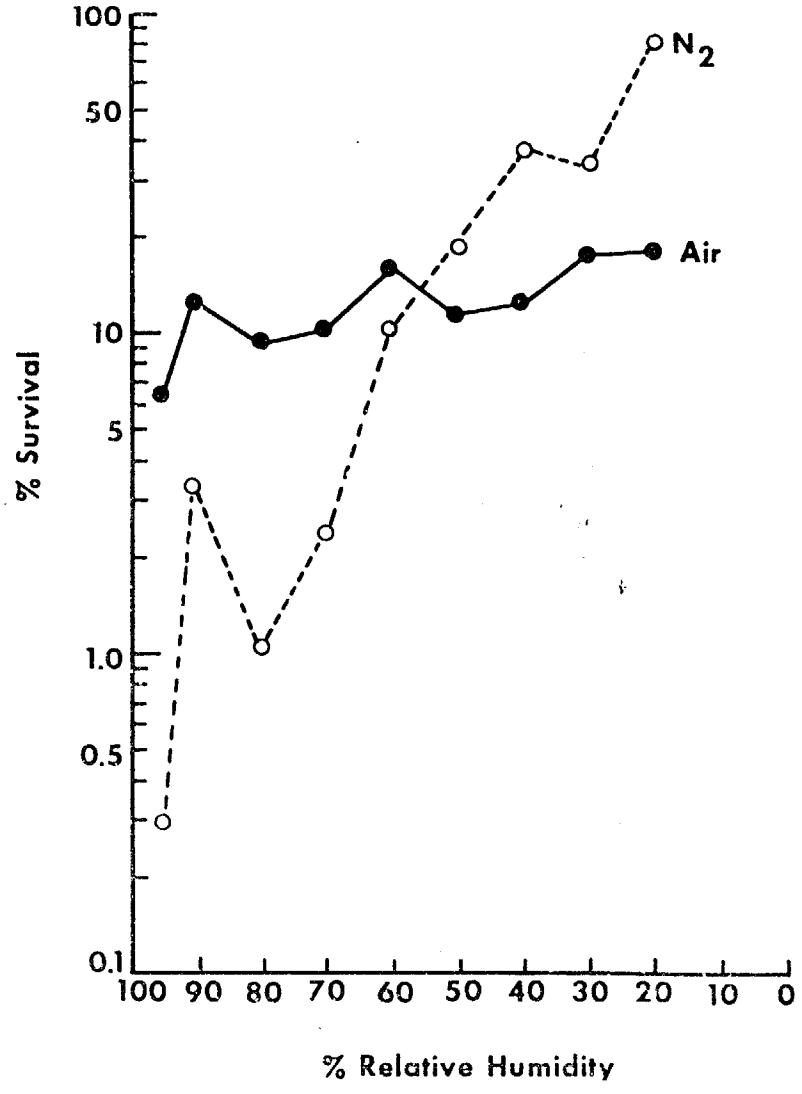

FIGURE 10. Effect of Air versus Nitrogen on Aerosol Survival of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 2 Minutes.

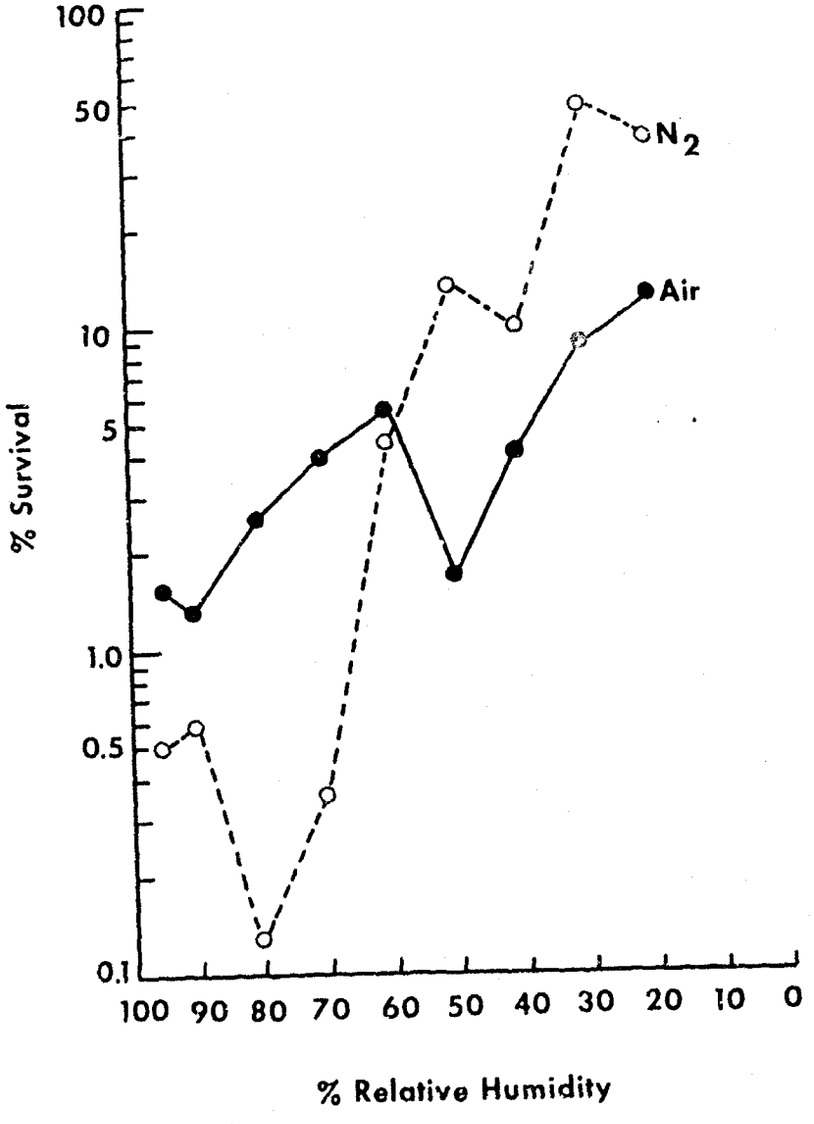

FIGURE 11. Effect of Air versus Nitrogen on Aerosol Survival of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 15 Minutes.

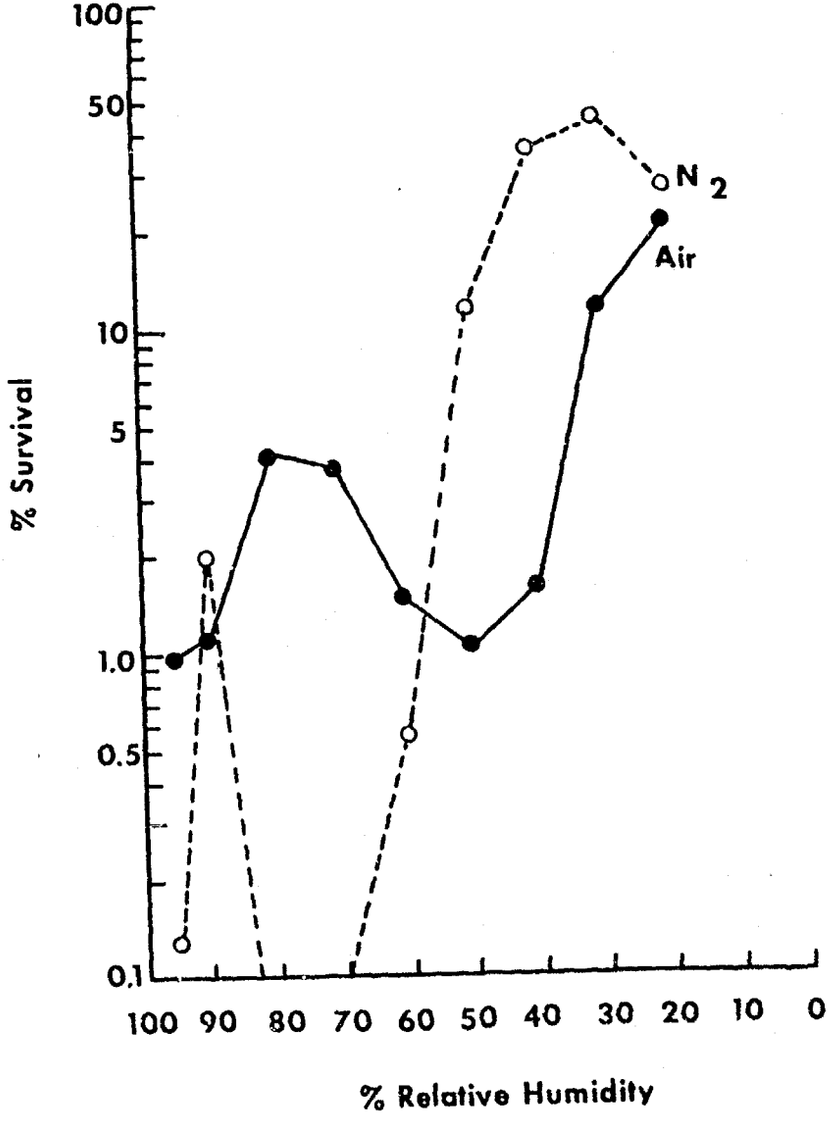

FIGURE 12. Effect of Air versus Nitrogen on Aerosol Survival of Escherichia coli B as a Function of Relative Humidity at an Aerosol Age of 30 Minutes.

IV. DISCUSSION

After showing that evaporation rate did not influence aerosol survival, it was suggested[3] 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[2-6] and the toxic nature of air due to oxygen below 70% RH.[3,7] 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.[2] 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, while for comparable wet dissemination, minima occurred at 100, 97, 86.8, and 85.7% RH.[2,3] If survival were truly related to water content, then the two sets of data should nearly coincide. Exact numerical equivalence would not be expected because of hysteresis in the sorption isotherm.[10] 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. 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;[3,7] for dry dissemination, air prevented the appearance of the minima. In addition, the results with sucrose do not agree with those for wet dissemination.[11] Hence, the conclusion is that wet and dry disseminated aerosols are not equivalent, i.e., the handling of E. coli B before aerosolization must affect its aerosol stability characteristics.

As stated earlier, the evaporation phase is absent for dry dissemination and therefore, in nitrogen, death must occur through rehydration, as previously suggested.[2-6] 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.[2-6]

The protecting effect of air at high RH suggests that oxygen can inhibit the occurrence of critical minima in the survival as a function of RH. It was found that, for wet dissemination, the occurrence of critical minima was associated with failure of aerobic metabolism.[12] Anaerobic metabolism was also inhibited.* Since the same cause of loss

* Unpublished data.

of viability probably occurred for dry dissemination, then oxygen must be able at least partially to inhibit the mechanism causing loss of aerobic and anaerobic metabolism. This inhibition may well be a free-radical-induced phenomenon.[7]

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13. ABSTRACT

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14. Key Words

Escherichia coli B	Relative humidity
Powder	Oxygen
Survival	
Nitrogen	
Additives	
Aerosols	

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