THE SURVIVAL OF BACTERIA IN DUST

I. THE DISTRIBUTION OF BACTERIA IN FLOOR DUST

By O. M. LIDWELL and E. J. LOWBURY

Medical Research Council Group for Research in Industrial Physiology and the Common Cold Research Unit, from Harvard Hospital, Salisbury

(With 3 Figures in the Text)

It is generally assumed that resistant pathogens can be transmitted by dust, and are likely to follow this route where people congregate with a minimum of physical contact—in dormitories and hospital wards, for example. The relative importance of this route, however, is obscure.

Evidence for the transfer of infection by dust may be summarized under the following headings:

(1) *The source of infection.* Massive contamination of textiles by infected persons and of air by agitation of textiles has been demonstrated repeatedly (Brown & Allison, 1937; Cruickshank & Godber, 1939; Hamburger & Green, 1946; Hamburger, Green & Hamburger, 1945; Dumbell, Lovelock & Lowbury, 1948; Duguid & Wallace, 1948).

(2) *The presence and persistence of pathogenic organisms in dust.* Virulent streptococci, diphtheria bacilli and tubercle bacilli have been found and shown to persist for long periods in hospital and house dust (Winslow & Kligler, 1912; Pressman, 1937; Thomas, 1941; Crosbie & Wright, 1941; Garrod, 1944). Influenza virus has been recovered from dust near infected ferrets, and will survive in the dry state for as long as 6 weeks (Edward, 1941); smallpox virus has been found after long periods in dry crusts (Downie & Dumbell, 1947).

(3) *Infection caused by dust.* There is little direct evidence. White (1936) described tonsillitis in someone who had swept out a cubicle previously occupied by a puerperal sepsis case. Experimental infection with tubercle bacilli has been demonstrated in guinea-pigs exposed to infected dust (Lange & Nowosselsky, 1925).

(4) *Epidemiology.* Contradictory reports exist on the hygienic value of dust suppression by oiling floors and blankets (Begg, Smellie & Wright, 1947; Commission on Acute Resp. Dis. 1946; Rountree, 1947). Dust-borne infection has been thought to account for the frequent outbreaks of acute respiratory disease arising in the dormitories of residential schools (Dudley, 1926; Hamburger *et al.* 1945), but on this matter also there is no agreement (Cook & Munro-Ashman, 1949).

This uncertainty is not surprising in view of the extremely variable composition of dust, a factor which has limited study of its bacteriology, but is in itself of interest. We have attempted a quantitative technique, primarily to observe the viability of dust flora under various environmental conditions. This paper is

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limited to an account of the technique and of the distribution of bacteria in dust which it reveals.

EXPERIMENTAL

Materials

Floor dust was obtained from the scarlet-fever wards at several isolation hospitals, from a flat at Harvard Hospital, and from two schoolrooms. A list of these dusts is given in Table 8. From each source the total sweepings collected on several consecutive days were pooled, and the coarser fluff was removed by a sieve of $\frac{1}{15}$ in. mesh. The finer dust, a mixture of blanket fibres and granular material, was thoroughly mixed by sifting twelve times.

Extraction and culture

Preliminary tests showed extreme variability in the flora of replicate samples of the same dust, even after thorough mixing of the sample. As the degree of variability was unknown, we decided to adopt an arbitrary sampling number, twenty portions of 10 mg. each being extracted each time bacterial counts of the dust were required. This represented the most detailed examination which appeared practicable. It was unusual to obtain from a single source at any given time a larger quantity of sifted dust than 5–10 g., and this had to be divided into 20–25 lots to enable sufficient observations to be made of the survival of the flora of a given dust under various conditions. With such a variable material as dust it was considered necessary to examine the effects of several conditions on different portions of the same dust simultaneously. Subdivision of a given lot into twenty portions required at least sixty Petri plates for a single complete estimation. Four simultaneous lots needed 240 plates, which was as many as could be handled in a single day. The weighing was done roughly with a 'balance' consisting of a folded strip of paper pivoted on a pin and cut short at one end so that a weight of 10 mg. at the extremity of the short end would restore counterpoise (Nash, Powell & Ubbelohde, 1948). Weighing appeared accurate to 1 mg. or better.

The weighed portions of dust were placed in 1 oz. screw-cap bottles containing glass beads, the dust being well mixed after each portion was taken, so that as representative a sample as possible should be obtained each time. 5 ml. of sterile Ringer's solution (physiological saline in the earlier experiments) were measured into each bottle, and groups of ten were thoroughly shaken by hand for a minute. The contents were poured into sterile test-tubes, in which they were allowed to stand for a few minutes so that coarser debris should settle. 0·1 ml. amounts of the supernatant fluid, undiluted and at a dilution of 1 in 20 (1 in 10 in a few experiments), were spread on culture plates.

Dust containing haemolytic streptococci was cultured on 5 % horse-serum agar, undiluted and 1/20 dilution of extracts being used; undiluted extracts were also spread on 5 % horse-blood agar containing one part in a million of crystal violet. Dust in which streptococci were not under study was cultured on serum agar only.

Surface-viable counts of total organisms and of β-haemolytic streptococci were made after 22 hr. incubation at 37° C. Serum-agar plates were allowed to stand on

the bench for a further 24 hr., and typical colonies of chromogenic *Staphylococcus aureus* were counted. A random selection of streptococci and staphylococci from each dust was picked for grouping and coagulase testing respectively.

Table 1. *Powder and fluff. Colonies developing per plate from a series of portions of dust*

	Totals (1/20 dilution)		β-haemolytic streptococci (undiluted)		*Staphylococcus aureus* (undiluted)	
No.	Powder	Fluff	Powder	Fluff	Powder	Fluff
1	20	0	0	0	0	0
2	35	0	0	0	0	0
3	41	1	0	0	0	0
4	46	1	0	0	0	0
5	49	2	2	0	1	0
6	56	3	3	0	3	0
7	58	3	11	0	4	0
8	61	3	17	0	5	0
9	71	5	20	0	9	0
10	75	7	25	0	13	0
11	78	9	28	0	14	0
12	79	17	30	0	15	0
13	86	19	38	0	21	1
14	105	42	72	0	22	1
15	138	59	93	0	23	1
16	144	64	95	0	28	4
17	151	143	111	1	31	6
18	153	177	126	2	33	10
19	188	487	263	10	95	10
20	204	544	559	13	320	410
Median (logarithmic)	76	10	18	0·05	8·0	0·3

The figures in each column have been arranged in order of increasing magnitude; the horizontal rows do not relate to any given portion of dust.

The log-median of each series has been determined graphically, as described in the text, and its anti-logarithm given in the table as the median (logarithmic).

Each figure in the table is the count on a single Petri dish.

Flora of different fractions of dust

When a Petri dish containing dry floor dust is gently oscillated for some time, two fractions separate—a granular powder and matted fluff. Bacterial sampling of each fraction was carried out on three dusts to assess the distribution of flora. Table 1 shows the results of one such experiment. It is immediately apparent that portions of the powder fraction usually carry many more organisms than similar portions of the fluff. Occasional portions of fluff, however, gave as high or higher counts than any of the comparable portions of powder. Since the separation of the dust fractions was not complete and the fluff certainly contained some entrained powdery material, the difference may have been even greater than these figures show. The other two experiments gave essentially similar results.

Tests on the uniformity of the dust suspension

(1) *Replicates of the supernatant suspension.* A 20 mg. portion of each of two dusts was extracted by shaking with glass beads in 10 ml. of sterile physiological saline. The supernatant suspension was removed in the usual way, and ten replicate samples from each portion, undiluted and at 1/20 dilution, were plated out on serum agar; undiluted suspensions were also plated out on crystal-violet blood agar. The variance in each case for all three groups of organisms was consistent with a Poisson distribution together with additional variance attributable to a 10–15 % error in plating out the replicates.

Table 2. *Comparison of supernatant with 'mixed' suspensions. Colonies developing per plate from a series of portions of dust*

	Total organisms (1/10 dilution)		β-haemolytic streptococci (undiluted)		*Staphylococcus aureus* (undiluted)	
No.	Supernatant	Mixed	Supernatant	Mixed	Supernatant	Mixed
1	(53)	—	0	0	0	0
2	57	49	1	0	0	0
3	58	56	2	2	0	0
4	58	70	4	4	0	0
5	63	73	4	8	0	0
6	73	80	10	11	0	0
7	78	103	13	16	0	0
8	90	111	20	53	0	0
9	93	130	41	116	1	1
10	98	153	198	140	1	1
11	149	165	—	—	1	1
12	160	174	—	—	2	2
13	168	174	—	—	3	2
14	168	194	—	—	4	3
15	308	400	—	—	4	3
16	408	450	—	—	4	3
17	560	630	—	—	5	5
18	570	700	—	—	8	6
19	650	888	—	—	9	14
20	—	—	—	—	10	20
Median (logarithmic)	151	173	7·0	8·0	1·2	1·2

The figures in each column have been arranged in order of increasing magnitude; the horizontal rows do not relate to any given portion of dust.

Each figure in the table is the count on a single Petri dish.

(2) *Flora of the supernatant suspension and the sediment.* Sampling was done in the routine manner and, after plating out from the supernatant suspension, the sediment was shaken up and all the plate samples repeated using these 'mixed' suspensions. Table 2 shows the results of one such experiment. There is some indication of a slightly higher count in the 'mixed' samples, but the differences are not statistically significant. Examination of the original records suggests that such differences as appear were due to an occasional considerably larger count in the 'mixed' one of a pair of samples. Presumably some aggregate which had

escaped dispersion in the original treatment was occasionally broken up subsequently in the mixed suspension.

Attempts to break down residual bacterial aggregates

The distribution of the counts in successive portions of a given dust suggested that all three groups of organisms, i.e. 'total' organisms, β-haemolytic streptococci, and *Staphylococcus aureus*, occurred in the dust in aggregates of large numbers which, while stable to sifting, were broken down by agitation with glass beads in saline. These aggregates might be held together by, for example, nasal mucus, saliva or an epidermal scale, and the following methods were used in attempts to break down any aggregates which might have survived.

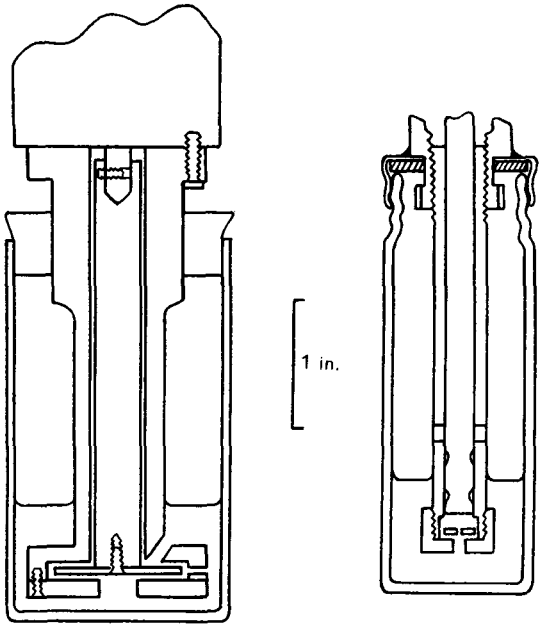

Fig. 1. Mechanical homogenizers. On the left rotating disk pattern, on the right piston type.

(*a*) *Mechanical homogenizers.* Two types were constructed and used (see Fig. 1). In the first the suspension circulated past a 1 in. diameter disk spinning at 12,000 r.p.m. with 1 mm. separation from fixed plates above and below the disk. The rate of circulation was limited by enclosing the disk in a cylindrical chamber having three small peripheral holes in addition to the axially or near axially disposed inlet apertures. The surrounding beaker containing the suspension was held in an ice-bath during operation to limit the temperature rise of the liquid. The second type of mechanical homogenizer consisted of a small piston operating in a cylinder closed at its lower end by a non-return valve pierced with a fine hole. The valve lifts in the up-stroke to allow the cylinder to fill freely but closes on the down-stroke, so that the liquid is forced out through the small orifice. The piston was driven at 1400 strokes per minute.

(*b*) *Papain.* If any bacteria were aggregated in dry mucus a mucolytic enzyme might be expected to disperse them. 1 ml. amounts of dust suspension were incubated for 30 min. in a water-bath at 37° C. with equal volumes of freshly prepared, Seitz-filtered, 1 % solution of papain in distilled water buffered to pH 7 with M/250 double phosphate buffer. After incubation the extracts were plated out. Four tests were carried out in which suspensions treated in one or other of the above ways were plated out in parallel with equivalent dilutions of untreated suspensions. The most comprehensive of these tests is exhibited in Table 3. It will

Table 3. *Breakdown of bacterial aggregates. Colonies developing per plate from a series of portions of dust. Dust no.* 3

	Total organisms (1/10 dilution)			β-haemolytic streptococci (undiluted)			*Staphylococcus aureus* (undiluted)		
No.	Control	Homo-genizer	Papain	Control	Homo-genizer	Papain	Control	Homo-genizer	Papain
1	11	11	6	0	0	0	0	0	0
2	15	15	11	0	0	0	0	0	0
3	20	22	11	0	0	0	0	0	1
4	32	24	16	0	0	0	0	0	4
5	33	24	22	4	0	0	1	0	9
6	36	31	22	7	0	0	5	5	10
7	41	35	25	16	3	0	10	5	14
8	50	40	26	18	7	1	20	19	17
9	74	43	33	23	9	3	170	170	140
10	185	105	187	28	13	7	1500	820	1400
Median (logarithmic)	34	28	18	4·5	1·5	0·4	2·0	2·0	8·0

The figures in each column have been arranged in order of increasing magnitude; the horizontal rows do not relate to any given portion of dust.

The homogenizer used in this experiment was that with a rotating disk.

Each figure in the table is the count on a single Petri dish.

be seen that, on this occasion, the two methods employed did not appear to break down any significant number of residual aggregates, the counts after treatment (except in one series) being unaffected or slightly lower than before. With one of the dusts examined a definite increase in count was observed with both types of mechanical homogenizer. This increase was obtained after 1 min. treatment; further treatment, up to 10 min., did not appear to produce any additional increase in the count. In general, the results obtained suggested that if any aggregates survived the shaking with glass beads they were either few or so strongly bound together as to resist the homogenizing methods employed here.

The numerical assessment of the results

Table 4 shows a typical set of results. It is obvious that the arithmetic means of such series are not suitable parameters owing to the dominating influence of a few high counts, or even a single one. Examination of a large number of such sets of data showed that the individual series approximated to distributions of the

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log normal type and in Fig. 2 a group is shown plotted on log probability paper. If x_n is the count of the nth observation in a series of observations arranged in ascending or descending order of magnitude of x_n, and n_0 is the total number of observations, then the points plotted are log x_n against $100\,\frac{n-\frac{1}{2}}{n_0}$ as the cumulative percentage under or over value. The plotted points lie reasonably closely about straight lines, and all the data have been treated by plotting in this way and then drawing, by eye, the apparent best straight line through the points. Zero values of x_n cannot, of course, be plotted in this way, and these observations are therefore

Table 4. *Colonies developing per plate from a series of portions of dust. Dust no.* 13

No. of portion (10 mg.)	Total organisms (1/20 dilution)	β-haemolytic streptococci (undiluted)	*Staphylococcus aureus* (undiluted)
1	140	1	0
2	107	83	30
3	600	29	1
4	380	6	46
5	131	1	0
6	208	95	4
7	154	104	6
8	60	38	17
9	79	8	380
10	320	12	7
11	167	20	18
12	101	26	2
13	400	13	0
14	89	5	0
15	139	260	40
16	128	1	13
17	190	26	194
18	250	25	1
19	280	18	0
20	720	65	3

Each figure in the table is the count on a single Petri dish.

omitted from the graphs. The lines through the rest of the points have been drawn on the assumption that zero values of x_n are best regarded as indicative of values lying between 0 and 1. The log-median value of the count (log m) and the standard deviation of the logarithms of the observed counts (s) for each series have then been estimated from the straight lines. The graphical method has been adopted, since it avoids the difficulties otherwise associated with zero values (not necessarily truly zero but only indicating no colony observed in the portion spread on the Petri plate) and values so large as to render accurate counting impossible. Throughout this paper and in the subsequent papers of this series the term log-median count refers to the median of the logarithms of the counts (to the base 10) graphically estimated in this way, and the standard deviations of the log-counts are the graphically determined parameters of the untruncated log-normal distributions

from which the observations are assumed to derive. It should be pointed out that if the distributions were truly log-normal then the log-median would coincide with the log mean or geometric mean. In a number of series, where difficulties with apparent zero readings or excessively high counts did not appear, the log-mean has been directly computed and found to give values close to those obtained graphically for the log-median.

The degrees of approximation of the data to a truncated log-normal form when the individual series include an appreciable number of zero counts has been

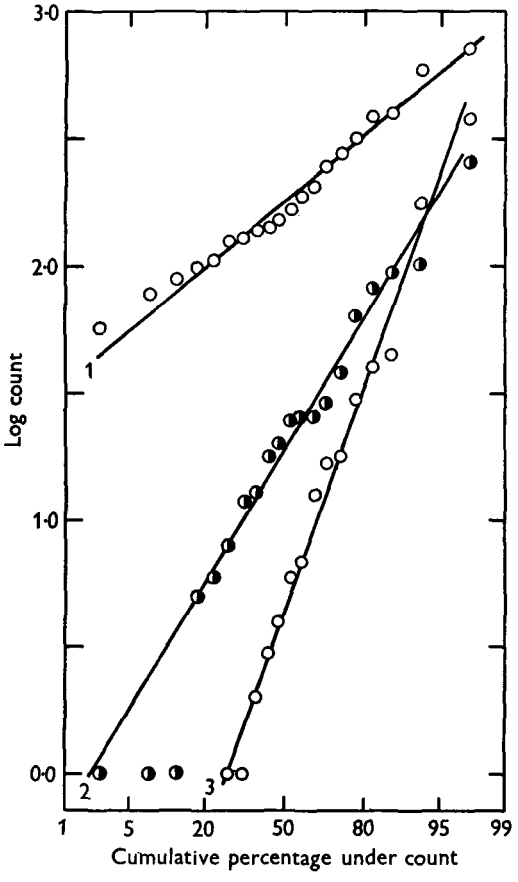

Fig. 2. The distribution of counts in series of 10 mg. portions from dust no. 13. Line 1 is for total organisms, line 2 for β-haemolytic streptococci and line 3 for *Staphylococcus aureus*.

examined for a group of ten such series. Five of these were series of counts of *Staphylococcus aureus*, the remaining five were series of counts of β-haemolytic streptococci. The recorded number of zero counts ranged from five to eleven out of the twenty observations comprising each series. Table 5 shows the observed distributions compared with those calculated from the graphically determined parameters of the assumed log-normal distributions. The agreement is quite adequate, particularly since no discontinuous series can be represented exactly by a log-normal distribution. It appears that the log-normal form, truncated at a log-count of zero, adequately describes the experimental data.

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Table 5. *Comparison of calculated with observed frequencies in a set of truncated distributions*

No.	log m	S	Range of log x					χ^2
			<0	0–0·50	0·50–1·0	1·0–1·50	$>1·50$	
1	0·45	1·38	7·5	2·8	**2·8**	**2·4**	4·5	0·72
			7	**4**	**3**	**1**	**5**	
2	0·96	0·93	**3·0**	**3·2**	4·1	4·1	5·6	1·63
			5	3	2	4	6	
3	0·55	1·31	6·7	3·0	**3·0**	**2·6**	4·7	0·61
			8	2	2	4	4	
4	0·40	0·81	6·2	4·7	4·4	**2·9**	**1·8**	0·96
			$7\frac{1}{2}$	$5\frac{1}{2}$	3	2	2	
5	$\bar{1}$·85	1·24	11·0	3·0	2·5	**1·7**	**1·8**	0·27
			$10\frac{1}{2}$	$3\frac{1}{2}$	3	1	2	
6	0·35	1·51	8·2	2·6	**2·5**	**1·9**	4·8	1·37
			9	1	2	2	6	
7	$\bar{1}$·95	1·70	10·2	2·3	**2·1**	**1·8**	3·6	1·92
			11	2	1	1	5	
8	$\bar{1}$·67	1·80	11·4	2·1	**1·9**	**1·5**	3·1	1·24
			11	3	$1\frac{1}{2}$	$\frac{1}{2}$	4	
9	$\bar{1}$·40	1·40	13·3	2·4	1·8	**1·2**	**1·3**	0·62
			13	3	1	2	1	
10	$\bar{1}$·74	1·08	11·9	3·3	2·4	**1·4**	**1·0**	0·29
			12	4	2	1	1	
						Aggregate χ^2		9·63

The observed frequencies in each group are shown beneath the expected frequencies, calculated from the graphically determined parameters of the assumed log-normal distributions. Observations on the range boundaries are divided equally between the two ranges. In order to reduce the number of groups with very small expected frequencies those pairs of groups shown in heavy type have been combined for the calculation of χ^2. The values of χ^2 are therefore based on four groups and involve a single degree of freedom only in each case. The corresponding values of P lie between 0·15 and 0·60. The aggregate χ^2, for ten degrees of freedom, corresponds to a value of P about 0·50, i.e. the agreement with the truncated log-normal assumption is as close as would be expected in 50 % of such trials.

Correlation between the counts for different bacterial species

The observations made on a series of dusts (nine in all) have been examined for correlation between the counts of the groups of organisms differentiated in these observations, i.e. β-haemolytic streptococci, *Staph. aureus* and other species taken together. The results of these computations are given in Table 6. Only for dust no. 8 are the correlation coefficients individually significant. The series of coefficients for the correlations of β-haemolytic streptococci and *Staph. aureus* separately with other species do not show significant mean values. The series of correlation coefficients between β-haemolytic streptococci and *Staph. aureus* does, however, show a significant, if small, mean value, 0·25 with a standard deviation of 0·09. It would appear that in these specimens of dust the different species were distributed largely independently except for a slight, easily understandable, association between β-haemolytic streptococci and *Staph. aureus*.

Table 6. *Correlation between counts for different groups of organisms in successive portions of a given dust*

Dust no.	No. of portions	r_{12}	r_{13}	r_{23}	$r_{sig.}$
1	15	—	−0·02	—	0·51
2	23	+0·34	+0·32	+0·07	0·41
3	10	−0·36	−0·04	+0·58	0·63
4	19	—	+0·10	—	0·46
6	17	−0·12	−0·32	+0·22	0·48
8	19	+0·64	+0·45	+0·54	0·46
9	29	+0·07	−0·17	+0·19	0·37
12	18	−0·07	+0·15	−0·22	0·47
13	20	+0·10	−0·34	+0·24	0·44

r_{12}, correlation coefficient between counts of β-haemolytic streptococci and 'other species'.
r_{13}, correlation coefficient between counts of *Staphylococcus aureus* and 'other species'.
r_{23}, correlation coefficient between counts of β-haemolytic streptococci and *Staphylococcus aureus*.
$r_{sig.}$, the value of the correlation coefficient for a significance level of 0·05.
'Other species' are all colonies other than β-haemolytic streptococci or *Staphylococcus aureus* growing as visible colonies on a serum agar medium after 24 hr. incubation at 37° C.

Source of the variation in count in successive portions from the same dust

As has already been pointed out, the variation in count in successive portions from the same dust was very great, even though the dust had been thoroughly mixed mechanically. This variation was much greater than could be accounted for by the technique of dilution and the errors of sampling. Errors due to sampling might be expected to follow a Poisson distribution with a standard deviation approximating to the square root of the median count. The observed standard deviations (see Fig. 3) are many times greater than this. Replicates from the extract of a given portion of dust, however, as has been stated above, varied in a manner consistent with errors distributed according to a Poisson distribution. Since the variation from portion to portion of dust was so much higher than the variation in replicates from the same portion, the limited number of plates possible were best employed on the maximum number of portions from a given dust sample rather than in replicates on individual portions.

That the variation is principally a property of the dust sample and not of the sampling technique is further confirmed by a comparison of the counts obtained on plating out the undiluted dust suspension with those obtained on plating out a 1/20 dilution of this suspension. Over a limited range of count of total organisms, reasonable numerical values can be obtained in both series. Over this range the standard deviations (of the logarithms of the counts) of the undiluted series are not significantly different from those for the 1/20 dilution series as is shown by the figures presented in Table 7.

Relation between the variation in count in successive portions of the same dust and the median count of that dust

In the course of the observations recorded in this and the succeeding papers, well over 100 samples of dust, representing the results of exposing thirteen original dust specimens to various illuminations and humidities for various times, have

Table 7. *Standard deviations of the log-counts of 'total' organisms at two dilutions*

log m (undiluted)	S (undiluted)	S (1/20 dilution)	Ratio
2·57	0·64	0·61	1·05
2·46	0·39	0·37	1·05
2·30	0·65	0·67	0·97
2·28	0·53	0·55	0·96
2·27	0·52	0·50	1·04
2·20	0·60	0·58	1·03
2·18	0·51	0·52	0·98
2·18	0·52	0·46	1·13
2·15	0·51	0·56	0·91
2·13	0·41	0·50	0·82
2·10	0·75	0·65	1·15
2·10	0·48	0·37	1·30
2·06	1·08	0·78	1·39
1·98	0·55	0·59	0·93
1·97	0·61	0·48	1·27
1·90	0·25	0·38	0·66
1·89	0·51	0·60	0·85
1·87	0·75	0·55	1·36
1·86	0·52	0·78	0·67
1·77	0·42	0·57	0·74
1·76	0·57	0·50	1·14
1·74	0·53	0·46	1·15
1·70	0·81	0·67	1·21
1·63	0·72	0·75	0·96
1·61	0·44	0·48	0·92
1·58	0·33	0·57	0·58
1·53	0·51	0·45	1·13
1·34	0·54	0·52	1·04
1·30	0·40	0·51	0·78
1·25	0·63	0·72	0·88
1·24	0·23	0·57	0·40
1·24	0·58	0·54	1·07
1·15	1·00	0·54	1·85
1·10	0·66	0·52	1·27
1·08	0·58	0·40	1·45
0·76	0·58	0·58	1·00
0·76	0·58	0·45	1·29

The mean value of $\frac{S \text{ (undiluted)}}{S \text{ (1/20 dilution)}} = 1{\cdot}04$ (S.D. 0·27).

been examined. Including the dusts as collected, 339 series, each representing successive portions of the same dust examined for single groups of organisms, have yielded values for the log-median and the standard deviation of the logarithms of the observed counts. The logarithms of these standard deviations have been plotted against the corresponding values of the log-medians with the rather unexpected result that all the points lie about a single line, not necessarily a straight line, irrespective of differences in the origin and treatment of the samples, or in the groups of organisms concerned. This is illustrated in Fig. 3, which shows the distribution of these points together with the median line through

the points and lines delimiting the outlying 16 % of the distribution on each side of the median line, i.e. at a distance from the median line approximating to the standard deviation. The deviations about the median line are close to those to be expected on the basis of the values of the standard deviations (s), but appear to be somewhat larger although the difference is doubtfully significant.

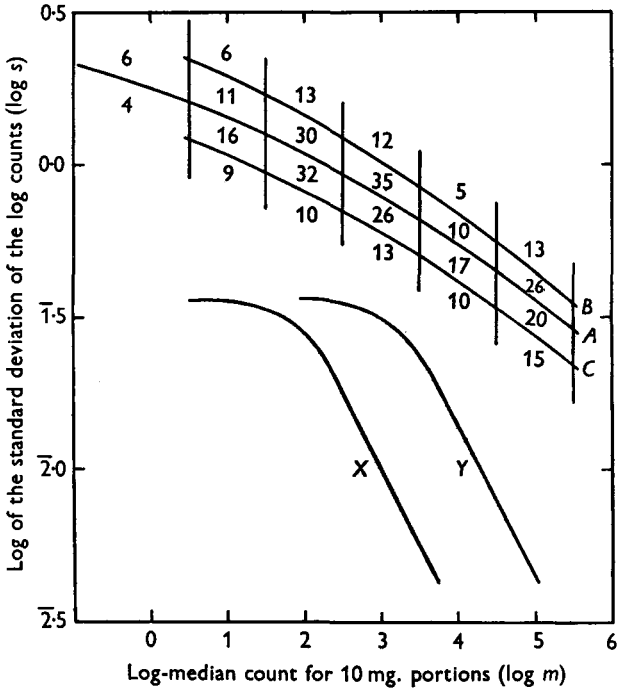

Fig. 3. Correlation diagram for the standard deviation of the log counts and the log-median count. The figures on the diagram show the number of series with values falling in the regions delimited by the adjacent lines. Curve A approximates to the median of the distribution, curves B and C lie at a distance from A corresponding to the standard deviation of the distribution about A, i.e. approximately 16 % of the observations lie above curve B, 34 % between B and A, 34 % between A and C and 16 % below C. Curves X and Y show the values of the logarithm of the standard deviation of the log-counts which would result from Poisson distributions with expectations of m/50 and m/1000 respectively, if the standard deviation were estimated graphically as described in the text.

DISCUSSION

The general facts of the distribution of all three groups of organisms in the dusts examined in this investigation, and in particular the extremely high degree of variability from portion to portion of the same dust even when this has been thoroughly mixed mechanically, appear to be compatible only with the hypothesis that these organisms are largely present in clumps or aggregates often containing many thousands of individuals, which are relatively easily dispersed in aqueous media. This conclusion is not an unexpected one, since multiplication of most species will have occurred in some watery menstrum, dried portions of which are subsequently found in the dust. Less expected and needing further investigation before any general conclusion can be drawn from it is the apparent homogeneity

of the dusts in respect of the relation between variance and median counts in successive 10 mg. portions of a given dust. This homogeneity appears to hold before and after exposure to the various circumstances examined and to cover the three groups of organisms differentiated. Strict analysis is difficult, but it seems possible that some such result might derive from a distribution of particle size in the original scattering of the infected material. In this connexion it is perhaps worth noting that the results of Duguid (1946) for droplets expelled by coughing, talking and sneezing show a similar size distribution for particles produced by all three activities, and that when the cumulative percentages over or under a given particle volume are plotted on log probability paper, in the same way as the counts from the 10 mg. dust portions have been, the points lie reasonably close to straight lines with a standard deviation of about 0·7, which is comparable in magnitude to the standard deviation of the log-counts of the dust portions. (This is true for particles over 50 μ diameter plotted separately; when particles of all sizes are plotted together, the smaller sizes, less than 50 μ diameter, show a similar variance, but there is discontinuity in the data close to a diameter of 50 μ, which is the size level at which a change-over took place in the technique of measurement.) In spite of the very large variance in the number of bacteria in successive portions of a given dust, the results obtained with the technique described in this paper are adequate to trace the general pattern of phenomena when the dusts are exposed to various environmental conditions, although the numerical estimates of the bacterial death-rates are necessarily subject to considerable errors. The additional number of portions required even to halve the standard errors of the log-medians makes improvement in accuracy in this way generally impracticable.

The irregular distribution of bacteria in dust and their presence in aggregates containing many thousands of organisms may have some bearing on the modes of transfer of infection. Boyland, Gaddum & McDonald (1947) have shown that in normal respiration very few particles greater than 5 μ in diameter pass through the nasal passages. Where the site of the primary infection is in the lungs, only very small particles carrying, at the most, tens of organisms can reach this point, and the work of Wells, Ratcliff & Crumb (1948) confirms this in demonstrating how much more readily a pulmonary tuberculosis is developed in rabbits after inhalation of small numbers of tubercle bacilli, suspended as small particles, than by inhalation of far larger numbers carried on particles too large to pass the nose. On the other hand, when we come to consider the much commoner upper respiratory infections, their relatively higher incidence being in part a tribute to the filtering efficiency of the nasal passages, it may be that aggregates of potentially infecting organisms have a much higher chance of initiating an active infection than singletons or small groups. Aggregates of pathogenic organisms in dust may be derived from discharging suppurative lesions via dried dressings or skin, or various processes of tactile transfer, as well as from such activities as coughing, sneezing, spitting or talking.

Although there was no close correlation between the occurrence of *Staph. aureus* and β-haemolytic streptococci in portions of dust, the highest counts of both organisms sometimes occurred in the same portion. This is not unexpected, since

Table 8. *List of dusts examined with their bacterial content*

No.	Source	Date examined	Total organisms (1/20 dilution)	Log-median counts β-haemolytic streptococci (undiluted)	*Staphylococcus aureus* (undiluted)
1.	A flat in Harvard Hospital	15. iii. 48	2·26 (0·22)	None found	1·06 (0·67)
2.	A country hospital scarlet-fever ward	1. iv. 48	1·86 (0·34)	1·08 (1·21)	0·42 (0·96)
3.	As no. 2 after 6 days' storage in refrigerator	7. iv. 48	1·47 (0·34)	0·70 (1·08)	0·58 (1·20)
4.	A London elementary school classroom	12. iv. 48	1·02 (0·31)	None found	1·37 (0·85)
5.	A London elementary school classroom	12. iv. 48	0·55 (0·60)	None found	$\bar{1}$·90 (–)
6.	As no. 2	19. iv. 48	2·21 (0·40)	0·85 (1·42)	1·00 (1·00)
7.	A London hospital scarlet-fever ward	12. x. 48	1·82 (0·34)	$\bar{1}$·77 (1·12)	0·19 (1·13)
8.	Another London hospital scarlet-fever ward	20. x. 48	1·90 (0·23)	1·80 (0·90)	1·86 (0·76)
9.	As no. 7	10. xi. 48	1·89 (0·45)	1·00 (0·79)	0·52 (1·00)
10.	As no. 8	29. xi. 48	1·28 (0·27)	0·35 (0·70)	$\bar{1}$·59 (0·97)
11.	As no. 7 after 21 days' storage in the dark	1. xii. 48	1·65 (0·43)	0·65 (1·51)	0·37 (0·88)
12.	As no. 2	27. i. 49	1·98 (0·26)	0·36 (1·32)	0·96 (0·64)
13.	As no. 7	12. ii. 49	2·26 (0·30)	1·30 (0·63)	0·65 (1·10)

Samples were usually collected over several days and were several days more in transit or store before examination.

The standard deviations of the log-counts are given in brackets.

To convert to log-median counts per 10 mg. portion add 3·0 to the figures at 1/20 dilution and 1·70 to those for the undiluted suspensions.

a large proportion of both organisms found in the environment probably came from the nose (Moss, Squire & Topley, 1948; Hamburger & Green, 1946). In all the samples of dust from continuously occupied places examined *Staph. aureus* was present, often in enormous numbers. No *Staph. aureus* was found in a limited study of the dust of ten rooms which had not been occupied for some months at least. Extensive sampling would be necessary to confirm a suggestion that *Staph. aureus* may be useful as an index of human (or animal) pollution of dust. It is worth noting, by contrast, that very small numbers of *Staph. aureus* were found in air samples from occupied rooms.

Although carried out for a different purpose and by slightly different methods, the observations of Williams (1949) on the distribution of β-haemolytic streptococci in the floor dust of elementary schoolrooms and day nurseries are in substantial agreement with those reported here.

SUMMARY

The viable bacteria in 10 mg. portions of dust were estimated by shaking with 5 ml. of Ringer's solution in 1 oz. bottles containing glass beads. The extract was allowed to stand for a few minutes, and 0·1 ml. amounts were then plated out at suitable dilutions. Successive portions from the same sample of dust showed very

great variation in the bacterial counts, and this variation has been examined in series of twenty such portions. The distribution of the counts for three groups of organisms appears to approximate to a log-normal form, and the graphical estimate of the log-median is taken as a suitable parameter for the study of the influence of environmental factors on the survival of dust flora.

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