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Survival of microbial aerosols: experimental observations and calculations

By J. D. MORTON*
Microbiological Research Establishment, Porton, Salisbury, Wilts

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INTRODUCTION

Investigators of the survival of airborne micro-organisms use a wide variety of ways to express their results. Unfortunately, experiments are often planned and reported in a manner that fails to make the best use of the experimenter's work. The meaning of the results may be obscured or even misleadingly distorted, or lack of some simple observation may prevent direct comparison with work elsewhere. I propose to discuss the observations which may be made and the ways in which they may be reported and analysed. In so doing I shall be discussing established practice in this and one or two other laboratories, in the belief that it could with advantage be generally adopted. My illustrative material will be drawn from the comparatively simple type of experiment in which a bacterial suspension is sprayed into a closed space, the cloud is held at constant temperature and humidity, and samples are withdrawn at intervals so that survival may be reported.

There is no need to discuss here all the means by which such an experiment may be done; these have been adequately covered in the literature (e.g. Wolfe, 1961). On the other hand, we need to mention the kind of measurements that give us 'viable concentration' and 'viability', so that we can be quite clear what these terms mean in our discussion. When a sample from a bacterial cloud or suspension is plated and incubated on nutrient agar (after any necessary dilution), the 'viable concentration' of the original material may be calculated from the visible colonies formed. The proportion that this bears to the total concentration of 'viable' and 'non-viable' units may be followed during an experiment by various means such as direct observation of the percentage that forms colonies (Postgate, Crumpton & Hunter, 1961); using a physically similar but resistant material as datum (Harper, Hood & Morton, 1958); or (for cloud samples only) measuring total cloud concentration by optical means (Dimmick, 1960). This is the 'viability', usually expressed as a percentage. (We are fortunately able here to evade the philosophical pitfalls in any simple definition of viability.)

The viability of a suspension before spraying is often assumed to be 100%; the viable concentration is measured and this is taken to be the total population. Since this is the usual practice in this laboratory, I must justify it. First, if we are handling a familiar bacterial species, there is a large amount of experience to assure us that the suspension is not heavily loaded with dead cells (for example, by

* Present address: Microbiological Associates, Inc., 4813 Bethesda Avenue, Washington 14, D.C., U.S.A.

comparing opacity and viable count, or from knowledge of the maximum viable concentrations observed in favourable conditions). Secondly—and this is probably the more important consideration—the object is usually to study the response of a bacterial population to the processes of spraying, equilibration, cloud holding and sampling; this response is unlikely to be much affected by dead cells in a proportion that does not make their presence obvious (as, for example, by low viable counts), and in any case our concern is usually with normal populations and not ideal ones. Whether the subsequent viabilities are referred to this assumed 100% viability in the suspension or to its true viability does not affect the subsequent argument.

Before the period of cloud holding, the bacteria are exposed to two processes, each of which may reduce viability. The process of spraying may damage the cells by mechanical stress. The more critical stage is the period of equilibration, during which the droplet loses water rapidly; this may take, say, 1 sec., and during this time the bacteria sometimes show enhanced sensitivity to sampling by impingement. As a result of the stresses of equilibration, the earliest cloud sample may show a large reduction of viability compared with the suspension viability. The subsequent viable decay of the airborne particles during the holding period shows a wide variety of forms. If log survival is plotted against time (without any implication that this is a meaningful relation), the most familiar patterns are: (1) straight line decay; (2) curve, convex towards the origin; (3) precipitous initial decay, followed fairly abruptly by a much reduced rate (sometimes fitting the log linear plot). Attempts have been made to find generalized mathematical expressions to describe viable decay. It would seem that the variety of behaviour of bacterial clouds alone (not to consider viruses), and the evidence of quite different processes dominating the behaviour at different times during an experiment, considerably reduce the prospects of success. The simplest treatment of this sort is exponential or logarithmic decay, and—as we have already noted—some experiments fit nicely to a linear semilog plot, describable in terms of slope or half life. The slope is often called the 'decay constant' and expressed in % loss of viability per minute. This is convenient and unobjectionable, so long as the observations fit reasonably well; but there is a tendency to extend it to the description of the slope between two arbitrary times in the life of the cloud, without regard for the niceties of fit in between, and this is of course quite indefensible.

Let us now look at two examples from experiments done in this laboratory which will illustrate the importance of making the appropriate measurements and reporting them suitably. In each case, the bacterial suspension was atomized by a Collison spray and held in a rotating drum (Goldberg, Watkins, Boerke & Chatigny, 1958) at the given temperatures and relative humidities. Cells of the same species, grown on a medium containing radiophosphorus and then killed, were added to the suspension as 'tracer' (Harper, Hood & Morton, 1958). The ratio of viable count to radioactive count in the suspension was taken to represent 100% viability; subsequent ratios are expressed as a percentage of this. The first cloud sample at 'zero' time was taken at about 1 sec. after spraying. All samples were taken in capillary impingers (May & Harper, 1957).

These figures fit excellently on a semilog plot (Fig. 1).

Table 1. *Viability of* Brucella suis *aerosols at* 54° *F.*

R.H. (%)	Time (min.) 0	5	30	120	240	360
87	106	91	100	95	82	86
60	66	63	67	56	43	41
50	76	78	76	62	54	47

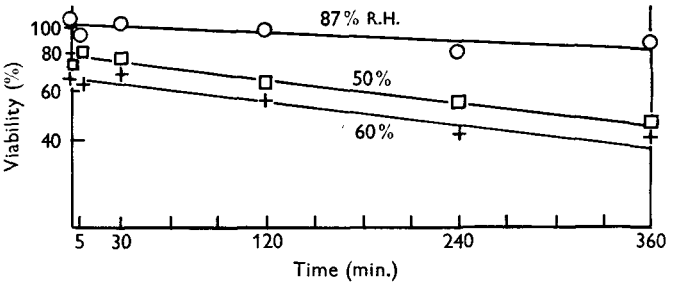

Fig. 1. Viability of *Brucella suis* aerosols at 54° F.

Table 2. *Decay rate of* Brucella suis *aerosols at* 54° F.

(R.H.) (%)	Decay rate (%/min.)
87	0·16
60	0·13
50	0·13

These results can be compactly expressed as in Table 2. Here however we can see clearly that this compact statement though accurate is incomplete, and has concealed an important feature of the results which is obvious in Table 1 and on the graph. At 87 % R.H., the viability has evidently been unaffected in the processes of spraying and equilibration. But at 60 % and 50 % R.H. it has been reduced to about two-thirds and three-quarters respectively of its original value (and we may reasonably conclude that since this did not happen at 87 % R.H. it was the equilibration and not the spraying which killed the bacteria). The decay rate does not show this. To put it another way, the decay rate misleadingly suggests that the viability at 360 min. would be about 60 % at both the lower humidities, whereas the actual viabilities related to the suspension are 40–45 %.

Experiments with *Bacterium tularense* give a different picture. The figures for viability in Table 3 show, and the graph (Fig. 2) vividly illustrates, the influence of relative humidity. The zero time viability is little affected, quite possibly not significantly. However, the earliest subsequent reading (at 5 min.) shows a very big and progressive effect of lowered humidity, which leads to a precipitous initial decay. At 20 % R.H. the decay from 30 to 240 min. can be represented closely by a straight line corresponding to a decay rate of 0·42 %/min., and this is very similar

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to the 30–240 min. decay rate at 85 % R.H. (though the actual curve at 85 % is not a straight line).

Table 3. *Viability of* Bacterium tularense *aerosols at* 45–50° F.

R.H. (%)	Time (min.) 0	5	30	120	240
85	58	42	29	18	14
50	74	(26)	8·9	4·9	(4)
20	54	(1·9)	1·2	0·8	0·5

(Some interpolation has been necessary because all experiments were not sampled at the same time; this does not affect the argument.)

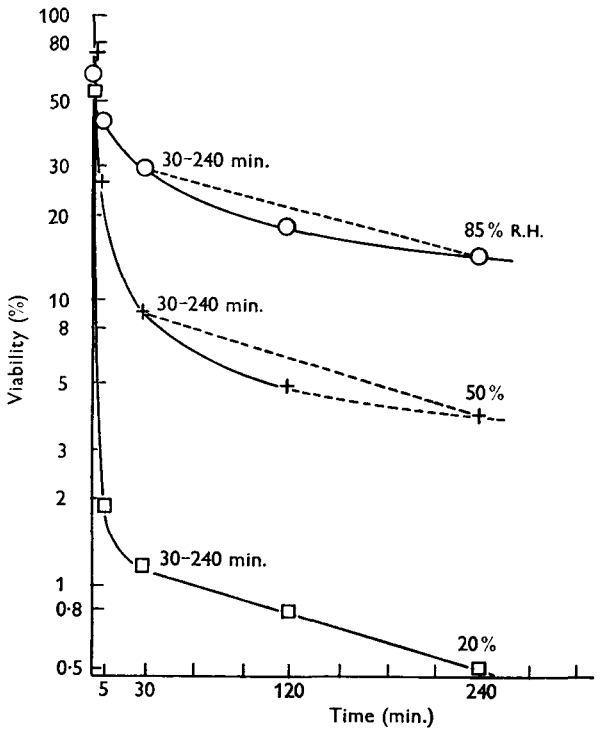

Fig. 2. Viability of *Bacterium tularense* aerosols at 45–50° F.

The decay rates from 30 to 240 min. in Table 4 illustrate particularly well the way in which the actual results can be obscured. On their own they suggest little difference between 85, 50 and 20 % R.H., but in fact the viabilities at 240 min. are 14, 4 and 0·5 %. The decay rates from 0 to 240 min. give a better idea of the influence of R.H. but obviously bear very little relation to the course of the decay curve. The extreme example of 20% R.H. might be described by two rates, from 0 to 5 min. and from 30 to 240 min., but there seems to be nothing to recommend this as compared with a table of observed viabilities supplemented—for those who are not adept at visualizing curves—by a graph.

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These results illustrate another point. We saw with the first example how the assumption that zero time cloud viabilities were all 100 % would obscure the true extent of the differences in viability at 360 min. In the present case, zeroing 58, 74 and 54 % viability to 100 % would make very little difference to the inter-relationship of the 240 min. viabilities. If, however, we were to take the 5 min. viabilities as our zero, a very different picture would be seen (Fig. 3).

Table 4. *Decay rate of* Bacterium tularense *aerosols at* 45–50° F.

R.H. (%)	Decay rate (%/min.) between times shown (min.) 0–240	5–240	30–240
85	0·60	0·44	0·35
50	1·17	0·75	0·38
20	1·86	0·63	0·42

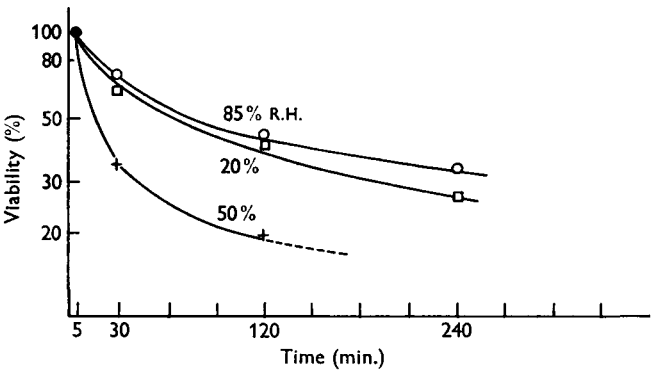

Fig. 3. Fig. 2 re-drawn with 5 min. viabilities as 'zero'.

This would suggest that viable decay is similar at 85 and 20 % R.H., and more rapid at 50 % R.H. A glance at Fig. 2 shows how wrong such a conclusion would be. The unfortunate fact is, however, that experiments are done and reported in this way.

The results at 20 % R.H. in this experiment show that there has been a relatively small loss in viability on spraying and a very large one in the first 5 min. of cloud holding time. The zero time sample is necessary to this observation. Some techniques preclude taking a sample until several minutes after the cloud is formed; they are therefore unable to discriminate between losses in spraying and in the early life of the cloud.

It is perhaps necessary to make it clear that the use of a technique such as the radioactive tracer method, which provides an internal datum in each sample by which the decay of viability may be followed, does not of itself guard against such errors of procedure. The ratio of viable bacterial concentration to radioactive tracer concentration must be measured in the suspension before spraying.

Experiments must be done and reported so as to distinguish clearly between single viable units and aggregates. For example, a particle containing several bacteria, deposited on solid medium, will form a single colony whether one or all are viable. This simple point is sometimes neglected.

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In our opinion the necessary measurements in an experiment on the survival of microbial aerosols include viabilities from the earliest practicable moment in the life of the cloud, and these viabilities must be related to the viability of the suspension before spraying. These figures must be reported in full, regardless of any mathematical treatment or presentation that is to be used, and a graph is usually of considerable help; this may conveniently be on a semilog plot. It is particularly to be noted that our procedure imposes no treatment that may hamper or mislead a reader in comparing and analysing various investigations. It is of course applicable to other experiments, as for example with viruses or dry bacteria.

SUMMARY

In experiments on survival of microbial aerosols, viability should be measured as early as possible in the life of the cloud, but this must not be used as the datum to which subsequent viabilities are related; all viabilities must be related to that of the material from which the cloud was generated. Mathematical models may be used as a convenience in expressing results, but observed viabilities must also be quoted.

I am indebted to the many colleagues whose work over the years has consolidated this approach, and particularly to Mr G. J. Harper whose experiments and thinking form the backbone of my discussion.

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