

A simplified application of the Andersen sampler to the study of airborne fungus particles

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An application of the Andersen sampler to the study of potentially allergenic, culturable, airborne fungus particles is described. For this purpose, a highly modified technique with a single culture plate beneath stage 6 (S_6 arrangement) was evaluated and found to produce a "telescoped" collection qualitatively comparable with growth obtained by conventional 6 plate arrays. The S_6 arrangement also appeared to yield quantitatively valid results if optimally short sampling periods were chosen. Up to 3 per cent of particles, capable of growth on the media used, were noted to bypass the S_6 plate after entering the intake orifice; however, these losses were not necessarily related to particle size. Significant impaction of particles upon sampler hardware was not suggested, and withdrawal of plates from the upper stages appeared to obviate adventitious deposition at those points. Increased particle recoveries were obtained when the sampler was made wind oriented, with the advantages being shown most clearly for genera having relatively large spores. Qualitative comparisons between collections made simultaneously by the open-culture plate method and by the wind-oriented Andersen sampler confirmed the latter's major theoretical advantage in recovering small-spored genera.

Airborne emanations of fungi may be classified according to several characteristics that determine their accessibility to study by specific sampling methods; these attributes include relative aerodynamic size, viability, uniqueness of microscopic appearance, and capacity to grow and sporulate on various laboratory media. At present, most available data are restricted to forms capable of growth on synthetic media and are derived from the exposure of open culture

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This work was supported by Research Grant AP-00001 from the National Air Pollution Control Administration, Consumer Protection and Environmental Health Service, United States Public Health Service.

Received for publication March 5, 1969.

plates. Unfortunately, even for these types, the familiar limitations of gravitational collection¹ are highly significant. Commonly encountered spores and hyphal fragments comprise a wide range of sizes; and, since small particles are recovered poorly by sedimentation and turbulent impaction, they tend to escape collection, leaving larger types to dominate the "catch." These unequal losses may be augmented by eddies produced when moving air flows past the vertical sides of Petri dishes, an effect that varies markedly with changes in atmospheric motion. However, even for particles of equivalent aerodynamic size, concentrations per unit volume of air, suitable for spatial and temporal comparisons, cannot be deduced from open-plate samples.

To provide regional data to which clinical observations might be related with greater confidence, a volumetric study of airborne fungus particles at Ann Arbor was begun in 1964. For the cultural phase of this work, the Andersen sampler² has been of considerable value, confirming a prediction of Gregory.³ Although, initially, this device was used in the conventional fashion, an attempt was later made to simplify the procedure in accord with our specific goals. Brief allusion to this modification has been made elsewhere¹; however, in view of its usefulness where facilities for processing samples are limited, a fuller exposition of our experience with the abridged technique seems pertinent.

MATERIALS AND METHODS

The Andersen sampler used was the Model 0101 (Andersen Samplers and Consulting Service, 1074 Ash Avenue, Provo, Utah). It consists, essentially, of a cone-shaped entrance orifice, a nesting series of 6 sieve plates, and a basal plate permitting attachment to a source of suction (Fig. 1). All components are clamped together into an airtight sequence during operation by spring-loaded hooks. Each sieve plate (stage) contains 400 holes of defined size which serve

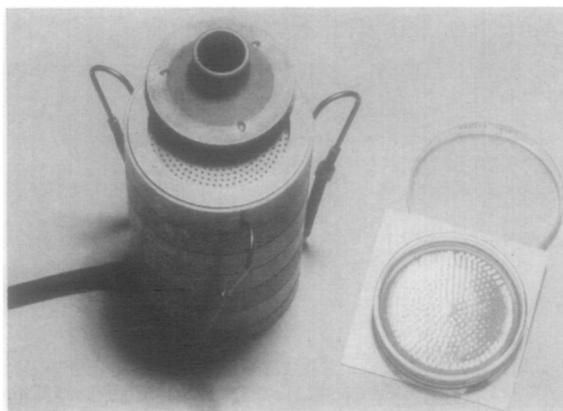


Fig. 1

The Andersen sampler partially disassembled to show the entrance cone, the first sieve plate (stage), and the retaining hooks. An exposed plate, shown in the foreground, demonstrates a pattern of shallow depressions formed by air jets impinging on the semisolid medium.

to impinge particle-bearing air upon the surface of semisolid culture medium in a plate positioned below. Since the diameter of the holes diminishes from stage 1 (0.0465 inch) to stage 6 (0.010 inch), the velocity of air flow increases as the sampler is traversed and the momentum of any single particle grows progressively. For this reason, the largest particles impinge on the surface of medium in the first plate, while smaller ones escape and pass on until, at a lower stage, they are accelerated sufficiently to effect their deposition. When 6 plates are used, the particle collection is separated into 6 size-related categories.

Special flat-bottomed Petri dishes, obtained from the supplier of the sampler, were filled with 27 ml. portions of medium with the use of a warmed, pre-calibrate, wide-mouthed pipette and a Propipette attachment (Arthur H. Thomas, Philadelphia, Pa.). Upon cooling, the unexposed dishes were inverted and stored at 4° C. in stainless steel Petri dish canisters to which several drops of a volatile fungicide, propylene oxide, were periodically added.⁴

The primary sampling medium employed in these studies was prepared from commercially obtained potato dextrose agar, dehydrated, Bacto (Difco Labs., Detroit, Mich.), with the use of 42 Gm. of the dried material per 1000 ml. of water. To each liter of this medium (PDA), 30 mg. of the dye, rose bengal, a bacteriostatic agent capable of limiting the lateral spread of fungus colonies,⁵

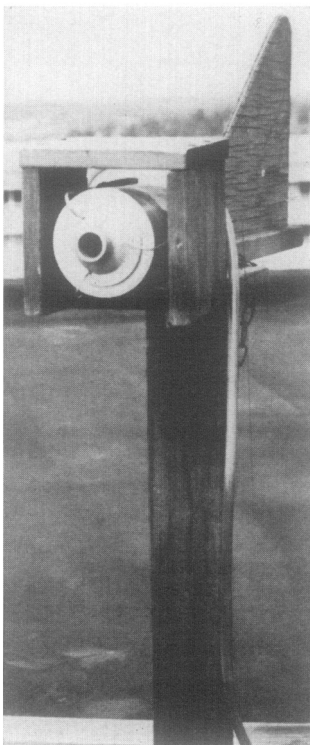


Fig. 2
The sampler in operation on its wind-responsive support.

was added; the resulting material (PDA-RB) was autoclaved for 30 minutes at 121° C. and 15 pounds of pressure.

All exposure periods were carefully timed. Air was drawn through the Anderson sampler at one cubic foot per minute with a rotary vane pump obtained with the sampler or an oilless diaphragm pump (Model EF-C, Air-Shields Inc., Hatboro, Penn.) with a critical orifice. The latter device was required when a length of $\frac{5}{16}$ inch (inside diameter) tubing greater than 2 feet was employed between the sampler and the suction source. Flow into the loaded Andersen sampler was monitored frequently with a rotameter (Fischer & Porter Co., Warminster, Pa.) of appropriate range adapted to the entrance orifice. Unless otherwise stated, all studies were carried out on an unobstructed rooftop, 50 feet above grade, at the University of Michigan Medical Center, Ann Arbor, Michigan. Open plates of PDA, containing roughly 15 ml. of medium were exposed on the upper plate of a standard Durham sampler 42 inches above the surface of the roof. The Andersen sampler was operated either in a fixed upright position or on its side in a homemade support fitted with a windvane and mounted on a low-friction bearing (Fig. 2); in either case, the height of the entrance orifice above the rooftop was approximately 42 inches. The feasibility of using a single plate of PDA-RB below stage 6 with none beneath stages 1 to 5 (S_6 arrangement) was tested and compared with the collection capability of the sampler when fully loaded with 6 plates (S_{1-6} arrangement). With the sampler either upright or wind oriented, the following sequence was followed: (1) An exposure was made, usually for 2 minutes, with a single plate below stage 6. (2) With 6 plates in place, the sampler was operated for a period 3 times the duration of the single plate exposure. (3) Phase one of the sequence was repeated.

The effect of wind orientation on sampler performance was assessed through several series of exposures; each series consisted of six 3 to 5 minute collections with the sampler alternating between an upright and a lateral wind-oriented position. A separate S_6 plate was used for each of the three replicates in each position, and the plates S_6 were changed as quickly as manual operation permitted.

The retentivity of the sampler for viable fungus spores was also studied with the use of an S_6 arrangement. During 5 to 15 minute exposures, a sterilized molecular membrane filter, with a pore size of 0.45μ , was interposed between the sampler and its suction pump using a plastic filter holder with nipples adapted to $\frac{5}{16}$ inch (inside diameter) tubing (Metricel filters and in-line support obtained from the Gelman Instrument Co., Ann Arbor, Mich.). Following each collection period, the membrane filter was removed aseptically and applied, face-upward, to the surface of 15 ml. portions of semiliquid medium in conventional Petri dishes; the medium was prepared by adding 250 ml. portions of water to each 250 ml. of hot PDA-RB prior to autoclaving. After cooling, the mixture was still sufficiently liquid to permeate the filter pores and support the growth of any germinating spores present on the upper aspect. After 5 to 7 days of incubation, counts of grossly visible colonies were made and the filter scanned for patches of hyphae with $200 \times$ magnification.

In an attempt to evaluate the extent of adventitious particle deposition on the internal hardware of the sampler, washings were taken from sieve plates 1 and 6 on several occasions. As a preliminary, the rounded outer aspect of the sieve plate was wiped thoroughly with xylene to minimize contamination from that surface. The entire plate was then immersed for 2 hours in a dilute solution of Hemosol (Aloe Medical, St. Louis, Mo.), which was agitated continuously by a magnetic stirrer. The washings were then passed through membrane filters with pore size 0.45μ , which were subsequently stained with cotton blue dye and examined microscopically for identifiable spores.

All culture media containing rose bengal were protected from direct sunlight both before and after use.⁶ Following exposure, all plates were provided with sterile glass covers and incubated, in an inverted position, at 20°C . and high relative humidity. At Days 5 to 7 following exposure, the plates were examined grossly and on the stage of a compound microscope with 200 power; easily recognizable colonies were counted with a grid inscribed on a transparent plastic overlay, and slide cultures were inoculated from initially "unknown"

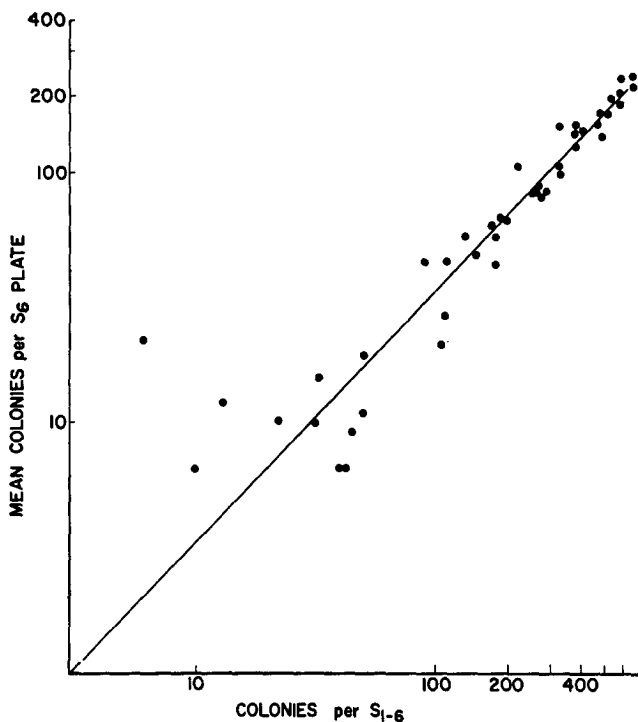


Fig. 3

Total colonies obtained on a full array of plates (S_{1-6}) in relation to the mean total colonies from single S_6 plates exposed before and after the S_{1-6} collection. The data are plotted on logarithmic axes. The volume of air sampled by the fully loaded sampler was exactly 3 times that of each single plate exposure. The diagonal line represents a plot of the relationship:

$$\frac{\text{Total colonies } S_{1-6}}{\text{Mean total colonies } S_6} = 3.$$

colonies. The plates were then stored at 4° C. until Days 17 to 21 when they were re-examined and the slide cultures, which had been maintained at room temperature, were scrutinized. Persistently sterile colonies were usually subcultured on additional substrates such as water agar and cornmeal agar in an effort to induce sporulation. Where indicated, colony counts were corrected for the probability of two or more identical particles initiating growth at a single site with the factors proposed by Andersen.²

RESULTS

Initial collections, made with a fully loaded Andersen sampler (S₁₋₆) in the summer of 1965, were dominated by species of *Cladosporium* (*Hormodendrum*)

Table I. *Studies of the ability of viable fungus particles to bypass a single S₆ plate*

Test No.	Total particle impactions on S ₆ plate	Colonies on membrane filter		% Slippage
		Number	Type	
1	32	0	—	—
2	60	1	Sterile growth	1.66
3	296	2	Cladosporium Alternaria	0.67
4	33	0	—	—
5	242	0	—	—
6	>2,628*	79	Penicillium	<3.00
7	601*	14	Penicillium	2.33
8	1,014*	12	Penicillium	1.18
9	180	1	Cladosporium	0.56
10	271	1	Epicoccum	—
11	104	0	—	—
12	116	0	—	—

*Exposures made indoors.

Table II. *Comparative recovery of several particle types by the Andersen wind speed*

Series	Wind speed (m.p.h.)	Alternaria + Epicoccum			Cladosporium	
		Wind oriented (W-O)	Upright (U)	$\frac{W-O}{U}$	Wind oriented (W-O)	Upright (U)
1	11	21	4	5.25	38	38
2	14	121	20	6.05	435	285
3	6	7	3	2.33	22	17
4	7	12	7	1.71	87	44
5	7	34	17	2.00	144	82
6	6	14	13	1.08	216	204
7	Calm	67	41	1.63	106	80
8	12	19	4	4.75	88	80
9	8	11	2	5.50	61	50
10	9	71	26	2.73	616	402
11	6	12	12	1.00	67	74
12	8	41	16	2.56	640	294
13	10	60	24	2.50	908	681
14	12	13	1	13.10	231	140
15	10	40	13	3.08	192	130
Mean		36.2	13.5	3.68	256.7	173.4

and showed lesser concentrations of *Alternaria*, *Epicoccum*, and *Fusarium* species, assorted yeast forms, and at least 29 additional types. It was noted early that plate 4 produced relatively few isolates and that plates beneath stages 5 and 6 rarely showed growth, although hundreds of colonies might be present on plates 1, 2, and 3. However, even with growth on only 4 plates per sample, it became technically unfeasible to process the materials resulting from regular daily collections, and a simplified procedure was sought. The apparent simplicity of the use of a single plate of medium below stage 6 to "telescope" the collection of viable spores prompted a critical evaluation of this alternative.

Fig. 3 shows data from 45 separate comparisons of collection by the fully loaded sampler (S_{1-6}) with the mean of results obtained by exposing, before and after, a single S_6 plate. In each instance, the exposure time for the S_{1-6} sample (and, hence, the total volume sampled) was 3 times that for each S_6 collection. When ambient concentrations were low, considerable variation was encountered in the ratio between the S_{1-6} collection and the mean of the S_6 samples; at higher particle levels, however, the proportion usually approached a value of 3 rather closely. With the use of a chi square test, the proportional representation of *Cladosporium*, *Alternaria*, *Epicoccum* and large-spored species of *Fusarium* and of other genera in the S_6 and S_{1-6} samples did not differ at a 95 per cent level of significance.

Of 19 studies in which a molecular membrane filter was interposed between the sampler and its vacuum source, 7 produced less than 30 colonies on the S_6 plate, and in all of these the filter remained sterile. The results in 12 other experiments involving larger collections are shown in Table I and suggest that less than 3 per cent fungus spores capable of growth on PBA-RB medium bypass a single S_6 plate after entering the sampler.

Washings from sieve plates 1 and 6 were obtained at intervals of 2 to 3

sampler, operated with and without wind orientation, in relation to ambient

$\frac{W-O}{U}$	Other types			Total colonies		
	Wind oriented (W-O)	Upright (U)	$\frac{W-O}{U}$	Wind oriented (W-O)	Upright (U)	$\frac{W-O}{U}$
1.00	50	67	0.75	109	109	1.00
1.53	112	89	1.26	668	394	1.70
1.29	76	56	1.36	105	76	1.38
1.98	40	19	2.10	139	70	1.98
1.76	92	62	1.48	270	161	1.68
1.06	16	23	0.70	246	250	0.98
1.32	141	89	1.58	314	210	1.50
1.10	116	90	1.29	223	174	1.28
1.22	19	12	1.58	91	64	1.42
1.53	23	20	1.15	710	448	1.58
0.90	174	203	0.86	253	289	0.88
2.18	21	23	0.91	702	333	2.11
1.33	14	9	1.55	982	714	1.38
1.65	117	68	1.72	361	209	1.73
1.48	40	26	1.54	272	169	1.61
1.42	70.1	57.1	1.32	363.0	244.6	1.48

weeks during which S_6 samples were taken daily. Although hundreds of colonies had developed on plates exposed during each of these periods, in many instances the washings yielded no identifiable spores. In all, 12 washings of stage 1 produced a total of 23 spores, all but 3 of them either rust ureidospores or spores of *Alternaria* or *Epicoccum* species, while 6 washings of stage 6 produced a total of 3 spores of *Cladosporium* species.

With the use of an S_6 arrangement exclusively, the results of collections made using the sampler upright and those obtained with the sampler wind oriented were compared. Fifteen series, each comprising 6 alternating exposures, are available for analysis, and mean values from these studies are summarized in Table II. It is evident that wind-oriented samples exhibited higher colony counts in 12 of the 15 series and that this advantage was especially prominent for large-spored types. Fig. 4 displays ratios between colonies present in wind-oriented samples and those of the corresponding upright samples in relation to

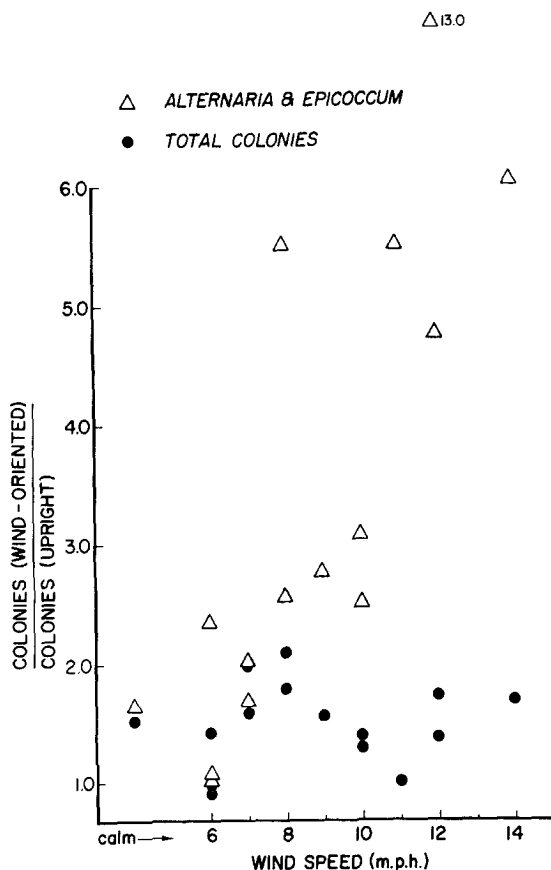


Fig. 4

Ratio of colonies obtained with the sampler wind oriented to those recovered when the sampler remained upright during collection; these data are plotted in relation to the mean wind speed during the sampling period. *Calm* denotes a speed of 5 miles per hour or less.

mean wind speed measured with a Robinson cup anemometer. The higher colony counts obtained in wind-oriented samples and the increase in the collection "advantage" gained by wind orientation with rising wind speed are both consistent with the known behavior of suction devices. Although it was also predictable that large-spored types would demonstrate this effect most clearly, the progressively greater increase in recovery of *Alternaria* and *Epicoccum* isolates than of other types, as wind speed rose, was no less striking.

The relative ease of processing single S_6 plates facilitated a comparison of this method with concurrent open-plate sampling; 110 paired samples were collected during the spring and summer of 1966 with the use of PDA without rose bengal. Open plates were exposed for a total period of 7 minutes, and, during the middle 3 minutes of those 7, a wind-oriented S_6 sample was taken. These collection periods were used throughout the study, and, except when heavy rain was falling, samples were taken without regard to prevailing weather. Unfortunately, by mid-June 3 minute sampling periods often proved too long, and the resulting S_6 plates became overloaded and were excluded from consideration. The comparative data shown in Table III are derived from periods when submaximal spore levels prevailed. Therefore, they do not describe the "average" concentration or relative prevalence of culturable fungus particles in air during the total period specified.

Table III. Results of 110 paired comparisons between an open culture plate and the wind-oriented Andersen sampler (S_6 arrangement)

Genus or type of isolate	S_6 samples			Open Petri plates		
	Colonies recovered	% of Total catch	Samples showing isolate	Colonies recovered	% of Total catch	Samples showing isolate
Cladosporium (Hormodendrum)	646	34.2	99	519	31.4	84
Yeast forms*	259	13.7	101	272	16.5	105
Fusarium†	144	7.6	60	27	1.6	21
Sterile colonies	204	10.8	87	91	5.5	51
Alternaria	84	4.5	31	152	9.2	46
Penicillium	82	4.4	56	94	5.7	49
Geotrichum	79	4.2	40	24	1.5	8
Pullularia	79	4.2	59	258	15.6	47
Rhodotorula	77	4.1	37	94	5.7	42
Phoma	53	2.8	36	61	3.7	19
Monilia‡	42	2.2	16	3	0.2	3
Aspergillus spp.	24	1.3	16	26	1.6	13
Epicoccum	14	0.8	7	4	0.2	3
Cephalosporium	9	0.5	4	0	—	0
Aspergillus niger	7	0.4	4	4	0.2	2
Sporobolomyces	7	0.4	3	0	—	0
Stemphylium	6	0.3	4	2	0.1	1
Miscellaneous§	69	3.6	—	21	1.3	—
Totals	1,885	100.0		1,652	100.0	

*Excludes *Sporobolomyces* and *Rhodotorula*.

†Most colonies isolated produced only microconidia in culture.

‡*Monilia* sp. (not *Monilia sitophila*)

§The miscellaneous group comprises 21 genera with species recovered five or fewer times.

DISCUSSION

Of the several devices developed to collect viable airborne microorganisms volumetrically, the Andersen sampler appears to be the most practical and aerodynamically sound. However, this sampler was conceived primarily to study relatively small infectious particles and, by determining their size distribution, to assess their capacity for lung penetration. The allergist, anxious to define the true prevalence patterns for a variety of potential allergens in- and out-of-doors, must deal with a broader range of particle dimensions—many considerably over 10 μ . Furthermore, until clinical significance can be attached to the respiratory level at which allergenic spores are deposited, the additional effort required to obtain size discrimination in spore collections seems difficult to justify. While the use of a single plate of medium below stage 6 is a radical change from established procedure, it appears to be highly defensible from the standpoint of practicality; the data presented suggested that this modification is also quantitatively valid.

In evaluating the S_6 arrangement, it seemed especially important to document particle losses that might occur due to deposition on the internal walls of the sampler. Gregory³ had emphasized the probability that large particles might be lost on the front of the first plate with the sampler fully loaded (S_{1-6}). More recently, May⁷ has documented these losses and defined zones of relatively low flow in the centers of stages 1 and 2 that appear to be responsible when the entrance cone is used. Such losses were less probable with an S_6 arrangement eliminating the presence of plates beneath the offending stages but may have been a source of bias in comparisons between the S_{1-6} and S_6 samples. The admittedly crude procedure of washing stages 1 and 6 did not suggest losses at these points. However, more recent washings from stage 1 after prolonged S_{1-6} exposures have consistently produced several of the larger spore types. Where the S_{1-6} arrangement is to be used in sampling fungus emanations, the use of a preimpinger⁸ or the revised radial jet pattern devised by May⁷ might be seriously considered.

With the use of an S_{1-6} arrangement, Andersen² determined that "slippage" (passage of a particle completely through the sampler) was negligible for micron-sized particles, and others^{7, 9} have derived collection efficiency curves providing theoretical support for his observation. Total retention of spores by a single S_6 plate was not found, although the proportion lost was small. Since it could not have been predicted that fungus particles would bypass the S_6 plate, other possible sources of filter contamination, such as a worn gasket between stage 6 and the metal base plate or direct air leaks into the filter holder, were considered. However, "mock" exposures (without flow through the sampler) and exposures in which air entering the sampler was first passed through a membrane filter did not result in contamination of the filter beyond stage 6. Table I does not suggest that slippage is confined to a specific particle size range. Although the source of this effect is unclear, it might result if a rapidly moving particle was to strike and rebound from one already deposited upon the sampling medium.

Results summarized in Table II reflect the substantial theoretical advantage

gained by wind orienting any suction sampler in situations of active horizontal air motion. Even with isokinetic flow,* a 90 degree angle between mean wind direction and the sampler orifice was shown by Mayhood and Langstroth¹⁰ to decrease by more than half the recovery of 12 μ particles of diethyl phthalate aerosol and to diminish collection of 37 μ particles by a factor of 10: similar findings have been recorded by Sheldon and Hewson¹¹ with the use of ragweed pollen grains. It is uncertain, however, whether the sole effect of wind orientation is to remove sources of sampling error that operate when the device is in a fixed upright position. Since the velocity at the intake orifice is approximately 2 miles per hour, ambient wind speeds which generally exceed this figure may be expected to factitiously increase the over-all capture of particles—particularly of larger types.¹ The magnitude of this effect, which increases with rising wind speeds, is not readily calculable for nonspherical particles of uncertain density. However, data summarized by Watson¹⁰ suggest that, even for particles in the size range of *Alternaria* and *Epicoccum* spores, “oversampling” due to anisokinetic conditions should introduce appreciably smaller errors than those resulting from nonalignment of an upright sampler with horizontal air flow. To evaluate the actual advantage gained by wind orientation would require a cultural isokinetic standard device with perfect responsiveness to minute fluctuations in wind velocity; such a sampler has not been developed even for investigative use. Although the effect of anisokinetic conditions upon collection by the wind-oriented sampler cannot be precisely determined for any particle type, its relative magnitude is probably small. Based upon purely theoretical considerations, a greater disparity between the upright and wind-oriented collections might actually have been expected for both large and small particles. It is likely that suboptimal responsiveness of the sampler support to rapid shifts in wind direction prevented fuller expression of these potential differences. We have continued to wind orient the Andersen sampler during all collections made in outdoor air while using the device in an upright position in most enclosed situations. By adding an orifice tube of smaller diameter, it has been possible recently to increase the intake velocity to approximately 6 miles per hour, thereby further reducing errors due to anisokinetic flow.

It is of some interest that concentrations of large-spored types derived from simultaneous 30 minute rotobar¹² samplers were, generally, in close accord with cultural data from the wind-oriented collections. Particle collection by such a rotating-arm sampler is not readily influenced by long- and short-term variations in the speed and direction of ambient air flow. Unfortunately for such comparisons, however, the percentage viability of spores enumerated in microscopic deposits is always uncertain. In addition, the contribution of hyphal fragments to culture plate data is equally obscure, and, although these particles are often abundant in air,¹³ their generic affinities are seldom obvious. No attempt was made to relate Andersen sampler data for colonies of *Cladosporium* species to

*Isokinetic conditions prevail when the intake velocity of the sampler is identical to that of the free air stream from which particles are collected. In this situation, air enters the sampler without deflection of its streamlines and the entrained particles undergo no accelerations at the orifice.

particle counts derived from rotobars; in addition to the problems previously mentioned, the tendency of these spores to be airborne in aggregates of variable size makes such comparisons essentially meaningless.

In comparing wind-oriented S_6 samples with open Petri dish exposures, the times of collection were chosen so that total colonies recovered by the two methods might be comparable. Since the collection capability of the Andersen sampler far exceeds that of the open culture dish, comparisons based upon the absolute numbers of colonies recovered by both methods have limited significance, being primarily a function of exposure duration. However, differences in the composition of the two collections are meaningful, and these are shown most clearly in Table III by comparing data for "per cent of total catch" obtained by the two methods. It is evident that the relative recovery of small-spored types (e.g. *Fusarium* species producing microspores, *Geotrichum* sp., *Monilia* sp., etc.) by the open culture plate is markedly diminished when compared with that of the Andersen sampler. Data for *Cladosporium* and *Pullularia* appear as exceptions to this generalization since, although their individual spores are relatively small, these types were readily recovered by open plates. Such apparent discrepancies can be explained in part by the high absolute concentrations in free air of particles derived from representatives of these genera. An unknown proportion of colonies are undoubtedly derived also from complex spore aggregates or hyphal fragments with relatively large aerodynamic sizes. Furthermore, it is common for isolates of *Pullularia* and those of unicellular yeasts to develop on culture plates in association with minute soil particles. The significance of the large group of "sterile colonies" is unclear, although this may comprise predominantly single mating types of heterothallic fungi, particularly certain ascomycetes.

Continuing experience with the Andersen sampler has emphasized several technical points that deserve mention. Especially with an S_6 arrangement, care is necessary to prevent the 0.01 inch diameter holes in stage 6 from becoming occluded; a fine strand of wire will usually serve to remove obstructing materials. Several drops of propylene oxide suffice to kill viable spores within the sampler, and, unless the interior of the device has become soiled, additional routine decontamination measures are probably unnecessary. The rate of air flow into the sampler should be determined frequently, and an alternative source of suction may, at times, be essential. Optimal function of the sliding vane pump has required rather frequent flushing of this device with small volumes of gasoline. With attention to these operational details, the Andersen sampler has provided a valuable source of clinically applicable volumetric data. Undoubtedly, the major deficiency of the S_6 approach is the tendency of a single plate to become overloaded, and, during summer months in Ann Arbor, even 30 second exposures have occasionally proven excessive. This is not a unique problem since an estimate of the particle load to be encountered is implicit whenever a sampling period is chosen. The wide applicability of the S_6 modification deserves emphasis. However, there is still need for a simple volumetric device, specifically designed to sample small volumes of air and to concentrate the entrained particles, without regard to their sizes, in deposits on growth media. The

sieve sampler of duBuy and Crisp,¹⁴ which first employed the principle of multiple jet impingers, might also serve as the basis for such a simplified collector, although other methods may prove equally adaptable.

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