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Physiological Responses of Airborne Bacteria to Shifts in Relative Humidity

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Most information currently available on the behavior of airborne cells has been collected by investigators studying aerosols held in static environments. Wells and Riley (15), for example, showed that survival of bacteria was markedly influenced by humidity and temperature, and that the effects varied between bacterial species. Death of airborne bacteria has been observed to increase with a rise in humidity (2, 17), but contrary findings have also been reported (6, 16). Maximal death rates were found between 50 and 60% relative humidity (RH), and death has been reported to occur at more than one rate (5). There are more recent reports of multiple-stage death rates (12, 13, 14).

Few reports have been published, however, describing possible experimental techniques for subjecting microorganisms in air to shifts in RH, although it is well understood that such shifts do occur in natural airborne environments. Brown (1) regulated moisture in static chambers with salt solutions and sprayed water to produce intermediate changes. Hemmes (9) reported similar experiments with shifts in RH produced by spraying water into the aerosol chamber. One may also effectively produce limited rehydration of airborne particles by permitting the incoming air of the atomizer to be at a higher humidity level than that of the final humidity condition (12). The principle of the adiabatic expansion of a gas has been used successfully by Druett (*personal communication*), who found that a rapid decrease in viability occurred if the expansion raised the humidity sufficiently to cause moisture condensation onto the particles. No effect was observed at low relative humidities. Other unreported experiments have apparently been performed, as discussed by Wolfe (cited in 8), wherein pressure changes were produced within an aerosol chamber. A change in temperature would effect a change in RH, although the task of ascribing noted biological effects to humidity alone would be difficult.

Our purpose in studying the effects of sudden shifts in RH on airborne bacteria already equilibrated to one humidity condition was twofold: first, we were interested in applying laboratory findings to natural environments where temperature and humidity are constantly changing; and second, we were interested in possible death mechanisms—noting effects of shifts on subsequent biological behavior might furnish us with additional clues to such mechanisms. It is the purpose of this paper to report our findings and to discuss some of the implications of our results.

DUAL AEROSOL TRANSPORT APPARATUS

We achieved an abrupt shift of humidity in an air stream by diluting it with a second air stream at a different humidity; air temperature was held constant at 21 C. Only a brief description of the equipment and methodology will be made, since details have been previously reported (8). A 45-ft (13.7-meter) duct, 6 inches (15.2 cm) in diameter, was inserted 2 ft (61 cm) into another 45-ft duct, 8 inches (20.3 cm) in diameter. Each duct was equipped with numerous sampling ports. The point of juncture, where mixing of two air streams occurred, was called the confluence point. Linear air flow through both ducts was equal, the transit time per duct was about 5.7 min; total aerosol time was about 11.3 min. The calculated dilution of the primary air stream at the confluence point was 0.56, or approximately 50%. Humidity could either be increased or decreased by the dilution effect. Figures 1 and 2 illustrate the apparatus.

Bacteria were sprayed into the primary air stream. Concentration of particulate matter was

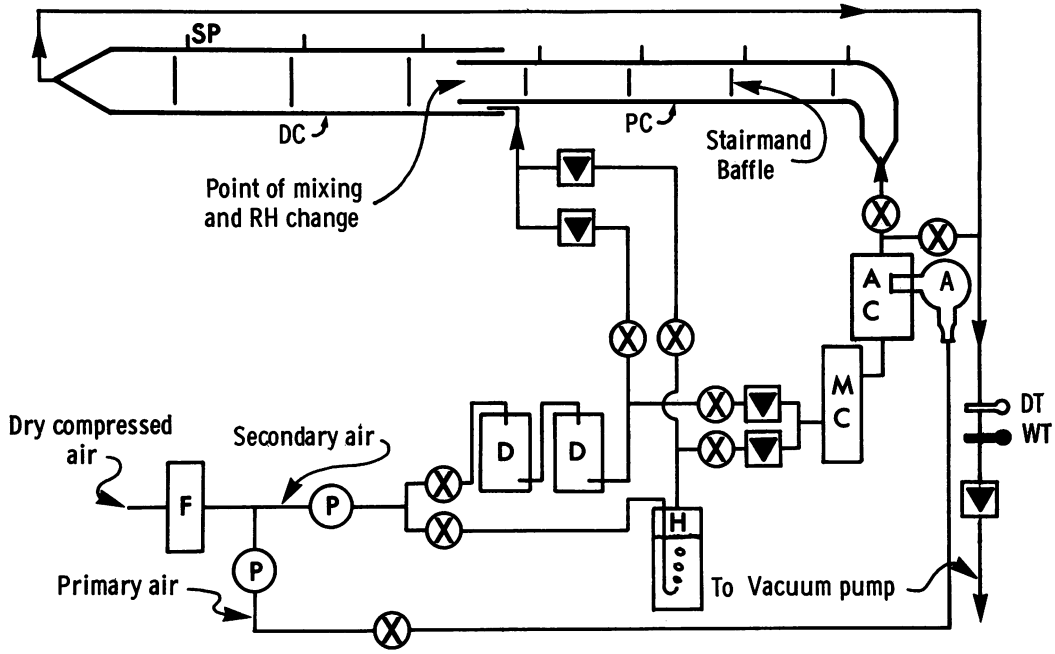

FIG. 1. *Schematic diagram of the dynamic aerosol transport apparatus. PC, primary aerosol chamber; DC, diluted aerosol chamber; A, reflux-type Wells' atomizer; AC, atomization chamber; MC, mixing chamber for conditioning of air; D, Lectrodryer; P, pressure regulators; F, filter; SP, sampling ports; H, humidifying chamber; DT, dry-bulb thermometer; WT, wet-bulb thermometer; ⊗, valve; ▼, rotometer.*

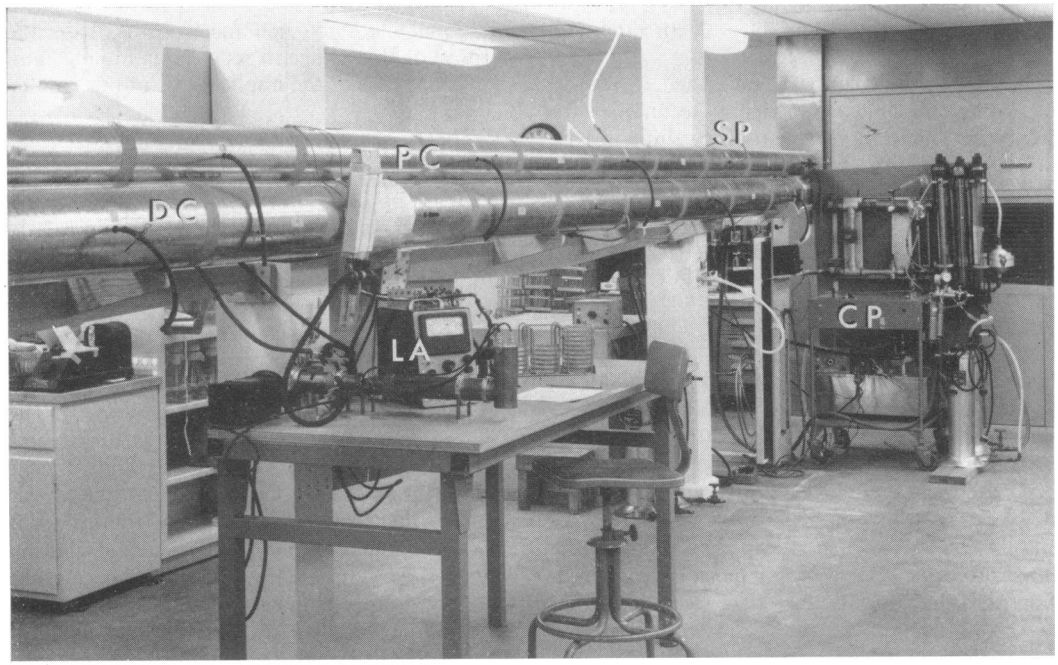

FIG. 2. *Lateral view of the dynamic aerosol transport apparatus and ancillary equipment. PC, primary aerosol chamber; DC, diluted aerosol chamber; SP, sampling port; CP, control panel; LA, light scatter apparatus.*

measured by forward-angle light scatter, and biological assay was made on samples collected either by slit samplers or impingers. Unless noted otherwise, we cooled 21.5-hr cultures to 4 C and then sprayed them from a refluxing atomizer, wherein the temperature usually increased to 15 C.

Figure 3 shows a hypothetical example, typical of observed results, for the purpose of defining parameters. The physical decay always followed first-order kinetics. The measured concentration of particles in the primary air stream immediately before dilution, compared with that in the secondary stream immediately after dilution (i.e., the apparent dilution ratio, ADR), was usually slightly higher than 0.56; sections A and B of Table 1 list some observed mean ADR values. Analysis of variance of three sets, 20 runs for each condition of shift-up, shift-down, or no shift, and disregarding other variables, indicated a 95% confidence interval of ±0.01 for all sets; differences between these three sets exceeded the 0.1% level of significance. These data were interpreted as indicating that the particles either increased or decreased in volume as a function of shift in RH. For example, if particles decrease in size, they scatter less light; therefore, the apparent dilution seems to increase and the ADR becomes smaller than without a shift in RH, and vice versa.

Since physical decay in the duct system was small and consistent, we refer to the sum of physical and biological loss as biological decay. The latter was always greater than physical decay and, in the primary air stream, usually followed first-order kinetics; "tailing" sometimes occurred after a humidity shift, but for comparative purposes we assumed first-order kinetics in all instances. Usually the biological loss, or biological dilution ratio (BDR), as a result of dilution at the confluence point, corresponded to the ADR (Fig. 3); important exceptions are noted below. Biological loss observed under the final conditions, as compared with loss under the initial condition, was defined as the dynamic-humidity-death (DHD) ratio (Fig. 3). If no change occurred, the theoretical ratio was 1.00; less than this number indicated enhanced death, and a number larger than 1.00 indicated that death process had decreased as a result of the shift. The mean DHD of 21 aerosols subjected to no shift in RH was 1.04 with a 95% confidence interval of ±0.05. We assume from this that DHD ratios greater than 1.10 or less than 0.90 are significant.

Serratia marcescens grown in, and sprayed from, dilute Trypticase Soy Broth (BBL) evinced an increased death rate (sorbed death, 11) when the RH was shifted from low to high values, but this effect was decreased if cells were sprayed

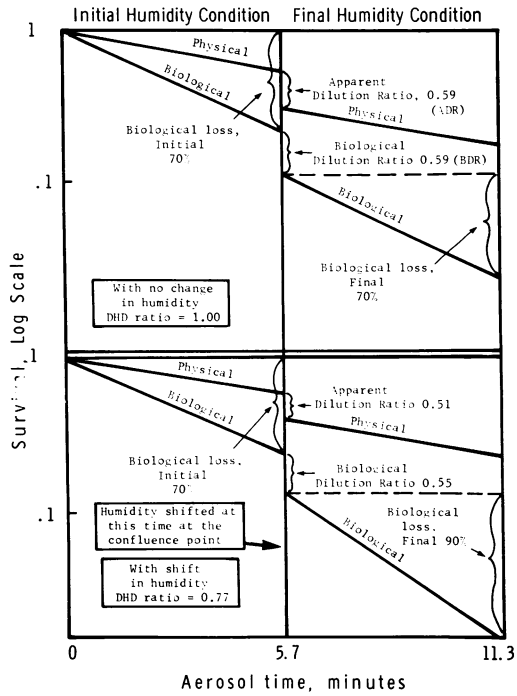

FIG. 3. *Theoretical behavior of bacteria aerosolized in the Dual Aerosol Transport Apparatus. There are two distinct intervals of both biological and physical loss, and there is loss caused by dilution. The theoretical dilution ratio, based on the geometry of the system, is 0.56. The apparent dilution ratio (ADR), measured by light-scatter, was approximately 0.59. A shift-up in humidity increased the ADR, whereas a shift-down decreased it. If no biological loss occurred at the confluence point, then the biological dilution ratio (BDR) was approximately equal to the ADR. We have frequently observed the BDR to be as much as 10 times the ADR. The initial biological loss divided by the final biological loss has been defined as the dynamic-humidity-death (DHD) ratio. In the upper example, with no change in humidity, the DHD ratio is approximately 1.00. In the lower, hypothetical example, where the change in humidity is unspecified, the DHD ratio shown is 0.77, indicating that the change was detrimental to survival. We have occasionally observed DHD ratios greater than 1.00.*

from a temperature-controlled, nonrefluxing (TCNR) atomizer at 4 C (section A, Table 1).

No sorbed death was noted (section B, Table 1) when *S. marcescens* was grown and sprayed in a chemically defined medium (3). Initially, there appeared to be a "toxic" effect of dilute Trypticase Soy Broth, because the DHD ratio was low (section C, Table 1) when cells were grown in chemically defined medium and resuspended in dilute Trypticase Soy Broth medium. It is of interest to note, however, that sorbed death was eliminated at humidity values above

TABLE 1. *Summary of results obtained when airborne bacteria were subjected to shifts in relative humidity*

Pertinent test conditions[a]	Per cent relative humidity conditions		ADR or BDR[b]	DHD ratio[b]
	Initial	Final		
Serratia marcescens				
(A) Grown and sprayed in dilute Trypticase Soy Broth (DTS)	24	51	ADR 0.61	0.71
	50	50	ADR 0.59	0.97
	24	47[c]		0.89
	90	57	ADR 0.48	1.10
(B) Grown and sprayed in chemically defined medium (CDM)	27	37	ADR 0.60	1.10
	51	51	ADR 0.57	1.08
	93	72	ADR 0.52	1.10
(C) Grown in CDM, resuspended and sprayed in DTS	22	54		0.62
	59	72		1.00
	24	55[c]		1.00
Atomizer fluid at 21 C	25[d]	53		$-\infty$[e]
Atomizer at 21 C with 1 mg/ml of chloramphenicol	25[d]	53		0.91
(D) Grown in DTS, resuspended and sprayed in CDM	25	52		0.90
(E) Grown in CDM and stored at 4 C for noted times, then resuspended in DTS at 4 C for 30 min and sprayed			Initial loss[b]	
(0 hr)	22[d]	52	71%	0.75
(1 hr)	22[d]	52	50%	0.86
(5½ hr)	22[d]	52	46%	1.20
(7 hr)	22[d]	52	21%	0.53
Pasteurella pestis A1122				
(F) Grown in Heart Infusion Broth	28	46	BDR 0.28	0.44
	28	28	BDR 0.59	1.00
	39	26	BDR 0.70	3.40
	87	61	BDR 0.16	1.00

[a] Refluxing atomizer; fluid chilled to 4 C before spraying, unless otherwise noted.
[b] See Fig. 3.
[c] Sprayed with modified, nonrefluxing atomizer with temperature control at 4 C.
[d] Single experiment; all other data are mean value of three or more aerosols.
[e] No survivors after shift in humidity.

59% RH, and when cells were sprayed from the TCNR atomizer at 4 C, the DHD ratio was 1.00. If cells were grown in dilute Trypticase Soy Broth and sprayed from chemically defined medium (section D, Table 1), the DHD ratio was higher than above; hence, dilute Trypticase Soy Broth was not toxic. Further evidence for nontoxicity is shown in section C, Table 1; cells grown in chemically defined medium and sprayed from dilute Trypticase Soy Broth at room temperature were so sensitive to sorbed death that no viable cells were recovered from the second duct, but the addition of 1 mg/ml of chloramphenicol to a similar suspension practically eliminated the detrimental effects of sorbed water.

The latter effect might be considered a protective one, but a similar result was obtained when cells were grown in chemically defined medium and stored at 4 C for various times before being added to dilute Trypticase Soy Broth to be sprayed (section E, Table 1). Storage in the cold for 5.5 hr changed the DHD ratio from 0.75 to 1.20; additional storage caused the DHD ratio to decrease to 0.53. This decrease was more apparent than real, however, because of the marked change in initial loss that occurred as a result of the cold storage period (see Table 1); the final biological decay, as a result of the 7-hr storage period, was less than that of the final decay of cells stored for 5.5 hr and equivalent to the final

decay of cells grown and sprayed in chemically defined medium.

Pasteurella pestis A1122 was grown and sprayed in Heart Infusion Broth (Difco). Although this species exhibited sorbed death similar to *S. marcescens*, there were three distinct differences between the species (section F, Table 1): (i) there was a change in death rate as a result of a shift-down in RH, a phenomenon we never observed with *S. marcescens*; (ii) the DHD ratio as a result of this change was 3.40, i.e., the death rate decreased markedly rather than increasing, (iii) instantaneous death (i.e., the rate was too rapid to measure) often occurred after humidity shifts (note last BDR, section F, Table 1). Also, preliminary evidence indicated instances of dissonance (3), or dilution shock, and a dependence on both constituents in, and temperature of, the sampling medium. For example, in one experiment in which plates, before being incubated, were chilled for 2 hr after the sample from an aerosol was inoculated, a twofold increase in colony numbers was found over the number on plates incubated immediately. In the same experiment, the addition of 1% whole blood to the medium caused a fourfold increase in colony numbers. Whole blood did not increase the number of colonies produced by unstressed populations. The actual extent of these increases varied with aerosol age.

APPLIED INTERPRETATIONS

These findings show that changes in RH do influence subsequent surivival of airborne bacteria. The evidence indicates that this effect might be applied to air-sterilization processes. For example, air conditioning equipment might be cycled to lower the air contamination of public places, such as hospitals, schools, institutions, etc. (5). The study has not indicated specific or generally applicable RH changes, or rates of changes, that might be most lethal, nor is there direct evidence that survival after a shift in RH is different than it would have been in the second condition without a change. There is, however, presumptive evidence for this, in that we never observed *S. marcescens* cells to die as rapidly at 53% RH as they did when shifted from 25 to 53% RH (section C, Table 1). The spray temperature in this instance was more equivalent to natural conditions (21 C) than in other experiments (4 to 15 C). Further, little more than 10% of airborne *P. pestis* cells survived a shift-down in humidity from 87 to 61% RH, although the death rates before and after the shift were observed to be identical (section F, Table 1).

The difficulty of finding interpretations meaningful to natural situations lies in the obvious dependence of airborne behavior on the history of environment of the culture before aerosolization. Goodlow and Leonard (7) previously pointed out the importance of such conditions. Rigid standardization in an effort to attain replicability does not aid the interpretation, because we usually do not know the cultural history of bacteria found in nature.

THEORETICAL INTERPRETATIONS

There are additional difficulties influencing our attempts to interpret these data from a theoretical viewpoint. We can justifiably point out some previously suspected mechanisms that either are not applicable or act only indirectly. Dehydration alone does not kill cells, otherwise freeze-dried cells would not survive as they do (4). Moreover, Hess (10), in a most important contribution, showed that little or no loss of viability occurred at any RH tested if cells were held airborne in oxygen-free chambers. Our data show that rehydration can cause death; interpreted broadly, these facts imply that part of the observed death may be caused by the act of sampling. In some instances, additional colonies arose when sampling plates were cooled before they were incubated or when nutrients not required by unstressed cells were added. Since cells ruptured by osmotic shock are unlikely to repair such damage, osmotic shock cannot be solely responsible for death. In fact, we may reasonably suggest that no currently used assay is accurate for cells injured by aerosolization.

From these data we theorize that airborne cells may be metabolically active. Substances such as chloramphenicol, or conditions such as low temperature that decrease metabolic functions, tend to increase survival capacity. Dehydration undoubtedly decreases metabolic functions, but probably in a manner that leads to an imbalanced but slowly readjustable condition. Cells sampled before readjustment die as a result of further imbalance unless provided with a situation where additional slow change, or repair, can take place. Cells normally exist in a variety of "states" because of the division cycle and differences in microenvironments. Therefore, individual cellular responsiveness to aerosolization ought to vary, and this is apparently what happened in our studies.

The evidence indicates that no single structural injury (e.g., hydrogen bond breakage, deoxyribonucleic acid denaturation) can account for all death observed and that measured behavior (colony formation) is highly dependent on functional activities of the cell.

SUMMARY

In summary, the aerobiologist places a biological system, the bacteria, in a hostile and ill-defined environment, the atmosphere, for the purpose of studying air-bacterium interactions. Measurement of this interaction is in terms of survival. Survival has been shown to depend not only on physicochemical reactions of the somatic, structural components of the cell, but also on those functional, physiological, dynamic properties of all living systems, termed adaptability or responsiveness. The problem, whether one is assaying infectivity or is searching for clues pertinent to death mechanisms, is to separate the two effects.

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Discussion

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In the experiments which Dr. Hatch has described, the immediate effect of an abrupt change in relative humidity on an airborne microorganism was expressed as a deviation from the expected reduction in aerosol concentration due to the dilution by the additional air introduced at the confluence point. The biological dilution ratio, based on samples, was compared with the apparent dilution ratio based upon light scatter measurements. The biological loss observed during the 5.7-min aerosol transit time in the second half of the apparatus was compared with the equivalent loss observed in the first half, and was expressed as the dynamic humidity death ratio.

Regarding the immediate effects of an abrupt change in relative humidity on airborne microorganisms, one might suggest that not only are the effects dependent upon the direction and magnitude of the change but, perhaps, also upon the