STUDIES OF THE LOSS OF VIABILITY OF BACTERIAL AEROSOLS*

III. FACTORS AFFECTING DEATH RATES OF CERTAIN NON-PATHOGENS

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(With 6 Figures in the Text)

INTRODUCTION

In a recent paper, Ferry, Brown & Damon (1958) reported measurements of the death-rates of several bacterial species stored as aerosols.

The organisms studied, *Micrococcus candidus*, *Escherichia coli*, *Mycobacterium phlei*, *Corynebacterium xerose* and *Serratia marcescens*, themselves non-pathogenic, were selected because they resembled certain pathogens either in structural or cultural respects. It was found that death rates could be compared in terms of constants k_1, k_2 and b, representing, respectively, rate constants for rapid initial and slow secondary decay processes and the fraction surviving rapid initial decay. By k_1' we denote percentage decay per second; similarly k_2' gives percentage decay per minute. Although the constants are functions of such factors as pH of the bacterial suspension from which aerosols were generated, relative humidity (subsequently indicated as R.H.), and temperature of the ambient air, they can also, under comparable conditions, be roughly correlated with structural or cultural characteristics of the organism studied.

Subsequently, we attempted to scrutinize more carefully some of the factors affecting death-rates. The relevant experiments are reported in this paper and include studies of: (*a*) aerosols stored in nitrogen instead of air, (*b*) the effect of temperature on the death rate of *M. candidus* stored in nitrogen, and (*c*) aerosols generated from organisms subjected to different procedures. The first two approaches were the more fruitful.

The methods and apparatus were those recently described except as noted in relation to certain experiments (Ferry *et al.* 1958). Other useful methods have been described by Kethley, Cown & Fincher (1957).

DECAY OF BACTERIAL AEROSOLS STORED IN NITROGEN AND AT DIFFERENT TEMPERATURES

Although we had long suspected (Ferry & Maple, 1954) that rapid loss of viability in bacterial aerosols was associated with drying, and that the much slower secondary

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process might be associated with oxidative processes in the bacterial cell, the experiments reported here represent our first direct experimental approach to this question. In these experiments we have studied slow decay of viability of aerosols containing either *M. candidus*, *S. marcescens*, or *Esch. coli* in an atmosphere in which nitrogen replaced air. Studies of *Esch. coli* aerosols were most extensive, and include not only dynamic studies but the results of experiments in which prepurified nitrogen (N_2) containing $< 0{\cdot}002\,\%$ oxygen (O_2) was used instead of commercial N_2 with an O_2 content of about $0{\cdot}1\,\%$. *M. candidus* aerosols were also studied at 15°, 25°, and 35° C. in N_2. In a single experiment at 25° C., O_2 was substituted for air.

The results obtained with each of these organisms are separately given and discussed.

Micrococcus candidus

Introduction

Aerosols containing this organism were the first to be studied. Although chosen originally because it was small and could be obtained in nearly monodisperse suspension, it is more significant that a relatively large fraction of these organisms survive the initial rapid decay process, even over a rather wide range of R.H. This phenomenon, presumably related to the physical and chemical structure of the organism, facilitated comparisons of data obtained by means of the static storage system even before we became aware of the need for control of R.H. of mixing air and before a single stage dilution system was employed. It has permitted us to draw conclusions concerning the effect of oxygen and temperature upon the rate of secondary decay.

A single experiment in which humidified O_2 was substituted for air as the ambient medium was not repeated because an explosion occurred in the oil-containing vacuum pump.

Results and discussion

Fig. 1 illustrates data obtained from fourteen experiments with aerosols generated from bacterial suspensions in phosphate buffer. Data obtained under comparable conditions are here plotted directly without correction. This method of plotting was statistically analysed for us by Prof. Hugo Muench.* His first analysis, to assay the validity of this treatment, showed that in each set of runs under comparable conditions neither the values of the estimated survival at the start of slow decay, log b, nor the slopes of the lines differed significantly among themselves. It therefore seemed legitimate to combine each group.

He subsequently fitted trends to the series of observations in N_2 at 15°, 25° C., and in air and O_2 at 25° C. Results at 35° C., which because of condensation of water in the system seemed less reliable, were not included in this analysis. His values for log b and the slopes, S, (k_2 as used by us) and their standard deviations are given in Table 1, together with values for the derived constants b and k_2'; the

* Hugo Muench, Professor of Biostatistics, Harvard School of Public Health. (Personal communication.)

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latter is $S \times 230{\cdot}3$. For comparison we have also included values for b and k_2' derived from earlier experiments in air (Ferry & Maple, 1954).

Prof. Muench concludes that for the results statistically considered by him there was no significant difference between the values for log b, but that the slope, S,

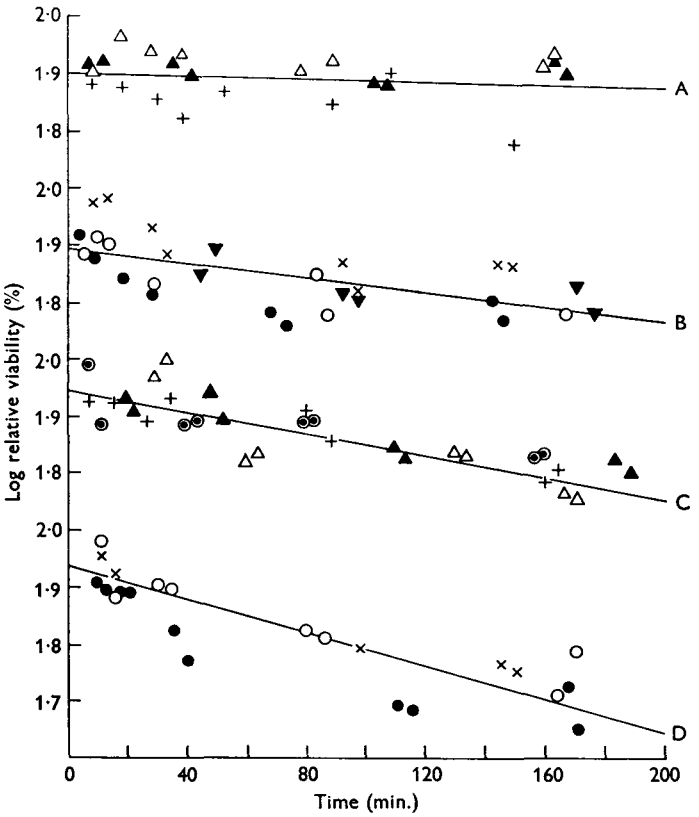

Fig. 1. Slow decay of *M. candidus* aerosols in air, oxygen and nitrogen.

Symbol	Date	R.H. %	pH	Symbol	Date	pH	R.H. %
	(A) Nitrogen $t = 15°$ C.				(C) Nitrogen $t = 35°$ C.		
▲	11. iv. 52	6·9	48	+	13. v. 52	6·9	48
+	16. iv. 52	6·9	48	⊙	15. v. 52	7·0	49
△	22. iv. 52	6·8	49	▲	19. v. 52	7·2	50*
				△	20. v. 52	6·8	47
	(B) Nitrogen $t = 25°$ C.				(D) oxygen and air at $t = 25°$ C.		
▼	25. iii. 52	6·8	46	×	24. iii. 52	6·8	52
×	9. iv. 52	6·9	50	○	31. iii. 52	6·6	50
●	9. v. 52	7·3	46	●	22. v. 52 (O_2)	7·1	45*
○	12. v. 52	7·3	49				

* Estimated from humidial reading.

Aerosols generated from bacterial suspensions in phosphate buffer. Ionic strength about 0·002.

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Table 1. *Loss of viability of* M. candidus *aerosols in air and nitrogen*

Conditions	pH 6·9 ± 0·4 Log b	R.H. about 50 % Slope S	b, fraction surviving rapid decay	k_2' % viability loss per min.
Air 25° C.*	1·936 ± 0·0586	−0·001444 ± 0·000218	0·86	0·33
Older measurements (1954) at about 55 % R.H.†				0·24
N_2 15° C.*	1·899 ± 0·0446	−0·000175 ± 0·000162	0·79	0·04
25° C.*	1·893 ± 0·0520	−0·000680 ± 0·000164	0·78	0·16
35° C.	1·945	—	0·88	0·23

* Analysed statistically by Professor Muench.

† The lower value is, presumably, in part due to higher R.H. (Ferry & Maple, 1954).

values had the following 'significance P levels'. (P, as is customary, denotes the probability that the observation was due to chance):

Air 25° C. against N_2 15° C. 0·000003
Air 25° C. against N_2 25° C. 0·005
N_2 15° C. against N_2 25° C. 0·03.

'It therefore seems reasonable to conclude that different conditions have produced real changes in the survival rates.'

Although log b does not vary significantly between single runs its standard error is not reduced by grouping, thus 'indicating some heterogeneity'.

The results obtained in the single experiment with humidified O_2 as the ambient medium appear to conform to the pattern for air storage.

The derived constant k_2' certainly increases with rise in temperature. A temperature rise from 15° to 35° C. produces a nearly sixfold increase in k_2'. Although the change in k_2' accompanying a change from 15° to 25° C. appears greater than that resulting from the change from 25° to 35° C., this may be due to experimental error in determining the constants.

We can therefore conclude that slow decay: (1) is decreased in the absence of O_2 by about 30 %; (2) in N_2 increases with rise in temperature, and may well be about doubled by an increase of 10° C., as might be expected on chemical grounds.

Escherichia coli

Introduction

The experimental results with *M. candidus* aerosols stored in N_2 instead of air have shown that although the rate of secondary decay was decreased in the former medium b was not changed. These observations suggested that the rate of primary decay was not affected and that studies with other organisms were desirable. Since we had extensive data concerning *Esch. coli*, and since the different magnitudes of k_1' and k_2' should permit ready comparison of values in the two media, the following experiments were made with *Esch. coli* aerosols generated from bacterial suspensions in phosphate buffer.

As with *M. candidus*, *Esch. coli* aerosols were stored in humidified N_2 containing less than 0·1 % O_2. Since it seemed possible that even this small concentration of

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O_2 might accelerate loss of viability, we made a few runs in which prepurified humidified N_2 served as the ambient medium. Although no adequate gas analyses were made, observations with a Pauling oxygen meter gave negative readings with the latter medium as contrasted with just detectable positive readings with commercial N_2. With the precautions taken to wash out the storage system, it seemed probable that O_2 concentration in the medium approximated that in the cylinder; it seemed certain that O_2 concentration was lower than that in commercial N_2.

Results

The results of static and dynamic storage studies are graphically shown in Figs. 2 and 3 respectively. In the former, the experimental points obtained at $67 \pm 1\%$ R.H. are referred to a mean value of log b, indicated by the intercept with the axis; the slope of the line $\times 230$ yields a value of k_2'.

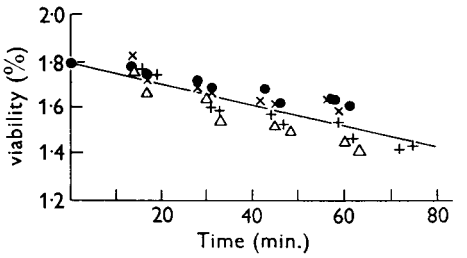

Fig. 2

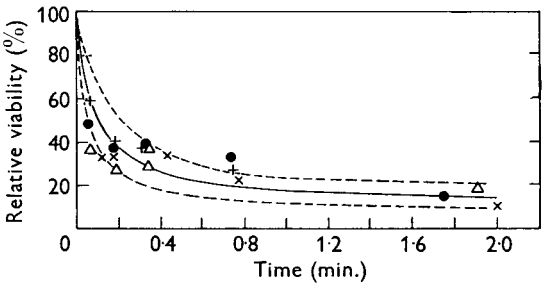

Fig. 3

Fig. 2. Slow loss of viability of *Esch. coli*, strain 75 aerosol stored in nitrogen at 25° C.

Symbol	Date	pH	R.H. % aerosol	Mixing air	Log b	Correction
●	8. xii. 53	7·1	68	59	1·72	+0·07
×	11. xii. 53	7·2	66	52	1·68	+0·11
+	14. xii. 53	7·1	66	53	1·92	−0·13
△	18. xii. 53	6·8	68	49	1·83	−0·04
				Mean	1·79	±0·11

Aerosols generated from bacterial suspensions in phosphate buffer. Ionic strength about 0·002.

Fig. 3. Rapid loss of viability of *Esch. coli*, strain 75 aerosol in nitrogen at 25° C.

Symbol	Date	pH	R.H. % aerosol	Mixing air
+	1. ix. 53	7·1	57	63
×	12. iv. 54	7·2	53*	—
△	14. iv. 54	7·2	53*	—
●	16. iv. 54	7·2	51*†	—

* Single stage dilution system.
† Prepurified N_2 used.

Solid curve drawn for air storage R.H. 50–60 % $b = 0{\cdot}10$ $k_1' = 20$.
Upper dotted curve for air storage $b = 0{\cdot}10$ $k_1' = 10$.
Lower dotted curve for air storage $b = 0{\cdot}10$ $k_1' = 40$.
Experimental points obtained during storage in N_2.
Aerosols generated from bacterial suspensions in phosphate buffer, ionic strength about 0·002.

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In the latter, experimental values at about 54 % R.H. have been plotted directly. In Table 2 appear values of the constants b, k_1', and k_2' derived from the results obtained at all humidities studied; for ready comparison, we include the constants obtained after storage in air under comparable conditions. The constant $k_1 \times 100 = k_1'$; it is, of course, an approximation (see Ferry *et al.* 1958, pp. 148–149).

Table 2. *Rapid and slow loss of viability of* Esch. coli, *strain* 75 *aerosol in air and* N_2; $t = 25°$ C., pH 6·8–7·3 *aerosols generated from suspensions in phosphate buffer*

	*a** Slow decay				*b** Rapid decay		
R.H. % ...	54–61	66–68	72–76		26–44	51–57	56–65
Nitrogen							
no. of experiments	2†	4	4		4	4	4
Log b (mean)	1·63	1·79 ± 0·11	1·98 ± 0·3		—	—	—
b	0·42	0·61	0·95	b	0·03	0·1	0·2
k_2' %/min. (mean)	1·06	1·06	1·2‡	k_1' %/sec.	35·0	20	10
Air§					R.H. 33–43		
b (fraction surviving first process)	0·1	0·42	0·44	b	0·05	0·1	0·2
k_2' %/min.	2·1	1·8	2·3	k_1' %/sec.	25·0	20	10·0

* R.H. of mixing air controlled.
† Prepurified N_2.
‡ Including aberrant run $k_2' = 2·1$.
§ Taken from Table 2, Ferry *et al.* (1958), p. 137.

Discussion

Despite a single aberrant run at about 73 % R.H., the results strongly suggest that: (1) slow decay is retarded 40–50 % in nitrogen; (2) decay rates in commercial and prepurified N_2 do not differ measurably; and (3) primary decay is not changed by the substitution of nitrogen for air as the ambient medium.

Static studies suggest that the fraction of all organisms surviving initial decay, b, is greater in N_2 than in air. This inference, however, is not supported by dynamic studies. It is, moreover, difficult simultaneously to assume that b is O_2 dependent and k_1', O_2 independent, since these two constants seem to be inter-related. Although scrutiny of individual experiments suggests that b is a characteristic of different preparations, the phenomenon deserves further study.

Serratia marcescens

Aerosols generated either from bacterial suspensions in water, gel, or phosphate buffer were stored in humidified N_2. The latter suspending medium was at about pH 7·1, the others at about pH 6·3. The earlier experiments, using essentially unbuffered suspensions, were made before we had learned to control R.H. of mixing gas, and all were completed before single stage dilution was adopted. The data refer to the results of static storage in humidified nitrogen.

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Results

Data from a series of experiments at about 49 % R.H., pH 7·1, mixing gas about 60 % R.H. appear in Fig. 4, in which log R.V. % is ordinate, time in minutes, abscissa. For more ready comparison, we have adjusted all points to a mean value of the intercept, b (see Ferry *et al.* 1958, p. 135). The slope of the line k_2, $\times 230 = k_2'$, percentage loss of viability per minute.

These results, as well as those obtained under other conditions, are summarized in Table 3. For comparison we give values of the constants k_2' and b when air at comparable humidities was the ambient medium.

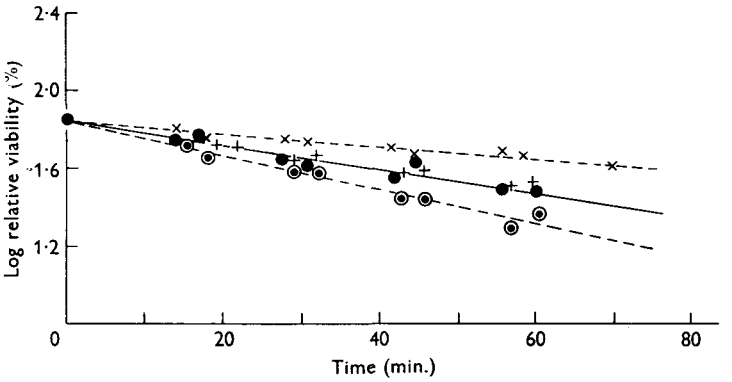

Fig. 4. Slow loss of viability of *S. marcescens* aerosol in nitrogen at 25° C.

Symbol	Date	R.H. % aerosol	Mixing air	pH suspension	Log b	Correction
		Mixing air controlled, R.H. aerosol 46–51 %				
●	23. xi. 53	46	56	7·1	1·82	+0·01
×	28. ix. 53	49	65	7·1	2·00	−0·17
+	30. ix. 53	51	62	7·2	1·77	+0·06
⊙	20. xi. 53	48	56	7·2	1·74	+0·09
					Mean 1·83	+0·16

Aerosols generated from bacterial suspensions in dilute phosphate buffer.

Discussion

Since the experiments summarized in Table 3*a* were completed before we had learned to control experimental conditions more carefully, it is not surprising to find that the results scatter, especially when R.H. of mixing gas was uncontrolled. Despite this, and the fact that aerosols stored either in N_2 of air were never subjected to exactly the same conditions, the data, especially those summarized in Table 3*b*, strongly suggest that secondary decay is retarded in N_2, and are consistent with the hypothesis that oxidative processes have a considerable share in this phenomenon. Values for b appear higher for aerosols stored in N_2.

The comparison of results at about 85 % R.H., but with humidity of mixing gas uncontrolled, can, we believe, be discounted, since R.H. of air taken directly from a general supply line, or from an independent compressor, was always low in mid-

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Table 3. *A comparison of the secondary decay rates of* S. marcescens *aerosols stored in air or nitrogen*

	*a**				*b*†	
	R.H. of mixing gas uncontrolled pH 6·2 ± 0·3 In nitrogen				R.H. of mixing gas controlled pH 7·1 ± 0·2	
R.H. aerosol	30	40–45	56–65	80–85	46–51	63
R.H. mixing gas	?	?	?	?	56–65	67
No. of experiments	2	4	6	4	4	1
b_1, fraction surviving primary decay						
Range	—	0·30–0·56	0·15–0·42	0·05–0·14	0·55–1·00	—
Mean	0·24	0·41	0·29	0·10	0·68	1·0
k_2' %/min.						
Range	—	5·2–12·5	2·5–(17·0)	—	0·69–1·97	
Mean	1·3	8·9	9·2	13·4	1·48	1·6
	In air‡					
R.H. % aerosol	32	45	51–61	80–86	47	54–62
Mixing air	—	—	—	—	47	Controlled
b (Mean)	0·17	0·13	0·12	0·2	0·18	0·52
k_2' (mean)	9·7	13·4	14·5	11·1	2·3	3·0

* Aerosols generated from bacterial suspensions in distilled water or gel buffer pH 6·2 ± 0·3.
† Bacterial suspensions in phosphate buffer used; pH 7·1 ± 0·2.
‡ Taken from Table 5*a*, Ferry *et al.* (1958), p. 146.

winter, when this series was completed. The latter source regularly supplied air at about 12–15 % R.H. When N_2 was used, gas emerging from a cylinder was always very dry. We have, moreover, already suggested that the rate of secondary decay may be conditioned by even a very short exposure to atmospheric R.H. differing from that finally attained (Ferry *et al.* 1958, p. 145). Although this may account for the rapid decay observed at the higher R.H., it is also possible that the organisms were not completely dry but were surrounded by a concentrated salt solution (see Ferry & Maple, 1954) which hastened decay.

STUDIES OF AEROSOLS CONTAINING TREATED MICRO-ORGANISMS

That the viability of bacterial aerosols decreases in two stages, the first relatively rapid, the second slow, seems clear. Our recent studies (Ferry *et al.* 1958), moreover, suggest that quantitative differences in the constants, k_1' and k_2', which characterize these processes, may be related to differences in chemical and physical structure. Evidence presented in an earlier part of this paper, moreover, suggests that the two processes differ not only in rate, but in character; the secondary process is retarded when O_2 is absent from the ambient medium, but not the first.

The experiments reported in this section would, we hoped, result in accelerating the primary process with *Myco. phlei*, and in retarding this process with *Esch. coli*, thereby directly correlating change in structure with rate of viability loss.

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Archer & La Mer (1955) observed that long-chain fatty acids spread on water surfaces greatly reduced the rate of evaporation. C. N. Davies, in conversation with us, had also reported that water droplets evaporated much more slowly when they contained long-chain (16–20 or more C atoms) primary alcohols. It therefore seemed likely that k_1' would be reduced if it was indeed dependent on evaporative rate, provided organisms could be coated successfully with one of these latter substances.

A. *Escherichia coli*

Methods

(A) *Esch. coli* were either grown, resuspended, or both grown and resuspended in media saturated with C_{16} or C_{18} alcohols. Aerosols were generated in the usual way. It was hoped that alcohol dissolved in the medium or incorporated into the bacterial cell might change the properties of the surface surrounding the organisms as the droplets containing cells evaporated.

(B) To a concentrated *Esch. coli* suspension were added different proportions of either (*a*) petroleum ether, or (*b*) petroleum ether containing about 5 × the weight of alcohol needed completely to cover the bacterial surface. This was readily calculable from: (1) well-established molecular dimensions, (2) approximate bacterial dimensions, and (3) bacterial concentrations.

A similar procedure was used in attempting to cover the organisms with material recovered from petroleum ether extraction of *Myco. phlei*.

Subsequently small bubbles of sterile filtered air emerging from a fine capillary at the rate of 0·6 l. per min. served to stir and cool the suspension, as well as to evaporate the ether. When evaporation of ether was complete in about 3 min., the residual aqueous suspension in phosphate buffer was used to generate aerosols. Longer periods of aeration reduced the viability of the suspension even in the absence of petroleum ether, but more rapidly in its presence.

Results and discussion

Method A. Although the results, summarized in Table 4, do not show that k_1' was decreased because of treatment, it is possible that the increase in b is significant. Estimates of the volume of liquid in a droplet enclosing an organism, solubility of the higher polyatomic alcohols, perhaps 5 mg./l., and molecular weight and cross-sections of the latter taken as $20{\cdot}6 \times 10^{-16}$ sq.cm. served in deriving an estimate of the area covered by a monomolecular layer. It turned out to approximate 1×10^{-8} sq.cm. Since we estimated the surface of *Esch. coli* taken as a cylinder with hemispherical ends to be about 5×10^{-8} sq.cm., not over 20 % could have been covered by alcohol. The negative results should be expected.

Method B. The method seemed promising, since L. C. Sack, working in our laboratory in 1956, had shown that *Esch. coli* in suspensions treated with polyatomic alcohol extract were no longer monodisperse and appeared under a phase contrast microscope to be more highly refractive, especially at the cell boundary; viability of the suspensions was not reduced beyond the experimental error. Suspensions treated with petroleum ether alone showed no morphological change.

These observations suggested that the solute, concentrated at phase boundaries, was deposited on the bacterial surface as the solvent evaporated.

Table 4. *Rapid loss of viability of* Esch. coli, *strain* 75 *aerosol generated from treated suspensions* pH 6·9, $t = 25°$ C. *in phosphate buffer*

	A*	B			
		Petroleum ether			
Method of treatment	Medium saturated with polyatomic alcohol	Containing C_{18} alcohol	Control	Containing *Myco. phlei* extract	Control
R.H.	56–62	63	62	74·5	73
b	0·30–0·63	0·29		0·49	
k_1	12–26	67		51	
Untreated controls†					
R.H.	50–60	60–66		70–74	
b	0·10	0·22–0·55		0·20	
k_1	20	10–15		10	

* Six experiments. † From Ferry *et al.* 1958, Table 2.

Nevertheless, the results, illustrated in Fig. 5, show no differences between behaviour of aerosols generated from buffered suspensions treated with petroleum ether alone, or treated with petroleum ether containing polyatomic alcohol. Similar results were obtained when a petroleum ether solution, containing what

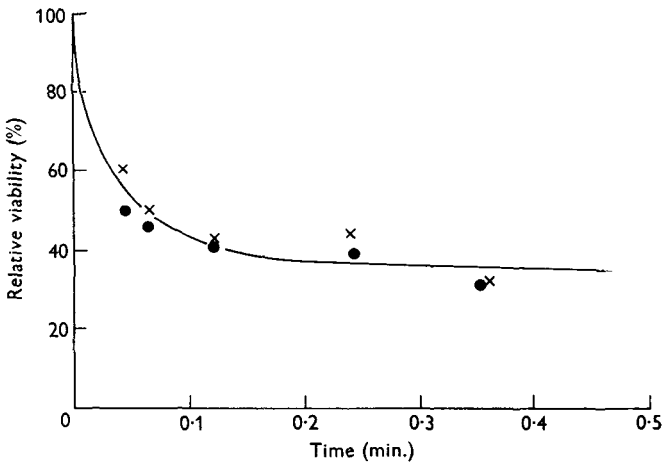

Fig. 5. Rapid loss of viability of aerosols containing treated *Esch. coli* at 25° C., R.H. 62 %.

Symbol	Date	Treatment
×	6. iii. 56	Petroleum ether alone
●	7. iii. 56	Petroleum ether + C_{18} alcohol

$k_1' = 67 \quad b = 0{\cdot}29$

Aerosols generated from bacterial suspensions in phosphate buffer.

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was believed to be an excess of *Myco. pheli* waxy material, was substituted for C_{18} solution.

The latter measurements, made at about 73 % R.H., show considerable scatter, but a curve could be roughly interpolated to describe the data. In Table 4 the results are summarized and the derived constants compared with those for aerosols containing untreated organisms.

Quite contrary to anticipation, the rate of primary decay appears to have been accelerated by treatment. This phenomenon can perhaps be associated with our observation that the total particulate count of suspensions appears to decrease more rapidly during bubbling when petroleum ether is being evaporated than when it is absent. This suggests that increased cell fragility following treatment may result in a more rapid primary death rate. We cannot, moreover, be sure that coating, even if it occurred, was complete. In any case, increased cell fragility could well be a more important factor. And although these findings give only indirect evidence, they suggest that further investigation may be profitable.

B. *Mycobacterium phlei*

This organism, normally resistant to drying and to storage in air or otherwise, is known to contain a considerable proportion of lipid material, some of it readily extractable with petroleum ether (Bloch, 1950; Asselineau, 1951). Bloch (1950) reported, moreover, that mycobacteria remain viable after extraction. This we confirmed before undertaking experiments with aerosols.

Since it seemed possible that extraction of organisms with petroleum ether might be reflected in changes k_1, k_2, or both, these experiments were undertaken.

Methods

100 ml. aliquots of a culture in a modified Dubos medium were centrifuged. The sediment was extracted by stirring with petroleum ether in the cold. Centrifugation in refrigerated cups followed. After three similar cycles the sediment was resuspended in phosphate buffer for further treatment or atomization.

Since it was inconvenient and appeared unnecessary for our purposes, losses, inevitable at each step, were not determined.

Although ultrasonic irradiation alone did not appear to increase k_2, it seemed desirable that the effect of extraction be assayed in controlled experiments. Accordingly, we studied *Myco. phlei* aerosols generated from suspensions treated by: (1) sonic irradiation only; (2) sonic irradiation followed by petroleum ether extraction; (3) petroleum ether extraction followed by sonic irradiation.

Sonic irradiation for one minute was performed with a Raytheon Model Db 101 magneto-strictive sonic transducer operating at 10 kcyc./sec. at 25° C.

Discussion and results

Experimental results are plotted as usual in Fig. 6. The results of different runs made under the same conditions are in satisfactory accord. The lines drawn to represent averages were therefore rather easily placed; apparent differences in

their slopes suggest real differences in k_2. The derived constants b, denoting the fraction surviving primary decay, and k_2' death-rate expressed as percentage per minute, appear in Table 5. Although the results with petroleum ether followed by

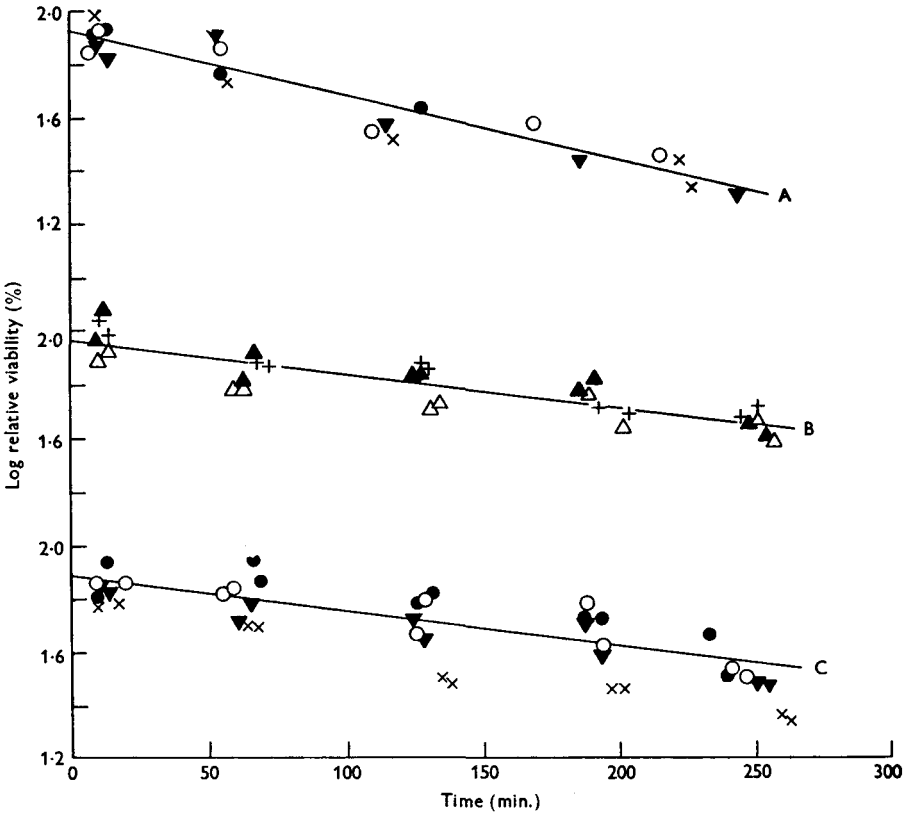

Fig. 6. Slow loss of viability of *Myco. phlei* aerosols generated from treated bacterial suspension; $t = 25°$ C., R.H. 28–30 %.

Symbol	Date	R.H. %	pH	MNP*	Age suspension (days)
	Upper diagram A. Extraction followed by sonic irradiation				
×	7. ii. 56	30	6·9	—	1
●	10. ii. 56	30·5	6·8	1·1	4
▼	14. ii. 56	29·5	6·8	1·05	8
○	17. ii. 56	28	6·9	1·1	11
	Middle diagram B. Sonic irradiation followed by extraction				
△	13. iii. 56	30	6·8	1·1	15
▲	14. iii. 56	30	6·8	1·2	16
+	23. iii. 56	30	6·8	1·1	23
	Lower diagram C. Controls sonic irradiation only				
×	2. iii. 56	30	6·9	1·1	4
○	9. iii. 56	30	6·8	1·1	11
●	16. iii. 56	29	6·7	1·1	18
▼	22. iii. 56	28	6·8	1·2	2

* MNP = mean number of organisms per particle.

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sonic irradiation were obtained with aerosols generated from a culture different from that used in other experiments, we have consistently found that slopes of lines describing loss of viability do not vary significantly with the culture used.

Table 5. *Secondary loss of viability of* Myco. phlei *aerosols generated from treated suspensions at* 25° C. R.H. 30 %

Treatment	Sonic irradiation	Sonic irradiation followed by extraction	Extraction followed by sonic irradiation
b, fraction surviving rapid decay	0·8	0·9	0·8
k_2' % per min.	0·3	0·3	0·6

GENERAL DISCUSSION

The results reported further support the validity of a quantitative experimental approach in which the concentration of viable organisms is always compared with total particulate concentration in an aerosol. In fact, our experience with some aerosols stored at R.H. 15 % in which 90 % of the particles originally present had disappeared within an hour, together with rapid changes in particle count resulting from pressure changes in the dynamic system, suggest that this comparison is essential if viability of particles is to be accurately determined. The present experimental limitation of concentration to a maximum of 2500 particles per l. can presumably be overcome by dilution of the aerosol for purposes of particle counts.

More restrictive, however, is the limitation of study to essentially naked organisms. Removal of this limitation will depend upon a method of atomization in which droplet size approaches bacterial dimensions, and droplets contain one and only one organism. Until both limitations are resolved, it is nevertheless possible to make effective comparisons of the resistance of different bacterial species to drying. Kethley *et al.* (1957) produced aerosols, containing about 1 organism per particle from different suspending media.

Within these limitations it is, however, possible quantitatively to compare decay rates under a variety of environmental conditions and experimentally to attain good reproducibility of results under fixed conditions. This is well illustrated by the results obtained with such different organisms as *Myco. phlei*, *M. candidus* and *Esch. coli*.

The studies reported here surely support the hypothesis that decay can be related to two different processes. That exposure to oxygen containing atmospheres accelerates the slower secondary process appears clear. Although these studies are not sufficiently detailed to analyse secondary decay in terms of partial pressure of oxygen in the ambient atmosphere, direct correlation would scarcely be expected, since the bacterial cell presumably contains oxidizing systems which are independent of molecular O_2. It seems reasonable to attribute residual secondary decay to them.

Primary rapid decay rates appear, certainly as an approximation, to be independent of oxygen. Although their velocity is low compared with that of the evaporation of water from droplets, this may well be related to characteristics of the cell boundary which delay evaporation from the cell body.

Although dynamic studies suggest that b, the fraction surviving primary decay, and k_1' the rate of primary loss of viability, appear to be unaffected by the absence of oxygen, the apparent increase in b observed especially in static studies is difficult to reconcile with this observation. The experiments are, however, limited, and it is possible that b derived from static studies is, as we have already suggested, related to characteristic properties of individual bacterial suspensions used for aerosol generation.*

The experiments with aerosols generated from extracted or treated organisms did not turn out as we had hoped. They do, however, support the hypothesis that the rate of viability loss is related to chemical and physical structure.

With *Myco. phlei*, the changes produced by petroleum ether extraction do not appear sufficient markedly to change resistance to atomization and drying. Weakening physical structure by this procedure appears to make the cells less resistant to physical trauma, revealed by more rapid secondary decay. Failure to induce measurable primary decay may tentatively be related to incomplete extraction from surface structure.

In the case of *Esch. coli* aerosols, the effects of petroleum ether appear to outweigh the possible effect of surface coatings.

SUMMARY

1. Additional quantitative studies of the survival of micro-organisms in aerosols are reported.

2. Substitution of nitrogen for oxygen in the ambient medium decreases k_2', the rate of secondary loss of viability of *M. candidus*, *Esch. coli* and *S. marcescens*, by 30–50 %.

3. The rate of primary decay (k_1') of *Esch. coli* does not appear to be affected by a similar change of atmosphere.

4. The rate of secondary decay, k_2', of *M. candidus* appears to increase two- to three-fold for 10° C. increments in temperature.

5. Treatment of suspensions of *Esch. coli* with petroleum ether alone or containing lipid material increases k_1' in aerosols generated from such suspensions.

6. Treatment of *Myco. phlei* with petroleum ether appears to lower resistance to subsequent sonic irradiation.

These observations are consistent with the hypothesis that differences in the physical and chemical structure of micro-organisms are reflected in constants k_1', k_2' and b.

* k_1 is, of course, an empirical approximation. Dynamic and static studies, previously reported, some of which were made during the course of single experiments, suggest that the approximation is useful despite the theoretical over simplification employed; b, derived from the simplified equation for rapid decay is also an approximation. The value derived from static studies is more accurate (see Ferry *et al.* 1958, pp. 148–149).

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