EXAMINATION OF FLOOR DUST FOR HAEMOLYTIC STREPTOCOCCI

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(With 3 Figures in the Text)

The epidemiological importance of contamination of floor and fabric dust with haemolytic streptococci has been stressed by many workers, but hitherto little work has been reported analysing the technique of collection or bacteriological examination of the dust. This paper records a study of methods suitable for use in epidemiological investigations; the study was made concurrently with that of Lidwell & Lowbury (1950) reported elsewhere, and differs from theirs chiefly in that the material has consisted of numerous small dust samples from many rooms, and that it has included a study of the serological group and type reactions of the streptococci.

There are two fundamentally different methods for the bacteriological examination of floor dust: (1) the dust may be collected, suspended in fluid, and then cultures made from the fluid; or (2) the dust may be transferred directly from floors and other sources to culture plates. When dust is to be collected at a distance from the laboratory there are clearly considerable advantages in the first method, and the work of Lidwell & Lowbury has shown that under ordinary circumstances haemolytic streptococci are unlikely to die off very rapidly in dry dust. Moreover, there is no difficulty in sampling dust from the whole floor of a room—a procedure that the present study has shown to be desirable. However, this first method has one great disadvantage, namely, that the suspension of the dust in the fluid almost certainly breaks up some of the bacterial clusters, and that to an unknown degree. The count derived from this method is probably, therefore, intermediate between a count of the total viable bacteria, and a count of the bacteria-carrying particles present in the sample. There would be obvious advantages in any method in which the dust was transferred directly to plates of solid culture medium, so yielding a true count of bacteria-carrying particles. It is, however, difficult to make a method of this sort convenient and accurate, if only because the very large number of bacteria-carrying particles present on most floor surfaces necessitates the collection of samples from very small areas of floor.

If the first method—the collection and subsequent cultivation of the bacteria-carrying dust—is adopted there appear to be two obvious ways of collecting the dust from the floor: (*a*) by sweeping, and (*b*) a 'vacuum-cleaner' type of instrument. If infection by haemolytic streptococci takes place by inhalation, the smaller dust particles, which can remain suspended in the air for some time, are presumably more important than the large particles, which fall rapidly to the floor. In this case the collection of the floor dust by an efficient suction device seems preferable to collection by sweeping, for in sweeping the small dust particles are distributed into the air and so are not collected, while it is only the larger particles that are not collected by suction. On the other hand, if contact with the floor is the more important mode of infection, collection of floor dust by sweeping may yield more relevant information.

METHODS

Collection of dust

In the present survey I have used three methods for the collection of floor dust: (1) a suction device referred to as the 'thimble sampler'; (2) sweeping with ordinary brooms; and (3) a device in which floor dust is blown directly on to the surface of a culture plate.

(1) *The thimble sampler.* This sampler (Fig. 1) was devised and constructed by Mr O. M. Lidwell, D.Phil. It consists of an aluminium cylinder 15 cm. long and 3·75 cm. in diameter, constricted to a tube 1 cm. outside diameter at one end, for connexion to a source of suction. Into the other end is screwed a nozzle, which is bored with a 1 cm. central hole and is turned at one end in the form of a cone. The collection 'bag' of the device is a paper Soxhlet extraction thimble (80 × 25 mm., double thickness), which is fitted over the cone on the inner end of the nozzle and is held firmly in place by pressure against a ring fixed to the inside of the barrel. A suction source giving an air flow of about 1 cu.ft./min. at 12 in. water gauge (such as a small domestic vacuum cleaner) is attached to the sampler. The barrel is then held vertically on

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the floor and moved about over the area to be examined. In routine work we have collected samples

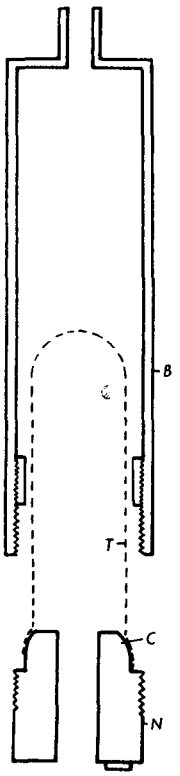

Fig. 1. Diagrammatic longitudinal section through the thimble sampler. B = cylindrical barrel with thread at lower end. N = nozzle screwing into barrel with cone (C) over which the thimble (T) can be fitted.

on areas roughly equivalent to one-quarter of the whole floor area of the room, and distributed as widely as practicable over it; owing to the small size of the nozzle only a small fraction of the dust in this area was collected. In schoolrooms this routine has commonly yielded about 1 g. of dust, which can be collected in a single thimble.

In a number of tests the thimble sampler has been found to pick up all visible particles present in samples of mixed house dust spread out over a flat surface; and an air sampler mounted in tandem with the dust sampler in the suction line showed that a negligible proportion of bacteria-carrying particles passed through the thimble. The sampler has been found convenient for collecting dust from fabrics—clothing, bedclothes, etc.—as well as floors. Before use the thimbles are wrapped in Kraft paper and sterilized by autoclaving; if necessary the hole in the nozzle can be sterilized with spirit between samples, though this was not done as a routine.

(2) *Sweeping.* The sample consisted of the whole of the dust collected during the daily sweeping of the floor of the room being examined. The brushes used for sweeping were not sterilized before use, but in no case was more than one sample examined from any one school on one occasion, and as some 20–50 g. of dust were collected it was considered unlikely that dust carried over on the brush would contribute greatly to the count.

(3) *The direct sampler.* This sampler was devised and constructed by Mr T. Nash, M.A., B.Sc., of this laboratory; with it an open Petri dish is held horizontally, agar surface down, about 3 cm. from the floor, and a 'puff' of air is projected obliquely on to the floor beneath the dish. Dust particles are blown off the floor and a proportion of them impinge on the agar and adhere to it. The proportion that is collected doubtless depends on many factors, such as the density and size of the particles, which are being studied. As a routine ten 'puffs' were used for one plate, each on a different area of floor.

Bacteriological examination of dust suspensions

Most of the work reported in this section was carried out on dust samples collected with the 'thimble' sampler; the technique used with the larger samples obtained by sweeping was substantially the same. The routine was as follows:

(1) The container and dust are weighed, the dust tipped into a stoppered bottle containing 10% broth-saline (50 ml. in a 200 ml. bottle for samples up to about 5 g., or 250 ml. in a 500 ml. bottle for larger samples) and the empty container weighed. (In six thimble samples examined, an average of 7 % of the weight of the dust collected was retained in the thimble.)

(2) The bottle is shaken vigorously for 2 min.; latterly a mechanical shaker was used, giving about 200 to-and-fro movements of 5 cm. pitch per minute.

(3) The bottle is spun lightly for 1 min. (The lowest speed on an 'International' centrifuge was used, the maximum reached being about 1000 r.p.m.)

(4) Using standard dropping pipettes (delivering 0·02 ml. drops) six drops of the supernatant suspension and six drops of a 1/10 dilution of the supernatant are plated on well-dried blood agar plates containing 1/500,000 crystal violet. The plates are rocked slightly to spread the drops over a greater area of agar, but not so much that the drops run together.

(5) After overnight incubation at 37° C. the colonies of presumptive haemolytic streptococci are counted, and a sample picked for group and type determination. To obtain strictly random samples, either all colonies are picked from a number of randomly chosen drop areas, or all the colonies from a sector of one randomly chosen drop area.

Effect of variations in bacteriological technique

The routine described is to a large extent arbitrary, but a number of tests have been made of various modifications. The results of these tests are described in some detail, and supported by statistical analysis, in the following paragraphs, since it is felt that such tests are of great importance in considering the implications of the results obtained in field studies to be reported elsewhere. The main findings may, however, be summarized briefly here. No noteworthy variation in the count of haemolytic streptococci was produced by slight variations in the time of shaking or of spinning; nor by leaving the suspension at room temperature for 2 hr., provided it was reshaken before plating. There was some indication that the counts were higher on surface-seeded plates than in pour plates.

In all the tests to be described the standard routine was followed except for the modification under test.

The addition of glass beads to the suspension made no obvious difference to the effect of shaking.

Time of spinning. In two experiments the suspension was divided into five portions and these portions spun 1, 2, ..., 5 min. before plating, the centrifuge being allowed to come to rest after each successive minute. The means of six drop counts from the five samples in the two experiments were: (i) 5·2, 3·3, 2·5, 4·0, 5·0; (ii) 11·3, 7·7, 8·3, 9·2, 7·7. Tested by analysis of variance, there is no significant difference between the successive counts, and it is evident, therefore, that within these limits the length of the period of spinning is not very critical.

Comparison of pour plates with surface inoculation. Fifty suspensions were inoculated, in 0·12 ml. quantities, into crystal-violet blood agar pour plates at the same time as the six drops (0·12 ml.) were seeded on to the surface of other similar plates. Twenty-four of the samples (col. 1–7, Table 2) had mean counts on the two plates of six colonies or

Table 1. *Counts of presumptive haemolytic streptococci, (a) before and (b) after an extra period of 1½–2 min. shaking*

No. of colonies per 0·02 ml. drop (mean of 6 drops)		Mean difference (d) between corresponding drops on a and b	S.D. of the array of six differences	$t=\frac{d\times\sqrt{6}}{\text{S.D.}}$
a	b			
5·3	7·2	1·83	3·55	1·26
8·7	11·5	2·83	7·06	0·98
86·2	91·6	5·50	16·05	0·84
0·8	1·5	0·67	2·16	0·76
1·0	1·5	0·50	1·76	0·70
64·5	67·0	2·50	12·60	0·49
55·8	55·5	—0·33	8·78	0·09
10·7	9·5	—1·17	4·58	0·63
15·7	12·3	—3·33	7·86	1·04
3·2	1·8	—1·33	3·00	1·09
1·7	0·3	—1·33	1·97	1·65
1·7	1·5	—0·17	1·72	2·42

$\Sigma t^2=15{\cdot}900$; $P=$ about 0·2.

Time of shaking. Dust suspensions were counted after (*a*) 1½–2 min. and (*b*) 3¼–4 min. shaking; twelve samples had a mean count of more than six colonies of presumptive haemolytic streptococci per plate (Table 1). There was no evidence that additional shaking materially increased the count, for in only half the samples was the second count greater than the first. Since the two series of suspensions were in each case seeded on to one set of six plates, the mean of the six differences between the suspensions *a* and *b* on the six replicate plates was tested for significant deviation from the expected value of zero. The values of t (column 5) indicate that only in one sample was the difference significant at the 5 % probability level ($t=>2{\cdot}2$). As an overall test for the absolute magnitude of the t's, Σt^2 was calculated; it did not differ significantly from the expected value, which is equal to χ^2 for 12 degrees of freedom.

more, and for these the mean ratio of surface count/pour-plate count was 1·52, suggesting that more streptococci were recognized on the surface plates than in the deep plates. To test the significance of this observation the differences between the counts by the two methods were calculated and expressed in terms of their standard errors (t, col. 5). If there were no real differences between the sets of counts, the frequency distribution of these values of t would be approximately normal, and tend to the 'expected' values in column 7. Although the number of samples is small, there was an excess of samples in which the surface plates gave a higher count than the pour plates; and this tendency is maintained when the counts of the forty-one samples yielding any number of streptococci are tabulated in the same way (cols. 8 and 9). Owing to the small numbers 'expected' for high values of t, a χ^2 test on the

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Table 2. *Comparison of surface count with pour plate count of presumptive haemolytic streptococci in dust suspensions*

No. of colonies from 0·12 ml. suspension					Frequency distribution of values of t				
					From samples given on left of table		From all samples (see text)		
Surface plate (1)	Pour plate (2)	Difference, S.−P. (3)	S.E. of diff.* (4)	t† (5)	Obs. (6)	Exp.‡ (7)	Obs. (8)	Exp.‡ (9)	t (10)
90	43	+47	11·5	4·1					
19	3	+16	4·7	3·4	3	0·05	3	0·1	>3
43	18	+25	7·8	3·2					
78	46	+32	11·1	2·9					
33	14	+19	6·9	2·8					
133	95	+38	15·1	2·5	4	0·55	6	0·8	2·1–3·0
177	135	+42	17·7	2·4					
48	32	+16	8·9	1·8					
77	58	+19	11·6	1·6					
20	12	+ 8	5·7	1·4	5	3·2	7	5·6	1·1–2·0
128	107	+21	15·3	1·4					
12	6	+ 6	4·2	1·4					
325	301	+24	25·0	1·0					
31	25	+ 6	7·5	0·8					
65	58	+ 7	11·1	0·6	5	8·2	10	14·0	0–1·0
72	65	+ 7	11·7	0·6					
15	12	+ 3	5·2	0·6					
27	28	− 1	7·4	0·1					
6	7	− 1	3·6	0·3					
91	95	− 4	13·6	0·3	4	8·2	6	14·0	0–1·0
254	264	−10	22·8	0·4					
5	12	− 7	4·1	1·7					
17	40	−13	7·5	1·7	2	3·2	7	5·6	1·1–2·0
81	122	−41	14·2	2·9	1	0·55	2	0·8	2·1–3·0
					0	0·05	0	0·1	>3·0
					24	24	41	41	

* Counts were assumed to conform to a Poisson distribution, and standard deviation calculated as the square root of the observed count.

† t = difference ÷ standard error of difference.

‡ Expected distributions calculated on the basis of a normal distribution of t.

frequencies as tabulated would be invalid. The frequencies for $t = > 1·0$ were therefore combined and the observed four frequencies of 16, 10, 6 and 9 compared with the expected frequencies of 6·5, 14·0, 14·0 and 6·5; the χ^2 value of 21·57 indicates a significant deviation ($P = < 0·01$) of the observed from the expected frequency distribution. It may be concluded therefore that, in this series, the surface plates differed significantly from the pour plates. An analogous result was obtained by Crone (1948) and by Reed & Reed (1948); but Snyder (1947) found pour plates to give the higher count. The surface-plating method is, of course, easier to perform than the pour plate, and offers much less difficulty in picking colonies for subsequent group and type identification.

Time of standing before plating. Suspensions were plated (*a*) immediately after spinning, and (*b*) after standing 2 hr. at room temperature in the light, being reshaken and spun before the second plating. The results from ten suspensions having a mean count of more than six colonies of streptococci per plate are set out as described in the previous section (Table 3). The variation in the differences exceeded that expected on a chance basis—in two samples the first count, and in one the second, showed a significant excess—but taken as a whole, there is no consistent evidence of rapid death or multiplication of the cocci in the suspension. Reshaking was omitted in a few other tests, and in these there was an indication that the second plating gave a smaller count than the first.

Effect of spreading drops with loop. In eight specimens, three of the six drops on the plate were spread over half the area of the plate with a loop; the total

Table 3. *Counts of presumptive haemolytic streptococci from dust suspensions (a) before and (b) after 2 hr. standing at room temperature*

No. of colonies per 0·02 ml. drop (mean of six drops)				
a	*b*	Difference, *a–b*	S.E. of diff.*	*t*
15·0	7·2	+7·8	1·52	5·1
3·2	0·2	+3·0	0·77	3·9
3·7	3·0	+0·7	0·42	1·7
11·0	9·8	+1·2	1·10	1·1
7·2	7·8	−0·6	1·58	0·4
2·0	2·2	−0·2	0·47	0·4
8·7	9·3	−0·6	0·94	0·6
5·2	6·2	−1·0	1·14	0·9
8·0	9·7	−1·7	1·52	1·1
2·2	4·5	−2·3	0·78	3·0

$\Sigma t^2 = 57{\cdot}02$; $P = < 0{\cdot}01$.

* Standard errors of the mean counts were here calculated directly from the pair of observed six-drop counts.

number of colonies on the spread area was compared with the total of the other three drops. The number of streptococci was small, and figures are therefore available only for counts of total colonies on serum agar plates set up in parallel. The mean ratio of drop/spread count was 0·93. The practice was stopped because it was found that spreading occasionally led to a single unobserved contaminant obscuring the whole count, whereas unspread it would at most have obscured one-sixth of the count.

Reproducibility of results

Once the dust has been shaken in the broth, the resulting suspension appears to be homogeneous. On general grounds, if the streptococci are distributed homogeneously, one would expect the individual drop counts in a sample to conform to a Poisson distribution. In 95 of 100 consecutive specimens yielding haemolytic streptococci the counts did show reasonable conformity with this expectation (Table 4).

As a test of the overall variability of the material the sum of the values of χ^2 from the 100 samples has been calculated. This should be distributed on 500 degrees of freedom. Tables of χ^2 are not available over this range, but as an approximate test we may calculate $\sqrt{(2 \times \text{total } \chi^2)}$, which is very nearly distributed normally about a mean of $\sqrt{(2 \times \text{total degrees of freedom} - 1)}$ and with unit standard deviation. When all the 100 counts are included this value differs significantly from expectation, indicating excessive variability; when, however, the five most variable counts are omitted, the remainder show no significant deviation from expectation. With one exception, the samples with excessive variability had mean drop counts of over 50.

Table 4. *Frequency distribution of values of χ^2 from the six replicate drops of 100 consecutive dust suspensions*

Range of values of χ^2	No. of counts with specified value of χ^2	
	Obs.	Exp.
0– 0·55	1	1
– 1·61	8	9
– 3·00	17	20
– 6·06	46	40
– 9·24	14	20
–15·09	8	9
15·10–	6	1

(*a*) On all observations:

$$\sqrt{(2 \times \text{total } \chi^2)} = 33{\cdot}95$$

$$\sqrt{[(2 \times \text{total degrees of freedom}) - 1]} = 31{\cdot}61;$$

difference = 2·34 (significantly excessive variability).

(*b*) Omitting the contribution to total χ^2 of the five most variable counts:

$$\sqrt{(2 \times \text{total } \chi^2)} = 30{\cdot}98$$

$$\sqrt{[(2 \times \text{total degrees of freedom}) - 1]} = 30{\cdot}81;$$

difference = 0·17 (not significant).

Replicate counts from one suspension show satisfactory agreement. Data from fourteen specimens in which two samples of the suspension were plated in parallel and which yielded an average of more than six haemolytic streptococci per six drops, were analysed (Table 5). There was no evidence of material differences between the counts, only one being significant at the 5% level, and the overall variation was well within the expected limits.

On the other hand, there were commonly great differences between the duplicate samples from the

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Table 5. *Replicate counts of presumptive haemolytic streptococci from one suspension of floor dust*

No. of colonies per 0·02 ml. drop (mean of six drops)		Mean difference (d) between corresponding drops on a and b	S.D. of array of six differences	$t=\frac{d\times\sqrt{6}}{\text{S.D.}}$
a	b			
19·7	17·0	2·67	6·09	1·07
6·7	4·5	2·17	5·56	0·96
7·7	6·2	1·50	4·43	0·83
2·2	1·7	0·33	1·21	0·67
4·5	3·8	0·67	3·21	0·51
2·5	2·1	0·33	1·63	0·50
278·2	276·2	2·00	62·57	0·08
293·2	296·8	−3·33	55·45	0·15
11·0	11·8	−0·83	6·34	0·32
1·2	1·3	−0·50	2·17	0·56
43·7	47·7	−4·00	11·22	0·87
0·7	1·3	−1·00	1·79	1·37
0·8	1·3	−0·83	1·17	1·74
1·5	2·0	−0·50	0·55	2·22

$\Sigma t^2 = 14{\cdot}749$; $0{\cdot}5 > P > 0{\cdot}3$.

Table 6. *Counts of presumptive haemolytic streptococci in duplicate dust samples (A and B) (colonies per mg. dust)*

'Thimble' samples from one area of floor about 3 sq.yd.		'Thimble' samples from total area of about 9–12 sq.yd.		'Sweeping' sample from whole floor divided into two samples	
A	B	A	B	A	B
7·0	2·2	0·4	1·6	0·3	0·2
15·9	28·1	0·9	1·3	0·8	2·8
17·0	60·5	1·9	1·6	4·3	93·4
341·9	20·5	9·9	125·5	19·4	27·7
—	—	13·1	30·8	27·8	4·7
—	—	31·6	5·1	58·5	47·0
—	—	66·0	119·3	—	—
—	—	231·0	69·0	—	—
—	—	428·6	178·6	—	—
—	—	591·0	739·7	—	—

same area, or different areas, of floor in a room, or from one sample of dust as collected by the thimble sampler or by sweeping (Table 6).

Simple comparison of the actual counts observed is not, however, very satisfactory owing to the nature of the frequency distribution of the counts (Fig. 2), which is very skew, with a preponderance of low counts, and very long 'tail' to the right, indicating a small proportion of very high counts. This means that an arithmetic average of a series of counts is liable to be unduly affected by the inclusion of a single very high count. Data of this sort are more conveniently dealt with when the logarithms of the counts are used; and in fact the logarithms of these counts are approximately normally distributed (Figs. 2, 3), i.e. the distribution is of the 'log-normal' type. Analysis may conveniently be carried out after transforming the counts by taking $\log_{10}(x+1)$, where x is the observed count, to allow inclusion of zero counts (Bartlett, 1947); or by graphical methods, as has been done by Lidwell & Lowbury.

Number of colonies to be picked for grouping and typing

Four colonies of presumptive haemolytic streptococci were picked at random from each of 185 specimens (Table 7); in 69 % of the specimens all four colonies proved to be either of the same group or of none of the three groups for which the strains were tested. More than four colonies were examined in a further twenty specimens (Table 7), and this sometimes led to the discovery of a second variety that was not recognized among the first four examined; nevertheless all the colonies were of the same group in ten, and all but one in a further four.

In routine practice, however, the examination of more than about four colonies may be impracticable, and it is perhaps of more interest to note the significance of the reaction of one or two colonies in indicating the group of the majority. This has been estimated from the results of the 185 specimens (first half of Table 7), with two assumptions: (1) that

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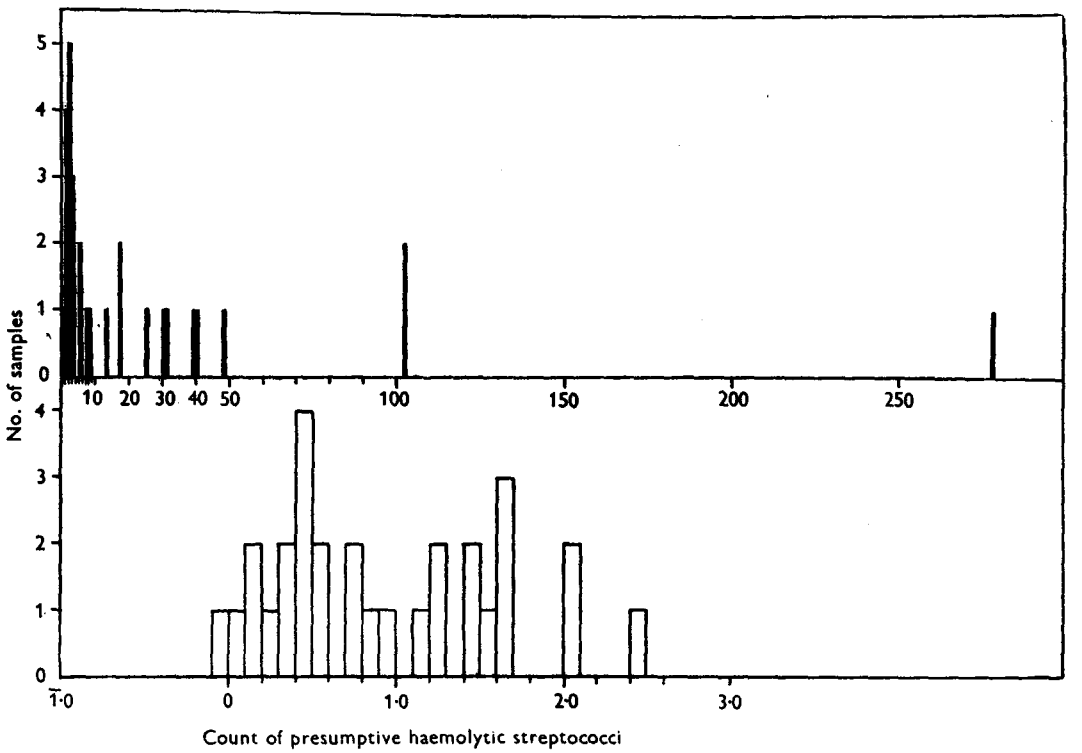

Fig. 2. Frequency distribution of counts of haemolytic streptococci in thirty positive samples of floor dust from schoolrooms. Ordinates = number of samples. Abscissa = count of presumptive haemolytic streptococci: above in number of colonies per mg. dust; below in logarithms of number of colonies per mg. dust.

the various frequencies observed are a valid estimate of the proportion in an unlimited population, and (2) that the estimates obtained from four strains are as satisfactory as those obtained from a greater number. Then, if a single colony is picked and proved to be group A, the probability that the remaining colonies are also group A is about 80 %. Similarly, if two colonies are picked and both prove to be A there is a probability of about 87 % that all the rest will also be group A.

With type reactions, the situation is complicated by the fact that some 16 % of the strains were untypable, and since most of the strains could be typed only by the agglutination method, the type reactions were not always clear-cut. In some cases strains have been classed as of one type when they gave slightly different, but closely related, agglutination reactions. The trend was similar to that noted for the group reactions (Table 8). In fifty-two (55 %) of ninety-four specimens where four strains were submitted for typing, all four strains proved to be of the same type; none of the four was typable in a further 5 %. More than one definite type was found in nine (20 %) of the specimens. Clearly it is more important with typing than with grouping to examine a number of strains, but with the assumptions made above it can be calculated from the figures in col. 4 of Table 8 that if, in a specimen yielding four group A strains, one of these strains is found to be a particular type, the probability is about 71 % that all four are the same type; if two strains are found to be the same type, the probability that all are the same is around 80 %.

RESULTS OBTAINED IN ROUTINE INVESTIGATIONS

Investigations of the streptococcal content of floor dust in elementary schoolrooms and day nurseries have been carried out as a routine over the past two years in connexion with a survey of the nose or throat carrier state of the children. This work cannot yet be reported, but one fact of interest has emerged: group A haemolytic streptococci were recovered from the floor dust of 50 % of sixty different rooms; and in none of these rooms was there a history of clinical streptococcal infection among the children. The mean count, estimated from the mean of the values of $\log_{10}$ (count per mg. + 1) was 2·32 per mg.; the range was from 0 to 278 per mg.

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Table 7. *Group reactions of streptococci in cultures of floor dust*

No. of colonies picked	No. of colonies of group A	C	G	Not A C or G	No. of samples		Percentage of the 185 samples in which four colonies were tested
4	4	0	0	0	124		67·1
	3	1	0	0	2		
	3	0	1	0	1	24	13·0
	3	0	0	1	21		
	2	2	0	0	4		
	2	0	2	0	1	18	9·7
	2	1	0	1	1		
	2	0	0	2	12		
	1	3	0	0	1		
	1	1	0	2	3	13	7·0
	1	0	3	0	1		
	1	0	0	3	8		
	0	1	0	3	1		
	0	2	0	0	1	6	3·2
	0	4	0	0	1		
	0	0	0	4	3		
5	3	0	0	2	1		—
6	6	0	0	0	2		—
	4	0	0	2	2		—
	2	0	0	4	1		—
7	7	0	0	0	1		—
	6	0	0	1	1		—
	0	6	0	1	1		—
8	8	0	0	0	3		—
9	9	0	0	0	1		—
	8	0	0	1	1		—
	0	0	0	9	1		—
10	10	0	0	0	2		—
	9	0	0	1	1		—
	7	0	0	3	1		—
16	16	0	0	0	1		—

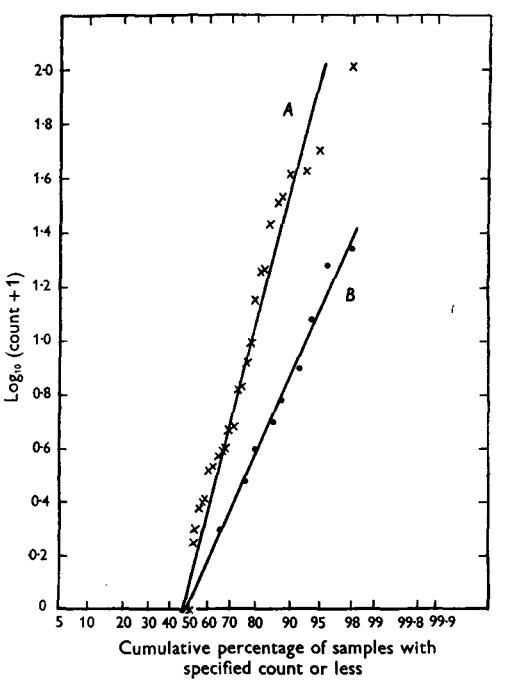

Fig. 3

Eighteen of the rooms were sampled by sweeping the floor and forty-two by the thimble sampler. The number of negative samples in the two sets of rooms was eight (44 %) and twenty-two (52 %) respectively; the mean counts were 3·56 and 1·88 streptococci per mg. The larger sweeping samples were positive rather more often than the thimble samples; this may reflect their greater size, or some contamination of the sample with streptococci carried over from another room. The number of rooms is small in relation to the range of counts observed, and, moreover, the schools were in different districts, so that no great importance can be attached to the difference.

Fifty rooms were sampled by the direct method, in which the number of streptococcal colonies indicates the number of infected particles impinging on

Legend to fig. 3

Fig. 3. Frequency distributions of counts of presumptive haemolytic streptococci in (*A*) thimble- or sweeping-samples from sixty different schoolrooms (same data as Fig. 2); and (*B*) 'direct' samples from fifty different schoolrooms and day-nursery rooms, plotted on 'probability' paper so that the points of a normal frequency distribution lie on a straight line. The unit of the count for *A* is colonies per mg. dust; and for *B* colonies per plate.

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the agar. The number of presumptive haemolytic streptococci on one plate from each of these rooms is plotted in Fig. 3. It will be seen that these counts fit a log-normal distribution better than those from the previous samples.

Table 8. *Type reactions of samples of four colonies of* Str. pyogenes *from ninety-four cultures of floor dust*

No. of colonies: Type A*	Type B*	Not typable	No. of specimens	Percentage of 94 specimens
4	0	0	52	55·4
3	1	0	8	8·5
3	0	1	9	9·4
2	2	0	4	4·3
2	1	1	4	4·3
2	0	2	6	6·4
1	1	2	3	3·2
1	0	3	3	3·2
0	0	4	5	5·3

* 'Type A' or 'type B' mean any particular but different types of streptococcus.

DISCUSSION

No originality is claimed for the bacteriological methods adopted in this work; but statistical analysis of the results obtained by their use on a considerable number of occasions gives a more precise idea of the limitations of the methods than has previously been available. The study has been concentrated on the internal consistency of the methods, and no formal comparison of the collection techniques has been attempted, partly because it is felt that practical considerations will generally determine which is used. There is no evidence that the results obtained from sweeping samples differ greatly from those obtained with the thimble sampler; but it is clearly difficult to make any direct comparison of these two methods with that of the direct sampler. It is planned to make some observations on this point subsequently.

One of the most striking features of the results obtained has been the lack of homogeneity in specimens of dust from one area of one room, an observation amply confirmed in the concurrent investigations of Lidwell & Lowbury. This has an important practical implication: in routine examinations of floor dust a true estimate of the degree of contamination with haemolytic streptococci will only be obtained by taking large samples. The heterogeneity in different floor areas also means that the sample should preferably be from the whole floor area, e.g. from a whole room or from all the area beneath and around a bed in a hospital ward; and that all the dust collected should be examined.

It is evident both from the results presented in this paper, as well as from those of Lidwell & Lowbury, that the frequencies of the counts of micro-organisms in floor dust are distributed in an approximately log-normal fashion (Figs. 2, 3). This appears to be true both of subsamples from one collection of dust from the floor of one room, and also of samples from different rooms. It was thought that this frequency distribution, with occasional samples giving exceedingly high counts, might reflect the presence in the floor dust of some large aggregates of streptococci that are broken up when the dust is suspended in fluid. The experience with direct sampling suggests, however, that this explanation is inadequate since the 'particle counts' obtained in this way had a distribution of the same sort; in fact they fit a log-normal distribution better than the earlier counts from the thimble or sweeping samples.

Two superficially plausible explanations can be suggested which would account for the log-normal distribution of counts in random sampling of a small area in each of a series of rooms (Fig. 3). It might be that, in rooms containing a carrier dispersing streptococci from one position, the number of infected particles on unit areas of floor decrease logarithmically with the distance from the carrier. Or it might be that following an initial, even, contamination of the floor, the count decreases logarithmically with time. The general form of the distribution would not be changed if the two possible causes were both acting. Some few indications have been obtained supporting the idea that floor-dust contamination is commonly localized to particular areas of floor.

The observation that the distribution of haemolytic streptococci in subsamples from a sample of the dust from the whole of one room is also of the log-normal type might be reconciled with these explanations if, in the neighbourhood of the carrier, there are present not only a large number of particles, but also a greater proportion of large particles. Such large particles, being heavier than others, would fall more rapidly to the floor and so could not be dispersed far from the carrier; they might also disintegrate in broth to yield a greater number of streptococci than the smaller particles. The collection and mixing of a sample of dust from the whole floor would yield a specimen containing particles of all sizes, and subsamples from it might well show the log-normal type of frequency distribution. Investigations into these points are continuing.

The fact that, in some 55 % of specimens, all of four group A streptococci examined gave identical typing reactions, or were untypable, suggests that the floor contamination is commonly from a single source. It may be noted that none of the specimens came from rooms in which an outbreak of clinical streptococcal infection had been observed; repeat

specimens (at 2–3 week intervals) from single classrooms were included. The relation of this dust contamination to the presence of carriers among the schoolchildren will be the subject of a separate paper.

With the bulk samples the results have been expressed as the number of presumptive or proven haemolytic streptococci per milligram of dust, with a note of the weight of dust collected. The use of the milligram rather than the gram has the advantage that the figure given is then generally of the same order as the number of colonies counted on the plate. The reference of the count to the weight of the dust rather than to the area of floor sampled may be questioned; but it has been our routine to sample approximately the same area of floor on each occasion, and we have thought that the amount of dust collected has reflected variations in the assiduity of collection as much as variations in the amount of dust present.

Lastly, the concentration of streptococci in the suspension has to average at least 10 per ml. (equivalent to 1 per mg. in 500 mg. specimen) before they are recognizable by the technique outlined; and at such low concentration false negative counts will commonly be observed. In view of the fact, however, that some 50% of dust specimens from normal schoolrooms have yielded some haemolytic streptococci, and only 25% of them had counts of more than 10 per mg., it is clear that low counts can be of no great epidemiological significance.

SUMMARY

Floor dust has been collected for bacteriological examination by three methods: (1) with a miniature vacuum cleaner in which the 'bag' consists of a paper Soxhlet extraction thimble, (2) by sweeping, and (3) by blowing the dust on to the surface of a culture plate. The dust from the first two methods was shaken with broth and an aliquot sample plated out on crystal-violet blood agar plates.

Surface inoculation of the plates was found to give somewhat higher counts than the use of pour plates, but otherwise minor variations in the routine were without great effect on the count.

Analysis of the results obtained in examining a number of specimens from schoolrooms and day nurseries showed that, though the counts of streptococci in successive samples from one dust suspension agreed well, there was wide variation between the counts from duplicate samples of dust, however collected; the frequency distribution of the counts in rooms was of the log-normal type. It is clear that in routine work, all the dust collected from the whole floor of a room should be examined, in order to give the most reliable result.

Of 185 specimens in which four colonies of haemolytic streptococci were grouped, 67% had all four strains of group A. Of ninety-four samples in which four group A strains were typed, 55% had all four strains of the same type; more than one type was found in 20%.

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REFERENCES

BARTLETT, M. S. (1947). The use of transformations. *Biometrics*, **3**, 39.

CRONE, P. B. (1948). The counting of surface colonies of bacteria. *J. Hyg., Camb.*, **46**, 426.

LIDWELL, O. M. & LOWBURY, E. J. (1950). The distribution of bacteria in floor dust. (In the Press).

REED, R. W. & REED, G. B. (1948). Drop plate method of counting viable bacteria. *Canad. J. Res.* (Sect. E), **26**, 317.

SNYDER, T. L. (1947). The relative errors of bacteriological plate counting methods. *J. Bact.* **54**, 641.

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