OBSERVATIONS ON THE BACTERICIDAL ACTION OF HEXYL RESORCINOL AEROSOLS

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(With 3 Figures in the Text)

INTRODUCTION

The provision of an efficient and practical barrier to cross-infection in the form of a bactericidal vapour is an attractive idea, to which much research effort has been devoted, and on which there is now a considerable literature. We do not yet feel in a position to produce a critical reappraisal of this, but it suffices to say that past experimental findings have been both disappointing and contradictory. This is particularly noticeable in the case of hexyl resorcinol (1-*n*-hexyl-2:4-resorcinol), which has given some promising laboratory results during the past 15 years, but has apparently failed in practice (Lidwell & Williams, 1954). As the result of a large number of experiments conducted by us, we have formed the opinion that past controversy is attributable at least in part to lack of knowledge of the mode of action, and hence the limitations on the successful application of this substance as a bactericidal vapour. This opinion is supported by the recent work of Kethley, Cown & Fincher (1957).

The experiments discussed below, though performed on artificially produced bacterial clouds, tend to clarify the nature of the action of hexyl resorcinol and the circumstances under which this action occurs and under which it might be of practical value. We hope that this will help to explain past misunderstandings, and stimulate further interest in the possible uses of bactericidal vapours. Although we are concerned here only with hexyl resorcinol, it will be clear that some of the general considerations we advance may apply equally to other appreciably volatile germicides. Some parallel desultory experiments with phenolic substances suggest that they do.

METHODS AND MATERIALS

Physical conditions

For the most part the experiments were carried out in two square laboratory rooms of identical lay-out and approximately 3000 cu.ft. volume, when stripped of all superfluous apparatus and furniture. The internal surfaces of the rooms consisted of glossy enamel, polished teak, polished linoleum, glass and aluminium. The ventilation (a filtered plenum and extract system) was sealed off during experiments, and opened afterwards for scouring purposes. The temperature was uncontrolled in the absence of ventilation, but for the short period of the experiments remained equal to the controlled temperature (about 70° F.) of the remainder of the building. Humidity was partly dependent on ambient conditions, but it was found possible to maintain abnormally high, constant humidity for the duration

of an experiment by slowly releasing steam into the room. The atmosphere was mixed by means of fans arranged after preliminary tests to give the most rapid stirring. Once established, the relative positions of the aerosol apparatus, samplers and mixing fans remained unchanged for all experiments with a few specified exceptions. The experiments were carried out by two persons wearing protective clothing and respirators.

One of the two rooms was reserved exclusively for control experiments, as it was found that, unless a room was allowed to lie fallow for several days after a trial, hexyl resorcinol which had condensed on the surfaces in the room produced an appreciable background level of vapour as soon as the ventilation was sealed off. We have every confidence, however, that the physical characteristics of the two rooms were approximately identical, and that the performance of control and germicidal trials in separate places has had no appreciable effect on the experimental results. A series of tests was in fact carried out to determine whether a difference in the intensity of illumination in the two rooms at different times of day would have any effects on the viability of bacterial clouds. No appreciable difference in survival was found.

A second type of chamber, employed for some experiments, consisted of a hollow steel sphere of 6000 cu.ft. capacity, fitted internally with mixing fans and appliances for aerosol production. Sampling was carried out through a tube in the wall of the sphere, and for technical reasons could be performed only by means of impingers. Temperature and humidity could be regulated with ease, and the chamber scoured out with steam and fresh air after trials.

Test organisms and methods of aerosol production

The test organisms were selected for ease of cultivation, identification, or spraying, rather than as representative of common airborne species, and were as follows: *Chromobacterium prodigiosum* (syn: *Serratia marcescens*, strain M 1/44), *Micrococcus caseolyticus* (strains M 9 and M 10), *Streptococcus salivarius* (strain NCTC 8606), *Bacterium coli* (a faecal strain of Type I, derived locally), *Brucella abortus* (strain S 19), *Brucella suis* (strain P.S. III. *k*), and a rabbit-adapted strain of vaccinia virus, originally obtained from Prof. Downie. The *Micrococcus* strains were isolated from air samples, and were selected because of the absence of clumping, and hence the ease with which they could be sprayed as single organisms.

The suspending fluids are listed in Table 1, but some of them require further explanation. Vaccinia virus was sprayed as rabbit-skin pulp suspended in 0·004 M McIlvaine buffer, adjusted to pH 7·2; otherwise 'buffer' refers to a phosphate buffer consisting of KH_2PO_4 (4·5 g./l.), $(NH_4)_2SO_4$ (0·5 g./l.) and NH_4Cl (0·5 g./l.), adjusted to pH 7·6 by the addition of NaOH (approximately 3·0 ml. (40%, w/v)/l.). Saliva was treated by heating for one hour at 60° C., and filtering through glass wool. *Brucella suis* was suspended in the supernatant liquor of autoclaved and spun-down whole culture.

Except where specifically stated, all bacterial aerosols were produced by means of a modified Collison spray (Henderson, 1952), and hence consisted principally (95%) of single-organism particles. Though such an aerosol is not representative of

Table 1. *Steady decay rate of various bacterial aerosols in the presence of various amounts of hexyl resorcinol*

Organism	Suspending fluid	Hexyl resorcinol concentration (μg./l.)	Relative humidity (%)	Decay constant, k (min.$^{-1}$)
Chromobacterium prodigiosum	Water	0	70	0·095
	Water	0·12	70	0·87
	Saliva	0	55	0·071
	Saliva	0	60	0·13
	Saliva	0·034	55	0·94
	Saliva	0·086	60	0·65 (0·11; 2)
	Buffer-glycerol	0	35–45	0·099 (0·006; 4)
	Buffer-glycerol	0	50–60	0·150 (0·015; 6)
	Buffer-glycerol	0	70–95	0·108 (0·011; 6)
	Buffer-glycerol	0·04–0·08	50–60	1·17 (0·19; 10)
	Buffer-glycerol	0·033	70	0·38 (0·02; 3)
	Buffer-glycerol	0·08–0·11	70–85	0·89 (0·19; 6)
	Buffer-glycerol	0·11	70	0·62 (0·11; 2) H
	Buffer-glycerol	0·57	55	0·76 H
Micrococcus caseolyticus M9	Water	0	60–90	0·042 (0·008; 4)
	Water	0·033	55	0·036
	Water	0·051	55	0·047
	Water	0·086	60	0·094
	Water	0·09–0·80	50	0·056 (0·015; 3)
	Buffer	0	50	0·025 (0·004; 2)
	Buffer-glycerol	0	40	0·033
	Buffer-glycerol	0	55–65	0·023 (0·001; 2)
	Buffer-glycerol	0·04–0·09	50	0·095 (0·006; 2) H
	Buffer-glycerol	0·6–0·7	45–50	0·025 (0·000; 2) H
	Saliva	0	35–55	0·061 (0·005; 2)
	Saliva	0·03–0·04	35	0·131 (0·011; 2)
	Saliva	0·18	60	0·37 (0·15; 2)
	Saliva	0·07–0·09	70	0·59 (0·13; 2)
Streptococcus salivarius	Water	0	35	0·038
	Water	0	65–70	0·050 (0·013; 3)
	Water	0·04–0·09	35–40	0·050 (0·010; 4)
	Water	0·08–0·12	70–75	0·98 (0·35; 6)
	Buffer	0	40	0·038
	Buffer	0·06–0·09	35	0·054 (0·010; 2)
	Buffer-glycerol	0	40	0·052
	Buffer-glycerol	0·07–0·08	40	0·060 (0·004; 2)
	Saliva	0	40	0·069
	Saliva	0·06–0·07	40–50	0·091 (0·009; 2)
Bacterium coli	Water	0	50	0·194
	Water	0	70	0·047
	Water	0·10	70	1·02 (0·10; 2)
	Buffer-glycerol	0	45	0·092 (0·026; 2)
	Buffer-glycerol	0	55	0·21
	Buffer-glycerol	0·05–0·13	30–40	1·17 (0·16; 4)
	Saliva	0	40	0·167
	Saliva	0	60	0·062
	Saliva	0·06	40	0·31 (0·02; 2)
	Saliva	0·06–0·07	60	0·38 (0·03; 2)
Brucella suis	Culture fluid	0	65–70	0·021 (0·001; 3)
	Culture fluid	0	75	0·0086
	Culture fluid	0·09	70	0·39
	Culture fluid	0·09	75	0·17
Br. abortus	Saliva	0	75	0·063
	Saliva	0·08	70	0·93
Micrococcus caseolyticus M10	Buffer	0	50	0·052 (0·011; 2)
Vaccinia virus	Buffer	0·21	75	0·10

Notes. (i) Some of the k are means derived from several experiments. In such cases the values are followed, in parentheses, by the standard *deviation* and the number of the component values.

(ii) Letter H following a k value means that the experiment was conducted by vapourizing hexyl resorcinol into an established aerosol of organisms.

(iii) Decay of vaccinia aerosols in absence of germicide was not certainly detectable during 1 hr.

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naturally occurring floras, it is of a predictable constitution and results are not complicated by appreciable fall-out. The air supply to the spray was provided by compressed air bottles, to avoid possible contamination of the spray fluid with germicide.

Aerosols of vaccinia virus were generated by the same method, and were considered to consist of aggregates of about bacterial dimensions. No data, however, were available on the number of virus particles per aggregate, nor on the extent to which such aggregates disintegrate during subsequent manipulations. Quantitative results were, therefore, of limited value.

Methods of aerosol sampling

The majority of bacterial cloud samples were taken using two Bourdillon slit samplers, running either alternately for similar successive sampling periods, or independently for unequal sampling periods at regular intervals. The samples were taken at a rate of 1 cu.ft./min. for periods ranging from $\frac{1}{2}$ to 2 min.

During some experiments, including all those carried out in the 6000 cu.ft. chamber, impinger samples were taken at a rate of 10 or 20 l./min. for periods ranging from 1 to 3 min. The impinger fluids were diluted where necessary, and plated on the appropriate media by a modified Miles and Misra technique, or, in the case of vaccinia virus, inoculated into eggs.

It was found that prolonged incubation, beyond the normal period for the organism concerned, produced no increase in the colony count. Tests were carried out to establish the concentration of hexyl resorcinol required in agar and impinger fluid to produce effects on colony count and size. This was found to be greatly in excess of any possible concentration obtainable during sampling procedures.

Vaporization of hexyl resorcinol and estimation of vapour concentration

In those experiments requiring the maintenance of a constant concentration of hexyl resorcinol, volatilization was achieved by means of one or more 'Aerovaps' (Cx type) supplied by Messrs Shepherd's Aerosols Ltd. The output of a unit of this type is about 0·04 g./hr. as measured by weight loss, though this tends to decrease as the contents become exhausted. The apparent output can be twice as great over a short period, owing to the loss of water vapour absorbed during a period of disuse. The weight loss was found to bear little relation to the ultimate vapour concentration, not only on this account, but also owing to condensation, droplet fall-out, oxidation, variable background concentration and other factors. It was found that one 'Aerovap' unit per 3000 cu.ft. ensured a reasonable working concentration of hexyl resorcinol after one hour's operation at temperatures near 120° C.

In those experiments in which a sudden peak concentration was required, a known quantity of hexyl resorcinol was dropped on to a hotplate placed in front of a mixing fan. Again, the amount used bore little relation to the vapour concentration, but its adjustment ensured that the latter was of the desired order.

Air samples were taken for the estimation of hexyl resorcinol before and at several times during each experiment, care being taken to avoid atmospheric

contamination with other phenolic substances such as Lysol and tobacco smoke, which produce false positive readings.

The hexyl resorcinol content of the test atmospheres was determined by the method described by Lovelock (Bourdillon, Lidwell & Lovelock, 1948, p. 64), modified by the substitution of impingers for the bubblers previously employed for sample collection. Approximately similar readings were obtained when the two methods were compared.

When hexyl resorcinol is volatilized by heat, it will be present in the atmosphere in three forms, as vapour, as particles, and adsorbed on to dust and bacteria. The bubbler (or single impinger) measurements will include all three. When two impingers were used in series, the first containing no collecting fluid, it was found possible to effect a partial separation of vapour and particles, a high proportion of the particles being retained in the dry impinger. The sum of the quantities thus captured equalled, or slightly exceeded, the amount retained in a single fluid-filled impinger. The amount of hexyl resorcinol in particulate form was generally in excess of that in the gaseous phase, and investigation with a cascade impactor showed that the particles consisted mainly of very small supercooled droplets. In view of this and the known absence of dust from the test atmospheres, the majority of measurements were made with a single fluid-filled impinger.

Method of calculation

Curve fitting was carried out in all cases by the method of moments. This is not the best method in the maximum likelihood sense, but it is free from personal bias and it is the only method which could be applied uniformly without great arithmetical labour. In some of our experiments the aerosol counts were varying so rapidly that appreciable changes occurred during a sampling period; then a correction was made: if k is the rate constant for an exponential growth or decay, and a mean value $\bar{n}$ is found for a sampling period δ, the actual value of the ordinate at the beginning of the period is

$$n = \bar{n} \frac{k\delta}{e^{k\delta} - 1},$$

and so is proportional to $\bar{n}$ for fixed k and δ. Hence, if the sampling periods are the same throughout a run, the estimate of k will not be affected by the use of the average counts; k need not be known in advance, but the correction must afterwards be applied to determine the initial concentration.

RESULTS

The number of variables in investigations of this kind is great, the phenomena in detail are complex, and the quantitative accuracy of the technique is poor. We have therefore arranged the work to cover a variety of organisms under a variety of experimental conditions, so as to justify, as we hope, a broad qualitative conclusion. The conclusion has in any case only an interim value as a guide to the conduct of animal experiments and field observations.

The normal death rate of airborne bacteria; some general remarks

The decay of a bacterial aerosol is usually found to follow a roughly exponential law over the greater part of its course. This is true both for the concentration of viable organisms (as estimated for example by means of an impinger) and for the concentration of viable particles (as estimated by means of a slit sampler. We use the expression 'viable particle' to mean an aerosol particle containing one or more viable organisms). There is no significant difference between these two concentrations when, as in the aerosols produced by a Collison spray, the proportion of particles containing more than one organism is small. Approximately the same estimates of concentration are obtained by different methods of sampling over their common range of applicability.

We can therefore write

$$n = n_0 \, e^{-kt}, \tag{1}$$

where n is the concentration of viable particles at time t, n_0 their concentration at $t = 0$ and k is the decay constant. The value taken by k is, for any kind of organism, dependent on the environment of the particles (temperature, humidity, etc.), on the presence of extraneous substances derived from the fluid in which they were originally suspended, and to a lesser extent on the way the samples are treated.

When a liquid suspension is sprayed into an empty chamber at a constant rate corresponding to an input of N organisms per unit chamber volume per unit time, the concentration rises to a definite maximum. The concentration in the chamber is given by

$$\frac{dn}{dt} = N - kn$$

and if $n = 0$ when $t = 0$ we have

$$n = N(1 - e^{-kt})/k. \tag{2}$$

For large t, say $t \gg 1/k$, n is nearly equal to

$$N/k = n_\infty$$

say. When spraying from a bacterial suspension is long continued, the viable count in the liquid gradually changes; usually it falls: loss of viability is approximately compensated by evaporation of water. Equation (2) can be refined so as to take account of this circumstance (Lidwell, in Bourdillon *et al.* 1948). Since the change is small we can assume that nearly enough

$$N = N_0 \, e^{\lambda t},$$

where N_0 is the initial spraying rate, and determine λ (which may be positive or negative) from the viable count in the liquid before and after spraying. The result is

$$n = \frac{N_0}{k + \lambda} \, (e^{\lambda t} - e^{-kt}). \tag{3}$$

It is then convenient to work with modified values

$$n^* = n \, e^{-\lambda t}, \tag{4}$$

so that $$n^* = \frac{N_0}{k+\lambda}\{1 - e^{-(k+\lambda)t}\} \qquad (5)$$

and $$n^*_\infty = N_0/(k+\lambda).$$

Equation (5) can be fitted to a set of modified data by exactly the same numerical procedure as is used for (2), since it is of the same functional form.

It is not usually a good plan to estimate k by fitting equations (2) or (5) to the values of n obtained during spraying because the points are more scattered than they are after spraying has ceased. The reason for this is the imperfection of mixing in the chamber. As long as spraying continues, a source of inhomogeneity is present; it is to be expected—and has been found conspicuously to be true—that the higher the decay constant and the shorter the sampling periods the more erratic are the individual sample counts. When spraying has ceased, the fans quickly reduce the aerosol to uniformity. However, if spraying is continued for a long time (compared with $1/k$), satisfactory estimates of n^*_∞ are obtained since the mean concentration is constant except near the beginning.

Example I

Micrococcus M9 suspended in water at a concentration of 9×10^6/ml. was sprayed in the experimental room for 5 min. Air samples ($\frac{1}{2}$ min. duration) were then taken at 5 min. intervals. The relative humidity was 60 %. The decay curve

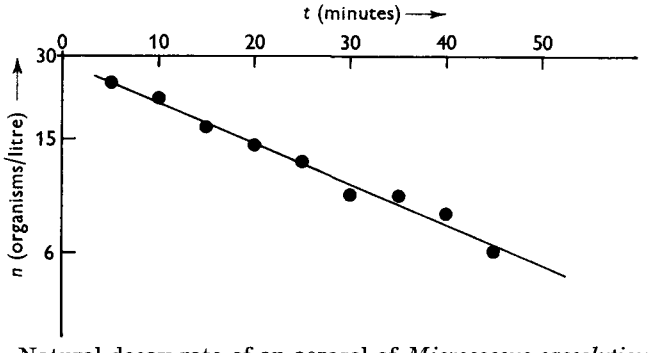

Fig. 1. Natural decay rate of an aerosol of *Micrococcus caseolyticus* M9. (Logarithmic vertical scale.)

is illustrated in Fig. 1; the estimated parameters are $k = 0{\cdot}0325$ min.$^{-1}$, n_0 (concentration at the end of spraying period) $= 27{\cdot}8$/l. For comparison a value of n_0 was also calculated from the nominal spraying rate by means of equation (3). The viable count in the suspension before spraying was $9{\cdot}64 \times 10^6$/ml.; afterwards, $8{\cdot}82 \times 10^6$/ml. Hence $\lambda = \log(8{\cdot}82/9{\cdot}64)/5 = -0{\cdot}01778$ min.$^{-1}$. The initial nominal output of the Collison spray was equivalent to

$$N_0 = \frac{9{\cdot}64 \times 10^6 \times 0{\cdot}1}{8{\cdot}94 \times 10^4} = 11{\cdot}4 \text{ viable particles/l./min.}$$

Insertion of these values in equation (3) gives $n_0 = 50{\cdot}0$/l.—about twice the previous estimate.

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Example II (*illustrating the application of equation* (5))

Brucella abortus suspended in saliva at a concentration of about 2×10^6/ml. was sprayed for 25 min. in the experimental room. Relative humidity 75 %. Samples ($\frac{1}{2}$ min. duration) were taken every minute. The value of λ was found as above to be about $-0{\cdot}00442$ min.$^{-1}$. The modified values n^* (equation (4)) are plotted in Fig. 2; it can be seen that the modified counts are less regular than those in Example I. The estimated decay constant is $k = 0{\cdot}0633$ min.$^{-1}$, and $n^*_\infty = 12{\cdot}3$/l. From the initial nominal spray output $n^*_\infty = 36{\cdot}0$/l.—about thrice the former value.

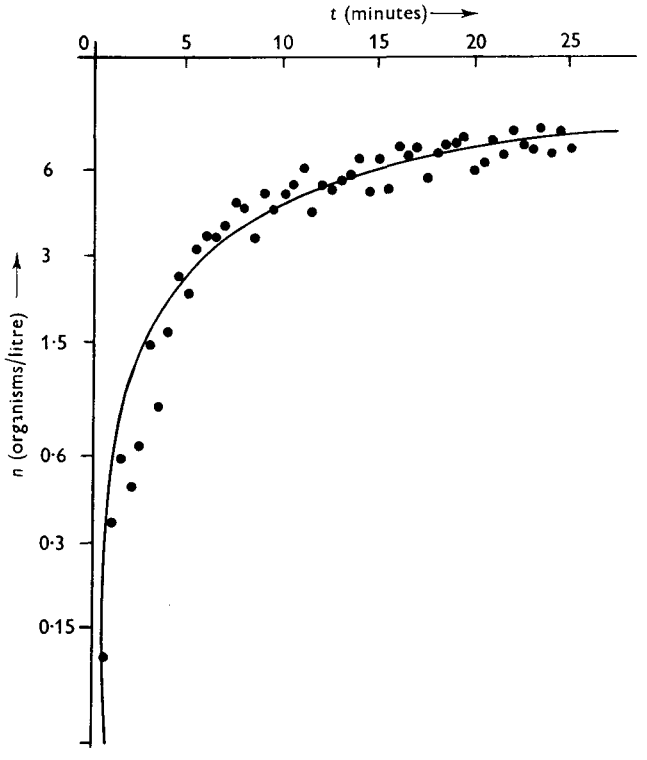

Fig. 2. Rise in count of *Brucella abortus* aerosol produced by steady spraying into an experimental room. (Logarithmic vertical scale.)

The anomaly in n_0 and n^*_∞ exhibited in these two examples is not unexpected. Previous experience has shown that the output of viable organisms from a Collison spray is less by a factor of 2–10 than the output reckoned from the loss in fluid volume. Part of the difference is due to evaporation, but part is a genuine loss of viability; the factor depends greatly on the species of organisms and on the nature of the fluid in which they are suspended. The loss of viability may well be the result of mechanical trauma—the factor is on the whole less for Gram-positive cocci (whose cell wall is mechanically the stronger) than for Gram-negative organisms. (Cf. Henderson, 1952, who uses the expression 'spray factor ratio' for the reciprocal of this factor.)

Since, then, the true N is usually unknown, equations (2), (3) and (5) are useful only for comparisons in which the viable spray ratio may be assumed to be the same. Our conclusions about the action of hexyl resorcinol are in nearly all cases based on experiments and controls carried out on the same suspensions of organisms in the same apparatus within a few hours of each other.

Other examples of the normal decay rate are included in Table 1. These include a component due to the loss of organisms by sedimentation. With such small particles as we used in the majority of our experiments this component is negligible, except in quite small enclosures, or where the air is very violently agitated.

The decay rate in the presence of hexyl resorcinol.

If a bacterial suspension is atomized into an established aerosol of hexyl resorcinol (or vice versa), the law of decay is again usually found to be exponential, and equations (1)–(5) apply, though with an increased value of k, depending on the concentration of the germicide. In practice it is found that k is not much dependent on concentration over a wide range above 0·04 μg./l. of hexyl resorcinol (roughly. See Table 1).

Variations in humidity had little effect on the death rate of most of our organisms over the middle range. Greatly increased rates were sometimes detectable but not easily measurable in atmospheres of R.H. above 80 %. *Streptococcus salivarius* was an exception; the rate changed from about 0·05 min.$^{-1}$ at R.H. 40 % to about 1 min.$^{-1}$ at R.H. 70 %. In this instance the numerical results were unusually erratic, probably because the control of humidity in the experimental rooms was inadequate, but their trend was repeatedly confirmed.

It is sometimes found that the viable decay in the presence of hexyl resorcinol is not exponential; the plot of log n against t is concave upwards. We have not been able to identify with certainty the circumstances which give rise to the curvature; it seems to occur when the humidity is high, or when the concentration of the germicide is low, and that of the organisms is high.

Decay rates in the presence of hexyl resorcinol are listed in Table 1. All have been calculated on the assumption that the decay is exponential, but some of the figures may well be erroneous because of a possible real but non-significant departure from this law. There are not enough examples under any one set of experimental conditions to enable formal statistical tests to be applied, and we submit the results as representative only. The rates of kill are such that sterility is approached only after some minutes, exposure of the organisms, at best: it would seem that hexyl resorcinol is of little value as a safeguard against immediate cross-infection, but a further factor, which may be of considerable practical importance, is involved.

When organisms are sprayed into an established aerosol of hexyl resorcinol, the concentration of viable particles quickly approaches the limiting value of $n_\infty = N/k$; if k is large the level attained is much the same for spraying times greater than $1/k$. MacKay (1952) noted that after spraying had stopped the time required to approach sterility was nearly independent of the period for which the spraying had continued; this observation is now seen to be a natural consequence of the law (2).

Example III

In three separate experiments *Chromobacterium prodigiosum* suspended in buffered 1% glycerol was sprayed for 5, 2½ and 1 min. into an atmosphere containing about 0·078 μg. hexyl resorcinol per litre. The same suspension of organisms was used throughout. The viable decay after spraying was followed for 10 min. in each run. The numerical results are given in Table 2; the values of n_0 estimated from the fitted curves are approximately constant because the k are large—here $1/k$ is less than 1 min.

Table 2. Chromobacterium prodigiosum *atomized into an atmosphere containing hexyl resorcinol: effect of different spraying times. See Example III in text*

Initial concentration of liquid suspension (organisms/ml.)	Spraying time (mins.)	Rate of subsequent decay, k (min.$^{-1}$)	Concentration of organisms in air at end of spraying, n_0 (viable particles/litre)	
			From fitted decay curve	Calculated from nominal spraying rate
$6{\cdot}12 \times 10^8$	5	1·14	25·9	656
$6{\cdot}23 \times 10^8$	2½	1·18	25·9	594
$6{\cdot}28 \times 10^8$	1	1·08	21·2	453

The initial kill

The figures in Table 2, corresponding to Example III, will serve to introduce a phenomenon which we think may be of great importance in the practical application of hexyl resorcinol. The n_0 estimated from the fitted curves are less by a factor of about 25 than those calculated from the nominal spraying rate. In control experiments without hexyl resorcinol the ratio is about 3. Another more carefully controlled example follows.

Example IV

(*a*) *Bacterium coli* suspended in water at a concentration of about 7×10^8/ml. was sprayed for 5 min. into an atmosphere containing 0·1 μg./l. of hexyl resorcinol at a relative humidity of 68%. Samples were taken every ½ min. for 15 min.

(*b*) The same suspension in the same apparatus was sprayed for 2 min. into an atmosphere free from hexyl resorcinol at a relative humidity of 69%. Samples were taken every minute.

(*c*) Still with the same suspension, experiment (*a*) was repeated.

The numerical results are given in Table 3; they show the same kind of anomaly as does Example III (Table 2). In IV*a* and IV*c* (hexyl resorcinol present) the ratio of the n_0 is about 30, whilst in IV*b* (hexyl resorcinol absent) it is about 8. The good agreement in (*a*) and (*c*) makes it unlikely that any great change occurred in the resistance of the organisms or the behaviour of the spray apparatus, and although in this as in other experiments we cannot be certain of the actual output of viable organisms from the spray, we can with confidence deduce that the germicide effectively caused a large reduction in output. And this reduction could scarcely have occurred until the organisms had left the spraying apparatus.

Table 3. *Bacterium coli aerosols: effect of spraying into atmosphere containing hexyl resorcinol. See Example IV in text*

Hexyl resorcinol concentration (μg./l.)	Decay constant, k (min.$^{-1}$)	Concentration of organisms in air at end of spraying, n_0 (viable particles/litre): From fitted decay curve	Concentration of organisms in air at end of spraying, n_0 (viable particles/litre): Calculated from nominal spraying rate
(*a*) 0·099	1·08	29·3	783
(*b*) 0	0·0473	224	1640
(*c*) 0·11	0·938	29·1	940

It will be convenient at this point to introduce the distribution function $F_{-}(t)$ of the 'life' of viable particles in an aerosol, i.e. $F_{-}(t)$ is the proportion of particles which still contain viable organisms at a time t after injection.† The frequency function of 'life' is

$$-dF_{-}(t)/dt.$$

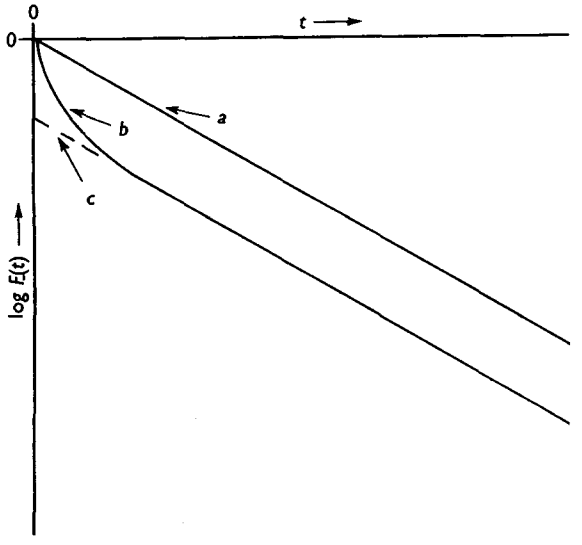

Fig. 3. Diagram illustrating initial kill. Curve *a*: simple exponential decay; curve *b*: postulated form of decay when initial kill occurs; curve *c*: asymptote of *b*.

In order to describe the discrepancy exhibited in Examples III and IV we now make the hypothesis that in the presence of hexyl resorcinol $F_{-}(t)$ is of the form

$$F_{-}(t) = e^{-kt}\{(1-\alpha)+\alpha\phi(t)\}, \tag{6}$$

where α is a constant and ϕ is some function which falls from an initial value $\phi(0)=1$ to a negligibly small value in a time short compared with $1/k$. The effect of the function ϕ can be seen in Fig. 3. Curve *a* is a pure exponential of slope $-k$.

† It is more usual in statistics, but less convenient here, to call $F_{+}(t)=1-F_{-}(t)$ the distribution function.

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Curve b is a possible form for equation (6); it falls steeply from $F_-(0)=1$ to become indistinguishable from a third curve c which is also a pure exponential of slope $-k$ but displaced vertically from a. Evidently c cuts $t=0$ in $F_-(t)=1-\alpha$. The 'resolution' and accuracy of our sampling methods are quite insufficient to determine a functional form for ϕ. Accordingly, we make no attempt to assign any kind of rate constant to the initial rapid fall in $F_-(t)$; instead we take as a measure of the integrated effect a quantity q_i which we call the 'initial kill' and define in the following way.

In such an experiment as that of Example II, where a constant input to the chamber is maintained, we have in general

$$n = N\int_0^t F_-(t-\tau)\,d\tau$$

$$= N\int_0^t F_-(\tau)\,d\tau. \tag{7}$$

(Equation (2) is equivalent to the assumption $F_-(t)=e^{-kt}$.) After a sufficient time n becomes constant:

$$n_\infty = N\int_0^\infty F_-(\tau)\,d\tau$$

$$= N\,[\tau F_-(\tau)]_0^\infty - N\int_0^\infty \tau F'_-(\tau)\,d\tau. \tag{8}$$

We can certainly assume that $F_-(t)$ diminishes for large t at least as fast as a negative exponential, if only because of sedimentation. Hence, the first term on the right of (8) vanishes. But the integral in the second term is simply the mean life of viable particles, $\bar{t}$ say. So

$$n_\infty = N\bar{t},$$

and this result is independent of the form of $F_-(t)$. Now, if $F_-(t)$ were a pure exponential, $\bar{t}$ would be simply $1/k$. We therefore write

$$q_i = 1-k\bar{t}.$$

Thus defined, q_i is a pure number which is actually equal to α in (6) if ϕ is of the form

$$\left.\begin{aligned}\phi\,(t)&=1 \quad (t=0),\\ \phi\,(t)&=0 \quad (t>0),\end{aligned}\right\} \tag{9}$$

and does not differ much from it for any other admissible ϕ. We can then say roughly that if bacteria are injected into an aerosol of hexyl resorcinol, a fraction q_i will quickly be rendered non-viable, and $1-q_i$ will be rendered non-viable at a much lower and nearly constant rate, k. It will be seen later that the initial kill is at least qualitatively explicable in terms of known physical processes.

To estimate q_i it is not actually necessary to make a determination of n_∞. It is easy to show that if (9) is true (or sensibly so), the effect of α and ϕ is merely to reduce the spraying rates by a factor $1-\alpha \doteq 1-q_i$. In Table 3 we estimate from the control (b)

$$\text{Actual spraying rate} = \frac{224}{1640} \times \text{nominal rate};$$

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and from experiment (*a*)

$$\text{Effective spraying rate} = \frac{29{\cdot}3}{783} \times \text{nominal rate.}$$

Hence $$q_i = 1 - \frac{29{\cdot}3}{783} \times \frac{1640}{224} = 0{\cdot}73.$$

Many experimental errors enter into the estimation of q_i, and no significance can be attached to values below 0·5 unless they are well confirmed by repetition. So far as we know an appreciable initial kill can occur only when the organisms are sprayed into an established aerosol of hexyl resorcinol. If organisms are first sprayed into an atmosphere free from germicide, which is then introduced by rapid volatilization (for example), no initial kill occurs.

Example V

(*a*) *Chromobacterium prodigiosum*, $2{\cdot}4 \times 10^8$/ml. in buffered 1% glycerol was sprayed for 5 min. into a fresh atmosphere, and then hexyl resorcinol was volatilized from a hotplate so as to reach a concentration of 0·11 μg./l. From the subsequent decay the parameters were estimated to be $k = 0{\cdot}702$ min.$^{-1}$, $n_0 = 331$/l. From the nominal initial spraying rate, n_0 should have been 1054/l.

(*b*) The experiment was repeated with a diluted suspension ($4{\cdot}9 \times 10^7$/ml.) and without addition of hexyl resorcinol. The decay constant was 0·124 min.$^{-1}$, and n_0 was 66/l. from the decay curve, and 185/l. from the nominal spraying rate.

The ratios of the n_0 do not differ appreciably in (*a*) and (*b*), i.e. $q_i = 0$. But in experiments conducted like Example III with the same organism and suspending medium a large initial kill (*ca.* 0·9) occurs.

A list of estimated q_i is given in Table 4; it will be noticed that the incidence is erratic, and depends especially on the liquid in which the organisms are suspended for spraying.

We now describe some ancillary experiments which bear on an explanation of the initial kill discussed below.

(A) *Chromobacterium prodigiosum* (final concentration *ca.* 10^6/ml.) was suspended in buffered 1% glycerol containing dissolved hexyl resorcinol at concentrations of 500, 50 and 5 μg./ml. The mixtures were plated out at intervals so that the death rate of the organisms could be measured. At 500 μg./ml. the kill was very rapid; at 50 and 5 μg./ml. the rate was 0·030 and 0·011 min.$^{-1}$, respectively.

Then some of a suspension ($8{\cdot}8 \times 10^9$/ml.) containing 50 μg./ml. hexyl resorcinol was sprayed into an atmosphere free from germicide, and the viable decay of the aerosol was determined in the usual way. The death rate was 0·237 min.$^{-1}$—about twice the normal control value and much higher than in the liquid suspension. Furthermore, by comparison with a control there appeared to be initial kill q_i of about 0·87. At 5 μg./ml. of hexyl resorcinol k was not appreciably enhanced in the aerosol, but an initial kill ($q_i = 0{\cdot}76$) still took place.

When the three experiments were repeated with *Micrococcus* M 9, no appreciable initial kill could be detected. Neither does this organism show any initial kill in

the usual form of experiment, when it is sprayed from a suspension in buffered glycerol.

(B) (i) *Chromobacterium prodigiosum* ($8{\cdot}8 \times 10^8$/ml.) suspended in buffered glycerol was sprayed into an atmosphere containing about 0·08 μg./ml. of hexyl resorcinol. The decay constant was determined in the usual way to be $k = 0{\cdot}85$ min.$^{-1}$, and by comparison with a control the initial kill was $q_i = 0{\cdot}95$.

Table 4. *Representative values of the initial kill q_i which occurs when bacteria are sprayed into established aerosols of hexyl resorcinol*

Organism	Suspending fluid	Relative humidity	Hexyl resorcinol concentration (μg./l.)	q_i
Chromobacterium prodigiosum	Water	70	0·1	0·83
	Buffer-glycerol	50–90	0·08–0·10	0·93
	Saliva	60–70	0·09–0·20	0
Micrococcus caseolyticus M 9	Water	60	0·04–0·08	0
	Saliva	60	0·2	0·46
	Saliva	70	0·08	0·84
Streptococcus salivarius	Water	35	0·03–0·06	0·46
	Water	40	0·07–0·09	0·89
	Water	65–75	0·08–0·12	0
	Buffer	35	0·06–0·09	0·50
	Buffer-glycerol	40	0·08	0·88
	Saliva	40–50	0·07	0
Bacterium coli	Water	70	0·10	0·75
	Buffer-glycerol	30–40	0·06–0·12	0·88
	Saliva	60	0·06	0

(ii) The experiment was repeated, using the same suspension, but the fans were turned off during the spraying period and for 10 min. afterwards. The decay rate, determined from samples taken after the fans had been turned on was $k = 0{\cdot}91$ min.$^{-1}$ —not significantly different from the previous value—but now q_i was 0·79 only, corresponding to a fourfold difference in effective spraying rate.

In the second part of this experiment, the two aerosols mixed slowly and the organisms were given more opportunity to dry before coming into contact with the germicide. The result must be regarded as suggestive only; not enough experiments of this kind have yet been carried out to establish the generality of the effect.

All these results indicate that the initial kill is associated with the simultaneous presence of germicide and (liquid) water.

DISCUSSION

So far as k is concerned our findings differ little qualitatively from those of previous workers in this field, but we have extended the list of test organisms to include pathogens and a virus, and have tested them under a wider range of conditions. Our experiments show that the action of hexyl resorcinol depends not only on the species of organism, but also on the nature of the liquid in which the organisms were originally dispersed, and the physical condition of this liquid at the time of exposure to the germicide.

In addition we have demonstrated a phenomenon, which we have called the initial kill. If this is irreversible *in vivo*, it must surely diminish the chances of cross-infection, since its action is extremely rapid. The magnitude of this effect will of course depend on the infective dose, the aerosol concentration, and the existing physical conditions of the particles.

We believe that two processes account for the initial kill.

(i) There is a wide distribution of size in the droplets formed by the Collison spray. Most of the smaller droplets do not contain organisms; if they did, the larger would contain several or many, and in fact droplets containing more than one particle are rare (less than 5% in the aerosols we used). Thus most organisms leaving the spray are surrounded by a relatively large bulk of water. Now from the theory of diffusion, just as the rate of evaporation from a sphere is proportional to its radius, so is the rate at which a sphere can absorb a vapour. Hence, when droplets leave the atomizer, they will immediately begin to absorb the germicide vapour at a rate higher than an isolated organism could do. But the bulk of the water evaporates very rapidly, so that the accumulated hexyl resorcinol, which evaporates very slowly even into an empty atmosphere, becomes more concentrated, and the organism therefore receives a powerful dose of germicide while it is still damp. Whether the organism 'dies' or not will depend on the size of the original drop, on its own specific properties, and on the physico-chemical nature of any residue from the suspending fluid. It is evident, also, that the way in which the two aerosols mix will affect the evaporation of the aqueous droplets. Some will probably dry before they come into effective contact with the germicide.

This mechanism cannot occur when hexyl resorcinol is admitted to an already established aerosol of organisms; it can occur when the germicide is not present as vapour, but is supplied at a non-lethal concentration in the fluid used to suspend the organisms. These two inferences are in accord with experiment.

(ii) We think it likely—but this is more speculative—that hexyl resorcinol can be absorbed rapidly by an organism only from an aqueous phase. Calculations based on known physical data (Powell & Pearce, 1955) indicate that diffusion of vapour alone could, in our experiments, supply roughly 10^{-16} g./sec. of hexyl resorcinol to a single organism. Of course the surface of the organism quickly comes into equilibrium with the atmosphere, and this rate is not maintained; if it were, the observed k would be much higher. Over a wide range of germicide concentrations k is found to change very little; this suggests that over this range the supply of vapour is always in excess of the demand, and that the death rate is conditioned by physical and chemical processes occurring within the bacteria-carrying particles. For a further discussion of these processes, see Nash (1951).

The bulk of the quantitative work cited here is based on slit sampler counts: a particle is effectively defined to be non-viable if it is incapable of giving rise to a macroscopic colony when deposited directly from the air on to a 'suitable' solid medium. This is merely conventional; it by no means follows that a pathogenic organism, non-viable in this sense, is not potentially infective.

We have carried out a few less systematic experiments in which samples were

taken also with impingers; unfortunately the common range of concentration over which both methods are trustworthy is rather short. Like MacKay (1952), we found that the two agreed well in the absence of hexyl resorcinol, but there were some large differences between them in its presence; control experiments showed that these differences were certainly not simply caused by the collection of hexyl resorcinol, either particles or vapour, in or on the sampling medium. In discussing one of his experiments, MacKay stated that 'the impinger result is greater by 1000-fold than the slit sampler counts'. Examination of his original data shows that this is a slip; the correct ratio is 100:1. But even this ratio is much larger than any we found (the largest was about 10:1).

In our experiments, the relation between slit sampler and impinger counts was to some extent systematic; the two agreed not only in the absence of hexyl resorcinol, but also in its presence if the organisms sampled suffered no initial kill in the particular circumstances. Where an initial kill occurred, the impinger counts were intermediate between the corresponding slit sampler counts and the counts which would have been expected in the absence of initial kill; that is to say the decay curve took a course intermediate between the lines *a* and *b* in Fig. 3. It appears, then, that organisms which by the slit-sampler standard have been rendered non-viable in the initial kill process may still be viable when a different standard is adopted. We tentatively conclude that the vigorous washing in the impinger frees the organisms from associated hexyl resorcinol which would otherwise remain long enough to inhibit reproduction.

We have not yet accumulated sufficient data to give a more precise description of a phenomenon which depends so markedly upon a number of non-metrical variables. It is safe to say that the initial kill (as indeed the steady decay) will be important in practical applications only in as far as laboratory criteria of viability correspond with potentiality for survival in a host animal.

Using a Collison spray aerosol of *Chromobacterium prodigiosum*, MacKay (1952) obtained a killing rate of about twenty-eight per hour. Using the same organism under similar conditions, we obtained a maximum killing rate over twice as great (at least sixty per hour). This may well have been due to a strain difference, since MacKay's strain was chosen for its resistance. Twort & Baker (1942) record a killing rate of the same order as this using an aerosol of *Corynebacterium xerosis* in broth, but in their experiments the humidity was much higher than in ours, and the hexyl resorcinol concentration was fifteen times as great. Using the same hexyl resorcinol concentration as in our experiments Lidwell & Lovelock (Bourdillon *et al.* 1948, p. 150) obtained a rate of only ten per hour with an aerosol of mixed salivary organisms ('sprayed spit'). This slow rate could be accounted for by the survival of organisms in the centres of large aggregates, and by the generally more resistant nature of Gram positive bacteria. However, provided that such a low killing rate is preceded by an initial kill, the ultimate result may be no less effective in the elimination of airborne bacteria than the high rates which can be obtained under certain favourable conditions.

SUMMARY

The bactericidal action of hexyl resorcinol vapour on airborne clouds of a variety of micro-organisms including pathogens and a virus has been investigated. In addition to killing rates comparable with those found by previous investigators, a phenomenon, which we have called the initial kill, has been demonstrated and experiments carried out to explain its mechanism. Under certain circumstances it enhances the elimination of viable bacteria very substantially.

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