THE SURVIVAL OF BACTERIA IN DUST

IV. ATMOSPHERIC HUMIDITY AND THE BACTERICIDAL ACTION OF ULTRA-VIOLET IRRADIATION

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(With 1 Figure in the Text)

Except in studies on airborne organisms, the effect of the ambient relative humidity has not generally been considered in evaluating the bactericidal action of ultra-violet irradiation. Most workers have used conditions, e.g. suspension in aqueous media or spreading on the surface of an agar plate, which are effectively 100 % relative humidity.

For air-suspended organisms, sprayed in a variety of media, it has usually been found that the bactericidal effect was increased at lower humidities (Whisler, 1940; Luckiesh & Holladay, 1942; Lidwell, 1946). Rentschler & Nagy (1940), however, reported that, in their experiments, humidity differences had no effect on the bactericidal action of ultra-violet radiation.

Our experiments with the flora of floor dust (Lidwell & Lowbury, 1950) gave a different picture. The bactericidal effect of radiation appeared markedly reduced under dry conditions. In an attempt to elucidate some of these rather confusing results we have exposed bacterial films to ultra-violet radiation over a range of humidities in a variety of suspending media.

METHODS

Throughout this work a strain of *Streptococcus pyogenes* (type 1) isolated from a sample of floor dust has been used. For exposure to radiation, 0·01 ml. portions of the well-mixed bacterial suspensions were spread on grease-free cover slips ($\frac{7}{8} \times \frac{7}{8}$ in). The cover-slips had previously been scratched with a diamond to facilitate subsequent fracture, and after spreading were dried for 15–30 min. in the dark over anhydrous calcium chloride. The prepared cover slips, in pairs on a microscope slide, were placed on wire grids in small baking trays ($8 \times 6 \times 1\frac{1}{2}$ in. deep). On the floor of each tray was a Petri dish containing about 30 ml. of a saturated solution of potassium bromide, sodium nitrite or calcium chloride to establish the required relative humidity i.e. 84, 66 or 36 % (O'Brien, 1948). In one tray the Petri dish of solution was replaced by a layer of anhydrous calcium chloride to produce dry conditions. Each tray could be covered with a cellophane sheet which was sealed down to the level rim of the tray with adhesive tape. The bacterial films were left for 1 hr. in the closed trays in the dark at laboratory

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temperatures before exposure to ultra-violet radiation in order to establish humidity equilibrium.

The intensity of ultra-violet radiation was the same as that previously used to irradiate floor dust (Lidwell & Lowbury, 1950), that is, about 2·6 μW./cm.^{2} at 2537 A., measured on the slides through the cellophane.

After exposure the cover-slips were removed from the trays, broken in two with forceps, and placed in 1 oz. screw-capped bottles where they were shaken for 1 min. with 10 ml. of sterile Ringer's solution and glass beads. The cover-slips were thoroughly crushed by this procedure. 0·1 ml. portions of a 1:10 and of a 1:100 dilution of the fluid were spread in duplicate on 5 % blood-agar plates containing one part in a million of crystal violet. Colony counts were done on all countable plates after 22 hr. incubation at 37° C.

Suspending media

Bacterial films were prepared and exposed, both in the dark and to ultra-violet radiation, with the following suspending media prepared as described below. For each period of exposure of any given suspension at a particular humidity a pair of cover-slips was used.

Suspension in distilled water

22 hr. cultures of the test organisms in Hartley broth were spun down, washed once and resuspended in distilled water.

Serum broth culture

The films were prepared directly from 22 hr. cultures in Hartley broth enriched with 10 % of horse serum.

Suspensions in aqueous dust extract

About 5 g. of dust were suspended in 30 ml. of distilled water and the mixture gently boiled, with stirring, for 15 min. The fluid fraction was centrifuged and the clear supernatant liquid autoclaved at 15 lb. for 20 min. The dark brown extract had a pH near 7·0. 22 hr. cultures of the organism in Hartley broth were spun down, washed, and resuspended in the extract.

Suspension in serum broth dust extract

About 5 g. of dust were extracted with 30 ml. of Hartley broth; the extract was enriched with 10 % horse serum, and the organisms were suspended in this extract. Extraction and suspension were carried out in the same way as for the aqueous dust extract.

Serum broth dust extract culture

A clarified extract of about 5 g. of dust in 30 ml. of Hartley broth was enriched with 10 % of horse serum and the organism was grown for 22 hr. at 37° in this fluid. Films were then prepared directly from this culture medium.

RESULTS

A typical set of results is given in Table 1 and another is illustrated in Fig. 1.

Table 1. *A typical set of experimental results. Films prepared from a culture of* Streptococcus pyogenes *in serum-broth dust extract. Counts of plates prepared from the* 10^{-1} *dilution*

Exposure time	(1)	(2)	(3)	(4)
0	130	107	86	79
	121	140	73	78

	Relative humidity							
	Dry		36 %		66 %		84 %	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
2 min.	173	135	53	94	57	32	44	55
	192	103	80	106	53	39	60	40
5 min.	141	55	54	28	42	38	24	12
	77	40	68	43	20	58	27	9
10 min.	45	84	13	74	10	15	2	4
	45	73	29	70	15	14	8	2
30 min.	8	22	12	34	4	1	7	8
	14	23	12	42	12	5	5	12
60 min.	14	13	3	14	8	5	1	5
	15	7	5	11	5	12	2	9

The numbers in brackets at the head of the columns refer to the replicate cover-slips. The counts of the replicate plates from a given cover-slip lie vertically above each other in the table.

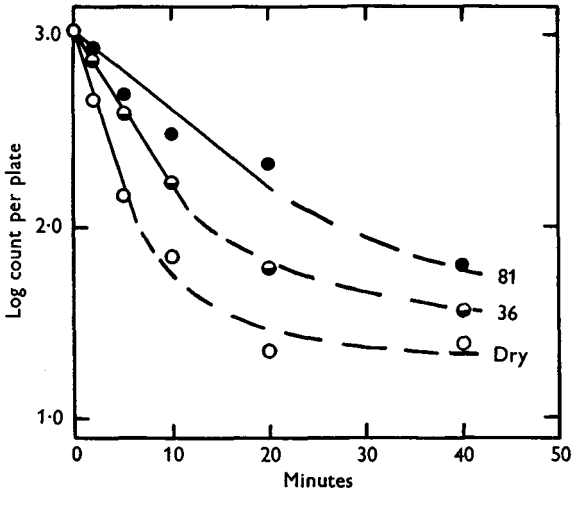

Fig. 1. The effect of relative humidity, given against each curve, on the bactericidal action of ultra-violet irradiation. The exposed films were prepared from a suspension of *Streptococcus pyogenes* in aqueous dust extract.

Replicate plates from the same cover-slip were generally in satisfactory agreement, although a proportion of pairs (perhaps 1:6) showed a difference that was excessive on the basis of random sampling errors alone. The proportion of such

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pairs was four times as great when the mean count per plate exceeded 100 as when it fell below this figure. The differences between pairs of cover-slips exposed simultaneously were much greater than could be accounted for by random sampling and appeared to be best represented as a standard deviation of 25 % on the mean count for a pair of slips. Part of this error will arise from the process of random sampling, and errors in measuring out and spreading the 0·01 ml. portions with subsequent errors in dilution will account for a further part. Irregular break-up of streptococcal chains may also contribute to the variance, and it is possible that some multiplication took place in some of the films either in the course of drying out at 37° C. or, particularly at the higher humidities, during the course of the 1 hr. equilibration period before exposure to radiation. In spite of this it was usually possible to fit a smooth curve to the results from any given experiment using the average value for the plates (normally four in number) prepared at the most convenient dilution.

Table 2. *Death rates of* Streptococcus pyogenes *in films of various media*

Medium	Radiation	Relative humidity				Resistant fraction
		Dry	36 %	66 %	84 %	
Distilled water	Dark	0·000021	0·000049	0·00054	0·0011	10 % (at R.H. 66 and 84 %)
	Ultra-violet (1)	0·60	0·19	0·15	0·15	1 %
	Ultra-violet (2)	0·46	0·11	(0·10)	(0·10)	1 %
Serum-broth culture	Dark	0·00004	—	0·00009	—	—
	Ultra-violet	0·15	0·08	0·08	0·08	10 %
Aqueous-dust extract	Dark	0·00004	—	0·00048	—	10 % (at R.H. 66 %)
	Ultra-violet	0·35	0·17	0·10	0·085	5 %
Serum-broth dust extract	Dark	0·00005	—	0·00048	—	1 % (at R.H. 66 %)
	Ultra-violet	0·10	0·03	0·03	0·03	10–15 %
Serum-broth dust extract culture	Dark	0·00006	—	0·0011	—	1 %
	Ultra-violet (1)	0·58	0·08	0·19	0·37	10 %
	Ultra-violet (2)	0·019	0·045	0·20	0·52	10–15 %

The death-rates are given per minute.

The resistant fractions are estimated very approximately by the point at which the apparent death-rate falls off rapidly.

A dash indicates no observation.

The repeated experiments with ultra-violet irradiation of films prepared from serum-broth dust extract culture, marked (1) and (2) in the table, were carried out with extracts of different dust samples.

Exp. (1) used dust from a fever-hospital ward. This dust was also used to prepare the aqueous dust extract. Exp. (2) used dust from a residents' flat at Harvard Hospital. This dust was also used to prepare the serum-broth dust extract.

Over the major part of the killing process, usually until at least 90 % of the original number of organisms had been destroyed, the action followed a logarithmic course, i.e. on plotting the logarithm of the count at any time against the time the resulting points could be adequately represented by a straight line. On a con-

siderable number of occasions the last fraction of the organisms to survive appeared to have a resistance to the action of the radiation (or of humidity in the case of the films exposed in the dark) of a considerably higher order than the majority. This is not an uncommon phenomenon and has already been noted in connexion with the bactericidal action on the flora of dust of various kinds of radiation, including ultra-violet (Lidwell & Lowbury 1950). Table 2 gives the death-rates in the films of *Strep. pyogenes* at the various relative humidities, obtained by plotting the logarithms of the counts at given times against the time and fitting, by eye, the apparent best straight lines to the resulting points over the first part of the process. The death rates have been computed from the slopes of these lines (death-rate $= \frac{1}{n}\frac{dn}{dt} = 2{\cdot}30\frac{d\log n}{dt}$, where n is the count at time t, given per minute when t is in minutes).

The death-rates in the dark are in every case very much slower than the corresponding death-rates for the irradiated films and can be neglected in considering the variation of these latter under the various circumstances. The values of the death-rates in the dark are of the same order as those for β-haemolytic streptococci in floor dust (Lidwell & Lowbury, 1950) but several times larger, especially for the resuspension in aqueous dust extract at higher humidities. In particular, in every case the death-rate increases with the relative humidity in a similar way to the death-rates in floor dust.

The death-rates under irradiation show an interesting picture. All the rates are very much faster than the corresponding rates for β-haemolytic streptococci in floor dust (Lidwell & Lowbury, 1950). With films of organisms resuspended in distilled water the killing action is most rapid under dry conditions, falling off, at first rapidly then more slowly, as the humidity increases. The absolute rate at the higher humidities corresponds to a total incident energy, for 50 % death, of approximately 100–125 ergs/mm.^{2}. This agrees well with the figures quoted by Duggar (1936) for various organisms under conditions which approximate to 100 % relative humidity. The same general pattern is followed by films prepared from serum-broth culture and by resuspension in aqueous dust extract or serum-broth dust extract. The death-rates are in all cases smaller than for the organisms resuspended in distilled water but fall off with increasing humidity in a similar manner. The effects of these media could be accounted for by absorption of the radiation; all are coloured (i.e. show absorption in the visible, and presumably in the ultra violet also), and the reduction in death-rate for the suspension in serum-broth dust extract is close to a summation of the effects of serum broth and aqueous dust extract as suspending fluids.

The effect of humidity in all the media discussed so far has been similar to that observed with airborne organisms produced by spraying cultures. The two sets of films prepared direct from cultures in serum-broth dust extract, however, show a reverse behaviour. The death-rate from irradiation under dry conditions is small, increasing at first slowly and then more rapidly as the humidity increases. This is concordant with the behaviour of β-haemolytic streptococci and other organisms in floor dust. The cause of the difference in behaviour is not apparent, but it would

appear possible that the organisms growing in the dust extract produce some metabolic product which becomes bactericidal under ultra-violet irradiation, and that the presence of water is necessary either for the photochemical reaction or for the action of the bactericidal substance on the organisms. The nature of the substance in the dust extract, and the material produced from it by the growth of the organisms, must be a matter for further study. If, as seems plausible, a similar compound is present in association with the organisms occurring in dust, it would seem likely that this must be produced from the substrate in which initial multiplication of these organisms takes place before their dispersion in the dust itself.

DISCUSSION

These experiments have shown that by varying the suspending medium it is possible to reverse the effect of ambient humidity on the bactericidal action of ultra-violet radiation on a strain of *Strep. pyogenes*. In view of the fact that dry conditions diminish the sensitivity to irradiation of both *Staph. aureus* and total organisms as well as β-haemolytic streptococci in dust, it seems likely that this phenomenon will be observed with these, and possibly other, species also.

While the details of the process which brings about the enhancing of the effect of radiation at high humidities are not apparent, the facts presented do throw some light on the apparently anomalous behaviour of the dust flora on irradiation and give an indication as to where a fuller explanation may be sought.

SUMMARY

The bactericidal effect of ultra-violet radiation on dried films of β-haemolytic streptococci has been shown to be dependent both on the relative humidity and on the nature of the suspending medium from which the films are prepared. In particular, while the bactericidal action is most often more rapid under dry conditions, films prepared from suspensions in dust extract culture show a similar behaviour to that of naturally occurring dust flora and are most resistant to the effects of radiation under dry conditions.

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