FURTHER STUDIES ON THE NATURAL TRANSMISSION OF THE COMMON COLD

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THAT the common cold may be transmitted from man to man by the instillation into the nose of bacteria-free filtrates of infective nasal washings has been established by several workers and fully confirmed at the Common Cold Research Unit, Salisbury. This information, however, throws little light upon the ways in which colds are transferred under natural conditions. The present report is concerned with attempts to study the transmission of colds under conditions closely resembling those of normal social intercourse.

EARLIER EXPERIMENTS

Earlier attempts to study the natural transmission of colds at this Unit had proved difficult. As already reported (Andrewes 1949), 19 normal persons in one trial were exposed for ten hours to others with early colds, and only one infection developed as a result; this was in a person exposed to another in the presymptomatic incubation period. In a second test none of 4 exposed people, and in a third attempt only 1 of 5, caught colds. Similar difficulty in producing naturally transferred infection was reported by Kerr and Lagen (1934).

In 1950 an experiment was made on an island off the north coast of Scotland (Andrewes et al. 1951):

> The hope was that people conditioned by three months' complete isolation would have acquired enhanced susceptibility and thus permit us to answer questions about the route of transfer of infection. The possibility of transfer by means of droplet nuclei or by contamination of the environment was explored by much the same techniques as are described below. 12 people thus exposed to 10 people with profuse colds failed, to our surprise, to contract infection. The colds to which they were exposed were of one of our "pedigree strains"—i.e., one which had been artificially passed in series from one person to another by nasal instillation of stored washings. When later, in contrast to earlier failures, a natural or "wild" cold in a crofter did "jump" naturally to 3 of 8 of the exposed "islanders," we were led to consider the possibility that "wild" colds might have better powers of "jumping" than had the "pedigree" ones which had been used not only in the experiment on the island but also in all our previous tests at Salisbury.

We therefore decided to make more transmission experiments, using people with natural colds as "donors" of infection. In several trials we used children, since Lidwell and Sommerville (1951) have produced evidence indicating that they spread colds more readily than do adults.

EXPERIMENTAL METHODS

The organisation of the Common Cold Research Unit and methods used in experiments with human volunteers have already been described (Andrewes 1949). In the present experiments all the volunteers were examined clinically on arrival at the Unit and on each morning of their subsequent stay. On the day of arrival the volunteers were placed in isolation. Most of them were accommodated in pairs, but some remained in single isolation. Those who were passed as fit to take part in the experiments were all in good general health, aged, with three exceptions, 19–45, with a preponderance of females (141 females, 90 males).

Any volunteer showing signs of a cold during the first three days of observation was excluded from the experiment, and if the volunteer was one of a pair in isolation both were excluded. Those remaining free from signs of a cold on the morning of the third day after arrival were exposed later that day to possible risks of infection by the experimental procedures under investigation. Clinical observations of the volunteers in isolation were continued until the ninth day after arrival. Allocation to the experimental groups was made by the bacteriologist, using random sampling numbers. The nature of the exposure was not made known to the clinical observer until he had written up his assessments at the end of each experiment. In the final analysis of colds induced as a result of the procedures only those volunteers developing typical symptoms and signs subsequent to exposure have been recorded as having had colds. Those with doubtful signs, or subjective evidence alone, have been classified with those having had no colds.

People with "wild" or natural colds of recent onset have been selected as "donors."

Comparison of Transmission of Colds by Different Routes

The object of these experiments was to determine whether or not colds could be transmitted by droplet nuclei alone or by indirect contact alone, these conditions being compared with full exposure allowing all normal routes of transference to operate. Five similar experiments were made, involving 82 volunteers and 22 donors. The details of the procedure are described below; not every method was used in every trial.

Exposure to infected droplet nuclei.—A room of about 2000 c.ft. was divided into two with a blanket extending to within about 1 ft. of the side walls, floor, and ceiling. A fan ensured good mixing of the air throughout the whole chamber. 5 or 6 healthy volunteers sat on one side of the partition; they were asked to read or to sew quietly throughout the experiment. After a short interval to allow particles in the atmosphere to settle again, the donors and one observer, wearing sterile gowns to minimise the secondary release of infected particles from clothing, entered and sat on the other side of the partition. The donors were encouraged to play games involving talking, shouting, or singing but little bodily movement. Sneezing-powder (*o*-dianisidine hydrochloride) was liberated half-way through the experiment. A wide tube, connected to a slit-sampler operated outside the chamber, enabled the bacterial content of the air to be measured at intervals. The number of colonies of *Streptococcus salivarius* grown on a selective medium (Williams and Hirch 1949) was taken as an indication of the degree of contamination of the atmosphere by secretions from the respiratory tract. After a 2-hour exposure the donors left the transmission chamber, removed their gowns, and went to another room, of similar size but without a central division, for the second part of the experiment. The volunteers returned to their flats and remained in isolation until the end of the trial.

"*Full contact.*"—The conditions of exposure in this group were those of normal social contact. The same donors as in the first experiment mixed freely with a second party of healthy volunteers for a further period of two hours, eating luncheon together and later playing cards and other games. No bacterial samples of the air were taken in this room, but an observer again noted outstanding incidents, such as sneezing, coughing, and violent nose-blowing. At the conclusion of this experiment the donors departed and the volunteers returned to isolation.

Indirect contact in contaminated environment.—The third group of volunteers was next exposed to the environment which had been contaminated by the donors in the full-contact exposure. Playing-cards and other objects handled by the previous occupants were again used, and once more the period of exposure was two hours. Before the volunteers entered the contaminated environment, the room was aired for 10 minutes by opening windows and doors, to diminish the likelihood that infective particles would remain in the air.

RESULTS

The results of these experiments are summarised in table I. In three instances children aged 8–13 years acted as donors, and in two others adults were used. Two

colds developed in 25 volunteers exposed to droplet infection, three in 32 exposed to full contact, and two in 25 exposed to a contaminated environment. The two colds in the last group were in volunteers occupying the same flat. One of these had minimal symptoms on arrival but remained clear during the quarantine period and was included in the trial. Symptoms returned on the day after exposure to the contaminated environment, and next day her partner showed signs of infection. Possibly a missed quarantine cold was responsible for both these infections; all the other colds shown in table I were separate incidents in different flats.

Another experiment is best considered separately; the results are not included in table I. Of 8 volunteers exposed to 5 children with colds 4 became infected. Conditions in this trial differed from those already described in two ways: the donors were, in part, younger children, their ages being 13, 11, 6, 5, and 4 years; also games were played, including blowing and nose-to-nose passing of a matchbox, rather a closer degree of contact, in fact, than is likely to operate commonly as a means of cross-infection. From all the experiments together we cannot conclude that children were more effective donors than adults.

Bacterial counts made during the droplet nuclei experiments indicated that there was considerable contamination of the atmosphere of the chamber with organisms from the respiratory tract, although the average for one trial was considerably lower than the other three. The number of colonies of *Strep. salivarius* per c.ft. of air sampled in one trial rose from an initial figure of 0·16 in the absence of children to 0·9 shortly after their entry, and to a peak of 3·2 per c.ft. immediately after the release of sneezing-powder. These figures are about twenty times as high as those found in recent trials in infants' school-rooms (Air Hygiene Committee of the Medical Research Council, personal communication).

TRANSMISSION BY MEANS OF MISTS

Since the results of exposure to infected droplet nuclei indicated that the common cold could spread by this means, studies were made with artificial mists of infected particles. In many instances material stored at −76°C in dry ice was used.

The apparatus consisted of a Collison-type spray, the metal parts of which were plated with gold. In the first two experiments power was supplied in the form of compressed air at a pressure of 10 lb. per sq. in., but it was found more satisfactory to use nitrogen from a cylinder at the same pressure. The droplet cloud was carried through a length of wide-bore rubber tubing to the nose-piece of a B.L.B. mask as used for the nasal administration of oxygen. When the source of power was nitrogen, a separate tube added pure oxygen to the inhaled vapour in the mask.

After the face-piece had been fitted and tested for leaks, the gas supplies were switched on, and the volunteer was instructed to inhale through the nose and to exhale through the mouth. A separate mask was used for each volunteer, and the virus suspension in the spraying chamber was changed after every 10 or 20 minutes' spraying. To reduce the inactivation due to the concentration of salt in the droplets, washings collected in 0·2% sodium chloride were selected in preference to those taken in physiological saline solution, and dilutions were made in distilled water. Protein, in the form of 0·2% bovine albumin, was also added to the spraying fluid. The true output of the spray under the conditions of the experiment was calculated to be 0·035 ml. per minute, and spraying for 5 minutes should deliver about the same dose of virus as that contained in 1 ml. of a 1 : 5 dilution of filtrate.

To investigate the possible effects of spraying on the infectivity of the material, the mist produced by the spray was drawn through 5 ml. of 10% broth-saline solution in a sampler containing small glass beads. After sampling for 5 or 10 minutes, the fluid was removed and the sampler was washed with a further 5 ml. of broth-saline solution. The spray sample was next tested in volunteers by the normal technique of inserting 0·5 ml. into each nostril in the form of drops.

As a further check on the output of the spray, and on the harmlessness of the process to a virus suspension, tests were made with influenza virus. Infected allantoic fluid from an A-prime strain A/Sweden/3/50 was diluted 10^{-4} in distilled water containing 0·2% bovine albumin and a small amount of radioactive iodine (I^{131}) was incorporated in the spraying mixture. The resultant mist was sampled in 10% saline solution, and the virus content of the original fluid and of the spray sample was determined by inoculating dilutions of both fluids into the allantoic cavity of developing 10-day hens' eggs. The dilution in the spray sample was estimated by measuring the radioactivity of both fluids with a Geiger counter. The results indicated that there had been no appreciable inactivation of influenza virus by spraying. It would, of course, be unwise to assume that the common cold virus is as stable as that of influenza.

TABLE II—SUMMARY OF SPRAYING TESTS

Test	Serial nos. of trials	Colds induced	No colds induced	No. of volunteers	Percentage positive
Spray 1/2 minute ..	114, 115, 116	3	13	16	20·0
Spray 3 or 5 minutes	114, 115, 120	4	11	15	26·7
Spray collected and given as drops ..	116, 120, 121	4	16	20	20·0
Drops 1 : 5 or 1 : 10	114, 115	4	4	8	50·0
Drops 1 : 50 or 1 : 100	114, 115, 116, 120, 121	11	19	30	36·7

The results of the spraying tests are shown in table II, which shows that the common-cold virus can survive the spraying, and that a cloud of infected droplets will produce colds in some of the persons exposed. The figures suggest, however, that the same volume of material was less effective as spray than as drops; they are not, however, highly significant (P = 0·07).

CONTAMINATION OF EXTERIOR OF NOSE

Three experiments were made in which infective secretions were applied only to the outside of the nose.

TABLE I—SUMMARY OF CONTACT EXPERIMENTS

Trial no.	Date of exposure (1951)	Donors	Droplet nuclei					Full contact			Contaminated environment		
			Volume of air sampled (c.ft.)	Av. count of *Strep. salivarius* per c.ft. of air	No. of volunteers	Subsequent colds		No. of volunteers	Subsequent colds		No. of volunteers	Subsequent colds	
						+	−		+	−		+	−
106	Jan. 31 ..	4 Children	12	2·8	6	1	5	6	1	5	5	0	5
108	March 3 ..	4 Children	48	1·7	8	1	7	7	0	7	6	2	4
112	April 28 ..	4 Children	48	0·35	5	0	5	5	0	5	6	0	6
122	Sept. 22 ..	5 Adults	60	2·2	6	0	6	7	0	7	8	0	8
124	Oct. 17 ..	5 Adults	..	..	..	..	..	7	2	5	..	..	..
				Total	25	2	23	32	3	29	25	2	23

TABLE III—SUMMARY OF TESTS OF APPLICATION OF VIRUS TO NOSE

Trial no.	Date	Nature of exposure	Duration of exposure (hr.)	Subsequent colds in volunteers			Comments
				+	−	No. of volunteers	
124	Oct. 17, 1951	Full contact	2	2	5	7	
		Contaminated handkerchiefs	24	0	8	8	
125	Nov. 28, 1951	Dry pack in nose	2	0	10	10	Material produced 6 colds in 10 volunteers in trial no. 123
		Wet pack in nose	2	2	7	9	
127	Jan. 2, 1952	Drops	..	5	3	8	
		Wet pack	2	2	5	7	
		Paint	1	0	7	7	
126	Dec. 12, 1951	Virus painted around external nares	..	0	7	7	Material produced 6 colds in 10 volunteers in trial no. 123
		Virus administered as drops	..	0	4	4	

No colds developed in 22 volunteers so exposed. The details of the experiments are as follows:

(1) Sterile cotton-wool swabs mounted on wooden applicators were soaked in infected material, and about 0·1 ml. was painted on the skin round the external nares. The 7 volunteers so treated were instructed not to touch or to wipe the nose for an hour after this procedure. Four volunteers received 1 ml. of a 1 : 10 dilution of the same material by the usual dropping technique. No colds resulted in either group, although the material, a filtrate of infected washings from a young adult with a "wild" cold, had previously produced six colds in 10 volunteers.

(2) Nasal blowings from 5 adults with recent colds were collected on 'Cellophane' handkerchiefs. These were washed off with 10% saline solution, and the pool of about 15 ml. of crude material was shaken with glass beads and then centrifuged to remove gross debris. The opalescent supernatant fluid was distributed in 1 ml. quantities, on the centre of clean linen handkerchiefs, which were refolded and issued to 8 healthy volunteers. Each volunteer was instructed to blow the nose on the contaminated handkerchief immediately, and to use the handkerchief throughout the succeeding twenty-four hours. The interval between contamination of the handkerchiefs and their first use by the volunteers was usually about half an hour, during which time most had dried sufficiently to be passed as clean handkerchiefs by the volunteers using them. No colds developed as a result of this procedure, although there were two colds in 7 other volunteers who were exposed to the 5 donors of the infected secretions for two hours under conditions of full contact.

(3) Eight-inch squares of sterile gauze, folded into pads eight layers thick, were contaminated by dropping 0·5 ml. of undiluted nasal washing into the centre of each. The impregnated pads were immediately handed to volunteers, who were instructed to spread the contents of the gauze over the outside of the nares and to repeat the process at short intervals for an hour, during which time they were neither to wash the hands nor to blow the nose. Concurrently 8 other volunteers received the same material, diluted 1 : 2 in broth-saline solution, by the usual dropping technique, and a third group of 7 volunteers was infected by means of packs inserted into the nose (see below). No colds developed in the 7 volunteers who applied the material to the outside of the nose, whereas 5 out of 8 receiving drops developed colds, and two colds developed in the third group.

It thus appears that the placing of infective secretions round the outside of the nose is unlikely to produce a cold. The absence of colds in the group of volunteers using contaminated handkerchiefs for twenty-four hours was unexpected. The result suggested that the virus might be particularly sensitive to drying. To test this hypothesis infective secretions were allowed to dry on gauze before being used for transmission studies.

COMPARISON OF MOIST AND DRY PACKS INSERTED INTO NOSE

Sterile ribbon-gauze strips 12 in. in length were soaked in 0·25 ml. of material known to be infective. Each strip was then kept over calcium chloride in a sealed container at room-temperature for an hour, after which the packs appeared to be quite dry. A second series of gauze strips was impregnated with the same amount of material shortly before the time of insertions. Volunteers were allocated in random fashion to one of two groups, and the appropriate gauze strip was inserted into one nostril with a sterile wooden applicator and remained in position for two hours. The results of this experiment are shown in table III. There were no colds in 10 volunteers exposed to infection by dry packs, and two colds among 9 exposed to moist packs. The numbers do not permit a statistical comparison of the two methods, but suggest that there has been inactivation of the virus as a result of the drying.

DISCUSSION

An analysis of the results in this Unit has shown that an average of 50% of volunteers may be expected to develop colds after the instillation of infected drops into the nose, irrespective of age, frequency of natural colds, or time since last cold. Though individual susceptibility varies considerably, the sensitivity of a group of 6–8 volunteers to infection with a given strain of virus may be expected to be rather more constant. Wherever possible such numbers have been used; otherwise duplicate experiments have been made on smaller groups.

Standardisation of the dose of infective material in transmission studies presents much greater difficulties. Although donors were selected on a history of recent onset of symptoms, together with nasal obstruction and discharge, it was impossible to assess the amount of secretion disseminated by different people other than by a crude estimate based upon direct observation. By using groups of 4 or 5 donors it was hoped that the effects of this variation might be lessened. As seen in table I, two groups of donors failed to pass their colds on by any route. In one of these the observer reported that the children acting as donors were quiet throughout the experiment, and this is reflected in the low figure for aerial contamination in this group. In the other trial no colds developed after exposure to 5 adults, although the plate counts indicated a considerable degree of contamination of the atmosphere.

With such a low rate of positives comparison between the three groups is clearly unjustifiable. There is definite evidence that colds may pass from person to person as a result of normal social contact, and that exposure only to infected droplet nuclei will produce colds in a small proportion of those exposed. The evidence for spread by indirect contact is, as explained above, less convincing, and further experiments are needed before the importance of this route can be estimated.

The fact that colds did develop as a result of droplet spread, in spite of the relatively small amount of material which can have reached the noses of the recipients, suggested that experimental infection of volunteers with a given quantity of virus might be more readily achieved by exposure to infected droplets than by other methods.

Comparative tests were therefore made with artificial droplet mists. Using strong virus suspensions we could induce colds in about 1 in 5 of persons inhaling the ærosol for $^1/_2$–5 minutes. When, however, about the same dose of virus was introduced into the nose in the form of drops, the rate of "takes" rose to 1 in 3 or 1 in 2. Experimentally, therefore, it appeared that infection by means of mists was a less effective method of inducing colds in volunteers than our customary method of applying drops to the nose. The technique has the further disadvantages of requiring more complicated apparatus, and of using much greater quantities of virus.

The transmission of any agent by indirect contact involves three stages. The environment must first be contaminated by an infected subject, the secretions must be in a state in which they retain their infectivity, and they must be picked up by a susceptible person and transferred by him to the nose. If we apply these ideas to our trials with the common cold, the available evidence suggests that infected secretions are rapidly inactivated by drying. Further, even when the route of transmission has been shortened to the extent of applying material of known infectivity to the outside of the nose, no colds have resulted.

Experiments made earlier, with fluorescent dyes used as tracers, had shown how readily secretions could be passed directly and indirectly from one person to another (J. E. Lovelock and K. R. Dumbell, personal communication). The present results do not support the idea that this transfer is necessarily effective, and indeed suggest that indirect contact is unlikely to play a major part in the spread of the common cold. Our tentative conclusions apparently conflict with a statement which has been made on several occasions: Arctic explorers are reported to develop colds when undoing bales of clothing which have been kept packed up for many months. The implication is that the agent concerned is very stable. One must assume either that their symptoms were due to something other than the common cold or that their isolation had conditioned them to respond to very small doses of virus. In the present experiments "takes" as a result of direct contact compare not unfavourably with the natural rate of cross-infection in homes (cf. Lidwell and Sommerville 1951); that they are rather fewer is not surprising in view of the fact that in our experiments there was a single period of exposure, whereas in the observations of Lidwell and Sommerville contact was for some days.

The question discussed here is important. We are very conscious that the low incidence of colds induced as a result of our experiments has meant that our findings are much less conclusive than we could have wished. It is probably true that the development of an overt cold in man is determined more by the varying susceptibility of the person than by the degree of exposure to the causal agent.

CONCLUSIONS

Though common-cold infection may spread from person to person by normal social contact, and through the air in the form of droplet nuclei, the rate of clinical cross-infection is low.

We found no evidence suggesting that spread by indirect contact is of major importance in the natural transmission of the common cold.

Our results suggest that the virus of common cold may be sensitive to drying.

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FURTHER INVESTIGATION INTO CAUSES OF THROMBOPHLEBITIS FOLLOWING INTRAVENOUS INFUSIONS

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IN a previous paper (Bolton Carter 1951) it was shown that the incidence of thrombophlebitis resulting from intravenous infusions could be diminished by limiting the duration of infusion to eight hours.

So far as practicable this procedure has been followed with all infusions set up in the surgical unit of University College Hospital over the last two years. During this period there has been no case of thrombophlebitis severe enough to cause the patient gross discomfort.

All the infusions have been carefully followed up, with inspection of the infusion sites, for a week afterwards, and all cases showing $^1/_2$ in. of venous thrombosis with $^1/_2$ in. of surrounding inflammation, or more, have been recorded as cases of thrombophlebitis.

From January, 1951, to August, 1951, the results were:

Infusions running for less than eight hours:
121 produced no reaction.
15 ,, thrombophlebitis.
Infusions running for more than eight hours:
3 produced no reaction.
7 ,, thrombophlebitis.

Thus 11% of the infusions running for less than eight hours produced thrombophlebitis.

All of these infusions consisted in some part of 5% dextrose. Since solutions of dextrose when autoclaved become acid, it was decided, as the next step in the investigation of infusion thromphlebitis, to study this factor. For three months the pH of every bottle of infusion fluid was measured electrometrically with a glass electrode, and it was found that the pH of whole citrated blood and of physiological saline solution was reasonably constant, being slightly on the acid side of normality, whereas that of the dextrose solution varied between 3·1 and 5·8, averaging 4·5. Having established that the dextrose solution was the only infusion fluid with an unstable and acid pH, a trial of buffered dextrose with a stable pH was started.

BUFFERED DEXTROSE

Previous work had shown some of the difficulties of buffering dextrose. Hudson and Tarlowski (1947) and Bryan and Grainger (1950) found that the addition of sufficient phosphate buffer to prevent the pH falling below 6 caused so much caramelisation as to render the solution unfit for use. Other methods tried by these workers are either impracticable or have the disadvantage of causing auto-agglutination and hæmolysis in vitro.

We have tested the effect of 0·2% and 0·5% of disodium hydrogen phosphate and 0·5% of trisodium citrate as buffers for 5% dextrose, and found the latter to give consistently a slightly higher pH. Such buffered solutions, however, have a well-marked brown colour due to caramelisation.

Since sodium metabisulphite has proved useful as an antoxidant in several injections, its effect was tested in preventing the darkening of buffered dextrose solutions. It was found that in concentration of 0·01% the colour was reduced from dark brown to pale yellow, and that with 0·1% the solutions were almost colourless or at the most pale yellow.

Although the dark-brown solutions have generally been regarded as unfit for use and pharmaceutically are