J. Hyg., Camb. (1975), **75**, 249
Printed in Great Britain

An investigation of the possible transmission of rhinovirus colds through indirect contact

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(*Received* 10 *March* 1975)

SUMMARY

Rhinovirus was recovered from the fingers of 16 of 38 volunteers and others, who were swabbed during the acute stages of their colds. Very low titres of virus were also recovered from 6 of 40 objects recently handled by infected volunteers, but not from the fingers of 18 non-infected subjects whose flat-mates were shedding virus. When rhinovirus from nasal secretions was dried on skin or other surfaces during laboratory experiments, approximately 40–99% of infectivity was lost. Virus could be transferred from surface to surface by rubbing, the transfer being more efficient if it was carried out while the inoculum was still damp.

Volunteers could infect themselves if a moderately heavy dose (88 TCD50) of virus was inoculated on the finger and then rubbed into the conjunctiva or nostril, especially if the inoculum was still damp. From estimates of virus titres in nasal washings and on fingers, and of amounts transferred by rubbing, it was concluded that spread of colds is unlikely to occur via objects contaminated by the hands of the virus-shedder, but that a recipient might pick up enough virus on his fingers by direct contact with heavily infected skin or secretions to constitute a risk of self-inoculation via the conjunctiva or nostril.

INTRODUCTION

Early studies on experimental colds suggested that spread was more likely via the airborne route than via contaminated objects and hands (Tyrrell, 1965). At the time of this early work it was not possible to identify either the virus used or the individuals who were serologically susceptible to it. However, later work on production of infectious aerosols from the respiratory tract confirmed the likelihood of droplet infection (Buckland, Bynoe & Tyrrell, 1965; Couch *et al.* 1966). Colds have been experimentally reproduced not only by instillation of drops into the nose (Bynoe *et al.* 1961) but also by inhalation of small-particle infectious aerosols (Cate *et al.* 1965). However, it has generally proved unexpectedly difficult to reproduce 'natural' transmission of colds from infected to susceptible subjects under defined conditions (Tyrrell, 1965; D'Alessio, Dick & Dick, 1972). Recently Hendley, Wenzel & Gwaltney (1973) presented evidence in favour of transmission of colds via infected hands and objects. They demonstrated that virus could survive on skin and on plastic surfaces, that subjects could pick up

virus from a plastic surface by rubbing with a finger, and that self-inoculation was possible if volunteers rubbed their conjunctivas or picked their noses with fingers contaminated with rhinovirus. However, detailed measurement of the virus transferred by such manipulations was not attempted, and some doubt remains as to the frequency of such happenings under natural conditions. This paper reports studies which in general confirm the findings of Hendley, Wenzel & Gwaltney, and attempt to measure the likelihood of spread of rhinovirus by contact.

METHODS

Virus strains

Two different preparations of rhinovirus type 2 (RV2), strain HGP, were used for experiments *in vitro*. A tissue culture adapted preparation (TC) consisted of tissue culture fluid from infected WI-38 cells. A second preparation (NW) had not been passaged in tissue culture but had undergone four man-to-man passages since isolation from a natural cold; this preparation consisted of nasal secretions from an infected volunteer, collected in phosphate buffered saline, diluted with an equal volume of bacteriological nutrient broth, and stored frozen in small volumes.

Other rhinovirus serotypes, namely RV4, RV5, RV9 and RV14, were used for inoculation of volunteers. RV4 and RV14 had not been passaged in tissue culture, and RV5 and RV9 had had a minimal number of passages in diploid human embryo lung cells. Viruses for inoculation of volunteers were prepared from pools of nasal washings of other infected individuals, suitably characterized.

Tissue culture methods

Rhinovirus-sensitive HeLa cells (Ohio HeLa) were used for all experiments. The media for growth and maintenance of these cells were as described by Stott & Tyrrell (1968). Experiments in this laboratory have shown that these cells are considerably more sensitive than WI-38 cells to rhinovirus (Strizova, Brown, Head & Reed, 1974). Virus isolations were carried out in roller tubes at 33° C., as were virus titrations measuring 50 % tissue culture infectious doses (TCD 50). Plaque-forming units (p.f.u.) were measured by a method based on that of Fiala (1968) using Ohio HeLa cells, which were grown in plastic tissue culture plates in wells 1·5 cm. diameter. The overlay medium consisted of 2 % fetal calf serum, 5 % tryptose phosphate broth, 0·03 M-$MgCl_2$ and 0·4 % agarose in Eagle's medium, with penicillin and streptomycin, 100 μg./ml. Cultures were incubated at 33° C. for 3 days (TC strain) or 4 days (NW strain), then fixed in formol saline and stained with gentian violet before the plaques were counted. Neutralizing antibody in volunteers' sera was measured by the micro-method described by Stott & Tyrrell (1968).

Sampling techniques

The transport medium (HH) described by Chaniot, Holmes, Stott & Tyrrell (1974) was used for sampling objects or skin. Cotton swabs were moistened in

2·2 ml. of the medium, and the area to be sampled was rubbed thoroughly four times in succession, the swab being rinsed and squeezed out in the sampling medium each time. Two vials, each containing 1 ml. of the sample, were stored at −70° C. until cultured for rhinovirus.

Survival and transfer of virus

Replicate inocula of 0·002 or 0·0055 ml. of virus suspension were applied to marked areas of skin or other surface and spread to an area of 2–3 cm^2. Drying took 2–10 min. depending on temperature, humidity and the volume of the inoculum. Transfer was carried out by firmly rubbing the inoculated area for 15 sec.

Volunteers

Healthy volunteers aged 18–50 were housed in isolation singly or in groups of 2 or 3. Most of the volunteers were taking part in other experiments involving challenge with rhinovirus. They were inoculated with rhinovirus suspension (usually as 1 ml. nasal drops) after a 3-day quarantine period, and were observed in isolation for six more days. Each was assessed daily and given a clinical score based on symptoms and signs, and at the end of the observation period a total clinical score and a clinical grade (nil, very mild, mild, moderate or severe cold) was allotted. Inoculation and assessment of volunteers were 'double blind'. Sampling from fingers and objects was generally carried out on the third day after virus inoculation, which is usually the day of maximal symptoms and maximal virus shedding, without warning the volunteers beforehand. Nasal washings for reisolation of virus were collected (after sampling fingers and objects) using 10 ml. Hanks's saline. Washings were stored at −70° C. after addition of an equal volume of nutrient broth.

RESULTS

Survival of rhinovirus on surfaces

Volumes of 0·002 ml. or 0·0055 ml. of suspension of the TC or NW preparations of RV2, containing a known titre of virus, were allowed to dry on marked areas of volunteers' skin, or on smooth surfaces such as stainless-steel, enamel or plastic table-tops. After the inoculum had dried thoroughly the inoculated areas were sampled. The samples were subsequently titrated, together with samples of the original inoculum, and the titre of virus recovered was calculated. Table 1 shows that survival ranged from 0·6 to 63% on the surfaces tested, the least good survival rate occurring when using a virus suspension of relatively high titre, from tissue culture. No attempts were made to define the effect on virus survival of variations of temperature, humidity, or suspending medium, the last being either tissue culture maintenance medium containing 2% bovine serum, usually further diluted in Hanks's saline with 50% nutrient broth, or nasal mucus diluted in Hanks's saline with 50% nutrient broth.

Table 1. *Survival of RV2 from nasal washings (NW) or tissue culture (TC) after inoculation of* 0·002 *or* 0·0055 *ml. on various surfaces*

Inoculum (p.f.u.) and source	Surface	No. of samples tested	Survival (%)	
			Mean	Range
220 (NW)	Skin	16	30	2–63
220 (NW)	Skin or plastic	11	17	3–33
550 (TC)	Skin	4	8	6–11
550–1500 (TC)	Enamel or steel	7	14	10–18
4×10^4 (TC)	Steel	3	5	2–7
5×10^4 (TC)	Steel	3	0·7	0·6–0·8

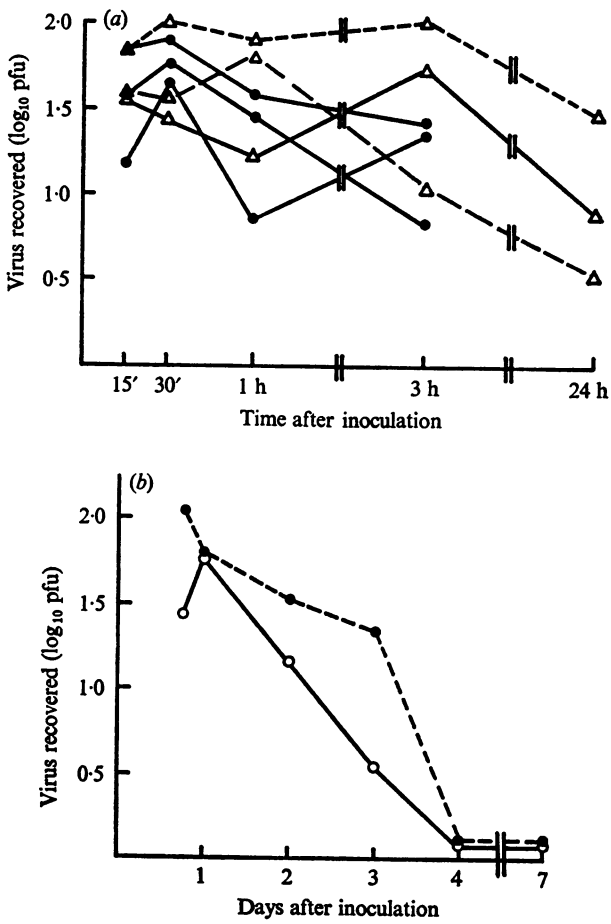

Fig. 1. (*a*) Survival of rhinovirus type 2 after inoculation on to skin and inanimate surfaces. ●——●, Backs of hands (three subjects); △---△, ball-point pens; △——△, table top; △— — —△, stainless-steel spoons. (*b*) Survival of rhinovirus type 2 on handles of stainless-steel spoons. ●---●, Spoons kept in the dark; ○——○, spoons kept in daylight.

Table 2. *Transfer of rhinoviruses between various surfaces by rubbing*

Starting titre (p.f.u.)	Attempted transfer from → to	Positive transfers effected	Percentage of starting titre transferred	
			Mean*	Range
33–120 (dry)	Fingers → {pen / steel}	0/29	—	< 3 % to < 13 %
20–120 (damp)	Fingers → {pen / steel}	8/12	14 %	5–42 %
70–150 (dry)	{Pen / Table / Steel} → finger	14/22	15 %	3–37 %
35–170 (damp)	{Pen / Table / Steel} → finger	16/24	21 %	3–46 %
20–50 (dry)	{Finger / Hand} → finger	14/18	17 %	0·6–31 %
30–100 (damp)	{Finger / Hand} → finger	10/12	34 %	19–49 %

* Calculation excludes the samples in which no transfer was achieved.

Duration of rhinovirus survival on surfaces

A series of marked areas of stainless steel, plastic or volunteers' skin were inoculated with virus, and the spots were sampled at various times after inoculation. Fig. 1(*a*) shows recovery of virus from marked areas on the backs of volunteers' hands, from a series of stainless-steel spoons, a series of plastic ball-point pens, and a series of areas on a plastic table top. During 3 hr. there was a possible slight loss of titre on skin, but little on the inanimate surfaces. After 24 hr. virus was still recovered from the various objects, though at lower titre. Fig. 1(*b*) shows virus survival on stainless-steel spoons allowed to lie on the laboratory bench, either beside a window or kept in a drawer in the same bench for a week. Virus was recovered until the third day, but not on the fourth, the initial recovery having been approximately $10^{1.75}$ p.f.u. per sample.

Recovery of rhinovirus from fabrics and paper tissue

Replicate spots were marked on cotton fabric as used for surgical gowns, heavy nylon fabric as used for working overalls, and paper handerkchief tissues. The spots were inoculated with 0·0055 ml. of suspension of RV2. At intervals up to 24 hr. attempts were made to recover virus by rubbing the inoculated area firmly with a finger for 15 sec. and then sampling the finger with a swab wetted in HH transport medium, or by cutting out the inoculated area and soaking and wringing out the fabric or tissue in HH medium. No virus was recovered from the fingers at any sampling time, although very small amounts were obtained by wringing out cotton or paper tissue at 1 hr. after inoculation. However, virus could be recovered from all samples of nylon up to 24 hr. by wringing out the fabric in transport medium: the average amount recovered was 30 % of the 200 p.f.u. inoculated.

Table 3. *Recovery of virus from fingers of subjects who shed rhinovirus from the nose*

	Virus recovered from fingers	
	Yes	No
Number of subjects (total 38)	16	22
Clinical status		
'Wild' colds	2	1
Experimental colds		
Moderate or mild	12	14
Very mild or symptomless	2	7
Mean clinical score (experimental colds)	36	22
Mean titre of virus in nasal wash*	$10^{2\cdot64}$	$10^{2\cdot07}$
Blew nose within previous 5 min.	8 (50%)	2 (9%)
Washed hands within previous 30 min.	5 (31%)	9 (41%)

* Log_{10} TCD 50 per ml. of nasal washing fluid.

Transmission of rhinovirus between surfaces

Replicate quantities of RV2 were inoculated on fingers or other areas of skin, or on plastic or steel surfaces, including ball-point pens or spoons. Samples were then taken as an indication of the initial virus titre surviving on the surface and attempts were then made to transfer virus by rubbing firmly for 15 sec. or, in the case of pens or spoons, by mimicking natural use for 3 min. The 'recipient' areas were then sampled and the frequency and amount of virus transferred (p.f.u.) was estimated. Table 2 shows that transfer was least efficient if the virus inoculum was allowed to dry on fingers, and attempts were then made to transfer virus to smooth objects by normal handling or rubbing. No transfer was detected in 29 such attempts using a dried inoculum, but some virus could be transferred if the inoculum was not completely dry at the time of contact. In the reverse experiments, when virus was dried on smooth surfaces including table tops, pens and stainless-steel objects, which were then handled normally or rubbed with a finger, transfer to the recipient finger occurred in 14 of 22 attempts (64%), up to 37% of the starting titre being picked up. Again, transfer was more efficient if the inoculum was still damp when handled. Rubbing of inoculated skin (fingers or backs of hands) by the fingers of other volunteers for 15 sec. also transferred virus successfully, especially if the inoculum was damp.

Recovery of rhinovirus from fingers of volunteers with colds

Samples were taken from the fingers of volunteers who had been inoculated with various serotypes of rhinovirus and from objects recently handled by them, small, smooth-surfaced objects being chosen for preference. A few subjects with natural colds were also examined. A single swab and 2·2 ml. of HH medium were used to sample both index fingers. The volunteers were also asked to say approximately when they last blew their nose, and when they last washed their hands or had them in water for other purposes. Nasal washings were taken, after the fingers had been swabbed. At least 1·2 ml. of HH medium stored from fingers and objects was subsequently cultured for rhinovirus and titrated, as

Table 4. *Recovery of virus from fingers of uninfected volunteers living in contact with subjects who shed virus from the nose*

Number of uninfected contacts examined	18
Number having virus on fingers	0
Washed hands within previous 30 min.	7 (39 %)
Status of 17 rhinovirus-infected partners	
'Wild' colds	1*
Experimental colds	
Moderate or mild	8
Very mild or symptomless	8
Mean clinical score	25
Mean titre of virus in nasal wash	$10^{2.17}$†

* This volunteer developed a 'wild' cold, from which a rhinovirus was isolated, during the first 3 days of observation, and she therefore received no experimental challenge. She lived in isolation with her flat-mate 'contact' for the usual 9-day period.

† Log_{10} TCD 50 per ml. of nasal washing fluid.

were the nasal washings. When dealing with any batch of samples in the laboratory, specimens from fingers and objects were always handled before nasal washings, and strict precautions were taken to avoid laboratory cross-contamination.

Results of finger sampling were evaluated according to whether or not the subject was infected with rhinovirus as judged by shedding of virus from the nose, this being a more sensitive index of infection than development of symptoms, or seroconversion. Table 3 shows results of finger-swabbing in those volunteers who were shedding rhinovirus from the nose. Rhinovirus was detected on the fingers of 16 of the 38 volunteers (42 %). In most cases titres were low, frequently being less than 1 TCD 50 per specimen (less than 3 of 6 inoculated tubes showing cytopathic effect), but ranging from this up to 20 TCD 50 in 12 cases, 60 TCD 50 in one case, and between 100 and 350 TCD 50 per specimen in three cases. Symptoms were rather more severe in those volunteers who had virus on their fingers than in those who did not, and the former had higher titres of virus in their nasal washings; they were also more likely to have blown their nose within the previous 5 min., and less likely to have washed their hands within the previous 30 min.

Forty objects recently handled by these volunteers were sampled, and virus was recovered from six, namely three coffee cups, a cigarette lighter, the handle of a domestic iron, and a small polythene bag used for collecting paper handkerchiefs. Each of these objects yielded less than 1 TCD 50 of virus.

In an attempt to determine the frequency with which rhinovirus is transmitted to the hands of non-infected contacts, a second group of volunteers was also assessed. These were uninfected as judged by absence of symptoms, absence of virus in two or more nasal washings, and absence of seroconversion. These contacts were generally serologically insusceptible to the challenge virus, as were flat-mates, sometimes husbands or wives, of volunteers who had become infected. They shared the same living accommodation, including living room, kitchen and bathroom facilities. As shown in Table 4, no virus was recovered from the fingers

of 18 such contacts, the swabs being collected on the day of the partners' maximal virus shedding. Table 4 also shows an analysis of the severity of the colds experienced by the infected partners of these contacts.

Inoculation of volunteers via their fingers

An attempt was made to compare the ability of an inoculum of RV9 to infect 'serologically susceptible' volunteers (i.e. those who had serum neutralizing antibody titres less than 1/8 before inoculation) when given as drops into the nose, as drops into the conjunctiva, or when inoculated on volunteers' forefingers, and thence applied to their conjunctivas or nostrils. Both forefingers were inoculated with 0·0055 ml. virus suspension, containing a total of 88 TCD 50 RV9, which was allowed to dry partially or completely. Volunteers then rubbed each conjunctival fornix for 30 sec. with the appropriate forefinger 'as if they were trying to pick out a piece of dust', or probed each nostril for 30 sec. with the appropriate forefinger. Fifty TCD 50 of RV9, when given as 1 ml. nasal drops by the standard method used at the Common Cold Unit, infected 12 of 15 'susceptible' volunteers, as judged by laboratory criteria (virus shedding on one or more days after inoculation, or seroconversion to RV9). By the same criteria two of four volunteers were infected when 88 TCD 50 of RV9 in $2 \times 0{\cdot}0055$ ml. volumes were dropped directly into the conjunctival fornices, one of five such volunteers infected themselves by rubbing their conjunctivas with fingers which bore a dried inoculum of 88 TCD 50 of RV9, and two of six became infected when the same inoculum was damp when transferred. In the case of those who inoculated their own nostrils, one of two became infected when the inoculum on their fingers was dry, and two of four when the inoculum was damp. Although the numbers involved in this experiment are small, the results suggest that a relatively large dose of RV9 inoculated indirectly is less efficient in producing infection than a smaller dose given directly into the nose.

DISCUSSION

These studies confirm the finding of Hendley, Wenzel & Gwaltney (1973) that viral contamination of the fingers is common in the acute stages of colds. Among our cases the rate of recovery of virus from fingers was 42 %, and low-grade viral contamination was also found on some of the objects with which the volunteers had recently been in close contact. In laboratory experiments it was shown that each individual step involved in transfer of virus from an infected person via intermediate objects to the nasal mucous membrane of a recipient can occur, at least under the somewhat contrived conditions of the laboratory.

However, an assessment of the significance of these findings in relation to spread of colds under natural conditions is not easy, and an attempt at measurement of surviving virus and the efficiency of its transfer from surface to surface is needed.

Rhinovirus evidently survives drying reasonably well; from 2 % to 63 % of infectivity of virus from nasal washings was found after drying, and this result is in reasonably good agreement with the loss of 0·2–0·9 $\log_{10}$ units of infectivity

reported by Buckland & Tyrrell (1962). Furthermore, virus was detected on steel surfaces for up to 3 days after inoculation.

Virus dried on surfaces could be transferred to other surfaces by rubbing (Table 2) and when this did occur, the amount transferred ranged from about 1 % to 50 % of the starting titre. The least efficient form of transfer was from contaminated fingers to objects. This could only be achieved if the inoculum was still damp – a circumstance which in daily life would imply very gross contamination. However, virus could be picked up with the fingers from smooth surfaces reasonably efficiently, and could also be transferred from the skin of one individual to another. In considering the likelihood of skin-to-skin transfer under natural conditions, it may be noted that we sampled only the pads of the index fingers of our volunteers, and we therefore have no information about contamination of other areas of skin, although the fingers and the skin of the face are probably the most heavily involved. In daily life, direct contact between finger pads of a potential recipient and a donor's contaminated face or fingers is probably not a particularly frequent happening except perhaps in the case of a person tending an infected child.

Although volunteers who were shedding virus from the nose frequently had virus on their fingers, titres were generally below 20 TCD 50, the maximum being 350 TCD 50. Bearing in mind the difficulty we encountered in transferring virus from inoculated fingers to another surface, the loss of infectivity which inevitably occurs even if transfer is successful, and the relatively large dose of virus required to infect by fingering the nostril or conjunctiva, the chance of successful transmission to a susceptible recipient by these routes must be low. The very small amounts of virus found on the objects handled by our volunteers bears out our laboratory finding that transfer from fingers to objects is inefficient; a potential recipient handling any of these objects would not have become infected. However, the experiments carried out *in vitro* suggest that if skin or another surface should become heavily contaminated by infectious droplets falling directly on it, a recipient might, by direct contact, pick up a sufficiently large inoculum to risk infecting himself by rubbing his eyes or picking his nose – events which Hendley *et al.* (1973) have shown to be very frequent in subjects who are not conscious of being observed.

The maximum titre of virus in nasal washings at the time of peak virus shedding is about $10^{4.5}$ TCD 50 per ml., and the mean about $10^{2.7}$ TCD 50 per ml. Secretions may be 30-fold more concentrated than this, i.e. a maximum of $10^{6.0}$ TCD 50 or a mean of $10^{4.3}$ TCD 50 per ml., giving $10^{2.0}$ TCD 50 or $10^{0.3}$ TCD 50 per droplet of 0·0001 ml. The larger dose, on skin or on objects, might perhaps initiate an infection if handled directly by a potential recipient, but not if further intermediate transfers were made.

It is clear that transmission of rhinovirus infection by indirect contact cannot be discounted, although our studies of the objects surrounding our volunteers, and their non-infected flat-mates, suggest that it is not likely to be of paramount importance. The present experiments do not provide convincing evidence of the importance of indirect contact in the natural transmission of rhinovirus infection,

but it is also hard to produce proof of the importance of airborne transmission. In attempting to assess the relative importance of these two routes of spread it may be remembered that even if production of infectious aerosols by coughing and sneezing is intermittent, the potential recipient is continuously sampling 10 l. air/min. In contrast, indirect contact spread must depend on the chance of picking up sufficient virus from an infected surface (an uncommon event, as judged by our volunteers' flat-mates) followed by probing the conjunctiva or nostril with the contaminated finger.

In order to obtain clearer evidence of the relative importance of the two routes of transmission in spread of rhinovirus infection, experiments similar to those reported by Tyrrell (1965) could be carried out using better virological control than was technically possible at the time of the original experiments. Serologically susceptible recipients could be exposed to donors either by the airborne route only, without manual contact, or to the objects and environment contaminated by these donors, without aerosol contact. Comparison of rates of virologically confirmed infection in these two groups of recipients should give useful information.

I am grateful to Dr J. W. Craig and Miss M. Bowden for care and clinical assessment of volunteers, to Mrs B. Head, Mrs P. Muir and Mrs K. Callow for skilled technical assistance, and to numerous colleagues for generously lending their time and their fingers for laboratory experiments.

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