STUDIES OF THE LOSS OF VIABILITY OF STORED BACTERIAL AEROSOLS*

II. DEATH RATES OF SEVERAL NON-PATHOGENIC ORGANISMS IN RELATION TO BIOLOGICAL AND STRUCTURAL CHARACTERISTICS

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(With 12 Figures in the Text)

INTRODUCTION

For several decades bacteriologists and epidemiologists have been interested in the survival of micro-organisms in the airborne state. Studies by many investigators, recently summarized by Wells (1955), have produced data concerning certain aspects of bacterial survival or death, but the information has not generally sufficed to permit quantitative analysis when a fluid environment was changed in the airborne state.

This paper presents the results of quantitative experiments with aerosols conducted by the author and his collaborators over a period of about 5 years. Studies of several different species of organisms suggest consistent relationships between the viability of a given micro-organism and certain controllable experimental conditions.

In earlier studies of *Micrococcus candidus* aerosols, Ferry, Farr, Rose & Blau (1951) and Ferry & Maple (1954) estimated the concentration of viable organisms, as number per litre of aerosol, from colony counts made on plate cultures of impinged samples taken from aerosols stored 0–300 min. These values, divided by the total particulate concentration, directly measured by a photo-electronic counter (Gucker, O'Konski, Pickard & Pitts, 1947), yielded the proportion of viable organisms. Comparison of the proportion viable at any time, t, with that in the freshly generated aerosol ($t=0$) yielded relative viability (subsequently indicated as R.V.) readily expressed as percentage. Relative viability was thus independent of settling or other effects which diminished total concentration. When logarithmic values of R.V. were plotted as a function of time, it appeared that attrition of viable organisms occurred in two stages, the first rapid, the second slow, characterized, respectively, by rate constants, k_1 and k_2, as already suggested by Dunklin & Puck (1948).

Although the method employed had certain experimental limitations, it seemed that the study of aerosols containing other bacteria might be relevant to an understanding of airborne infection. We first selected *Escherichia coli*, primarily

* This work was supported by a contract with the Chemical Corps, Fort Detrick, Frederick, Maryland.

an aquatic organism, for further studies. It soon turned out that, with this organism, k_1, the primary constant was large and measurable in seconds, not minutes, and that to evaluate this rapid process it was necessary to provide a modified system, termed by us a dynamic storage system. This is described in this paper.

Since use of the dynamic system, in conjunction with the older static method, made the study of aerosols containing very different bacteria possible, it seemed interesting to extend our studies to aerosols containing bacteria resembling certain pathogens. Although consideration for safety was responsible for the choice of non-pathogens, it appeared to us that the organisms chosen for investigation, *Micrococcus candidus*, *Serratia marcescens* (*Chromobacterium prodigiosum*), *Escherichia coli*, *Mycobacterium phlei* and *Corynebacterium xerose*, not only differed in their morphological, structural and cultural characteristics but sufficiently resembled certain pathogens, more sensitive cocci, the typhoid group of intestinal invaders, the causative organism of tuberculosis, and certain upper respiratory tract invaders, respectively, to make these studies useful. The results obtained do confirm early suspicions of some of the reasons underlying the epidemiology of certain diseases and may help partly to explain why some organisms usually invade the host by one route rather than another.

The methods used are first briefly described. Subsequently, the data obtained for the organisms enumerated are presented and briefly discussed. At the end we give a more general discussion, followed by a short summary.

APPARATUS AND METHODS

The experimental method used consisted of the atomization of bacterial suspensions generally in very dilute phosphate buffer (ionic strength about 0·0017), followed by dilution of the resulting mist with air at known temperature and humidity. For storage periods lasting from 2 to 300 min., the aerosol was aspirated into a storage balloon from which samples could be withdrawn at suitable intervals. This, we have termed the static storage system.

To obtain samples less than 2 min. old, we permitted aerosols to flow at a constant rate, either through flasks of known volume, or tubing of known diameter and length; the age of samples emerging from these containers was measured in seconds. This we have called the dynamic storage system.

Although the experimental methods used in collecting data for this paper do not, in principle, differ from those already described by us (1951, 1954), the static system has been modified, and the dynamic system is new. The apparatus, is, therefore, described briefly and illustrated in Figs. 1 and 2; cultural methods for the organisms used are also included. These block diagrams are not drawn to scale.

Apparatus

Generating system

A commercial compressor with a storage tank at 80 pounds per square inch (p.s.i.) delivered air through reducing valves at 15 p.s.i. and relative humidity (R.H.) ranging from about 20 % in summer to 10 % in winter. As indicated in Fig. 1, all air used in the system passed through

filters which removed all particles large enough to produce distinct photo-electronic impulses with the electrical system employed.

Some of the air, regulated by valve 4 (V_4), passed successively through one or more calibrated orifices, shown at the upper left of the diagram, and a thermo-regulated saturating column. To provide air at different R.H.'s, it was mixed with air taken directly from the compressor controlled by V_3. This mixed supply was then used either to dilute the bacterial mist generated by the atomizer, or to express stored aerosol from a balloon suspended in a water-jacket storage chamber, as shown at the upper right of the figure. Access of air flow to different parts of the system was controlled by manipulation of appropriate stopcocks; pressures and flow rates were observed on manometers and flowmeters as shown.

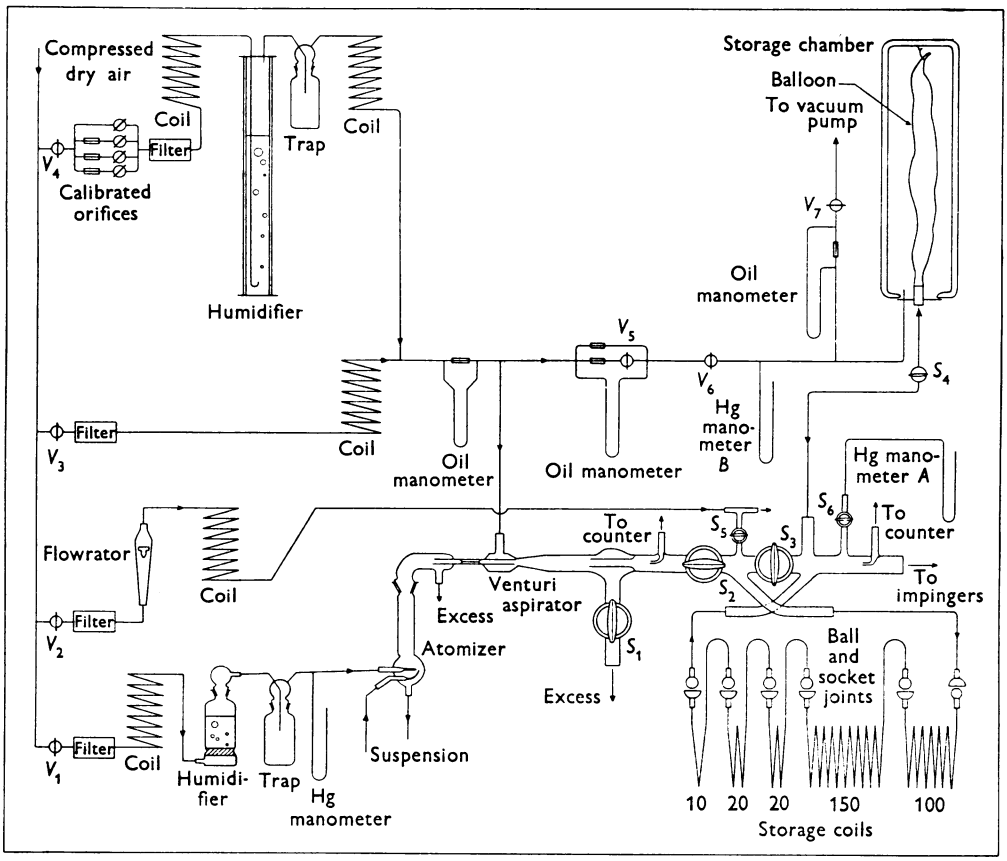

Fig. 1. Aerosol generation and storage system.

Another line controlled by V_2 supplied clean air to sweep out various parts of the system.

A fourth line was regulated by V_1, in conjunction with a mercury manometer, to deliver atomizer air at 5 p.s.i. and a flow rate between 5 and 9 l./min. according to the atomizer used. First passing through a bubbler and trap, it emerged from the atomizer nozzle at about 75 % R.H. and atmospheric pressure.

The atomizers used were either of the reflux type (Ferry *et al.* 1951, p. 268), or of a similar design, but so arranged that the bacterial suspension passed through the atomizer only once. With the reflux type, the initial concentration of the suspension was maintained by adding water from a syringe actuated by a motor-driven mechanical device; with non-reflux atomizers, the suspension was delivered to the nozzle at a rate of about 5 ml./min. by means of

a Sigmamotor pump.* Part of the mist from the atomizer was aspirated by Venturi action and immediately diluted with conditioned air at controlled R.H. at 25° C. to form an aerosol containing about 1500 particles per litre at the rate of about 42 l./min. Since the ratio of diluting air to mist was about 50:1, the R.H. of the aerosol approached that of the diluting air. Distribution of the aerosol to counter, storage balloon, or storage coils was directed by a number of stopcocks as shown and effected by aspiration with vacuum pumps. Connecting tubes were either copper or Tygon.†

Although excess mist and aerosol were always provided in order to prevent aspiration of adventitious particles from outside, the pressure in the manifold and beyond was approximately atmospheric, since excesses were vented into a stack. In it they could be sterilized by heat or ultra-violet light, if desired.

Significant changes from the system formerly used are: (1) omission of a second dilution stage; (2) a different manifold; (3) constant supply of suspension to non-reflex atomizer by a Sigmamotor pump; (4) use of a separate air compressor in order to maintain constant pressure and flow.

Static storage system

This consisted of a balloon suspended in a water-jacketed stainless-steel tank. Connexions for inserting balloon, for entrance of aerosol to balloon, for evacuation or filling outer space with conditioned air, and to a steam line for sterilization were included. Temperature was kept constant by circulation of water from a thermoregulated water bath.

Dynamic storage system

This development became necessary as soon as the rapidity of the first decay process became apparent. Initially we used a series of interchangeable round-bottom Pyrex flasks with capacities ranging from 0·5 to 22·0 l., which, at a flow rate of about 26 l./min., provided storage times varying from about 0·02 to 0·8 min. It was, however, soon observed that viabilities of stored samples were comparable only when flow rate was constant. For example, the viability of a sample stored dynamically in a 5 l. flask was not the same as that stored in a 10 l. flask with a flow rate twice as great.

Consequently, the tube system diagrammatically indicated in the lower right-hand corner of Fig. 1 was developed. It consisted of a series of coils of 0·5 in. copper tubing about 10, 20, 20, 150 and 100 ft. long, with corresponding volumes of 0·29, 0·60, 0·60, 4·41 and 2·94 l. The coils were connected by brass interchangeable ball and socket joints soldered to the ends of the tubing. The terminal joints were connected to the manifold by Tygon tubing to permit easy shifting of position. Successive changes provided coil combinations 10, 20, 30, 40, 50, 100, 150, 200, 250 and 300 ft. long; these, at a uniform flow rate of about 25·5 l./min., provided

* The Model T-6S Sigmamotor pump with electric motor and Revco Zero-Max speed changer was made by Sigmamotor, Inc., Middleport, New York. This device circulates fluid, through rubber or other flexible tubing by a series of cam-operated fingers which press against the tubing in sequence.

† Tygon is a flexible, translucent plastic tubing readily obtainable at supply houses in the United States and manufactured by U.S. Stoneware Company, Akron, Ohio.

storage times of 1·1, 1·8, 2·5, 3·2, 3·9, 7·3, 10·9, 14·5, 17·9 and 21·4 sec., respectively, since time of storage = (volume)/(flow rate), provided flow is turbulent. Under these conditions, the Reynolds numbers calculated suggested turbulent flow; calibrations in which time of transit was measured by means of abrupt changes in the counting rate when flow of clear air was abruptly changed to aerosol, or vice versa, confirmed the calculated times. With the flask system, the same expression can be shown to hold for average storage time, provided mixing is complete. Both assumptions have been tested and seem valid within the experimental error. Shorter storage times could readily be provided, but, with experimental error of about 5–10 % and decay rates observed thus far, they would not be useful. Flow through the coils was controlled by Kocher artery clamps; provision for by-passing the coils by stopcock S_3; for sweeping coils with clean air by S_5. Although the dynamic system was not surrounded by a water bath, it was situated in an air-conditioned room at $25 \pm 1°$ C. As suggested by our earlier studies (1954), this should not introduce significant errors.

The sampling tubes leading to the counter had cross-sections intended to make sampling nearly isokinetic. Although the sampling point more distal to the atomizer seemed satisfactory when used with the static storage system, introduction of the dynamic storage coils resulted in pressure changes at this point, leading to uncertainties in the flow rate through the counter and consequent variations in estimates of bacterial viability. These were, we believe, avoided by sampling up-stream where the pressure was approximately atmospheric.

With 300 ft. coils, comparison of counts obtained by adjusting the pressure at the more distal point to 1 atm. with those obtained by sampling up-stream at 1 atm. showed, moreover, that impingement was negligible as compared to other errors of measurement, particularly the error entailed by colony counts.

Impinger system

This is diagrammatically shown in Fig. 2. It differed from the one used earlier in that the volume of impinger samples was directly measured in a wet gasmeter after collection and saturation. A Pressovac* pump actuated the flow through impingers, by-passes, or the Schwartz tubes filled with magnesium perchlorate to absorb water. R.H. was calculated from sample volumes and gravimetric observations. Flow of aerosol or clean air through impingers, Schwartz tubes or by-passes was directed by the stopcocks or artery clamps as shown.

Counter

This instrument is similar in optical principles to a dark field microscope. Light scattered by airborne particles passing through a chamber is focused on a photo-tube (either RCA 931 or better the more sensitive IP 21). The electrical impulses generated can be recorded electrically or mechanically. We used a decade scaler coupled to a mechanical register in our more recent studies.

* Pressovac pump, manufactured by Central Scientific Company, Chicago, Illinois. It is intended either to evacuate or deliver air under pressure.

The impulses were monitored by a cathode-ray oscillograph. This not only graphically illustrated reasonable uniformity of impulses, but also, when unusually large pulses appeared, gave warning of leaks. It also indicated the efficiency of the electrical system by showing the signal to noise ratios.

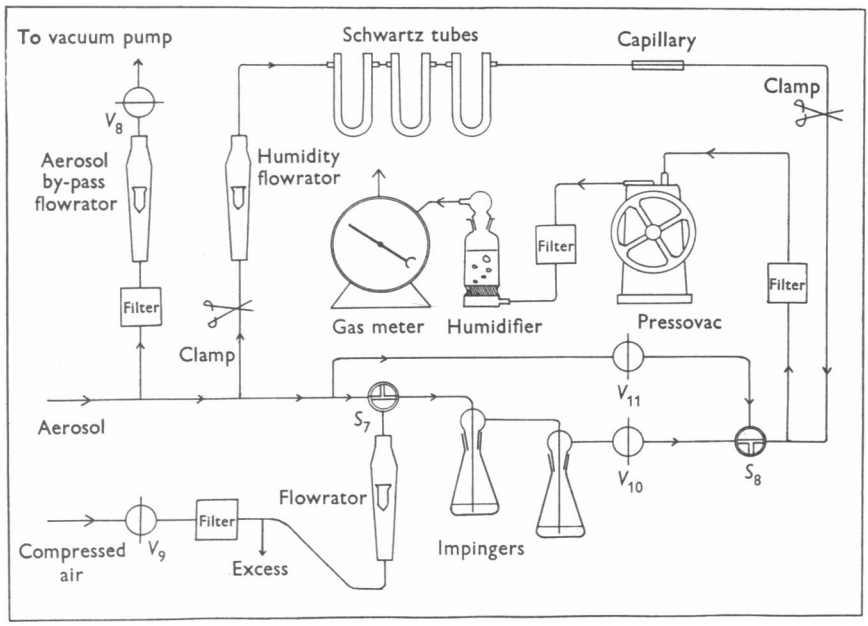

Fig. 2. Impinger system.

Bacteriological methods

Culture media

M. candidus—suspensions were prepared from cultures grown for 18 hr. at 25° C. in tryptose broth containing 0·3 % glucose. Uniformity of inoculum was attained turbidimetrically.

Esch. coli were incubated 24 hr. in 2 % yeast extract broth containing 0·5 % glucose and 0·0001 % $MgSO_4$. Strain no. 75, which is streptomycin resistant, was grown in a similar medium, containing 100,000 units of streptomycin per l. medium.

Myco. phlei was grown at 37° C. on a medium originally devised by Dubos (Pierce, Dubos & Middlebrook, 1947) and slightly modified by him (personal communication, 1954). After 24 hr. without stirring, it was continuously stirred with a magnetic stirrer for 5½ days, taking precautions to avoid overheating.

C. xerose was incubated 24 hr. at 37° C. on a beef heart infusion broth.

S. marcescens was grown in a medium containing 3 % Bacto-tryptose, NaCl 0·5 %, glucose 0·5 %. It was incubated for 18–24 hr. at 32° C. with mechanical shaking.

Seed organisms were stored on agar slants and transplanted about once a month. The slants were stored at 2–6° C.

Preparation of suspensions

After incubation, bacteria were harvested, centrifuged, and washed twice with distilled water and finally resuspended, except in earlier studies of *S. marcescens*, in dilute buffer at ionic strength about 0·002. These organisms were suspended either in distilled water or dilute gelatin buffer or dilute buffer.

A low concentration of buffer was selected in order to avoid counts arising from dried particles remaining after evaporation of buffer droplets. In earlier experiments a homomixer was used to break up clumps of *M. candidus*. Since *Esch. coli* and *S. marcescens* clumped little, with them this precaution was unnecessary; with *Myco. phlei* it was unsuccessful. Since it was subsequently found that ultra-sonic irradiation for 1 min. with a Raytheon sonic transducer did not materially impair viability and markedly reduced clumping with *M. candidus* and *Myco. phlei*, this procedure was later used when necessary to break clumps. Although the pH of the suspension was regularly measured and recorded, it should be remembered that the final pH of the bacterial milieu may have shifted nearly 1·0 pH unit more acid during the dying process.

Although fresh suspensions of *M. candidus*, *Esch. coli* and *S. marcescens* were used for aerosol generation in earlier work, it was later observed that shell-frozen concentrated suspensions were not measurably damaged by storage in the frozen state for periods up to 5 months.

With every organism studied, bacterial concentration of the suspension atomized was about 10^8 organisms per ml., as shown by direct count. The number of clumps containing, respectively, one or more organisms was separately recorded and the mean number of organisms per particle (M.N.P.) calculated. Clumps containing more than three organisms were rare, and have been neglected in our calculations. If M.N.P. exceeded 1·3, calculated viabilities were corrected using the graphs derived by Muench (Ferry & Maple 1954). When M.N.P. $< 1{\cdot}3$, the correction, which did not exceed the experimental error, was omitted. *Atomization* was, except for *M. candidus* suspensions, in which reflux atomizers were used, with non-reflux atomizers.

CHANGING VIABILITY OF *MICROCOCCUS CANDIDUS* AEROSOLS

Introduction

Although our previous work (Ferry & Maple 1954) had suggested that with this organism decay could be described in terms of two constants, the first k_1, large, the second k_2, relatively small, it was not until the dynamic storage system was devised that it was possible to evaluate k_1 satisfactorily. The method of evaluation subsequently used depends on two assumptions:

(1) As a first approximation, there are two processes involved, both logarithmic in character and characterized by constants k_1 and k_2.

(2) At the conclusion of the first rapid process, a fraction of the organisms originally viable, b, is subject to slow decay.

From a theoretical analysis developed here by W. F. Brown, it was shown that in the static storage system the following relationship might be expected to hold

$$\text{R.V.} = (1-b)\, e^{-(k_1+k_2)t} + b\, e^{-k_2 t}; \qquad (1)$$

where

$$\text{R.V.} = \text{relative viability at time } t = \frac{\text{viability at time } t}{\text{initial viability of aerosol}} \times 100,$$

b = fraction surviving rapid decay,

k_1 = constant defining rapid decay at first measured per minute, later per second,

k_2 = constant defining slow secondary decay per minute.

For purposes of calculation, the values used must be in appropriate units.

With some organisms, k_1 was much greater than k_2 so that the first term rapidly approached 0 during static storage periods measured in minutes. Consequently, k_2 could be estimated from the slope of curves in which log % R.V. was plotted against time in minutes as abscissa. The slope, multiplied by 230, yields k_2', a decay constant expressed as percentage decay per minute.

If, however, $k_2 \ll k_1$, and t is small (measured in seconds), equation (1) may be written in the form

$$\text{R.V.} = \frac{1-b}{e^{k_1 t}} + b, \tag{2}$$

which is a good approximation. Expansion of the denominator by Taylor's theorem yields the expression

$$e^{k_1 t} = 1 + k_1 t + \frac{(k_1 t)^2}{2!} + \frac{(k_1 t)^3}{3!} + \ldots.$$

Since higher terms can be neglected when both k_1 and t are small, we can use the approximation $e^{k_1 t} = 1 + k_1 t$. Under these conditions

$$\text{R.V.} = \frac{1-b}{1+k_1 t} + b. \tag{3}*$$

Although this approximation holds only when k_1 and t are both small, it provides a convenient and satisfactory method of comparing data. The constants k_1 and b can readily be computed from a series of paired values of R.V. and t, graphically, or, once their approximate values are known, by trial and error. For two sets of paired values for R.V. and t, it can be shown that

$$\frac{t_v}{t_1}\,\text{R.V.}_v - \frac{\text{R.V.}_1}{1-\text{R.V.}_1}(1-\text{R.V.}_v) = b\left(\frac{t_v}{t_1} - \frac{1-\text{R.V.}_v}{1-\text{R.V.}_1}\right). \tag{4}$$

This has the form $y = bx$. Using appropriate experimental values for R.V. and t, y and x can be plotted; the slope of a straight line approximating these points yields b, k_1 can then be evaluated by equation (3). For effective use of this method $1 > \text{R.V.} > b$, and t must also be appropriately chosen; the method of trial and error is, after practice, often more satisfactory.

To express k_1 in terms of percentage decay in the approximate form, we need only to multiply by 100. This empirical constant is denoted as k_1'.

Results

The data obtained over a period of about 2 years, either by means of the flask or tube storage system, were reasonably concordant and appear in Fig. 3 A–D inclusive, in which R.V. % is ordinate, t in seconds abscissa, together with the relevant constants k_1' and b. Two lower curves illustrate results obtained with an aerosol generated from a shell-frozen suspension 504 days' old.

Discussion

Consideration of equation (1) and the approximate equation (2) at once suggest that the proportion of all organisms which survive during the first rapid process varies with the product $k_1'(1-b)$. For comparative purposes this is included in Table 1.

* With the flask system, the storage time t is the average of an aerosol age distribution and mathematical analyses using equation (1) gives directly

$$\text{R.V.} = \frac{1-b}{1+k_1 t} + \frac{b}{1+k_2 t},$$

which for $k_2 t \ll 1$, approximates equation (3). With the coil system, t represents the actual storage time and the validity of equation (3) depends on the two approximations

$$e^{k_2 t} \simeq 1 \quad \text{and} \quad e^{k_1 t} \simeq 1 + k_1 t.$$

Over the range studied $1/(1-b)$ is approximately a linear function of k_1'. This, however, is only an approximation, if the first process is indeed related to drying, since as R.H. approaches 0, k_1 should approach a finite value determined by the

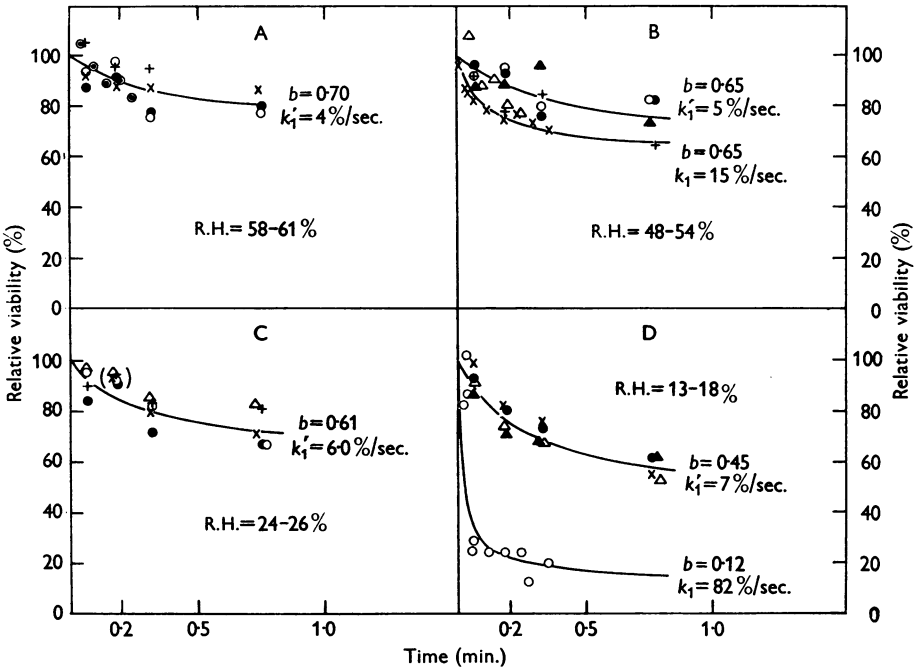

Fig. 3. Rapid decay of *M. candidus* aerosols; $t = 25°$ C., pH 5·3–6·5.

Symbol	Date	Age suspension (days)	M.N.P.	Symbol	Date	Age suspension (days)	M.N.P.
	(A) R.H. 58–61%				(B) R.H. 48–54%*		
●	11. x. 54	5	1·2	△	8. xi. 54	5	1·4
○	21. x. 54	1	1·0	●	6. xii. 54	12	1·3
+	3. xi. 54	5	1·2	+	13. xii. 54	19	1·3
⊙	5. xi. 54	2	1·2	▲	14. xii. 54	20	1·2
×	26. xi. 54	2	1·2	○	7. xii. 54	13	1·2
				×	31. vii. 54	504	1·2
	(C) R.H. 24–26%*				(D) R.H. 13–18%		
●	15. xi. 54	5	1·2	●	18. vi. 54	2	1·6
+	22. xi. 54	5	1·3	▲	21. vi. 54	5	1·7
○	30. xi. 54	6	1·3	△	28. vi. 54	5	1·9
△	1. i. 54	54	1·2†	×	21. vii. 54	5	1·2
×	18. i. 54	55	1·3†	○	27. i. 56	504	1·2†

* All suspensions in this series ultrasonically treated.
† Experiments with coil system. Derived constants appear in Table 1.

temperature of the particle, concentration of the aqueous ambient medium at time, t, and also characteristics of the cell itself including its geometry, chemistry and structure of the cell wall. Concentration and temperature of the particles

Table 1. *Rapid decay of* M. candidus *in air*

R.H. %	14–16*	18†	24–26	48–54	53†	58–61
pH	6·3	6·5	5·8	5·8	6·5	6·0
Age suspension (days)	2–5	504	5–54	5–20	504	1–5
b, fraction surviving rapid decay	0·45	0·12	0·61	0·65	0·65	0·70
k_1' % viability loss per second	7·0	82·0	6·0	5·0	15·0	4·0
$k_1'(1-b)$	3·8	72·0	2·3	1·8	5·2	1·2

* Corrected for mean particle size. † Stored in the shell-frozen state.

could not readily be measured, and we know of no theoretical treatment concerning the evaporation of droplets of solution. At saturation of the ambient atmosphere k_1' should presumably vanish; it does indeed decrease as humidity increases as is shown in Fig. 3. Inspection of this figure also shows that at higher R.H. values, the magnitude of the experimental error limits exact determination of k_1.

It is remarkable that the ancient preparation survived as well as it did, and especially at higher R.H.

ESCHERICHIA COLI

Introduction

The experiments reported here were made over the period 1952–56 on aerosols containing either strain nos. 17 or 75 of *Esch. coli*.* The data relating to the latter, which is streptomycin resistant, are both more extensive and, we believe, more reliable. The results of typical runs in the static and dynamic systems are illustrated in Figs. 4 and 6; those at other humidities are summarized in Table 2. Data from a number of faulty experiments in which humidity varied during a run or plate counts were low or some experimental error was known, have not been included. The results with strain no. 17 are not given in detail, but are summarized in Table 2. The results appear under two captions: Static Studies and Dynamic Studies.

Since early studies of this organism showing its rapid death under certain conditions, had made the development of the dynamic storage system virtually mandatory, it seemed proper to use *Esch. coli* for comparative studies during the development of this system. Data obtained with both flask and coil systems are numerous and in fair agreement; the former had disadvantages, but did provide information at storage periods somewhat longer than those obtainable with the soil system used. Static storage studies also abound, among them preliminary studies not reported here, which suggested that over the range pH 4·0–9·0, this organism is not pH sensitive. These were not pursued further.

STATIC STUDIES

Examination of the crude data showed that at any given R.H. the slopes of the lines describing logarithmic decay were generally comparable, even if the fraction surviving rapid decay, denoted as b, and derived from the intercepts with the Y

* Obtained from Dr C. F. Brown, Fort Detrick, Md.

axis varied, perhaps for reasons discussed later. To permit more ready comparison of the data at a given pH, we have used the following procedure.

First, we averaged the logarithms of the intercepts. Secondly, the positive or negative deviation relating to a simple run has been algebraically added to all points pertaining to that run. The points thus corrected have been plotted as usual with log R.V. % as ordinate, time as abscissa. This procedure slides lines up or down the Y axis without changing their slope, and by using a common intercept or b value, at a given R.H. facilitates comparison of slopes as well as increasing economy of space. The observed values of log R.V. %, of course, can be readily obtained by reversing this procedure, since values for log b are given in the legend.

Results

Fig. 4 illustrates data from a series of experiments at R.H. 62 ± 1 %, performed during an 18-month interval. At $t = 0$, log R.V. % = log b (mean). A solid line, drawn by inspection, yields an approximate mean slope; the dotted lines suggest the range of slopes. Slopes $\times 230{\cdot}3 = k_2'$, or percentage loss of viability per minute. Although the experiment of 13 November 1952 is clearly aberrant, a likely explanation will be given in the *S. marcescens* section.

The derived constant, log b, with its standard deviation, b and k_2', with ranges and means, at a number of humidities appear in Table 2. For b, the mean is taken from log b (mean). Fig. 5 illustrates b and k_2', obtained from single experiments, as functions of R.H. These charts, together with the summarized results show that b rises sharply and k_2' falls abruptly at about 50 % R.H.

The constants derived from experiments in 1956 are, however, quite different from the rest, but do not seem related to a change in method. A similar and abrupt change with strain no. 17, after several months of satisfactory experimentation, had occurred earlier. At present, we can only tentatively suggest a mutation, or alternatively the emergence of a bacteriophage as possible explanations.

Summarized data from experiments with strain no. 17 do not include the change. They do suggest that the characteristics constants for this organism differ somewhat from those for strain no. 75.

Dynamic studies

The experiments, summarized in Table 2, performed from 1952–56 with different modifications of the dynamic system, were helpful not only in characterizing this normally aquatic organism in the airborne state, but in developing the method.

Results

The results of a typical series of experiments are shown in Fig. 6 in which ordinates are R.V. % abscissae, time in minutes. For the solid curve we first averaged values for R.V. % over narrow time ranges. These points were next plotted and the curve, as depicted, constructed by means of equation (2) and the constants $b = 0{\cdot}10$, $k_1 = 0{\cdot}20$. The dotted curves are drawn through points representing, respectively, means plus or minus their standard deviations, using the

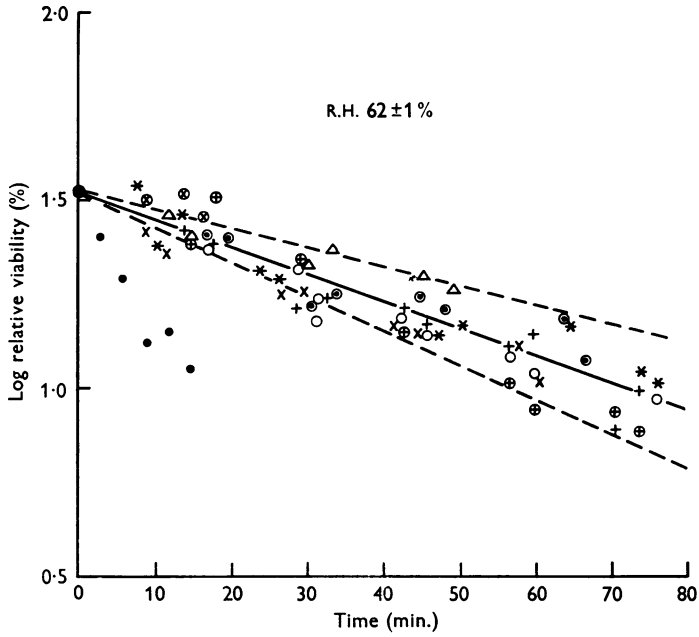

Fig. 4. Slow loss of viability of *Esch. coli*, strain 75 aerosol at R.H. 62 ± 1 %, pH 6·6–7·2, $t = 25°$ C.

Symbol	Date	R.H.	pH	Age suspension (days)	Log b	Correction
●	13. xi. 52	62	6·8	5	1·72	−0·20
⊗	18. iv. 53	62	6·6	5	1·54	−0·02
*	16. iv. 53	61	7·1	1	1·22	+0·30
△	18. v. 53	63	7·2	3	1·72	−0·20
⊙	25. i. 54	62	7·3	3	1·61	−0·09
×	28. i. 54	63	7·2	6	1·72	+0·20
⊕	8. iii. 54	62	7·2	5	1·58	−0·06
+	26. v. 54	62	7·2	5	1·26	+0·26
○	3. vi. 54	61	7·2	13F	1·28	+0·24
				Mean	1·52 ± 0·21	

The slope of the solid line × 230·3 gives an approximate mean value for death rate in per minute; slopes of the dotted lines represent extremes.

appropriate pairs of constants $b = 0{\cdot}10$, $k_1 = 0{\cdot}10$ and $b = 0{\cdot}10$, $k_1 = 0{\cdot}40$. The values in Table 2 were similarly obtained. Although we had previously used the method of paired values of R.V. and t to evaluate constants relating to individual experiments, the method of trial and error using the fractional form, $k_1 = 0{\cdot}01k_1'$, proved more convenient for an experimental series.

As might be expected, deviations are less at low R.H. and increased with R.H. as k_1 decreases. The progressive increase in scatter occurs because an error of measurement of 5–10 %, produces deviations directly proportional to the magnitude of R.V.

In dynamic studies, as in static studies, b and k are inversely related. It is possible, moreover, that the apparent rise in b and fall in k_1' at the lowest R.H. at

Table 2. *Derived constants for decay of* Esch. coli *aerosols; t* = 25° C., pH 6·5–7·5

(A) Strain no. 75, slow decay

									1956 data	
R.H. %	15	25–35	45	53–59	61–63	63–69	70–72	73–74	62–64	78–80
No. expts.	1	3	1	7	9	9	8	6	5	4
Log b*	1·0	1·17	1·06	1·0 ± 0·25	1·52 ± 0·21	1·62 ± 0·22	1·81 ± 0·19	1·64 ± 0·21	—	—
b (mean)†	0·1	0·15	0·11	0·10	0·33	0·42	0·65	0·44	0·06 (0·44 dynamic)	0·5
k_2' mean	36·0	29	3·5	2·1	1·6	1·8	1·2	2·3	27	16

(A) Strain no. 75, rapid decay

R.H. %	12–15	25–31	33–43	45–49	50–60	60–66		70–74	75–81
						Low	High		
No. expts.	4	7	6	7	14	9	7	6	8
b	0·1	0·05	0·05	0·06	0·10	0·22	0·55	0·20	0·55
k_1'	20·0	35·0	25	20·0	20	10	15·0	10·0	6·0
$k_1'(1-b)$	18·0	33·0	24·0	19·0	18·0	7·8	6·7	8·0	2·7

(B) Strain no. 17

R.H. %	19–22	30	37–51	59–61	46–65	64–87	90
b Static	—	0·22	—	—	0·2	0·5	0·8
b Dynamic	0	—	0·15	0·16	—	—	—
k_2'	—	30·0	—	—	16·0	6·9	4·6
k_1'	17·5	—	15	12·5	—	—	—
$k_1'(1-b)$	17·5	—	13	10·5	—	—	—

* ± indicates standard deviation. † Values given are antilogs of log b mean.

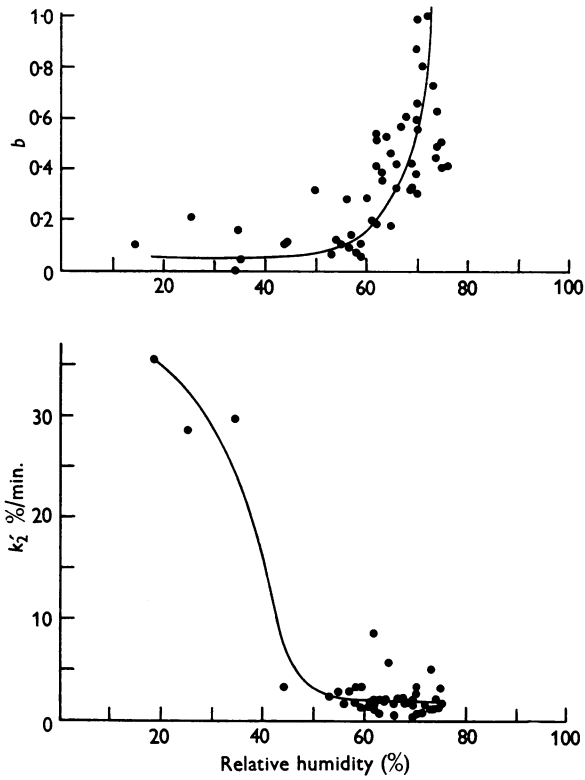

Fig. 5. b and k_2' as functions of relative humidity (see text).

which observations were made, are real. Under these conditions, evaporation may be fast enough to cool and freeze the droplets, thereby retarding drying, with consequent decrease in k_1' accompanied by an increase in b.

Discussion

Although the results strongly suggest a consistent and at least semi-quantitative dependence b, k_1 and k_2 upon R.H., variability at the same R.H. is conspicuous. It is not apparently referable to slight differences in preparation of suspensions for atomization, nor to the age of relatively fresh or older frozen suspensions. While the magnitude of scatter in dynamic runs is proportional to the magnitude of R.V., in static experiments the number of viable organisms recovered in a sample may frequently diminish so rapidly that large errors must be expected on statistical grounds. Even if these two sources of error account for some of the differences observed between individual runs at the same R.H. and certain differences in method (see *S. marcescens*, discussion) for others, variability especially that of b, is not completely explained. Tentatively and reluctantly, and despite our precautions to control conditions, we resort for explanation to biological variation, a euphemism for uncontrolled or uncontrollable experimental conditions.

In analysing dynamic studies at R.H. $> 60\%$, it turned out that, at about 63 % and between 71 and 80 %, R.V. values fell into two groups. At the lower R.H., the

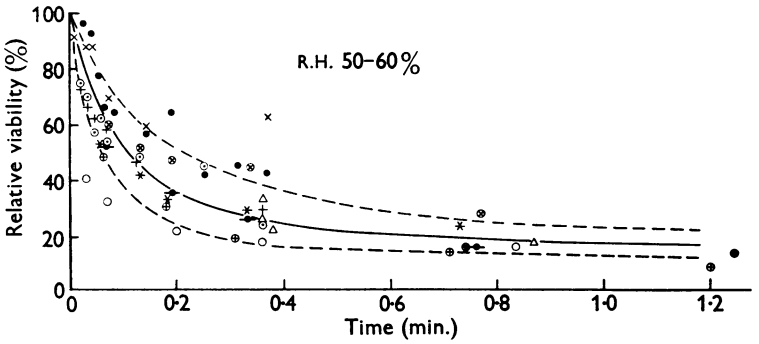

Fig. 6. Rapid loss of viability of *Esch. coli*; $t = 25°$ C., R.H. 50–60 % pH.

Symbol	Date	R.H. %	Age suspension (days)	Symbol	Date	R.H. %	Age suspension (days)
⊕	26. i. 53	51	3	○	9. vi. 54	59	19
△	9. iii. 53	54	5	●	28. iii. 55	53	59
△	10. iii. 53	50	6	×	6. vi. 55	56	59
⊛	5. v. 53	57	6	△	25. xi. 55	55	22
⊷	9. ix. 53	54	5	△	29. xi. 55	53	25
✱	15. i. 54	50	7	+	7. xii. 55	51	34
⊗	5. iv. 54	55	33	⊙	14. xii. 55	53	41

	b	k_1
Solid curve	0·10	0·20
Dotted curves:		
Upper + S.D.	0·10	0·10
Lower − S.D.	0·10	0·40

△ Have each only 1 point.

lower values were obtained either with flasks, or the most recent form of the coil system. This suggests that in an earlier version of the coil system, pressures and flow rates may not have been completely controlled. At the higher R.H. range, higher R.V. values could be associated with higher R.H., except for one erratic run. The results appear under 70–74 % and 75–81 % R.H., respectively.

It is, however, surprising to find that rapid decay in 1956 agreed with earlier observations. The results do indeed demonstrate that *Esch. coli* does not, when almost naked, survive long in the airborne state.

MYCOBACTERIUM PHLEI

Introduction

This organism, chosen not only because of its resemblance to acid-fast pathogens, but also because it contained lipids and long-chain alcohols (Asselineau, 1951), repaid study. Because *Myco. phlei* is markedly aerobic, counts of surface colonies were necessary. And even after practice, we felt less confident of their accuracy than with those from poured plates. Clumping of organisms in suspensions, which may result in false estimates of viability, was also a problem, eventually resolved with fair success by the use of ultra-sonic radiation to break up clumps. The data eventually obtained are reasonably consistent, at R.H. as high as 40 %.

Methods

In earlier experiments, aerosol generation and assay followed our ordinary routine. If clumping leading to a M.N.P. $\geqslant 1{\cdot}3$ was observed in suspensions before atomization, observed viabilities were corrected by means of the curves prepared by Muench (see Ferry & Maple 1954).

Studies were limited to the R.H. range 15–56 %, since lower limits were difficult to attain, and at higher R.H. changes in R.V. are small compared with the experimental error. Preliminary dynamic studies clearly showed that this approach would, at present, be unfruitful.

Results

Two diagrams in Fig. 7 represent data obtained at R.H. 29–33 % and 38 %, respectively. At the lower R.H. points are, as with *Esch. coli*, referred to a mean log b. The slope of the solid line, placed by inspection, represents a mean decay

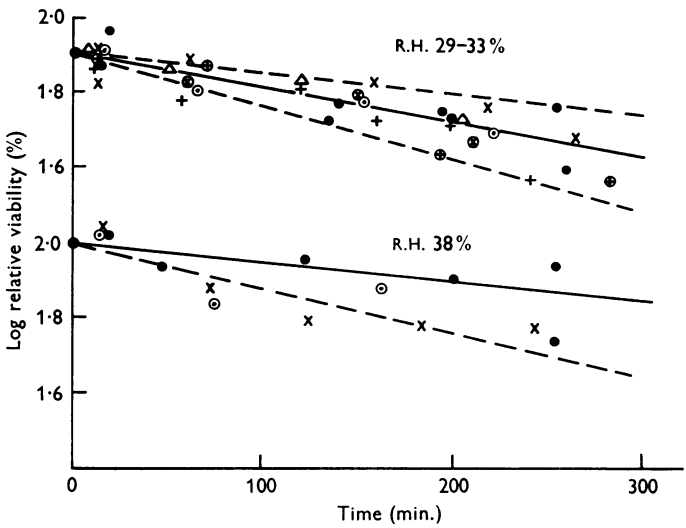

Fig. 7. Slow loss of viability of *Myco. phlei* aerosols; pH 7·0–7·4, $t = 25°$ C.

Symbol	Date	M.N.P.	Corrected for M.N.P.	Log b	Correction
		Upper diagram R.H. 29–33 %			
△	4. i. 54	?	No	1·97	−0·06
⊕	19. ii. 54	1·03	No	1·91	0
×	2. iii. 54	1·25	Yes	1·96	−0·05
⊗	27. iv. 54	—	No	1·86	+0·05
●	11. v. 54	1·0	No	1·94	−0·03
⊙	14. v. 54	1·0	No	1·83	+0·08
+	24. ix. 54	1·3	Yes	1·90	+0·01
			Mean	1·91 ± 0·05	
		Lower diagram R.H. 38 %			
●	19. iii. 54	1·15	No	—	—
×	21. xi. 54	1·4	Yes	—	—
⊙	9. iii. 54	1·2	No	—	—

rate; that of the dotted lines suggests its range. At the higher R.H., we have included two runs in which R.V. seemed unduly variable. But since we later observed that this was to be expected when decay rates are low and R.V. high, they are included here. Values for log b, and k_2' at different humidities appear in Table 3, as well as values obtained about R.H. 30 % after ultra-sonic irradiation.

There is no doubt that k_2' is low under the conditions of study and log b correspondingly high. It is possible that the lipids and higher alcohols in this organism may form a layer, which like long-chain alcohols spread on water (Archer & La Mer, 1955), greatly retards evaporation, and thus averts rapid death.

This organism is surely hardy and its viability in air is not materially reduced by ultra-sonic irradiation of suspensions.

Table 3. *Slow decay of* Myco. phlei

R.H. % ...	... 15–17	20*	24*–28	29–33		36–38	48*	56
				Untreated	Sonic treatment			
No. of runs	5	2	2	7	4	3	1	3
Log b	1·77 ± 0·09	1·94	1·83	1·91 ± 0·05	1·87 ± 0·07	1·9–2·0	1·93	1·98–2·0
b Range	0·50–0·73	—	—	0·72–0·92	0·63–0·87	—	—	—
b Mean	0·59	0·87	0·68	0·81	0·74	0·9	0·85	0·96–1·01
k_2' Range	0·27–0·62	—	—	0·12–0·33	0·16–0·45	0·11–0·28	—	0–0·11
k_2' Mean	0·44	0·23	0·46	0·22	0·30	0·20	0·48	—

* Less certain values.

CORYNEBACTERIUM XEROSE AEROSOLS

Introduction

The choice of this diphtheroid organism for study proved fortunate. Not only is it culturally and morphologically similar to certain pathogens, but it also grows readily in simple media, suspensions are nearly mono-disperse and study of aerosols with the static storage system yields reproducible data. Because of the magnitude of the experimental error, dynamic studies at R.H. > 25 % were unprofitable and even at 20 % R.H., the results are not sufficiently consistent accurately to define the constants b_1 and k_1'. It is probable that data at even lower humidity would be more consistent.

Results

Results obtained with the static system, plotted as usual, appear in Fig. 8. Those obtained with the dynamic system at 17–21 % R.H. appear in Fig. 9. The relevant constants have been assembled in Table 4.

Discussion

When R.H. > 30 %, this organism dies off very slowly and is virtually unaffected by the first rapid process; when R.H. < 21 %, the initial process becomes perceptible. Although the curve drawn through the data in Fig. 9 was based on the calculated

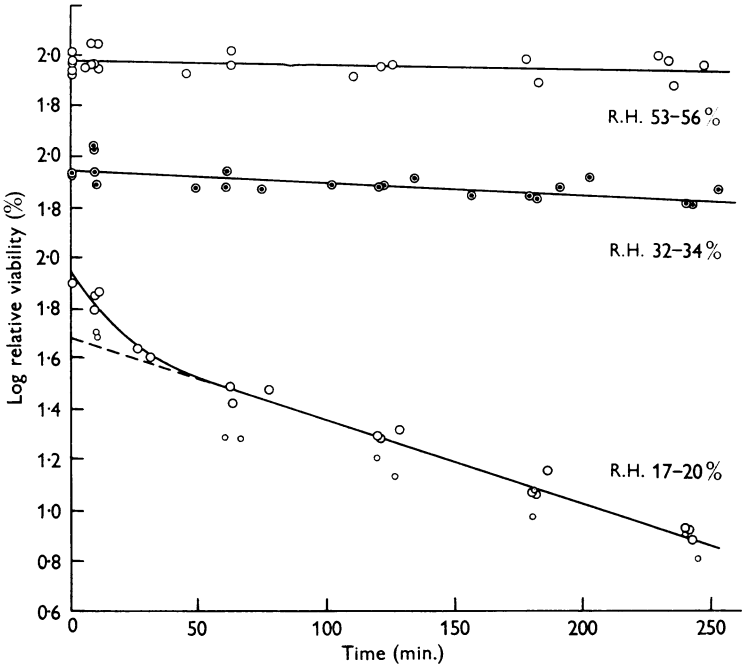

Fig. 8. Slow decay of *C. xerose* aerosols; air, 25° C., pH 6·5 ± 0·1.

Date	R.H. %	Symbol	
24. v. 56*	55	○	Plotted at top
29. v. 56*	53	○	
31. v. 56*	56	○	
5. vi. 56*	34	⊙	Plotted in middle
7. vi. 56*	34	⊙	
12. vi. 56*	32	⊙	
13. vi. 56	32	⊙	
19. vi. 56†	19	○	Plotted at bottom
21. vi. 56†	17	○	
25. vi. 56‡	18	○	
27. vi. 56‡	18	○	
2. vii. 56*‡	20	○	

* Combined dynamic and static run.
† Somewhat less reliable values.
‡ More reliable values.

Table 4. *Loss of viability of* C. xerose *aerosols at* 25° C.; pH 6·5 ± 0·1

R.H. %	k_1'%/sec.	*b* fraction surviving initial process	k_2'%/min.	*b* fraction surviving initial process
53–56	Not calculable		0·05	0·96
32–34	Not calculable		0·12	0·88
17–20	12·5	0·71	0·75	0·48
	4·0*	0·48*	—	—

* Using *b* value derived from static study.

constants, $b = 0{\cdot}71$ and $k_1' = 12{\cdot}5\,\%$/sec., it can be fitted equally well using $b = 0{\cdot}48$ (derived more exactly from static system measurements) and a decay constant $k_1' = 4\,\%$. Examination of the data for slow decay at 17–20 % R.H. suggests that the 'rapid' process is protracted. The data can, moreover, be fitted up to about 25 min. by means of the approximate equation (2) and the constants $b = 0{\cdot}48$, $k_1' = 4{\cdot}0\,\%$ which yield the values R.V. = 52, 50 and 49 % at 5, 10 and 20 min.,

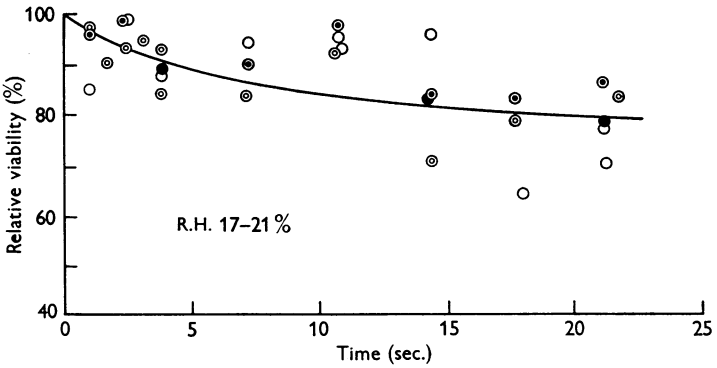

Fig. 9. Rapid decay of *C. xerose* aerosols; air, 25° C., pH 6·5 ± 0·1.

Date	R.H. %	Symbol
2. vii. 56*	20	●
5. vii. 56†	18	⊙
9. vii. 56†	21	◎
11. vii. 56†	18	○

* Dynamic and static storage. † Dynamic storage only.

respectively. Although the approximation is mathematically unjustified, since higher terms of the Taylor series expansion should be considered, k_1' might be expected to decrease with increasing concentration of the medium as evaporation proceeds to dryness. This partly justifies its use. Quantitative estimation of this change, which would necessitate consideration of the chemistry and physical properties of the cell wall, geometry of the cell, and changing temperature of the particles defies analysis, at least by us. As an approximation, decay of viability in terms of constants b, k_1 and k_2 still seems useful.

Further study of aerosols containing these organisms might well be profitable, since their behaviour represents a transition between sensitive forms like *Esch. coli* and *Myco. phlei*, which is resistant to drying.

SERRATIA MARCESCENS

Introduction

This Gram-negative chromobacterium turned out to die off rather rapidly in the airborne state. Pathogenic only in large doses, it morphologically resembles pathogens intermediate in form between cocci and bacilli. Consequently, studies of this organism have been helpful not only in development of methods, but in taking a place in the series for comparison with other organisms.

STATIC STORAGE STUDIES

Before we referred experimental points, obtained under the same conditions, to a mean value of log *b* (see *Esch. coli*, p. 135), it had been difficult to correlate the data. The experiments made at an early stage of this development, included a wide variety of conditions, such as (1) atomization of bacterial suspensions in water instead of buffer, (2) the use of a two-stage dilution system with R.H. of mixing air uncontrolled and low, and (3) different pH values of the suspension atomized.

Results

Two experimental series are illustrated in Fig. 10 and the data are summarized in Table 5*a*. A comparison of data obtained at R.H. about 45, 58, 65 and 70 % and either at pH 6·2 or 7·0, strongly suggest that (1) exposure to air at low R.H. for

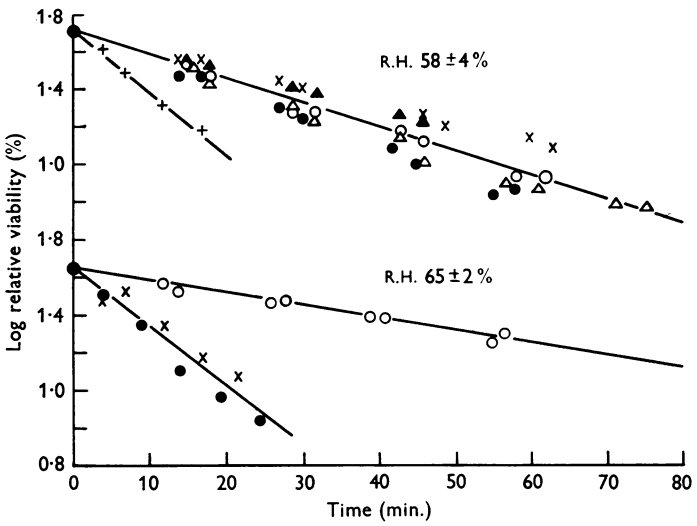

Fig. 10. Slow decay of *S. marcescens* aerosols at 25° C., pH 6·8–7·4.

Symbol	Date	Aerosol	Mixing air	Age suspension (days)	Log *b*	Correction
			Upper diagram R.H. 58 ± 4 %			
●	10. xi. 53	54	52	4	1·78	−0·07
×	24. xi. 53	56	59	4	1·76	−0·05
○	16. xi. 53	59	51	3	1·76	−0·05
△	18. xi. 53	62	65	5	1·70	+0·01
▲	9. ii. 54	58	Single stage	95	1·76	−0·05
+	18. iv. 52	62	?	7	1·52	+0·19
	Log *b*, mean 1·71 ± 0·22; $-k_2'$, upper line = 3·9; lower line = 9·9.					
			Lower diagram R.H. 65 ± 2 %			
●	7. iv. 52	67	?	5	1·64	—
×	17. iv. 52	66	?	6	1·65	—
○	10. viii. 53	64	70	4	1·97	−0·33
	$-k_2'$, upper line = 1·5; lower line = 7·3.					

even a fraction of a second markedly increases the fraction killed off by drying as shown by diminution of log b and increases the death rate k_2; and (2) that this organism is pH sensitive. The first effect is less pronounced when aerosol R.H. is relatively high. The first mechanism may well explain the aberrant run with *Esch. coli* (Fig. 4).

When R.H. of the aerosol was varied from 47 to 75% under more carefully controlled conditions, b appeared to rise up to about 65% R.H. The apparent drop at 70–74% R.H., though based on few experiments, may be real and due to the lethal effect of concentrated salt solutions, which may persist at higher humidity. This phenomenon, if real, would be hard to analyse with phosphate buffers since solubilities of the acid and basic salts differ. Their ratio would be expected to change with consequent changes in pH.

Dynamic studies

Data from a number of experiments are illustrated in Figs. 11 and 12. Table 5*b* gives condensed results of other experiments.

Fig. 11 tends to support the hypothesis that even very short exposures to air at a given humidity affect the properties of organisms subsequently exposed to a different humidity. Fig. 12 illustrates results of recent experiments. Although

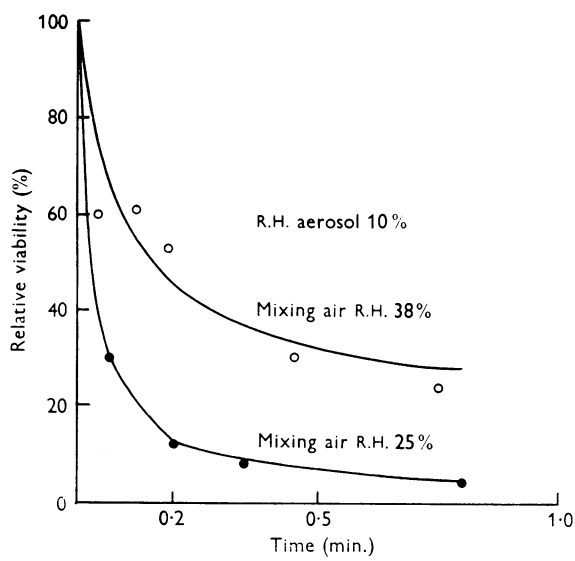

Fig. 11. The effect of humidity of mixing air on loss of viability of aerosol. The conditions are the reverse of those shown in Fig. 10. Upper curve R.H. aerosol 10%—R.H. mixing air 25%. Lower curve R.H. aerosol 11%—R.H. mixing air 38%. These experiments were made using the same preparation on two successive days with steady conditions during the experiment.

experimental points related to the same time or to a short time range have been average to calculate the drawn curve, they are too scanty to permit statistical treatment.

Table 5. S. marcescens

(*a*) Slow decay constants at different humidites and pH values

pH 5·8–6·4

R.H. %*	5	10	21	32	45	51–61	64–67	74	80–86	88–96
No. of expts.	3	2	2	3	4	6	3	2	7	3
Log *b* Mean	1·17	0·95	1·32	1·22	1·11 ± 0·21	1·22 ± 0·2	1·37	1·2	1·3 ± 0·14	1·81
Log *b* Range	0·1–0·2	0·07–0·1	0·21–0·22	0·16–0·18	0·08–0·2	0·1–0·3	0·1–0·3	0·1–0·3	0·14–0·3	0·4–0·98
Mean	0·15	0·09	0·21	0·17	0·13	0·12	0·23	0·16	0·2	0·65
k_2'	9·7	13·2	9·7	9·7	13·4	14·5	14·5	7·3	11·1	5·4

pH 7·1 ± 0·3

R.H. Final	47	54–62		64–67		70–74	
R.H. Mixing air	47	Controlled	No control	Controlled	No control	Controlled	No control
No. of expts.	1	6	1	1	2	3	1
Log *b* Range	1·25	1·70–1·78	1·52	1·97	1·64–1·65	1·48–1·52	1·86
Log *b* Mean	—	1·75 ± 0·03	—	—	—	1·50	—
b	0·18	0·52	0·33	0·92	0·44	0·32	0·72
k_2'	2·3	3·0	7·6	1·5	7·3	4·8	7·4

(*b*) Rapid decay constants

R.H. % Aerosol	10	10	17–19†	28–31	37–38	35–37†	47–56	52–54†	63
R.H. % Mixing air	25	38	—	38–47	49	—	48–53	—	73
No. of expts.	1	1	3	3	2	3	3	3	1
pH	7·0	7·0	6·6	6·9–7·2	7·1	6·6	7·2	6·6	7·2
b	0·02	0·18	0·06	0·02	0·20	0·10	0·3	0·10	0·55
k_1'	67	17	20	50	5·0	10	6·0	15	7
$k_1'(1-b)$	66	14	19	49	4	9	4·2	13·5	3

* Humidity of mixing air not controlled in this series and presumably between 10–20 % R.H.

† Single stage dilution and coil storage system used.

Results given in Table 5, with the exception of the series at about R.H. 30%, pH 7·0, suggest a pattern in which b increases and k_1 falls as R.H. rises. The results at pH 6·6 seem to fall in place, if pH is taken into account.

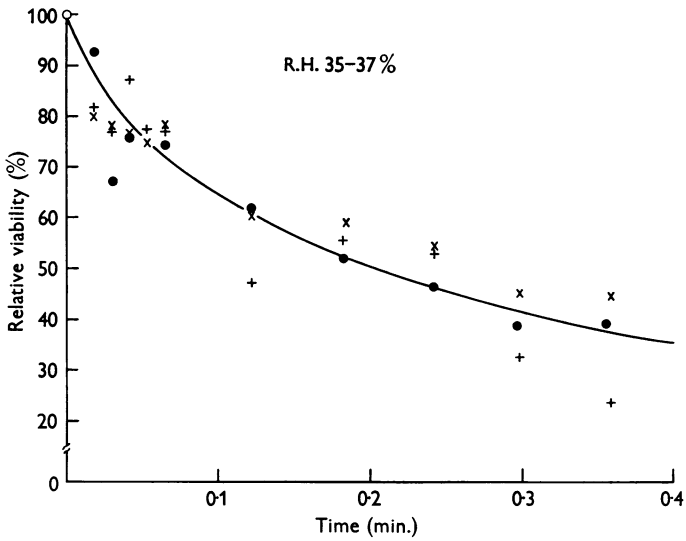

Fig. 12. Rapid decay of *S. marcescens* aerosols; $t = 25°$ C., pH 6·6, R.H. 35–36%.

Symbol	Date	R.H. %	Age suspension (days)	pH
×	20. vii. 56	36	7 frozen	6·65
+	24. vii. 56	36	11 frozen	6·63
●	1. viii. 56	35	19 frozen	6·66

The curve has been drawn through average values of the experimental points using constants $b = 0{\cdot}10$, $k_1 = 0{\cdot}10$.

Table 6. *A comparison of death rates of five micro-organisms at about* 15 *and* 50% R.H.

	Organism	*M. candidus*	*Esch. coli* Strain no. 17	*Esch. coli* Strain no. 75	*Myco. phlei*	*C. xerose*	*S. marcescens*	
R.H. 15%	pH	6·6	6·4–7·0	6·5–7·5	7·2	6·5	6·6	7·0
	b	0·45	0	0·10	0·59	0·48	0·06	—
	k_1	7·0	17·5	20·0	T.S.†	4·0	20·0	—
	k_2	1·0*	T.F.‡	T.F.‡	0·44	0·75	—	—
R.H. 50%	b_1	0·65	0·16	0·10	0·9	0·96	0·10	0·3
	k_1'	5·0	12·8	20·0	T.S.†	T.S.†	15	6·0
	k_2'	0·35	16	2·1	0·11	0·05	—	4·2

* From Ferry & Maple (1954), estimated or observed.
† T.S. = too slow for accurate measure, or data erratic.
‡ T.F. = too fast for satisfactory measurement.

Discussion

Like *Esch. coli*, *S. marcescens* is not resistant to drying. Although values for k_2 at pH 6·0 show no marked trend, this might be expected, since short exposure to uncontrolled mixing air, probably near 15% R.H. would surely affect the death rate. Under more carefully controlled conditions at pH 7·0, the range is limited and data scarce. One can conclude that b tends to rise with R.H. and that *S. marcescens* is an organism sensitive to low R.H. Our results appear to be in fair agreement with those of Griffin, Kantzes, Ludford & Pelczar (1956).

The observations that even a very short initial exposure to low R.H. is harmful, and conversely, that short initial exposure at R.H. greater than that finally attained in the aerosol, tends to protect the organism, seem significant. For quantitative studies, R.H. control must be complete. It is possible that initial exposure of bacteria coming from respiratory tract lesions to saturated air of the upper respiratory tract may enable them to survive longer than if they were immediately subjected to dry air, thus increasing the chance of spreading infection.

GENERAL DISCUSSION

The results presented are based on about 350 complete experiments, which, together with necessary calibrations and trial runs, were made during 5 years. The data appear to be reasonably consistent, especially after experimental conditions were more exactly controlled and illustrate the value of quantitative studies of airborne micro-organisms for significant comparisons of death rates of different bacteria. They also show the need for both dynamic and static storage systems; the former is essential when micro-organisms survive only for seconds.

Although the dynamic storage system is useful for estimating rapid loss of viability, measured in seconds, certain conditions must be satisfied. These are: (1) maintenance of a steady state, including aerosol concentration and flow rate, within the system; (2) a flow rate large enough to insure perfect mixing in flasks or turbulent flow in coils; (3) negligible impingement; and (4) controlled pressure in all parts of the system. We have experimentally observed that these conditions were satisfied, but it would have been difficult without adequate instrumentation, including flowmeters, pressure gauges and especially the photo-electric counter. In its present state, measurements covering storage periods from slightly less than 1 and up to 22 sec. are readily made; with flasks the upper limit is about 2 min. Since static storage for less than 3 min. is inconvenient for us, it may be desirable either further to modify the system or to use the simple device described by Griffin *et al.* (1956) for this intermediate period.

At present, analysis of the overall decay process is satisfactorily approximated by means of the hypothesis, first suggested by Dunklin & Puck (1948) that two processes are involved. Although equation (2) is an approximation holding only when t is small and $k_1 \gg k_2$, and based on the assumption that both processes are logarithmic, it has been helpful in characterizing the micro-organisms studied by us. By plotting log R.V. against time, it can be shown that this assumption,

apparently valid for the second process is an over-simplification with respect to the first, which is more complicated. This is scarcely surprising since factors other than R.H., such as changes in concentration of the ambient liquid medium, temperature of the particle, and rate of transmission of water through the cell boundary, all of them variables, must be involved. But we are unaware of any exact analysis of this difficult problem and are not able to make it ourselves. Indeed, the necessary measurements would be difficult. But despite the fact that the primary process is slow in comparison with the rate of evaporation of water droplets of about the same size, it seems possible that it is associated with drying of the cell, even if not due to evaporation of the surrounding medium.

But whatever the nature of the processes leading to death of airborne bacteria, Table 6 shows that the five organisms studied differ markedly with respect to k_2, b and the apparent constant k_1, even though these values are only approximations. The constant, b, in particular has been shown to vary in different experiments at the same R.H. with the same organism. An inevitable delay of 0·2–0·4 sec. in obtaining unstored samples is, at observed decay rates insufficient to account for differences beyond the experimental error. Careful study of values for unstored viability under different experimental conditions suggests that this measurement, as made by us, is satisfactory.

Differences in the constants are indeed real and can be associated with differences in bacterial structure, be it physical or chemical. *Esch. coli*, for instance, appears under the phase contrast and electron microscopes as a rather fragile shell with little inside. Similarly, *S. marcescens*, except for areas of nuclear material, is not optically dense. Both organisms are broken quite rapidly in a reflux atomizer. *Esch. coli* is, moreover, primarily an aquatic organism. And both organisms are very labile in the airborne state.

M. candidus, *C. xerose* and *Myco. phlei* are resistant. The former, nearly spherical, is optically dense; its shape is not only structurally strong, but provides an almost minimal surface; volume ratio, not likely to favour rapid evaporation. *C. xerose* also appears substantial and is known to survive and even grow on relatively dry surfaces; it also is relatively long-lived in the airborne state.

Although *Myco. phlei* has a high surface to volume ratio, it does contain lipids, and especially higher alcohols which like fatty acids are polar molecules. Since Archer & La Mer (1955) have found that monolayers of fatty acids enormously hinder evaporation of water from surfaces, it is possible that the resistance of this organism can be explained in similar terms.

Since k_1 and k_2 vary in the same direction, long-term survival may also be related to structure. And our experience with non-pathogenic organisms suggests an explanation for the observation that some organisms, notably *Mycobacterium tuberculosis*, gain entrance to the host more readily by the respiratory tract, and others, primarily aquatic, by ingestion through the alimentary tract. The constants can perhaps serve as quantitative measures of these properties.

The observations reported here do, however, concern essentially naked bacteria, and there is no doubt, as Fry & Greaves (1951) among others have shown, that survival characteristics will change as the surface is coated with different

substances. Nevertheless, they can perhaps be useful as a basis of comparison and to stimulate further investigation to show why organisms enter the body by different routes, even if they do not solve the problem of why infection does or does not occur after invasion.

SUMMARY

1. Quantitative studies of the survival of airborne bacteria permit significant comparisons of the death rates of different micro-organisms.

2. To permit accurate study of death rates measured in seconds, not minutes, we have devised a dynamic storage system, described in this paper.

3. The death rates of airborne *M. candidus*, *Esch. coli*, *Myco. phlei*, *C. xerose* and *S. marcescens* are compared in terms of constants k_1, k_2 and b.

4. Differences in the values of the constants are tentatively and partly ascribed to differences in physical and chemical structure.

5. Experience with these organisms suggests why some pathogens invade the host by one route rather than another.

6. Similar experiments with pathogens should give significant quantitative information concerning the transmission of disease.

We gratefully acknowledge the effective interest and help of our former assistant, T. Grant Maple and the careful, effective and conscientious work of our technical assistants, Cynthia Hartwig, Grace C. Leigh, Paulette Slaney, Martha Szerslip, Nora Galins, Ann Barr and Elizabeth Neidhardt who have, at different times, helped secure the data presented here. Doris Oldfield deserves particular thanks for care in the preparation of the manuscript.

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(*MS. received for publication* 1. X. 57)