THE SURVIVAL OF BACTERIA IN DUST

II. THE EFFECT OF ATMOSPHERIC HUMIDITY ON THE SURVIVAL OF BACTERIA IN DUST

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(With 2 Figures in the Text)

Artificial desiccation *in vacuo* is commonly used for the preservation of certain bacterial species (Hammer, 1911; Swift, 1921; Flosdorf & Mudd, 1935; Morton & Pulaski, 1938; Stamp, 1947). Some organisms, notably respiratory pathogens, will survive natural drying in air for varying periods (Kirstein, 1902; Buchbinder, Solowey & Solotorowsky, 1941). Dunklin & Puck (1948) have studied the influence on airborne streptococci, pneumococci and staphylococci of a range of humidities occurring in nature; these organisms, suspended in saline or saliva, died in a few minutes when sprayed into air at a relative humidity of about 50 % but survived for hours at both higher and lower humidities; in aqueous suspension there was no critical killing humidity, the death-rate being slightly higher in moist atmospheres than in dry.

Pathogens present in dust have undergone a natural drying process from mucus, pus or saliva, but their persistence may be influenced by the degree of dryness reached, which will vary with the atmospheric humidity. Experiments to determine the survival of dust flora, including pathogens, at controlled humidities are described in this paper.

METHODS

Dust from the scarlet fever wards of two hospitals in London (dusts nos. 8 and 11) and of one in the country (dust no. 12) were selected after preliminary cultures had shown that numerous β-haemolytic streptococci were present. A reference to the dust numbers is given in the preceding paper (Lidwell & Lowbury, 1950*a*).

Initial viable counts of total bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes* in sifted dust were made, using the technique already described (Lidwell & Lowbury, 1950*a*). The pooled sweepings were then divided into as many parts as were necessary and spread out in open 6 in. aluminium Petri dishes for exposure to controlled humidities. The Petri dishes were placed on metal grids in tin boxes measuring approximately 9 by 9 by 9 in. Atmospheric humidity in the boxes was controlled by saturated solutions of salts (O'Brien, 1948), about 250 ml. being placed in a bowl on the floor of each box.

The salts used and the humidities established in these experiments were as follows: (1) sodium sulphate, R.H. 93 %; (2) potassium bromide, R.H. 84 %

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(3) sodium nitrite, R.H. 66 %; (4) potassium carbonate, R.H. 44 %; (5) anhydrous calcium chloride to produce a dry atmosphere.

The lids of the boxes were secured all around with adhesive tape. The relative humidity, checked with a hair hygrometer, was shown to reach equilibrium in about 1 hr., and to maintain its level while saturated solutions were present. The boxes were kept in a room at 65–67° F.

Three experiments were done, a different dust being used in each. In Exps. 2 and 3 dust was spread on glass disks contained in the aluminium Petri dishes.

At intervals between the second and the thirty-fifth days, and in some samples after longer periods of exposure, dust was extracted and plated out in the routine manner, and viable counts were done.

Table 1. *Log-median counts after exposure of dust at various humidities. Dust no.* 8

Species and dilution	R.H. %	Days exposure 0	2	4	7	9
Total organisms 1/20 dilution	Dry	1·90 (0·23)	2·09 (0·50)	—	2·04 (0·40)	—
	44	—	—	1·97 (0·33)	—	1·55 (0·35)
	66	—	—	1·81 (0·28)	—	1·93 (0·53)
	93	—	1·82 (0·29)	—	3·21 (0·30)	—
β-haemolytic streptococci undiluted	Dry	1·80 (0·90)	1·65 (0·80)	—	1·50 (0·80)	—
	44	—	—	1·60 (0·50)	—	1·55 (0·67)
	66	—	—	1·50 (0·60)	—	1·85 (0·55)
	93	—	1·00 (0·65)	—	0·50 (1·10)	—
Staph. aureus undiluted	Dry	1·86 (0·76)	1·20 (0·94)	—	1·00 (1·05)	—
	44	1·8	—	1·29 (0·83)	—	0·96 (0·93)
	66	—	—	1·60 (0·56)	—	1·15 (0·90)
	93	—	0·53 (1·10)	—	**1·78 (1·30)**	—
		35	37	58	78	92
Total organisms 1/20 dilution	Dry	1·77 (0·35)	—	—	1·63 (0·48)	—
	44	—	1·60 (0·38)	—	1·15 (0·35)	—
	66	—	**0·60 (0·25)**	—	**$\bar{1}$·94 (0·23)**	—
	93	2·37 (0·55)	—	1·50 (0·55)	—	**0·76 (1·08)**
β-haemolytic streptococci undiluted	Dry	1·75 (0·72)	—	—	1·81 (0·58)	—
	44	—	0·70 (0·89)	—	$\bar{1}$·90 (1·13)	—
	66	—	[$\bar{3}$·5]	—	[$\bar{4}$·0]	—
	93	[$<\bar{4}$·0]	—	[$\ll\bar{4}$·0]	—	[$\ll\bar{4}$·0]
Staph. aureus undiluted	Dry	1·45 (0·86)	—	—	0·68 (0·85)	—
	44	—	0·00 (2·20)	—	[$\bar{4}$·0]	—
	66	—	[$<\bar{4}$·0]	—	[$\ll\bar{4}$·0]	—
	93	$\bar{1}$·90 (1·10)	—	[$\ll\bar{4}$·0]	—	[$\ll\bar{4}$·0]

In brackets ( ), the standard deviation of the log-counts.

In heavy type, these values have been obtained from plate counts at the alternative dilution and corrected to the dilution for the other values in the row by using the appropriate factor.

In square brackets [ ], on these occasions too few positive plates were obtained for any direct estimate of the log-median or the standard deviation of the log-counts to be made. Approximate values for the log-median have been estimated as described in the text.

Table 2. *Log-median counts after exposure of dust at two humidities.*
Dust no. 11

Species and dilution	R.H. %	Days exposure 0	2	7	15	49
Total organisms	58*	**2·95 (0·43)**	2·46 (0·39)	2·28 (0·53)	2·10 (0·75)	1·89 (0·51)
Undiluted	84	—	2·20 (0·60)	1·97 (0·61)	1·70 (0·81)	1·30 (0·40)
β-haemolytic streptococci	58*	0·65 (1·51)	0·19 (1·40)	0·35 (1·51)	$\bar{1}$·43 (1·31)	$\bar{2}$·95 (1·15)
Undiluted	84	—	0·20 (1·36)	$\bar{1}$·08 (1·50)	$\bar{3}$·90 (1·90)	[$<\bar{4}$·0]
Staph. aureus	58*	0·37 (0·88)	$\bar{1}$·67 (1·80)	$\bar{1}$·16 (1·36)	$\bar{3}$·40 (2·30)	[$<\bar{4}$·0]
Undiluted	84	—	$\bar{1}$·10 (1·90)	$\bar{1}$·20 (1·40)	[$<\bar{4}$·0]	[$<\bar{4}$·0]

Symbols in this table are used as described for Table 1.

* The potassium carbonate solution used for maintaining the humidity in this experiment was not saturated. 58 % represents the estimated mean value of the relative humidity during the experiment.

Table 3. *Log-median counts after exposure of dust at various humidities.*
Dust no. 12

Species and dilution	R.H. %	Days exposure 0	2	7	14	35	36	37
Total organisms 1/20 dilution	Dry	1·98 (0·26)	1·73 (0·42)	1·68 (0·36)	1·76 (0·29)	—	—	**1·62 (0·37)**
	44	—	1·98 (0·40)	1·70 (0·33)	—	—	—	—
	66	—	1·91 (0·41)	1·84 (0·26)	1·48 (0·28)	1·08 (0·38)	—	—
	84	—	1·75 (0·26)	1·61 (0·26)	1·38 (0·30)	1·12 (0·46)	—	—
	44*	1·68 (0·36)	1·60 (0·39)	1·70 (0·28)	1·94 (0·53)	—	1·52 (0·49)	—
β-haemolytic streptococci undiluted	Dry	0·36 (1·32)	0·36 (1·32)	0·16 (1·56)	0·03 (0·91)	—	—	$\bar{1}$·90 (2·0)
	44	—	0·63 (0·85)	$\bar{1}$·40 (1·4)	—	—	—	—
	66	—	$\bar{1}$·74 (1·08)	0·41 (0·95)	$\bar{1}$·17 (0·87)	[$\bar{3}$·5]	—	—
	84	—	0·50 (0·88)	$\bar{1}$·13 (1·37)	[$\bar{3}$·0]	[$<\bar{4}$·0]	—	—
	44*	0·16 (1·56)	0·82 (1·30)	$\bar{1}$·62 (1·80)	$\bar{1}$·05 (1·36)	—	0·12 (1·53)	—
Staph. aureus undiluted	Dry	0·96 (0·64)	0·71 (1·45)	1·19 (1·18)	0·88 (1·65)	—	—	$\bar{1}$·85 (1·24)
	44	—	0·71 (0·54)	0·31 (0·90)	—	—	—	—
	66	—	1·40 (1·22)	0·70 (0·92)	0·39 (0·82)	$\bar{2}$·60 (1·6)	—	—
	84	—	0·30 (0·90)	0·30 (1·10)	0·08 (1·30)	$\bar{2}$·90 (1·1)	—	—
	44*	0·31 (0·90)	0·92 (1·30)	1·15 (1·03)	0·92 (1·40)	—	$\bar{1}$·96 (1·28)	—

Symbols in this table are used as described for Table 1.

* Owing to the potassium carbonate solution used for maintaining a relative humidity of 44 % becoming unsaturated between the second and seventh day in the original series, a second series was started using part of the dust which had been kept in the dry container.

RESULTS

Tables 1, 2 and 3 give the values for the log-median counts and the standard deviations of the log-counts, in series of twenty 10 mg. portions, for the three experiments, in which separate lots of a given dust were kept in the dark for varying periods at several relative humidities. The values of the log-medians and of the standard deviations of the log-counts have been estimated graphically as described in the preceding paper (Lidwell & Lowbury, 1950*a*). The standard deviation of the log-median might be expected to vary from about one-quarter the standard deviation of the log-counts at the start of each experiment, when all or nearly all of the twenty plates examined will have been positive, up to about one-half the standard deviation of the log-counts when only four or five plates are still positive. When the number of positive plates falls below this, direct estimate

of the log-median and the standard deviation of the log-counts is not practicable; the estimates of the log-median values given in the tables in square brackets have been arrived at by using the positive plates available, and assessing the most likely combination of log-median and standard deviation of the log-counts by means of the general relation between these two quantities set out in the preceding paper (Lidwell & Lowbury, 1950*a*, fig. 3). The standard deviation of these estimates is not easy to assess but may easily be as large as 1·0. It will be seen from these considerations, and the standard deviations given in the tables, that quantitative assessment of the death-rates of the different groups of organisms at the various relative humidities must be subject to considerable error. Assuming that the values of the log-median count for any group of organisms diminish linearly with time, an assumption which conforms with the experimental results within the limits of error, the various death-rates have been estimated graphically and are given in Table 4. Part of the course of a particular experiment is illustrated

Table 4. *Death-rates of the different groups of organisms at various relative humidities*

		Death-rate per day		
Dust no.	R.H. %	Total organisms	β-haemolytic streptococci	*Staph. aureus*
8	Dry	0·012	0·005	0·030
12	Dry	0·015	0·015	0·040
13	Dry	0·019	0·030	0·043
8	44	0·024	0·056	0·12
12	44	0·015	(0·06)	0·040
6	55–60	0·054	0·079	0·078
9	55–60	0·046	0·075	0·098
11	58	0·040	0·088	0·37
8	66	0·066	0·25	0·26
12	66	0·063	0·20	0·15
11	84	0·075	0·39	0·53
12	84	0·073	0·63	0·18
8	93	(0·07)	0·45	(0·38)

The results for dusts nos. 6, 9 and 13 are obtained from a subsequent paper (Lidwell & Lowbury, 1950*b*).

Figures in brackets are of less accuracy.

in Fig. 1. Graphical estimation, drawing in the apparent best lines by eye, has been used in view of the difficulty and complexity of attempting to calculate regression lines with points of varying accuracy, while also making allowance for observations at other relative humidities. The death-rates have been given per day and derived as follows:

$$\text{Death-rate} = 2{\cdot}30\,\frac{d\,(\text{log-median})}{d\,(\text{time})} = 2{\cdot}30\,\frac{d\log m}{dt} = \frac{1}{m}\,\frac{dm}{dt}.$$

(The figure 2·30 $\log_e 10$) is introduced to allow for the use of common logarithms to the base 10 for the log-medians.)

The death-rates computed in this way are comparable to the velocity constants of monomolecular reactions and to the usual expression of such quantities as ventilation. The half-life (i.e. time to reduce the median count to one half) is

approximately $\frac{2}{3 \times (\text{death-rate})}$, expressed in days when the death-rates are given per day.

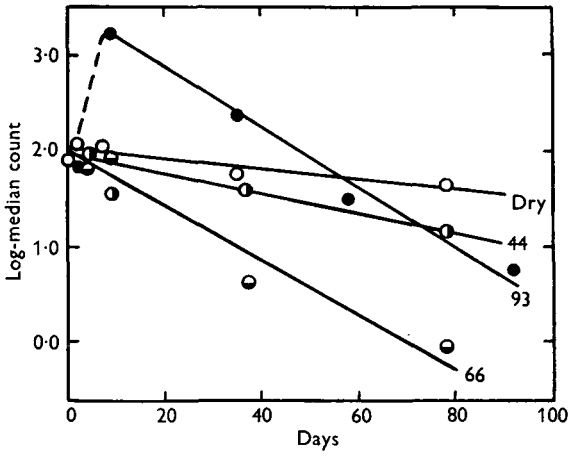

Fig. 1. The changes in the log-median count of 'total organisms' when dust no. 8 was exposed to various relative humidities; the actual percentage relative humidity is given against each curve.

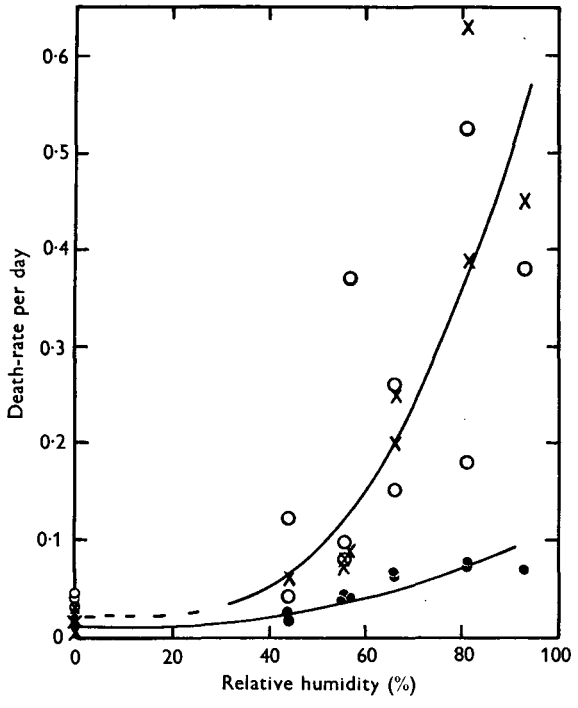

Fig. 2. Death-rates of dust flora at different humidities. Open circles, *Staphylococcus aureus*; crosses, β-haemolytic streptococci; filled circles, 'total organisms'. The lower curve is drawn as representing the trend of the results for 'total organisms'; the upper curve similarly represents the results for β-haemolytic streptococci and *Staph. aureus*, these being taken together since they do not appear to differ significantly.

As would be expected in view of the larger values of the log-median and the accompanying lower values of their standard deviations, the estimated death-rates

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for total organisms show a smaller scatter than those for β-haemolytic streptococci or *Staph. aureus*. The variations in the death-rate with relative humidity are illustrated in Fig. 2.

DISCUSSION

The results show a positive correlation between atmospheric humidity and the death-rate of bacteria. This agrees with expectation, and with the results of tests in which bacterial films were exposed to a range of humidities (Lidwell & Lowbury, 1950*c*). The relation was found to hold for total bacteria and for *Staph. aureus* and *Strep. pyogenes*, but our results do not exclude a different or contrary relation in sparsely represented bacteria, e.g. *E. coli*, which Heller (1941) and Bardsley (1948) have found to be more persistent in a moist than in a dry environment. Adaptation to survival in moisture might be expected of intestinal organisms, which are conveyed in food or water, while respiratory flora would benefit by adaptation to survival in the dry state if air carriage or persistence in dry places are important in their transfer from host to host.

The death-rate for each group of organisms increases more rapidly with relative humidity at high humidities. This is a similar form of relation to that found between the action of certain bactericides and the relative humidity (Lidwell, Lovelock & Raymond, 1948), except that in the present instance there is no suggestion of a maximum death-rate at any particular relative humidity. This relation is also paralleled by the variation with relative humidity of the free-water content of certain organic materials, e.g. saliva (Lidwell, 1949). These comparisons suggest that the variation of the death-rate of the flora of dust with relative humidity may be dependent on the proportion of free water absorbed by the matrix in which the bacteria are embedded.

Before starting the experiments described in this paper we had observed that the bacteria of dust survived longer in samples kept in a cupboard than in those stored at 4° C. in a refrigerator, where the relative humidity was found to vary between 75 and 90 %; the disadvantage of high humidity had evidently outweighed the advantage of low temperature. In other experiments (Lidwell & Lowbury, 1950*b*) we had noticed that the death-rate of bacteria in dust kept in the dark increased during the summer months. Indoor humidities are reduced during cold weather, which would favour the survival of streptococci, staphylococci, and probably of other respiratory pathogens in dust during the winter. Reasons for the seasonal variation of infectious disease incidence are not understood and are certainly complex. The concentration of pathogens in the environment is a likely extrinsic factor, and low humidity may be more important at this level than low temperature in determining a prevalence of respiratory pathogens during winter months.

Although the humidity levels in our experiments were too few to exclude it, there was no indication of a critical killing effect at R.H. 50 % or any other level. This is what we should expect, for in the experiments of Dunklin & Puck (1948), bacteria were exposed in sprayed droplets, and death occurred during the process of evaporation. The bacteria in dust, on the other hand, are those which have survived a drying process, and any damage caused by substances in solution (e.g. hygroscopic salts) would be more likely to occur at the higher humidities.

It is possible, as we have shown for *Staph. aureus* and total organisms (Table 1 and Fig. 1), that certain pathogens may actually multiply in dust when the atmospheric humidity approaches saturation point. Such conditions are uncommon indoors, but may occur in unheated rooms during the winter, or on floors which are kept moist. Dust in these conditions, however, is matted together and cannot be dispersed in the air. Moreover, saprophytic micrococci and fungi will multiply more rapidly and almost certainly crowd out the more sensitive pathogens.

Against the general assumption of the superior healthiness of a dry climate, then, we must emphasise that many bacteria and probably also viruses (Noguchi, 1918; Haagen, 1939) tolerate dry atmospheres better than moist. But the influence of humidity must not be assessed in isolation from temperature and light, which also vary with the season. We have made some preliminary studies on the bactericidal action of various forms of illumination and of ultra-violet irradiation on the organisms present in dust, and also of the action of ultra-violet irradiation at various relative humidities on β-haemolytic streptococci dried from culture and from suspensions in various fluid media. These are described in subsequent papers (Lidwell & Lowbury, 1950*b*, *c*).

SUMMARY

Dust from scarlet-fever wards was exposed to a controlled range of atmospheric humidities by enclosure in metal boxes containing anhydrous calcium chloride and saturated solutions of potassium carbonate, sodium nitrite, potassium bromide and sodium sulphate.

The death-rate of total organisms, *Staphylococcus aureus* and *Streptococcus pyogenes* in the dust was assessed by periodic sampling of series of twenty 10 mg. portions. A positive correlation between atmospheric humidity and death-rate was observed for the three groups of organisms counted in three specimens of dust.

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