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The size distribution of airborne particles carrying micro-organisms

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The ability of a particle to remain airborne, its ability to pass through filters, the site at which it may be deposited in the respiratory tract and the rate at which it will be removed from the air by sedimentation are all dependent on the size and density of the particle. In the course of a variety of investigations we have determined the size distribution of particles carrying various species of bacteria and fungi, using the size-grading slit-sampler described by Lidwell (1959). A few of the results obtained have already been quoted in part, but the majority have not been published previously.

The air sampler used separates the airborne particles into four size ranges, each of which is deposited on the surface of the agar medium contained in one of four 6 in. Petri dishes. This apparatus is constructed so that the air sample, entering through a slit 7 mm. wide, impinges on to the surface of the first Petri dish at such a velocity that only the larger particles, i.e. those having an equivalent particle diameter† greater than about 18μ, are deposited. The air stream carrying the smaller particles is then caused to impinge in turn on to the surface of the three remaining Petri dishes, each time at an increased velocity and through a narrower slit, so that the minimum particle size for 50% deposition is about 10μ for the second dish, 4μ for the third and less than 1μ for the fourth and last. When the plates have been incubated the colonies found will be derived from organisms which entered the sampler carried on airborne particles corresponding approximately to the four size ranges, greater than 18μ, between 18 and 10μ, between 10 and 4μ and less than 4μ. These size limits correspond to the value of equivalent particle diameter for 50% deposition so that there is, in fact, a considerable size overlap between the fractions. In spite of this, however, reasonably good estimations of the particle size distribution within the sample and hence of the median equivalent particle diameter and of the dispersion, expressed either as an interquartile range or, if appropriate, as a standard deviation, can be made by plotting on probability paper the cumulative fraction oversize against the 50% collection limits, namely 18·2, 9·6 and 4·2μ. As there are internal losses in the instrument it is necessary to correct the numbers of colonies counted in the later stages in order to arrive at a good estimate of the size distribution in the original sample. The numbers found on

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† The equivalent particle diameter is the diameter of a sphere of unit density which has a settling rate in air equal to that of the particle in question.

plates 2, 3 and 4 should be multiplied by 1·10, 1·20 and 1·25, respectively. These factors are derived from experimental observations.

CULTURAL METHODS

Samples for *Staphylococcus aureus* were collected on nutrient agar containing 5 % horse serum and 0·1 % phenolphthalein-phosphate (Barber & Kuper, 1951). Phosphatase positive colonies were tested for coagulase. Counts of total flora and of aerobic spore-bearing bacilli were made on the same medium but the phenolphthalein-phosphate was sometimes omitted.

Streptococci were grown on serum-sucrose agar containing tellurite and crystal violet (Williams & Hirch, 1950). The levan-producing colonies of *Str. salivarius* (Williams, 1956) were recognized by their colonial appearance and counted after 40–48 hr. incubation at 37° C. The other streptococci and the enterococci were estimated by picking a random sample of colonies from these plates and examining them by methods previously described (Air Hygiene Committee, 1954, Routine C, pp. 52–4).

Samples for *Clostridium welchii* were collected on a modified Nagler medium containing neomycin. Those colonies showing zones of serum opacity were regarded as *Cl. welchii* (Noble, 1961).

Sabouraud's dextrose agar containing antibiotics to suppress bacterial growth was used for isolating fungi. Cultures were incubated for 3 days at 37° C. for the *Aspergilli* and the *Candida* species and up to 3 weeks at room temperature for the remaining fungi. When sampling for *Candida albicans* or the dermatophyte fungi, Actidione (0·5 g./l.) was added to the medium.

RESULTS

The values deduced for the median equivalent particle diameters and for the inter-quartile ranges are given in Tables 1 and 2. These were obtained in the way described previously (Lidwell, 1959). Estimates of the inter-quartile ranges, the limiting diameters defining the 25 % smallest and the 25 % largest particles, are given, rather than standard deviations of the diameter, since the forms of the distributions are not known. In most cases an arithmetic-normal distribution appeared to fit the data reasonably well, where the median equivalent particle diameter was greater than 10μ; if the median diameter was smaller than this a log-normal distribution usually appeared the better. We have, however, no information about the tails of the distribution except that these must be truncated at or above about 1μ for the bacteria and at sizes corresponding to that of the single cells for the fungal species. Some environmental factors can be seen to affect the recorded values of median equivalent particle diameter. Ventilation preferentially removes the smaller particles so that the median diameter normally becomes greater when the ventilation is increased. The staphylococcal samples show a small but definite increase in median diameter with increasing activity during the sampling period. A similar, and partly associated, variation with the amount of air contamination is illustrated in Fig. 1.

Table 1. *Airborne bacteria*

Species	Place	Activity, etc.	Colonies counted	Median equivalent diameter of the airborne particles (μ)	Inter-quartile range (μ)
Total aerobic flora (grown at 37° C.)	Offices	Low ventilation	> 15,000	7·7	4–11
	Offices	Good ventilation	> 8,000	10·0	5–15
	Hospital wards	Moderate	> 50,000	12·8	7–18
	Hospital wards	Considerable	> 30,000	13·0	8–18
	Operating rooms	Unoccupied	> 30,000	12·3	7–18
Total mouth streptococci	Offices	Low ventilation	> 800	10·0	4–16
	Offices	Good ventilation	> 300	12·4	6–18
Streptococcus salivarius	Offices	Low ventilation	> 500	11·0	4–18
	Offices	Good ventilation	89	14·4	7–(22)
Beta-haemolytic streptococci	Offices	Low ventilation	29	11·7	8–15
	Offices	Good ventilation	22	12·5	8·5–16·5
Enterococci	Offices	Low ventilation	83	11·0	6–16
	Offices	Good ventilation	50	10·8	4–17
Staphylococcus aureus	Hospital wards	Light–moderate	> 6,000	13·3	8–18
	Hospital wards	Bedding disturbed	> 7,000	14·8	10–(19)
	Hospital wards	Bed-making	> 2,000	15·7	11–(20)
Bacillus sp.	Hospital ward	Moderate	> 300	(3·0)	(?)–8
Clostridium welchii	Outside air	Wet weather	186	11·0	5·5–16·5
	Outside air	Dry weather	> 500	17·2	10–(24)
	Hospital wards	Moderate (wet weather)	299	11·4	4–18

The inter-quartile range is given as the limiting diameters defining the 25% smallest and the 25% largest particles. Where the estimated diameters are below 4μ or above 18μ the values depend on extrapolation and have been given in brackets to indicate the greater possibility of error.

The samples of air from the offices were obtained in several different rooms of a large group of offices over a period of months.

The hospital samples came from a number of different hospitals.

Table 2. *Airborne fungi*

Species	Place and activity, etc.	Colonies counted	Diameter of spore or single cell (μ)	Median equivalent diameter of the airborne particles (μ)	Inter-quartile range (μ)
*Aspergillus fumigatus**	Hospital ward, moderate	> 100,000	2·5–3·5	(3·0)	[3–3]
Penicillium spp.	Hospital ward, moderate	> 750	2·5–4·5	(3·1)	[2–4]
Paecilomyces spp.	Hospital ward, moderate	105	(2·5–3) × 6	(3·4)	(2)–6
Rhodotorula spp.	Hospital ward, moderate	> 4,000	(3–5) × (4–7)	(3·8)	[3·3–4·3]
Aspergillus spp.†	Hospital ward, moderate	159	2·5–4	4·3	(2)–8
Cladosporium spp.	Hospital ward, moderate	> 2,500	(2–6) × (3–20)	4·9	(3)–7
Aspergillus niger	Hospital ward, moderate	125	2·5–10	5·5	(3)–9
Syncephalastrum spp.	Hospital ward, moderate	240	(2·5–5) × (5–20)	6·6	4–10
Rhizopus spp.	Hospital ward, moderate	45	3 × (6–9)	6·8	4–10
Monilia sitophila	Hospital ward, moderate	74	3 × (3–10)	9·5	7–13
Didymocladium spp.	Hospital ward, moderate	> 500	5 × (10–15)	10·7	7–15
Candida albicans	Hospital ward, moderate	67	(3–6) × (3–12)	13	5–(21)
Trichophyton mentagrophytes‡,§	Clinic, scraping and examination of skin	> 300	2 × 4 (*a*) (4–6) × (10–50) (*b*)	16	11–(22)
Rhodotorula spp.‖	Clinic, scraping and examination of skin	118	(3–5) × (4–7)	18	11–(26)
Epidermophyton floccosum§	College dormitories, little	18	(6–10) × (20–30)	19	12–(26)
E. floccosum	Clinic, scraping and examination of skin	172	(6–10) × 20–30)	(21)	15–(26)
Candida albicans	Clinic, scraping and examination of skin	> 1,000	(3–6) × (3–12)	(22)	18–(27)

The inter-quartile range is given as the limiting diameters defining the 25% smallest and the 25% largest particles. Where the estimated diameters are below 4μ or above 18μ the values depend on extrapolation and have been given in brackets to indicate the greater possibility of error involved. Substantial correction has had to be applied to the values of the quartiles for *Asp. fumigatus*, *Penicillium* spp. and *Rhodotorula* spp., found in hospital wards, on account of the diffuse cut-off of the stages in the instrument, and these are enclosed in square brackets. Where the spore or cell concerned is markedly non-spherical the approximate ranges of both the shortest and the largest axes are given in brackets, linked by a multiplication sign. In the case of *Trichophyton mentagrophytes*, (*a*) refers to the microconidia and (*b*) to the macroconidia.

* In seven separate evaluations the estimated median equivalent diameters ranged from 1·8 to 4·1 with an indication that the value tended to be larger in the winter.

† Including *Asp. gracilis*, *Asp. nidulans*, *Asp. terreus* and *Asp. versicolor*.

‡ This was derived from examination of an artificially contaminated arm.

§ Spores of these organisms were never seen in material obtained directly from patients. Only mycelium could be seen in the infected skins.

‖ This organism is not known to have been associated with any patient.

The hospital ward samples were obtained in various places on different occasions.

In comparing the figures for median equivalent particle diameter given here with those deduced from the ratio between volumetric and settling counts (Bourdillon, Lidwell & Lovelock, 1948) it must be remembered that these latter are primarily estimates of the mean settling rate, i.e. of the mean-square diameter. The relation between this quantity and the median diameter as determined here or by data

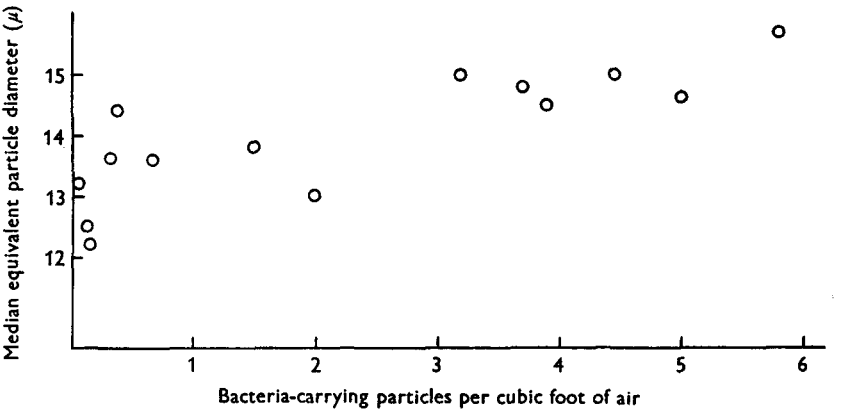

Fig. 1. Median diameters of airborne particles carrying *Staphylococcus aureus*.

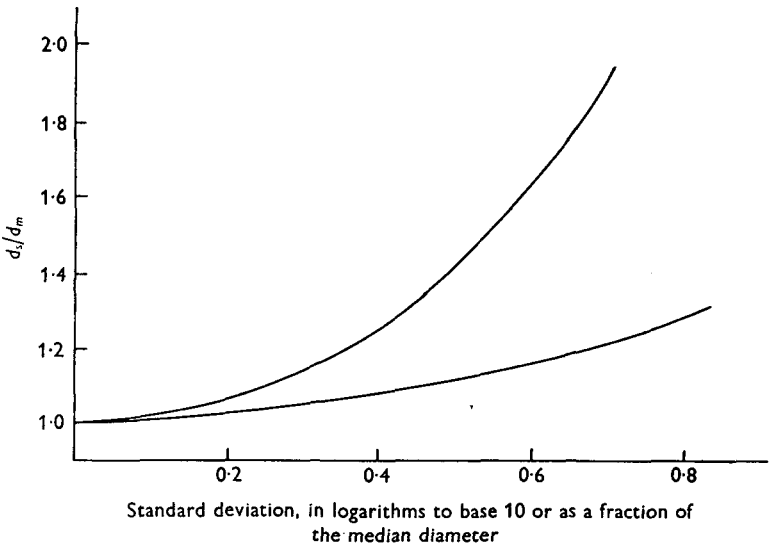

Fig. 2. Relationship between mean settling diameter, d_s, and median diameter, d_m. The upper of the two curves refers to log-normal distributions. The arithmetic-normal distributions, lower curve, are truncated at zero diameter and their standard deviations are expressed as a fraction of the median diameter.

obtained in a similar way, e.g. samples taken with the Andersen or Batelle samplers (Andersen, 1958; Mitchell & Pilcher, 1959), depends on the distribution of particle size in the sample. Fig. 2 gives values for the ratio of the two quantities for both arithmetic-normal and log-normal distributions over a range of values of the standard deviations of these distributions.

The following example illustrates these points.

At the same time as the samples of total flora were collected in the operating theatres with the size-grading sampler (see Table 1), settling plates were also exposed. The mean rate of settling over the whole series of experiments was 5·89 colony-forming particles per minute per square foot of surface exposed. The mean volumetric count over the same period was 4·90 cu.ft. of air sampled. This corresponds to a mean settling rate for the particles concerned of $5{\cdot}89/4{\cdot}90 = 1{\cdot}20$ ft./min. Using the figure of $0{\cdot}006d^2$ for the settling rate in feet per minute of a unit density particle d microns in diameter this corresponds to a mean equivalent settling diameter of $(1{\cdot}20/0{\cdot}006)^{\frac{1}{2}} = 14{\cdot}2\mu$.

The median equivalent diameter of the particles as determined by the size-grading sampler was $12{\cdot}3\mu$ and the distribution conformed well to a normal distribution in the arithmetic value of the diameter with a standard deviation of $8{\cdot}0\mu$. The ratio of this standard deviation to the median diameter is 0·65 and from the lower curve in Fig. 2 this would correspond to a median settling diameter 1·18 times the median diameter, i.e. $12{\cdot}3 \times 1{\cdot}18 = 14{\cdot}5\mu$, which agrees closely with the estimate obtained above by direct comparison of the settling and the volumetric counts, namely $14{\cdot}2\mu$.

The estimated standard deviations of the size distributions of the airborne bacteria-carrying particles which we have so far examined have almost always fallen within a fairly narrow range, about 0·3 for a log-normal distribution to the base 10 or about 0·6 times the median diameter for arithmetic-normal distributions. For standard deviations of this magnitude the mean settling diameter is about 15% greater than the median diameter and this value may be used when comparing particle diameters estimated by the two methods where detailed information on the size distributions are not available.

DISCUSSION

The most striking fact revealed by these data is the size of the median equivalent diameter associated with almost all the bacterial species and with those fungi that are probably derived from a human source, that is, the dermatophyte species, *Trichophyton* spp. and *Epidermophyton* spp. and *Candida albicans*. These diameters, which are much greater than the dimensions of the microbial cell, imply that the organisms are usually disseminated into the air in association with material derived either from the menstruum in which they originally multiplied or from some intermediate resting place. There is some evidence (Williams, Lidwell & Hirch, 1956) that the streptococci are derived directly from the mouth and that the particle therefore largely consists of dried saliva. Davies & Noble (1962) have recently presented evidence that many, if not most, staphylococci are present in the air attached to skin scales which could account for the observed diameters. It also seems likely that infected skin scales are responsible for the dissemination of the dermatophyte fungi. The size distribution of airborne particles carrying micro-organisms is determined by two opposing factors, gravity, which tends to eliminate the large particles, and the chance that a particle will carry a viable organism, which is likely to increase with the size of the particle. These factors combine to

confine the distribution within a relatively narrow range over the main part of which, at any rate, the distribution approximates closely to the arithmetic normal.

In contrast many fungi such as the aspergilli, penicillia, cladosporia, among those included in the observations reported here, possess a mechanism for direct dispersal of their spores into the atmosphere from their natural growth sites. This is reflected in the close correspondence for many of these species between the median equivalent particle diameters found for the air sample and the size of a single spore. Within the observed portion these distributions approximate to a log-normal form.

The most marked exception to the general pattern described above is the particle diameter associated with the aerobic spore-bearing bacilli. This is not much larger than the single cell although there is a very wide spread of particle size. It is possible that this is a result of the capacity of these organisms to multiply in extremely dilute media, in which sporulation commonly occurs, so that when the spores are dispersed by mechanical action they are only very loosely bound together and embedded in only small amounts of dried material.

SUMMARY

Values are given for the median equivalent diameters and for the inter-quartile range, of airborne particles carrying a variety of micro-organisms.

Organisms associated with human disease or carriage were usually found on particles in the range 4–20μ equivalent diameter.

Many fungi appeared to be present in the air as single spores.

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