Experiments on the spread of colds

1. Laboratory studies on the dispersal of nasal secretion

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It is now known that colds are due to infections with a number of different viruses and that it is therefore unwise to generalize about the epidemiology of the disease. We have, nevertheless, attempted to measure some of the processes which may operate when infected nasal secretion leaves the upper respiratory tract of a model 'patient' and reaches that of another subject.

This paper describes work using biological tracers; in this we have measured the clearance of foreign material from the nose, mouth and conjunctiva and the dispersal of material from the mucous surfaces of normal subjects on sneezing, coughing and so on.

METHODS

We wished to use a tracer which could be detected after great dilution, could be measured accurately, was completely unaffected by the activity of biological fluids, and could be detected in the presence of these fluids or of common house dust. It had, of course, to be completely non-irritating and harmless to man even when administered repeatedly. We considered the use of fluorescent or radioactive powders and tried suspensions of uniform plastic beads stained with fluorescent dyes, and detected by ultra-violet microscopy. We finally chose to use a suspension of spores of the non-pathogenic organism *Bacillus mycoides*, which was recommended and prepared for us by the Microbiological Research Establishment, Porton. This organism is a saprophyte which forms large colonies in 18 hr. at 30° C. on a medium (1 % peptone, 2 % agar) on which the normal human flora will not grow at all, and the saprophytes of the laboratory and home will grow very little. The colonies are so characteristic that they are immediately distinguished from the occasional contaminant (see Plate 1*a*). The suspension used contained 10^9 spores per ml.; it was stored at 4° C. and the viable count was unchanged at the end of 1 year. It was shown that the viable counts were quantitatively satisfactory provided that not more than fifty colonies on a 4 in. plate were counted; also the counts were unaffected by inoculating spore suspensions mixed with human saliva and nasal secretion. If spores were spread on glass, enamel and other surfaces and allowed to dry they could be completely recovered by washing the surfaces with 1 or 2 % of calf serum in isotonic saline buffered at pH 7·1 with 0·01 M sodium phosphate buffer (sampling fluid).

We had also to choose methods of collecting material for assay. We found that the absorbent cotton bacterial swab used in this laboratory took up about

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0·04–0·1 ml. when rubbed on to a moist mucous surface. This method was the best available for taking repeated samples from the respiratory tract. However, we also modified previous methods for studying the expulsion of droplets from the nose and mouth. In order to measure the total number of spores expelled by sneezing or coughing, the subject was seated on a chair with his head over the top of a large air-filled polythene bag (120 cm. × 60 cm.) of the type used for storing clothes, containing 190 ml. of sampling fluid. The air sampler, which was held in a retort stand, was lowered to the bottom. The plastic was drawn round the subject's neck while he coughed, spoke or stimulated his nose with a wooden applicator in order to induce a sneeze. The applicator was inside the bag, but grasped by the subject's fingers through a fold in the bag. Immediately after the experimental procedure the subject's head was withdrawn, and the neck of the bag was closed. The air pump was then switched on and in about 2 min. it removed through the sampler practically all of the air in the bag. The sampler was then extracted, the fluid or slides removed for assay (see below); the spores on the sides of the bag were removed by closing the neck again and swirling the sampling fluid all over the inside of the bag; an aliquot of the sampling fluid was removed for assay.

To sample air we used one of two methods. The first was a pre-impinger (type A) designed by May & Druett (1953) added to an impinger through which air was drawn in series at 11 l./min. (Plate 1 b); the pre-impinger collects in 4 ml. of sampling fluid particles of about the size trapped in the nose, i.e. those which behave like spheres of unit density, and a diameter of 4 μ or greater (Landahl & Black, 1947); the impinger collects smaller particles down to about 1 μ in diameter. It was necessary to wash the dome and neck of the pre-impinger to collect those spores which were not trapped in the fluid. Spores in the fluid were assayed as indicated above. In order to obtain fuller information about the size distribution of airborne droplets we used as a second method a cascade impactor as designed by May (1945). In this apparatus air is drawn at 17 l./min. through four slits of decreasing size and particles are trapped on glycerine-coated slides (Plate 1 c). The sizes of airborne particles trapped on each slide were determined by May (1945) and we used his data. The deposit was washed off each slide and assayed for spores. Only in a few preliminary experiments did we use settling plates. These give valuable information on how many infectious particles may drop on to a vulnerable surface, such as a wound, but we thought that the most important material epidemiologically would be what remained airborne and was therefore likely to be inhaled. We also wished to extrapolate from our results to the probable behaviour of virus-containing material of much lower infectivity; it was therefore less important to know the number of airborne particles which were carrying infectivity than the amount of infectivity which was being carried in particles of a certain size range. We therefore used impingers and impactors rather than apparatus such as the Bourdillon slit sampler, on which particles are impacted directly on to an agar surface.

Except where noted the experiments were reproducible although the results of only one of each type are presented. Several others, sometimes performed with minor modifications, have given similar results.

RESULTS

Transport of spores in the nose and mouth

We first studied the fate of spores placed in a small volume of fluid on various mucous surfaces of a normal subject. About 0·05 ml. of spore suspension was placed on both sides of the nasal septum just inside the nasal vestibule. The subject then
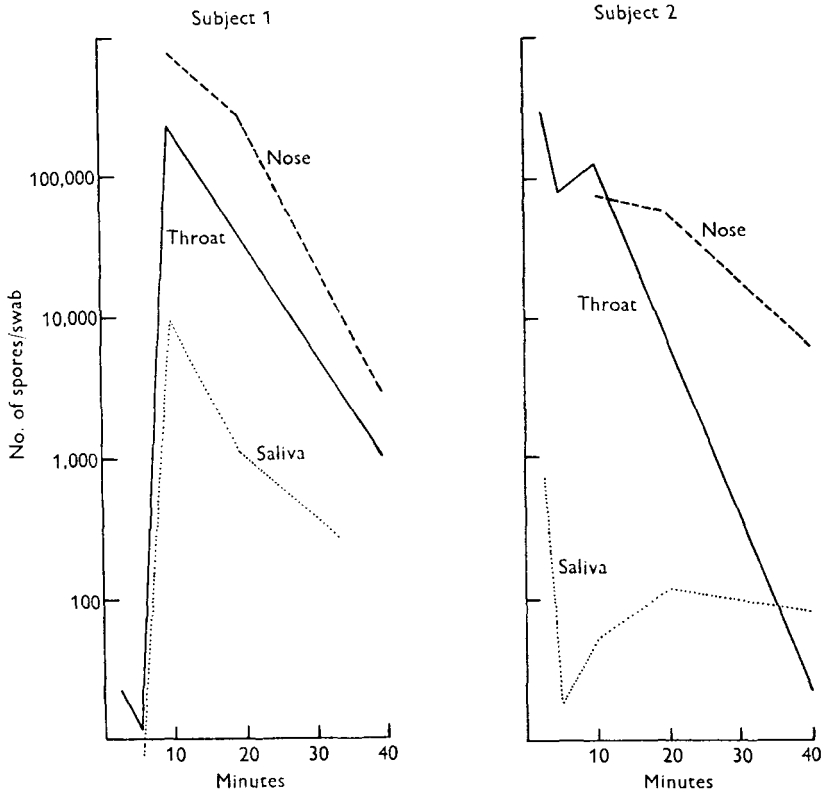

Fig. 1. The recovery of spores from the nose, throat and saliva following the inoculation of 10^8 spores in 0·1 ml. of fluid on to the anterior nasal septum of two normal subjects.

continued his laboratory work, speaking if necessary but avoiding sneezing or blowing the nose. Swabs were collected at intervals from the throat and mouth and after 5 or 10 min. from the anterior part of the nose too. The 'decay' curve of spore concentration was apparently exponential as can be seen from Fig. 1. The spores took 5–10 min. to reach the throat and at about the same time appeared in the saliva. The concentration in the saliva was lower than and followed that of the throat. This suggested that not only did ciliary activity move nasal mucus rapidly into the throat, but that some unknown mechanism moved throat secretions forwards into the mouth. Experiments on subject 1 always gave smooth curves, but in subject 2 the process of clearance seemed to be much less regular. Spores were placed under the tongue in the same way and swabs were collected

from the top and side of the tongue; they were steadily removed as can be seen from Fig. 2; this was thought to be due to the fact that they were being diluted with saliva and swallowed.
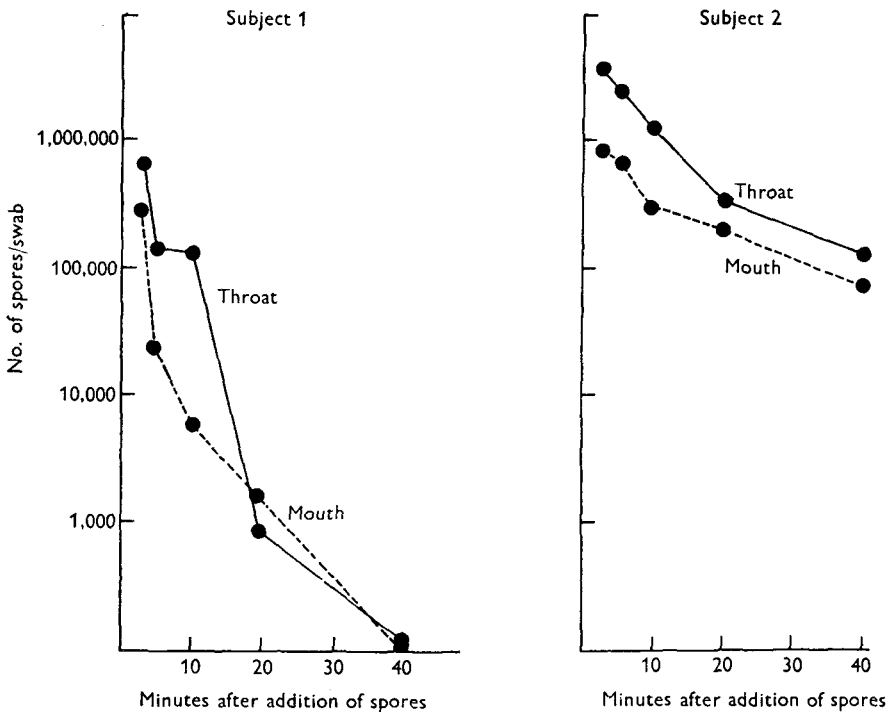

Fig. 2. Recovery of spores from the throat and mouth following inoculation of 10^8 spores into the mouth of the same two subjects as in Fig. 1.

Rate of clearance of spores

Assuming that spores were rapidly and uniformly diluted in saliva we calculated from the intercept and slope of the curve the apparent volume and rate of secretion of saliva. The subjects also dribbled saliva into a Petri dish and the rate of secretion was calculated directly. By direct measurement subject 1 produced 1·8 ml./min. of saliva and 2 produced 0·22 ml./min. By calculation from the spore counts 1 produced 1·8 ml./min. and 2 formed 0·27 ml./min. From the ratio of the concentrations of spores in the throat and saliva we calculated that throat secretion was being carried forward into the mouth at the rate of 0·09 ml./min. in subject 1 (Fig. 1) and 0·0012 ml./min. in subject 2 (data of Fig. 3). We tried to make similar calculations from the curves of nasal clearance, but found unreasonable results, presumably because most of the spores passed through the nose in a little 'packet' (as was suggested by experiments with dye powders, see below) and therefore the mathematical model did not apply. The 'half life' of spores in the nose was 3 and 7 min. and of spores in the mouth was 2 and 6 min. in subjects 1 and 2, respectively. These experiments were all repeated with bacteriophage T3 and almost identical results were obtained, indicating that a
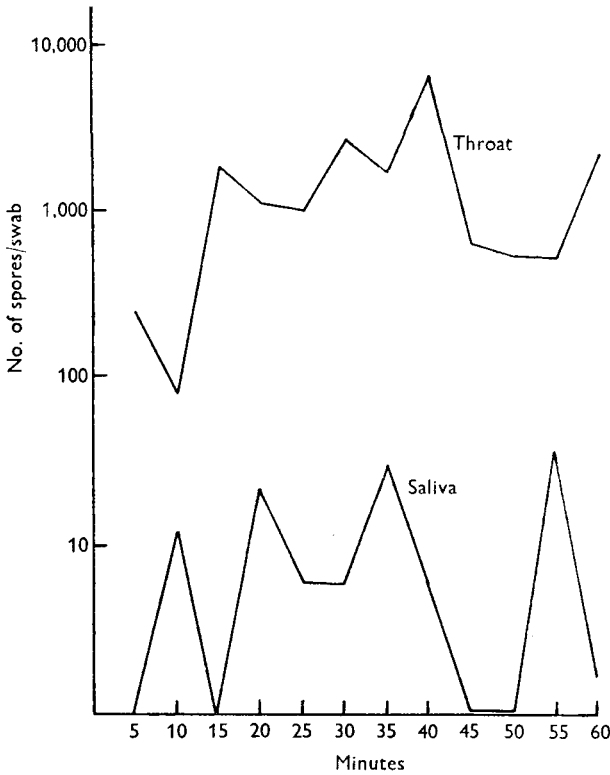
Fig. 3. Recovery of spores from throat and saliva during continuous infusion by nasal catheters of a suspension containing $10^{7.1}$ spores/ml. The concentration in saliva was at all times much lower than in the throat.
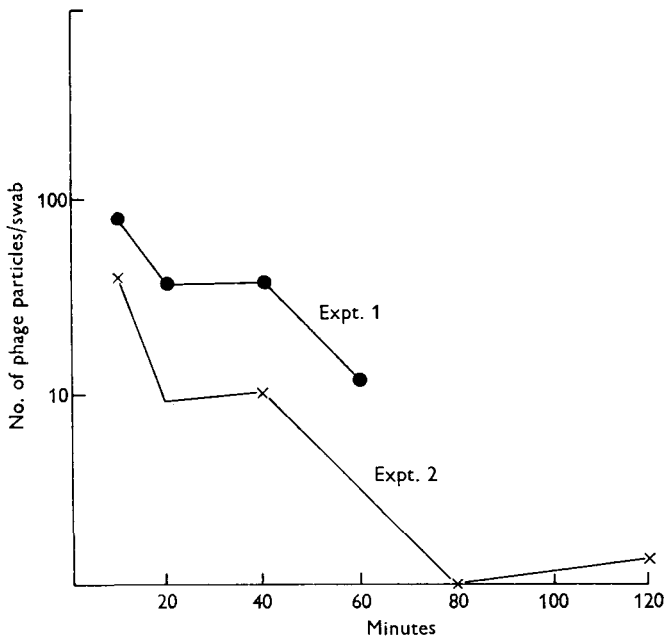

Fig. 4. Recovery of bacteriophage T3 from nose after instillation of $3 \cdot 3 \times 10^5$ pfu into conjunctival sac.

particle with the dimensions of a medium-sized virus might be expected to be transported in the same way as the spores. Bacteriophage particles were also added to the conjunctiva. They were removed rapidly (Fig. 4), but could not be detected regularly in the anterior nares.

It was desirable also to mimic the way in which infectious secretions are constantly being formed in the nose during a cold. By means of a sinusoidal pump 3×10^6 spores per min. were delivered in 0·25 ml. of phosphate buffered saline through two fine nasal catheters (Jacques E.G. 3 with one side hole) inserted 4 cm. into the nostrils. The fluid emerged as a slow trickle from each catheter which lay on the floor of the nose; the subject sat erect with his head tilted backwards just enough to prevent the suspension running out of the nose. The number of spores recovered from the throat and mouth swabs are shown graphically in Fig. 3. This shows that even when nasal material containing about 10^6 spores per 0·1 ml. was being constantly carried down into the throat, spores were detected only intermittently in the mouth of this subject although they were constantly present in the throat. We thought that spores were forced into the mouth by an occasional imperfection in the swallowing mechanism; this was supported by another experiment in which it was found that the concentration of spores in the mouth increased greatly when the head was tilted forwards. Negus says (personal communication) 'I see no reason why peristaltic contraction of pharyngeal constricters should not force material into the mouth after the pharynx has been elevated'.

We also followed nasal clearance non-quantitatively by placing a powder of edicol orange and calcium phosphate inside the anterior nares. This was usually observed on the pharyngeal mucosa between 5 and 15 min. later, but the rate of clearance could not be evaluated quantitatively.

The dispersal of tracers from the nose and throat

Spores were placed in the nose of a subject as described in the section on transport of spores, and those expelled were then recovered after three coughs, each of which was as deep and vigorous as the subject could make it; in another experiment he vigorously declaimed ten lines of Shakespeare, sneezed hard, or blew his nose into a cotton handkerchief 40×40 cm. The results shown in Table 1 indicate that over 1000-fold more spores were recovered after sneezing or blowing the nose than after the other manoeuvres. Hare & Thomas (1956) found that the snorting occurring in blowing the nose released more *Staphylococcus aureus* from the nose than did sneezing. It has been observed in high-speed photography (Jennison, 1942; Bourdillon & Lidwell, 1941) that the droplets expelled by sneezing come mainly from the mouth. This was confirmed in our experiments by weighing the secretion expelled into two separate small plastic bags held over the nose and mouth during an experimental sneeze. We recovered 0·15 g. from the mouth and 0·025 g. from the nose. However, there were more spores in the nasal secretion than in the saliva which apparently forms most of the droplets coming from the mouth; it was therefore thought likely that more spores would be shed from the

nose than from the mouth during a sneeze. This was confirmed in other experiments also shown in Table 1, in which the nose or mouth were closed during the sneeze and in which the secretions of the nose and mouth were collected separately with plastic bags. Immediately after sneezing into the two bags swabs were collected from the nose and mouth—190,000 spores were recovered from the former and 1500 from the latter. In five experiments carried out between 1 and 2 min. after putting spores on to the anterior nasal septum we recovered from the large plastic bag between 6 and 38 % of the spores added, the average recovery being 25 %.

Table 1. *Activities which disperse spores placed on the anterior nasal septum*

	Time between adding 10^8 spores and making experimental manoeuvre	Spores recovered from plastic bag after indicated activity*	
		Surface	Air
Experiment 1			
Talking	5 min.	380	40
Coughing	5 min.	380	0
Sneezing	$1\frac{1}{2}$ min.	5,700,000	1,910
Blowing the nose	2 min.	1,400,000†	4,100‡
Experiment 2			
Sneeze with mouth covered	10 min.	11,400,000	2,020
Sneeze with nose covered	10 min.	14,200	470
Experiment 3			
Sneeze with: 2 small bags	10 min.	—	—
Mouth bag	—	1,500	—
Nose bag	—	1,040,000	—

* Subject 1.
† 32,800,000 spores were recovered from the handkerchief.
‡ In experiments with subject 2 counts in this category were much lower.

It was thought that during talking or sneezing rapid airflows might move spores from the nose into the mouth or from the mouth into the nose. This was tested by swabbing one site after adding spores to the other and carrying out the manoeuvre. There was no evidence of any movement of spores at all in this way.

It was concluded that under the conditions of a common cold, in which infectivity was present mainly in the nasal secretion, the infectious particles would be expelled mainly during sneezing and mainly from the nose. A substantial proportion of fluid on the anterior part of the nasal septum would be expelled. Hamburger, Green & Hamburger (1945) found that patients who had throat and nose swabs which were positive for haemolytic streptococcus shed eighty times more of these organisms than patients in whom only the throat swab was positive.

The size of droplets in which spores are sneezed out

In many experiments the number of spores which could be recovered from the air in the $1\frac{1}{2}$–2 min. after sneezing into a bag, and the number of spores which were recovered from the wall of the bag were measured separately as indicated in

Table 1. This suggested that most of the spores were found in droplets which remained airborne for a very short time. A subject sat in front of a bench on which a large number of glass slides were spread. He sneezed in a horizontal direction over them and the droplets on the slides were allowed to dry and were then stained and measured with a microscope with a micrometer eyepiece. The size of droplets before drying was assumed to be 1/2·5 of the diameter measured (Liddell & Wootten, 1957), and it was calculated that the diameter of the dried droplets ranged from 50 to 860 μ—76 % were between 80 and 180 μ. The number of spores found in individual drops ranged from one or two to uncountable masses.

Table 2. *Number of spores carried in droplets of various sizes expelled into plastic bag by sneezing*

	Total number of spores recovered	
Sample collection from:	Expt. 1	Expt. 2
Bag surface	36,000,000	37,050,000
Impinger sampler	48,000	—
Impactor: (1) > 10 μ*	—	2,130
(2) 16–3 μ	—	24,000
(3) 6–1·5 μ	—	10,600
(4) 2½–0·8 μ	—	95

* Approximate size range of particles of unit density on numbered slides—there is some overlap in the sizes collected (see May, 1945). Stages 1 and 2 approximate to upper respiratory collection. Stages 3 and 4 approximate to lower respiratory collection.

We then studied the distribution of spores in the smaller sized particles. Some typical results are shown in Table 2. This indicated that about two-thirds of the spores were carried in particles collected on slides 1 and 2: and such particles were therefore in the larger size range which might be trapped in the nose. This was confirmed by other experiments with preimpinger and impinger samplers. It is, of course, impossible for many spores to be found on slide 4 of the cascade impactor because there is not room for a spore inside a droplet of less than about 1 μ diameter yet such droplets might well carry virus particles. The experiment was therefore repeated using T3 bacteriophage, but the results were very similar; from 2 to 6 % of plaque-forming units were recovered from slide 4.

Model experiment on possible routes of transmission to other subjects

We next repeated the experiment under conditions which approached more closely those in which sneezing normally occurs. The experiment was done in a quiet laboratory in which the windows were closed. The subject sneezed with his head just above the level of a bench on which was placed a rectangular glass plate 56 × 30 cm. This was intended to collect droplets which would normally drop to the floor. Beyond the plate a microscope slide was placed vertically, to represent the conjunctival surface of a person in the vicinity of a sneezer; next to this stood an impinger with attached preimpinger and a cascade impactor. The glass surfaces were all coated with a thin film of a mixture of glycerine and gelatine and the spores

Table 3. *Spores recovered after sneezing in an open room. Number of spores recovered by sampling method*

Method of dispersal	Horizontal glass plate	Vertical microscope slide	Pre-impinger	Impinger	Impactor			
					1	2	3	4
Sneeze	2,000,000	10	970 65%*	500 35%	500 24%†	1,000 48%	510 25%	30 3%
Artificial coarse spray	6,400,000	150	82,400 59%	60,000 41%	26,000 21%	95,000 55%	41,000 23%	1,270 1%

* Percentage of all airborne spores recovered in preimpinger–impinger sample.
† Percentage of all airborne spores recovered in impactor sample.

were recovered by washing the glass plate with 100 ml. sampling fluid and the slide with 5 ml. The arrangement of the apparatus is shown schematically in Fig. 5 and some typical results in Tables 3 and 4. The vast majority of spores (99·9 %) dropped rapidly towards the ground and only a small proportion remained airborne in the vicinity of the sneezer for as long as a minute. Rather over half the spores were carried on droplets which might be trapped in the nose. Very few spores were picked up by the vertical slide. It was possible that some of these results were due to difficulty in 'lining up' the sneeze when powerful reflex movements began, so the experiment was repeated with a hand spray specially made for us by Dr B. M. Wright. This device produced a rather coarse spray of droplets which resembled in size distribution that formed during a sneeze. As can be seen from Table 3 the results were the same as those of a sneeze.
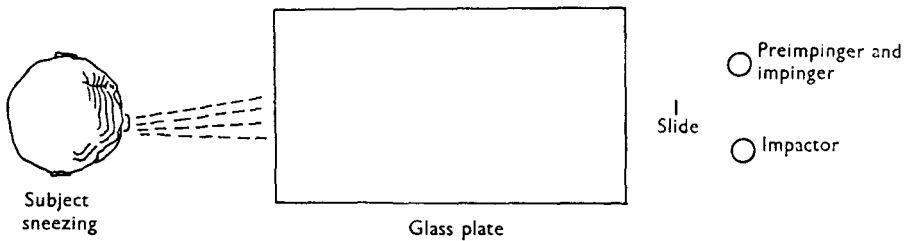

Fig. 5. Scale drawing of a subject sneezing in an open room, showing the position of the horizontal glass plate, the vertical slide and the impactor and the pre-impinger and impinger samplers. (Scale 1:12.)

Table 4. *Rate of dispersal of sneezed material in a quiet room*

Time of collection in minutes from time of sneeze	Spores recovered at indicated times		
	Horizontal plate	Vertical slide	Impinger
$0-\frac{1}{4}$	1,800,000	30	1,350*
$\frac{1}{4}-\frac{1}{2}$	2,800	0	90
$\frac{1}{2}-\frac{3}{4}$	600	0	518
$\frac{3}{4}-1$	5,000	0	330

* The neck of the impinger was not washed out.

We concluded that just after a sneeze only a tiny fraction of the expelled material would remain airborne in droplets near the patient, but that over half of these might be of a size which could be trapped in the upper respiratory tract.

We attempted to detect spores by swabbing the nose and conjunctiva after opening the eyes in or inhaling a droplet suspension produced by the spraying device. Small numbers of spores were recovered and we attempted to extrapolate from these to the number picked up, using the known clearance rates to account for the number which had disappeared. The results were unfortunately too erratic to be significant. The results obtained in the model system seemed to be a much more satisfactory way of estimating the uptake.

DISCUSSION

It has long been known that the mucociliary blanket clears particulate material from the nose (Proetz, 1953). Much early work on the disappearance of bacteria from the nose is difficult to interpret because some organisms might have been inactivated by lysozyme, but it is generally believed that bacteria and viruses would be treated like inert particles. Our results form, therefore, only a small addition to the many facts about the nasal mucous membranes which have been summarized by writers such as Rivera (1962) and Negus (1958). We measured the rate at which the normal nasal mucociliary blanket removes spores and bacterio-phage deposited in the nose and transfers them to the pharynx; in a patient with a cold the rate might be greatly modified by increases in the amount or viscosity of nasal secretion and damage to the cilia, but virus formed primarily in the nose would probably be found in high concentrations in the pharynx. Because of the flow of saliva and the efficiency of the mechanism of swallowing it seemed that virus being formed in or passing through the pharynx would usually appear in the saliva in low concentrations.

The dispersal of bacteria and secretions from the respiratory tract has been studied since the end of the last century, and it has been shown that although coughing and talking may disperse some droplets from the respiratory tract, sneezing produces many more, and that most of these droplets originate from the mouth. They range in size from coarse droplets which fall rapidly to the floor to fine ones which dry off while airborne and form droplet nuclei of Wells (1956). It is widely believed that bacteria which are present in the mouth become airborne directly, but that others such as streptococci and staphylococci contaminate the skin, or fabrics and then dry off and become airborne later. The number of coarse droplets bearing bacteria which are produced and the direction in which they travel varies with the activity which produces them (Hare & MacKenzie, 1946). It is also known that bacteria found in air are usually attached to particles 4–20 μ in diameter (Noble, Lidwell & Kingston, 1963) whether they are believed to have been formed directly from expelled secretions or resuspended after drying. All this and much other excellent research is summarized by Duguid (1945) and in reviews such as those by Williams (1960), and Hare (1964), but we felt that tracer experiments specifically designed to study the fate of materials arriving in or being produced by the nose would be helpful in interpreting the epidemiology of colds, the agents of which are readily destroyed by drying and can only be efficiently transmitted experimentally if infectious material is put up the nose (Lovelock *et al.* 1952); in addition virus is usually found in the nose and throat of patients with colds and rarely in their saliva.

The present experiments suggest to us that since virus particles, such as a bacteriophage, which have no affinity for human cells, are rapidly cleared from the nose infectious viruses must rapidly attach themselves to cells or perhaps to cilia because only a few tissue culture infectious doses can infect when given as a small intranasal drop or aerosol.

The artificial sneezes were probably comparable with those occurring naturally

during a cold, but direct experiments on subjects with colds will be needed to show whether the amount of infectious secretion shed, and the proportion of virus carried in small particles and coarse droplets are the same as those found in our model experiments. If our analysis is correct, saliva is likely to contain very little of the virus produced in the nose and throat: the number of droplets of saliva in the air and the salivary bacteria therein will therefore have only an indirect relationship to the transmission of colds.

Organisms which have been resuspended after drying apparently play a large part in the transmission of bacterial infections of the respiratory tract. It remains to be seen whether infectious virus particles can be dispersed in the same way. Volunteers do not catch colds from living in rooms in which others with colds have spent several hours (Lovelock *et al.* 1952), but colds are transmitted from person to person living in the same house. Therefore the airborne droplets of nasal secretions produced by sneezing or blowing the nose are likely to be the main route of transmission of viruses, although virus will probably not remain infectious in them very long. These droplets are relatively speaking so few in number that it is not surprising that colds are, on the average, transmitted relatively ineffectively, even within a household (Badger *et al.* 1953; Lidwell & Sommerville, 1951).

SUMMARY

An attempt has been made to study quantitatively the mechanisms by which infectious materials may enter the body by the upper respiratory tract and be shed during a cold.

The rapid clearance of tracers, spores of *B. mycoides* and bacteriophage type T 3, has been measured after adding them in small drops to the nose, conjunctiva and mouth.

Tracers placed in the nose pass rapidly down the throat, but are found in only small amounts in the saliva. They are dispersed by blowing the nose and, more efficiently, by sneezing. Nearly all are shed as coarse droplets. About 0·1 % are shed in droplets small enough to remain airborne and just over half of these are in the size range likely to be trapped in the upper respiratory tract. The droplets are apparently formed mainly in the nose. The larger amounts of droplets formed in the mouth carried relatively few infectious particles.

Testing by experiments in a model system it was concluded that most of the coarse droplets produced by a sneeze or by an experimental spray fall rapidly to the floor. A few of the larger droplets were trapped on a moistened microscope slide intended to mimic the possible trapping of droplets on the conjunctiva. The spores in these represented only a small proportion of those found in droplets which remained airborne and were collected by an air sampler.
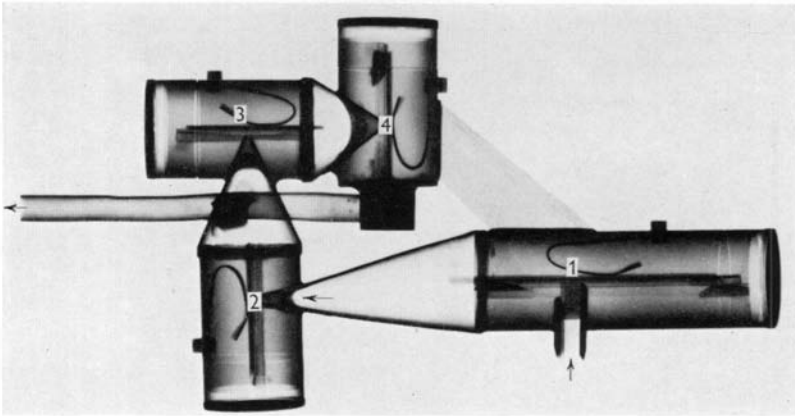
(a)

(b)

(c)

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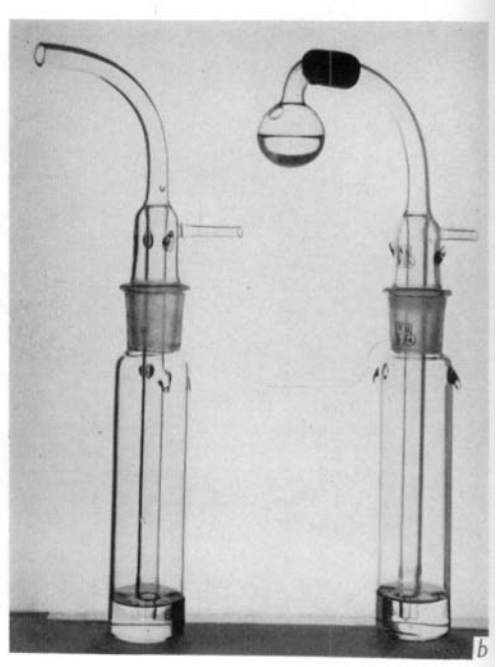
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EXPLANATION OF PLATE 1

(a) A plate showing *Bacillus mycoides* colonies growing up from a mixture of spore suspension and room dust.

(b) Photograph of a Porton impinger with and without an attached pre-impinger.

(c) Hard X-ray photograph of a cascade impactor. The arrows show the route by which air is drawn into and out of the apparatus. The numbers indicate the position of the four slides and the springs which hold them in place.