

THE SURVIVAL OF AIRBORNE MICROORGANISMS

I. EXPERIMENTS NEAR 0°C WITH SOME PSYCHROPHILIC BACTERIA

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Summary

The rate of death of airborne organisms was studied for one strain each of *Achromobacter*, *Pseudomonas*, and *Micrococcus* at relative humidities between 45 and 90 per cent. at 0°C. For all three organisms the rates of death were least and of a similar magnitude at 65-70 per cent. R.H.

I. INTRODUCTION

The possible importance of atmosphere transport of bacteria has been realized since the end of the last century when, among others, Carnelly, Haldane, and Anderson (1887), estimated bacterial populations in the air of streets, schools, and dwellings of a Scottish town by drawing air through glass tubes lined with "Koch's Jelly" and making colony counts after incubation at room temperature. Since then interest has increased in aerial bacteriology and considerable information is now available on rapid methods of killing airborne bacteria, and on techniques and equipment suitable for estimating their numbers. It is not surprising that nearly all of this work has been done with a medical motive and under conditions closely related to those encountered under human living conditions.

On the other hand, the amount of fundamental data concerning the behaviour of airborne bacterial cells in relation to atmospheric conditions is very limited and little agreement seems to have been reached among those people who have worked in this field. Papers have been published on this and related aspects by Williamson and Gotaas (1942), Wells and Zappasodi (1942), Edward, Elford, and Laidlaw (1943), Loosli *et al.* (1943), De Ome *et al.* (1944), Rosebury (1947), and Dunklin and Puck (1948).

These authors, except Dunklin and Puck, have claimed greatest death at either high or low humidities for airborne bacteria or virus particles. Dunklin and Puck observed maximum death of certain coccal types near 50 per cent. R.H. in the presence of sodium chloride but no correlation between humidity and survival in its absence.

The present paper submits results of experiments on the behaviour of bacteria when airborne at temperatures approximating those employed in the storage of chilled beef. Three bacterial genera have been employed, all of which may be found in association with chilled beef spoilage.

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II. MATERIALS AND METHODS

(a) Test Room

Throughout the work a refrigerated test room was used. The volume of this room, corrected for inclusions such as brine coils etc., was 370 cu. ft and total floor area 47 sq. ft. Air was circulated by an electric fan situated in one corner of the room. This fan produced an air speed of 670 ft/min at 1 ft in front of the blades and circulated the air round the room in 10-20 sec as judged by smoke tests.

(b) Temperature and Humidity Control

Room temperature was maintained by cold brine circulating through side coils and control was normally effected to $\pm 0.2^{\circ}\text{C}$ of the stated value. Under certain conditions, however (e.g. very cold brine), rather more variation than this was experienced. In the earlier experiments room temperature was maintained at approximately -0.5°C , but later this was raised to $+0.5^{\circ}\text{C}$ to reduce freezing of agar plates exposed within the room.

Humidity was controlled over the range 45-90 per cent. R.H. by adjustment of brine temperature and the use of a supplementary electric heater or trays of water in the lower and upper ranges respectively. Extension of this range was not possible with the available equipment. Fluctuations in humidity of up to 3 per cent. R.H. during a run were experienced under these conditions. In early experiments humidity was measured by placing wet- and dry-bulb thermometers in front of the fan and making readings by entering the room before and after a sampling run. Readings so obtained are treated with reserve and are indicated appropriately in the results. All other measurements were made from outside the room with an instrument-reading telescope and in such cases temperature and humidity were followed throughout a run.

(c) Introduction of Bacteria into the Test Room

Suspensions of bacteria in the appropriate aqueous media were introduced into the test room from an atomizing device, known by the commercial name of "Phantomyst," situated about 3 ft inside the test room and about 15 in. above floor level. This instrument is classified as a nebulizer insofar as its construction causes a "refluxing" of the liquid against baffles, resulting in the emission of a spray of very fine particle size. Nebulizer performance varied with the volume of liquid employed and hence for all runs a volume of 100 ml was used. Performance also varied with temperature. Under the conditions of use when the nebulizer, at $20-25^{\circ}\text{C}$, was placed in the test room, a mean output of 0.55 g water per min was obtained, giving an aerosol of particles approximately 45 per cent. of whose mass had a diameter greater than $2\ \mu$ when sampled immediately in front of the outlet. Particle size was determined with the Cascade Impactor (May 1945) using slides coated with soft vaseline. Impressions in the vaseline were measured.

(d) Air Sampling

Air was sampled by means of a slit sampler such as described by Bourdillon *et al.* (1948). The sampler was located at floor level outside the test room and

air was drawn from within through a wall pipe and two lengths of rubber hose of 1.5 in. internal diameter. The outside length (28 in.) was curved through 90° to fit over the sampler intake. The sampler was operated at a pressure reduction of 12 in. water (\approx 1 cu. ft/min) and the petri dish was rotated at 0.5 r.p.m.

(e) *Estimation of Settling*

The total number of viable organisms depositing on the floor in any experiment was estimated by exposing a number of petri dishes for the duration of a sampling run.

(f) *Organisms*

The organisms used in this section of the work were representative of bacterial types normally associated with the spoilage of chilled beef. All were isolated in 1933 and have been stored on nutrient agar at 0-0.5°C with transplants approximately every 6 months. The organisms have been identified generically and henceforth are referred to by their numbers in the stock culture collection of the Division of Food Preservation and Transport, C.S.I.R.O., as follows:

| | |
|--------------------------|---------|
| <i>Achromobacter</i> sp. | No. 483 |
| <i>Pseudomonas</i> sp. | No. 451 |
| <i>Micrococcus</i> sp. | No. 202 |

(g) *Media*

Media used for the growth, estimation, etc. of these and other organisms in this programme were exclusively "Difco" dehydrated media made up with glass-distilled water.

(h) *Experimental Procedure*

To enable rapid estimates of bacterial population to be made at the beginning of an experiment, the bacteria were grown at 20°C for periods of up to 8 days in nutrient broth (100 ml in 4-oz bottles) and the plate count was correlated with readings obtained on an E.E.L. photoelectric colourimeter using a green filter. Plate counts were made by the method recommended by Wilson (1935) for which petri dishes were incubated at 20°C for 3-4 days.

A typical run for the determination of death rate was made as follows.

The test organism was grown at 20°C for periods of 3-7 days in 100 ml quantities of nutrient broth. A measurement was made of the turbidity of the culture, which was then diluted 100-fold with the appropriate diluent. The diluted suspension was transferred to the sterile nebulizer and, together with sedimentation plates, placed in the test room. Entry of the room caused a slight disturbance of conditions but usually a stable state was re-established within 5-10 min after restarting the fan. Where possible, however, not more than 2-3 min was allowed to elapse between leaving the room and spraying the bacterial suspension, as it was sought to keep the suspension in its diluted state for as short a time as possible. This time was normally 5-7 min.

Bacteria were introduced into the air by running the nebulizer for a period determined by the turbidity of the original culture and the sampling require-

ments of the slit sampler. A period of 1 min was allowed to elapse between cessation of spraying and taking the first sample. Samples of 1.4 cu. ft air were taken every 7 min with a total exposure of 10 plates. These times were used in order to adhere to an earlier schedule employed with a different sampler which gave a throughput of 0.7 cu. ft/min for the same air velocity at the slit (i.e. 12 in. water pressure reduction).

At the conclusion of sampling the fan was stopped, the room again entered, and sedimentation plates covered and removed along with the nebulizer. The mean time at which settling plates were covered was recorded and regarded as the end of a run. All petri dishes were incubated at 20°C for 3 or 4 days (depending on the organism) and then counted. Counts so obtained were treated as described below.

III. EXPRESSION OF RESULTS

Rate of loss of cells from the air is expressed in terms of changes of air per hour and represented by the symbol K as defined and calculated by Bourdillon *et al.* (1948). This symbol, expressed as hr^{-1} , may also be defined as the number of viable particles disappearing from the air from any cause per unit time, per unit volume, per unit concentration, and this definition may be shown to agree with that of Bourdillon *et al.*

Sedimentation rate is expressed in similar units by the symbol K_s which is calculated from the equation

$$K_s = \frac{\Delta S}{\Delta N} K,$$

and not, as stated by Lidwell (1948), from the equation

$$K_s = \frac{\Delta S}{\Delta N} \cdot \frac{V}{A} \cdot K,$$

where the symbols are Lidwell's. Lidwell (personal communication) has recently indicated his agreement with the former equation. When K_s is calculated in this way, the death rate, K_d , may be calculated in the above units, from the equation

$$K_d = K - K_s.$$

* In all cases K has been calculated statistically with the assumption that deviation remained constant over a run. Similarly comparisons of results have been made statistically and statements of no difference are made on this basis.

IV. RESULTS

(a) Mechanical Factors

(i) *Removal from Walls.*—A preliminary survey was made to assess the importance of removal by air circulation of bacteria from the walls of the test room. The results of this survey showed that when a broth suspension containing 10^8 - 10^9 bacterial cells was smeared over 8 sq. ft on one wall and the fan directed at this wall, disturbance of cells became very significant. On the other

hand, when the same number of cells was distributed over the four walls (2 sq. ft per wall) and the fan left in its normal position, no increase in the number of airborne cells could be detected in the room up to 90 min after making the smears.

(ii) *Deposition on Walls*.—The rate of impingement of cells on the walls was measured by attaching to the walls microscope slides coated with nutrient agar. Impingement on the ceiling was not measured because it was assumed to be negligible and because the excessive time needed for the attachment would have resulted in marked disturbance of the atmospheric conditions.

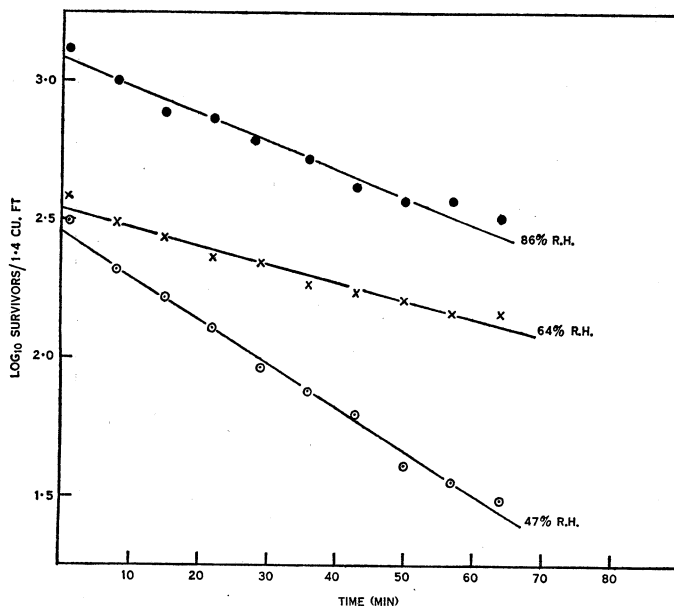


Fig. 1.—*Achromobacter* sp. (483). Regressions of log survivors v. time at three levels of humidity.

Mean results of two such runs at 87-89 per cent. R.H. with *Pseudomonas* No. 451 are: $K = 2.57$ $K_s = 0.10$, $K_w = 0.037$,

where K_w = the rate of deposition on the walls expressed in the same units as the other K values.

(iii) *Sampling Efficiency*.—Evidence was sought that all viable cells delivered to the test room could be accounted for. Accordingly, on a number of runs the bacterial suspension to be used was treated for 1 min in a Waring Blendor to break up clumps, and plate counts were made on the treated suspensions. Treatment in the Blendor was necessitated by the action of the nebulizer, which itself breaks up clumps and delivers an essentially mono-cellular aerosol. On the basis of a delivery from the nebulizer of 0.54-0.55 g water per min and the particle size characteristics already described, agreement to within 10 per cent. was usually obtained with the number of cells estimated by air sampling.

(b) Effect of Diluent

Rates of death of *Achromobacter* No. 483 at 65-72 per cent. R.H. were similar when either nutrient broth or tap water was the diluent used in preparing suspensions. Tap water was used subsequently for experiments with all three organisms. Such determinations in the 65-70 per cent. R.H. region are accompanied by disadvantages which will become apparent later.

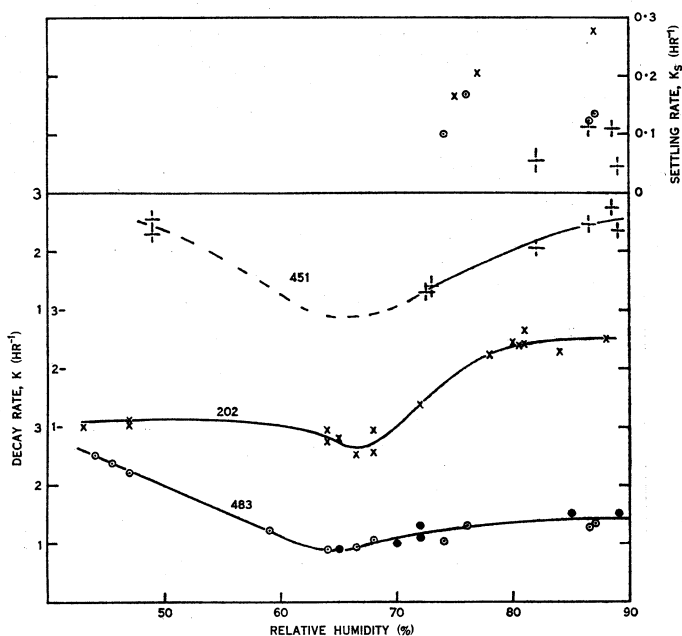


Fig. 2.— K /humidity relationships of *Achromobacter* No. 483, *Micrococcus* No. 202, and *Pseudomonas* No. 451. ●, Room entered for humidity readings.

(c) Effect of Age of Culture

Using *Achromobacter* No. 483 and *Micrococcus* No. 202, the effect on K of age of culture beyond the logarithmic growth phase was investigated between 63 and 72 per cent. R.H. No differences in death rates were found for cultures aged 3-7 days at 20°C. It will be seen from later results in this and the following paper that the selection of the above humidity range to test an age effect was not a happy one, but analyses of results of all three organisms at all humidities tested failed to reveal any variations that could be associated with differences in age within the range employed.

(d) Effect of Relative Humidity

Over the available humidity range, death of the three airborne organisms was always a simple logarithmic process and typical regressions of log survivors *v.* time are shown for the *Achromobacter* No. 483 in Figure 1.

The relationships between K , K_s , and R.H. of all three bacteria are presented in Figure 2, from which it will be seen that, for 483 and 202, K values were

least and of like magnitude at humidities in the region of 65-70 per cent. R.H. Similar behaviour by 451 may be inferred, although the absence of points between 49 and 73 per cent. R.H. prevents a definite conclusion.

V. DISCUSSION

The generic differences in sensitivity to changes in humidity shown in Figure 2 are of some interest and tend to discourage generalization about the effect of humidity on survival of airborne bacteria. It may be noted that the results obtained with either the *Achromobacter* (483) or the *Micrococcus* (202), considered separately, could be used either in support of or in opposition to the schools which have claimed either a positive (Williamson and Gotaas 1942; De Ome *et al.* 1944; Lidwell and Lowbury 1950) or a negative (Wells and Zappasodi 1942; Rosebury 1947) correlation between death rate and humidity.

One aspect of behaviour common to the three bacteria and which may lead to a generalization, however, was the occurrence of minimal death rate in the 65-70 per cent. R.H. region. One is tempted to think that two levels of humidity may prove to be potentially lethal to airborne bacteria over the range 45-90 per cent. R.H. and that the survival/humidity relation of any species is determined by its sensitivity at each of these levels. Further evidence on this concept is presented in the following paper.

VI. ACKNOWLEDGMENTS

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