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Effect of Route of Inoculation on Experimental Respiratory Viral Disease in Volunteers and Evidence for Airborne Transmission

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INTRODUCTION

Initiation of respiratory viral infection, with some possible exceptions, appears to depend upon deposition of infectious virus at some point on the respiratory tract. There appear to be two possible mechanisms of transmission, contact or airborne. The former term is meant to refer to transfer of virus by physical contact between an infected and a susceptible subject, or indirectly through personal articles or fomites. Transmission by this route would result in deposition of virus predominantly in the nasopharynx.

Airborne transmission is intended to mean transfer of infection by means of small-particle aerosols (11, 16). These particles are the evaporated residues of infected respiratory secretions which are of such small size (mostly less than 5 μ in diameter) that they will remain airborne for long periods of time. As a function of their small size, such droplets, when inhaled, deposit predominantly in the lower respiratory tract. Particles between 5 and 15 μ to 20 μ in diameter represent an intermediate stage, and most particles in this size range will be trapped in the nose, although some will penetrate to below the larynx. (Lower respiratory tract will refer to that portion of the respiratory tract below the larynx.) Still larger particles may be produced by coughing and sneezing, etc., but since, because of their large size, they do not produce stable aerosols, transmission will ordinarily occur only by direct impaction on the nasopharynx of persons in the immediate vicinity of an infected case. Such transmission would be difficult to distinguish from that resulting from contact, and is best considered under this category.

This report will describe studies of the transmission of respiratory viral diseases which were a joint undertaking of the U.S. Army Biological Laboratories, Fort Detrick, Md., and the Laboratory of Clinical Investigations, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

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The first part of the report will describe an investigation of the infectiousness of respiratory viruses given by methods which attempt to simulate natural contact and airborne transmission, namely, nasal drops and aerosols containing virus. Coxsackievirus A type 21, a strain of rhinovirus, and adenovirus type 4 were used in these studies.

The second part will describe recovery of virus from natural aerosols produced by coughing and sneezing and from air of rooms contaminated by such discharges. In addition, preliminary results of an experimental attempt to transmit respiratory viral infection in volunteers by the airborne route will be presented.

MATERIALS AND METHODS

Volunteers

Subjects were healthy adult male inmates from several federal correctional institutions and were selected on the basis of serum antibody determinations, willingness to participate, and demonstration of good health. For studies performed at the Clinical Center, National Institutes of Health, volunteers were isolated two or three to a room for 3 to 4 days prior to inoculation and 10 to 14 days after inoculation. Examinations were performed daily by physicians having no knowledge of which of several respiratory agents was administered to a particular volunteer.

An experimental transmission experiment was performed at the Federal Prison Camp, Eglin Air Force Base, Fla. Volunteers were housed in a converted barracks building, and were evaluated before inoculation and twice daily after inoculation by physicians who knew which volunteer was inoculated and which was an exposed susceptible. Complete separation of the two groups, as described in the text, was carefully maintained; however, only partial separation from the remaining camp population was maintained.

Inocula

Virus strains used in these studies were obtained from Marines with acute respiratory disease at Parris Island, S.C., or Camp Lejeune, N.C. (through the courtesy of K. M. Johnson, H. H. Bloom, and R. M. Chanock). Each inoculum had been passaged once or twice (see Results) in either human embryonic kidney (HEK) or human embryonic fibroblast (HEF, strain WI-26) tissue cultures (17). The harvests in each case were frozen and thawed, pooled, centrifuged at 1,000 $\times$ *g* for 20 min, and filtered through 800-mμ membrane filters (Millipore). The filtrates were stored at -60 C until used. Each inoculum was safety-tested for adventitious agents in a manner previously described (19). In addition to the above described procedures, the coxsackievirus A type 21 strain 48654 HEF_2 was submitted to vacuum concentration and trifluortrichlorethane (Gelman) treatment. Further details of these procedures have been described (6, 8, 9).

Inoculation Procedures

Volunteers received aerosol inoculation by means of a molded rubber face mask attached to a cylindrical chamber containing a continuous flow of aerosol generated by a Collison atomizer. Virus was approximately 10 sec old at the time of inoculation. This equipment and other necessary auxiliary components were contained in a mobile truck and semitrailer and have been previously described (15). Each man inhaled 10 liters ($\pm$5%) through the nose, and exhaled by mouth into a discharge bag. Each inoculation required 30 to 60 sec and usually followed a training period on a previous day with use of the same equipment. The size of particles in the aerosol ranged from 0.2 to 3.0 μ in diameter. Particles 1 to 2 μ in diameter comprised 54% of the total particle volume and contained 68% of recoverable virus. Further details of the aerosol will be described in a subsequent report in this symposium (14). Aerosol inoculations with particles 15 μ in diameter were performed with the same equipment, except that the vibrating reed method of Wolf was used to generate the aerosol (25). Volunteer doses for both aerosols were calculated from virus assays in simultaneously collected Shipe impinger samples of the aerosol.

Nasopharyngeal inoculations were performed by the instillation of 0.25 ml of virus inoculum into each nostril of the volunteer while he was prone. This inoculation was accompanied by a sensation of liquid in the nose but not by a desire to expectorate or swallow. In addition, some volunteers received 0.5 ml of inoculum into each nostril as well as 0.5 ml sprayed into each nostril by a no. 127 DeVilbiss (12) hand atomizer. Studies on the aerosol produced by this atomizer have shown that 99.95% of the inoculum is contained in particles greater than 5 μ in diameter and most would be deposited in the nasopharynx (*unpublished data*).

Collection of Cough, Sneeze, Talking, and Room Air Samples

Particles produced in selected expiratory events were collected for size analyses and virus

assay. In addition, room air samples were collected in a large-volume air sampler. Description and analysis of the methods used will also be described in a subsequent report in this symposium (14).

Virus Iolations and Identification Procedures

Specimens obtained varied with the virus being studied but included nose, throat, and anal swabs, nasal washes, and expectoration specimens. Specimens were collected prior to and subsequent to inoculation. Expectoration specimens were stored in that form until tested. Nasal washes were performed with 10 ml of Veal Infusion Broth (Difco) containing 0.5% bovine albumin with antibiotics; swabs were agitated in 2 ml of this medium and then discarded. All specimens were stored at −20 C until tested. Testing for virus was performed by inoculating 0.4 ml of specimen fluid into one HEK and HEF tissue culture tube that contained 1.5 ml of equal parts of medium 199 and Eagle's MEM, 2% inactivated calf or chicken serum, and antibiotics. The cultures were incubated in a roller drum turning at 12 rev/min at 33 to 34 C and were observed for cytopathic effect (CPE). This observation period was 14 days for coxsackievirus A type 21 and rhinovirus NIH 1734, but 60 days for adenovirus type 4. All the latter studies were performed in HEK cultures. Tissue culture fluid and cells were harvested when CPE involved 75 to 100% of the cell sheet. For coxsackievirus A type 21 and adenovirus type 4, the first and last isolates, as well as intervening isolates, when indicated, were identified by hemagglutination-inhibition (HI) with 20 antibody units of specific hyperimmune guinea pig serum or rabbit serum. HEF cultures were used for identification of comparable specimens of rhinovirus NIH 1734 by neutralization of 32 to 100 $TCID_{50}$ of virus with specific hyperimmune guinea pig serum. Further details of these procedures have been reported (6, 8, 9).

Serological Tests

Serial fourfold dilutions of inactivated serum were tested for neutralizing antibody for each virus by mixing equal volumes of the serum dilution with a test dose of virus, incubating at room temperature, inoculating each of two tissue culture tubes with 0.2 ml of the mixture, and observing thereafter for CPE.

All neutralizing antibody titers, calculated by the method of Karber, are expressed as the initial dilution of serum completely inhibiting CPE of 32 to 100 $TCID_{50}$ of coxsackievirus A type 21 and adenovirus type 4, but 10 to 16 $TCID_{50}$ of rhinovirus NIH 1734. Further details of the procedures have been reported (6, 8, 9, 13).

RESULTS

Response to Inoculation with Aerosol and Nasal Drops

Coxsackievirus A type 21: 50% human infectious doses (HID_{50}). Volunteers free of detectable antibody were inoculated with a range of doses of coxsackievirus A type 21 by small-particle aerosol (diameter of particles, 0.3 to 2.5 μ), large-particle aerosol (diameter of particles, 15 μ), and nasal drops (0.25 ml in each nostril). An example of the type of response obtained is shown in Table 1. Twenty-eight volunteers received strain 49889 HEK_1 in a small-particle aerosol, and 18 became infected. The doses, number of volunteers who received each dose, and the number who became infected, as determined by virus isolation and antibody rise, are shown. Based on these findings, the HID_{50} for this inoculum administered in this way corresponds to 28 $TCID_{50}$ (Spearman-Karber method; 13). Only two of the infected volunteers failed to develop illness, indicating that the 50% infectious dose and 50% illness dose are nearly the same.

In this experiment, three volunteers developed unexplained mild cases of rhinitis. Experience with over 300 volunteer inoculations indicates that such an illness is recorded in about 15% of uninfected individuals. The phenomenon occurs even though virus is inactivated with specific hyperimmune serum, in men with all levels of serum antibody, and irrespective of virus type or materials and methods used for inoculum preparation (8). Attempts to isolate a causative agent in HEK, HEF, and rhesus monkey kidney tissue cultures have been unsuccessful.

The HID_{50} for strain 49889 HEK_1 and another inoculum (strain 48654 HEF_2) of coxsackievirus

TABLE 1. *Response of antibody-free volunteers inoculated with 0.3 to 2.5-μ particle aerosol of coxsackievirus A type 21 (strain 49889 HEK_1)*[a]

Inhaled dose ($TCID_{50}$)	No. of volunteers	No. infected	No. ill
832	1	1	1
676	3	3	2
316	3	3	3
83	2	2	2
71	5	5	4
47	4	3	3
18	4	1	2[b]
6	6	0	2[b]

[a] HID_{50} = 28 $TCID_{50}$.

[b] Three cases of afebrile URI without infection.

TABLE 2. *HID$_{50}$ for coxsackievirus A type 21*

Inoculum	Inoculation method	No. of volunteers	No. infected	HID$_{50}$	95% Confidence limits
Strain 49889 HEK$_1$[a]	Aerosol, 0.3 to 2.5-μ particles	28	18	28 TCID$_{50}$	15–49
Strain 48654 HEF$_2$[b]	Aerosol, 0.3 to 2.5-μ particles	14	8	34 TCID$_{50}$	22–52
	Aerosol, 15-μ particles	29	12	32 TCID$_{50}$	13–78
	Nose drops	14	7	6 TCID$_{50}$	3–13

[a] One passage in human embryonic kidney tissue cultures.
[b] Two passages in human embryonic fibroblast tissue cultures.

TABLE 3. *Clinical response of antibody-free volunteers to coxsackievirus A type 21*

Inoculum	Inoculation method	No. of volunteers	No. infected	No. ill	Predominant illness		
					Afebrile URI[a]	Febrile URI	Febrile LRI[b]
Strain 49889 HEK$_1$[c]	Aerosol, 0.3 to 2.5-μ particles	28	18	16	1	3	12
	Coarse spray and nose drops	13	13	8	2	6	
Strain 48654 HEF$_2$[d]	Aerosol, 0.3 to 2.5-μ particles	14	8	8	1	7	
	Aerosol, 15-μ particles	29	12	11	1	8	2
	Nose drops	14	7	5	2	3	

[a] Upper respiratory tract illness.
[b] Lower respiratory tract illness.
[c] One passage in human embryonic kidney tissue cultures.
[d] Two passages in human embryonic fibroblast tissue cultures.

A type 21 administered by each of the described methods in shown in Table 2. As can be seen, the HID$_{50}$ is virtually identical for the three aerosol titrations; however, for virus administered by nasal drops, it is about fivefold less. Natural virus (virus recovered from naturally infected individuals, but not cultivated in vitro) administered by small-particle aerosol (not shown) produced infection in one of two volunteers at a dose of 28 TCID$_{50}$, and in none of six who received 7 TCID$_{50}$, suggesting a similar degree of infectivity (8).

The HID$_{50}$ for each aerosol inoculum is based on inhaled virus. Available information indicates that only 50 to 75% of particles of the size range in the small-particle aerosol would be retained and that the majority of these would deposit in the lower respiratory tract (11, 16). This indicates that the true HID$_{50}$ for the inocula administered in this way is appreciably less than that indicated in Table 2. All of the nasal drop inoculation was retained, and therefore the HID$_{50}$ for this method corresponds to the HID$_{50}$ given in the table. Since virtually all 15-μ particles would be retained, and the majority would be trapped in the nose, one would expect the HID$_{50}$ by this route of inoculation to be similar to that obtained by nasal drops. No explanation is presently available for the observed difference.

The clinical responses that correspond to the strains and inoculation methods in Table 2 are shown in Table 3. In addition, the responses to 3,000 TCID$_{50}$ of strain 49889 HEK$_1$ administered to the nasopharynx by coarse spray and drops are included (22). The frequencies of occurrence of illness in each of the five categories were not significantly different. As can be seen, the predominant clinical response to strain 49889 inoculated by small-particle aerosol was febrile lower respiratory tract illness. All 12 volunteers with this response were clinically diagnosed as having acute tracheobronchitis. The pertinent data obtained on a volunteer from a more recent experiment, but typical of the syndrome, are shown in Fig. 1. Characteristic of this syndrome was the occurrence of pain in the neck (tracheal) and chest, the latter usually being substernal. Cough, often paroxysmal, was usually non-

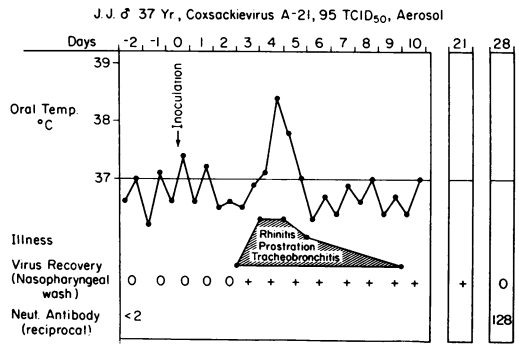

FIG. 1. *Case report of an antibody-free volunteer inoculated with coxsackievirus A type 21 by small-particle aerosol.*

productive, although auscultation of the chest occasionally revealed scattered rhonchi, and, in two cases, there was X-ray evidence of pneumonia. These lower respiratory tract symptoms were accompanied by malaise, myalgias, chilly sensations, sweats, headache, and anorexia. Illness was not limited to the lower respiratory tract, however, since 9 of the 12 volunteers with tracheobronchitis also had upper respiratory tract illness that was characterized by rhinorrhea and nasal obstruction. Four of the remaining six infected volunteers had upper respiratory tract illness only, and the other two had infection without apparent illness.

In contrast to the small-particle aerosol response, 8 of 13 volunteers who received nasopharyngeal inoculation developed upper respiratory tract (nasopharyngeal) illness only. The fact that virus was deposited in the nasopharynx in this case and predominantly in the lower respiratory tract in the former suggested that virus deposition site accounted for this difference and that it might be the factor that determines the clinical response. However, when strain 48654 was administered by small-particle aerosol, the lower respiratory tract illness, which was characteristic of strain 49889 given in this way, was not seen. The predominant clinical response to strain 48654 in a small-particle aerosol was febrile upper respiratory tract illness (Table 3). Thus, virus deposition site and inoculum differences both appeared as important factors in determining the type of clinical response.

Febrile upper respiratory tract illness was also the predominant clinical response for strain 48654 administered by 15-μ particle aerosol and by nasal drops (Table 3). Not shown are clinical responses to natural virus and to still another strain of virus administered by small-particle aerosol (8, 20). For these inocula, febrile upper respiratory tract illness also predominated. This combined experience suggests that virus deposition site may be an important factor in determining the type of clinical response that occurs. However, for coxsackievirus A type 21, most strains appear to lack the capability of producing lower respiratory tract illness when presented such an opportunity by virus deposition at this site.

In all other aspects, the clinical responses were similar for each inoculum and inoculation method. The incubation period was 2 to 5 days, illness usually lasted 2 to 3 days, fever rarely exceeded 38.5 C, and fever usually persisted less than 1 day.

The effect of pre-existing serum neutralizing antibody on the responses following inoculation of volunteers with coxsackievirus A type 21 (strain 49889) has not been completely evaluated, but the data available are shown in Table 4. As can be seen, all individuals with intermediate titers of antibody were infected after nasopharyngeal inoculation, but infection occurred in only 5 out of 11 with high titers. A similar suggestion of reduction in infection also occurred in the small-particle aerosol groups.

Rhinovirus NIH 1734: HID_{50}. Volunteers free of detectable antibody to this virus were inoculated with a range of doses of rhinovirus NIH 1734 by small-particle aerosol and by nasal drops. The HID_{50} for each inoculation method is shown in Table 5. Nasal drop doses of 1 $TCID_{50}$ and less were extrapolated values based on dilutions of a pool with known virus concentration, and aerosol doses of 2 and less were extrapolated from measured concentrations of virus in aerosols produced, during the inoculation period, by higher concentrations of virus. Repeated tests of several dilutions of virus run in sequence have been shown to produce proportionate changes in aerosol virus concentration. Assays for virus were performed in HEF (WI-38) tissue cultures, in a

TABLE 4. *Response of volunteers with pre-existing antibody to inoculation with coxsackievirus A type 21*

Level of antibody	Nasopharyngeal inoculation			Aerosol, 0.3 to 2.5 μ particles		
	No. of volunteers	No. infected	No. ill	No. of volunteers	No. infected	No. ill
Intermediate (1:4–1:128)	6	6	4	5	3	2
High (1:256 or greater)	11	5	0	3	0	3[a]

[a] Each illness was mild rhinitis.

TABLE 5. *HID_{50} for rhinovirus NIH 1734*

Inoculation method	No. of volunteers	No. infected	HID_{50}	95% Confidence limits
Nasal drops	17	11	0.032 $TCID_{50}$	0[a]
Aerosol, 0.3 to 2.5 μ particles	26	20	0.68 $TCID_{50}$	0.2–2.0

[a] Indicates no intermediate response between 100 and 0% infection.

manner described previously (6). Other types of tissue culture [HEK and HEF (WI-26)] and tissue culture assay [HEF (WI-38) plaque assay] were tested and found to be equal to or less sensitive than the cultures and methods used.

As can be seen in Table 5, the HID_{50} for both inoculation methods was below the practical limits of detection. Failure to infect all volunteers with small-particle aerosol inoculation first occurred at an inhaled dose of 2 $TCID_{50}$, and none of three who inhaled 0.06 $TCID_{50}$ became infected. The HID_{50} for this inoculation method was 0.68 $TCID_{50}$ (Spearman-Karber; 13). In contrast, all volunteers who received 0.1 $TCID_{50}$ by nasal drops became infected, although none became infected at two lower doses. The HID_{50} for this method corresponded to 0.032 $TCID_{50}$. These results indicate an approximately 20-fold disparity between infectivity for the virus given by the two methods. The disparity could be accounted for by assuming that the 10 to 20% of small-particle aerosol particles that deposit in the nasopharynx are responsible for all infection in volunteers inoculated in this way. However, the fact that this is not the case is suggested by the occurrence of lower respiratory tract illness in some of these volunteers. In any event, the data suggest that the nasal mucosa is somewhat more susceptible to rhinovirus NIH 1734 than is the lower respiratory tract. Although the difference was less for coxsackievirus A type 21, it was similar in direction.

The clinical responses of all volunteers who have received either nasal or small-particle aerosol inoculation with rhinovirus NIH 1734 are shown in Table 6. As can be seen, the characteristic response to either method of inoculation is an upper respiratory tract illness which in all respects is a common cold. The pertinent data obtained from one of the volunteers inoculated by nasal drops are shown in Fig. 2. His response consisted of a common cold syndrome characterized by nasal obstruction and discharge, and was accompanied by throat irritation and systemic symptoms. The extent of the rhinorrhea

TABLE 6. *Clinical response of volunteers to inoculation with rhinovirus NIH 1734*

Inoculation method	No. of infected volunteers	No. ill	Illness		
			URI	URI-LRI[a]	LRI
Coarse spray and nose drops	48	43	41	2	0
Aerosol, 0.3 to 2.5 μ particles	41	33	23	5	5

[a] Upper and lower respiratory tract illness.

is shown in the figure. Fever was absent in this volunteer and occurred in less than 10% of the volunteers, regardless of method of inoculation.

As can be seen in Table 6, lower respiratory tract illness (acute tracheobronchitis) was predominant in five volunteers who received small-particle aerosol inoculation, and diffuse respiratory tract disease without a predominant localization was seen in five others. Predominant lower respiratory tract illness was not seen in men inoculated by nasal drops, although two volunteers exhibited a combination of upper and lower respiratory tract illness. These findings suggest that aerosol inoculation may produce lower respiratory tract involvement, but the characteristic response to infection produced by either method is an upper respiratory tract illness.

The incubation period of the illnesses produced by both inoculation methods was 2 to 4 days, the illness usually lasted 2 to 3 days, and fever, when it occurred, was usually 1 day in duration.

The effect of pre-existing serum neutralizing antibody on responses to inoculation with rhinovirus NIH 1734 is shown in Table 7. As can be seen, no significant reduction in frequency of infection occurred unless high levels of serum antibody were present. This reduction in frequency of infections occurred for both methods of inoculation and was accompanied by a similar reduction in illnesses. [Data are grouped for convenience. Individual values were tested in Spearman's rank correlation or Yates mean score tests (13). Reduction in infection and illness with increasing serum antibody was statistically significant ($P < 0.05$) for both inoculation methods.]

Adenovirus type 4: HID_{50}. Nine volunteers free of detectable antibody to adenovirus type 4 received small doses of this virus by small-particle aerosol. Six volunteers received the virus by 15-μ particle aerosol. The results of these

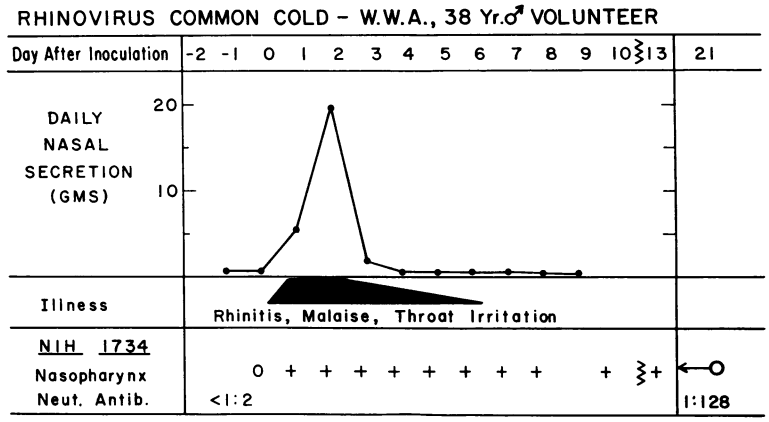

FIG. 2. *Case report of an antibody-free volunteer inoculated with rhinovirus NIH 1734 by coarse spray and nasal drops. (Reproduced with the permission of the Journal of Clinical Investigation.)*

studies are shown in Table 8. As can be seen, all volunteers who received doses of 11 and 5 $TCID_{50}$ by small-particle aerosol became infected, but only one out of three became infected at a dose of 1 $TCID_{50}$. Other volunteers were inoculated in this way, and, although the data are incomplete, the studies indicate that the HID_{50} for small-particle aerosol inoculation is about 1 $TCID_{50}$. It should be stated that these virus assays were performed in HEK tissue cultures, the most sensitive tissue available for adenovirus, and the cultures were observed for 60 days for CPE with subpassage as needed. This time period was shown to provide maximal detection of adenovirus (9).

Only one dose level of adenovirus type 4 has been administered by 15-μ particle aerosol, and this was 1,000 $TCID_{50}$. All six volunteers who received this dose became infected. Preliminary results on inoculation of volunteers by nasal drops indicate that the HID_{50} by this method is about 20 $TCID_{50}$. This combined experience with adenovirus type 4 suggests that a greater dose of this virus is required to initiate infection in the nasopharynx than in the lower respiratory tract.

Also shown in Table 8 are the clinical responses seen in the volunteers inoculated by aerosol. As can be seen, all volunteers infected by means of small-particle aerosol inoculation became ill, and the illness was usually febrile. Three volunteers had predominantly upper respiratory tract illness, and, in three others, illness was predominantly in the lower respiratory tract. The latter included one instance of mild pneumonia. Only three of the six volunteers infected by 15-μ particle aerosol inoculation became ill, two with febrile upper respiratory tract illness and one with pneumonia. The incubation period for these

TABLE 7. *Response of volunteers with pre-existing antibody to inoculation with rhinovirus NIH 1734*

Level of antibody	Nasopharyngeal inoculation			Aerosol, 0.3 to 2.5 μ particles		
	No. of volunteers	No. infected	No. ill	No. of volunteers	No. infected	No. ill
Low (1:2–1:8)	3	3	2	5	4	4
Intermediate (1:16–1:64)	9	8	7	8	6	4
High (1:128 or greater)	13	8	4	4	1	1

illnesses varied between 6 and 13 days, duration of illness varied between 2 and 10 days, and fever between 1 and 8 days. In addition, the severity of illness, as manifested in respiratory tract involvement and constitutional symptoms, also was quite variable. Upper respiratory tract findings occurred in all men in the 15-μ particle aerosol group, whereas this finding was variable in the small-particle aerosol group. The pertinent data obtained on one of the volunteers who exhibited the syndrome described as acute respiratory disease (ARD) of military recruits are shown in Fig. 3. Bacteriological cultures were negative for pathogens, and spontaneous recovery occurred without antibiotic therapy.

It is notable that the syndromes of febrile respiratory tract illness that occurred after aerosol inoculation resemble the naturally occurring type 4 adenovirus diseases of military recruits (3, 7, 21). Previous studies by others, in which volunteers were inoculated in the nasopharynx, usually resulted in asymptomatic infection or mild afebrile upper respiratory illness (1). Inocula-

TABLE 8. *Response of antibody-free volunteers to adenovirus type 4*

Inoculation method	Dose[a]	No. of volunteers	No. infected	No. ill	Illness		
					Afebrile URI[b]	Febrile URI	Febrile LRI[c]
Aerosol, 0.3–2.5-μ particles	11	3	3	3	1	1	1
	5	3	3	3		1	2
	1	3	1	1		1	
Aerosol, 15-μ particles	1,000	6	6	3		2	1

[a] Expressed as $TCID_{50}$.
[b] Upper respiratory tract illness.
[c] Lower respiratory tract illness.

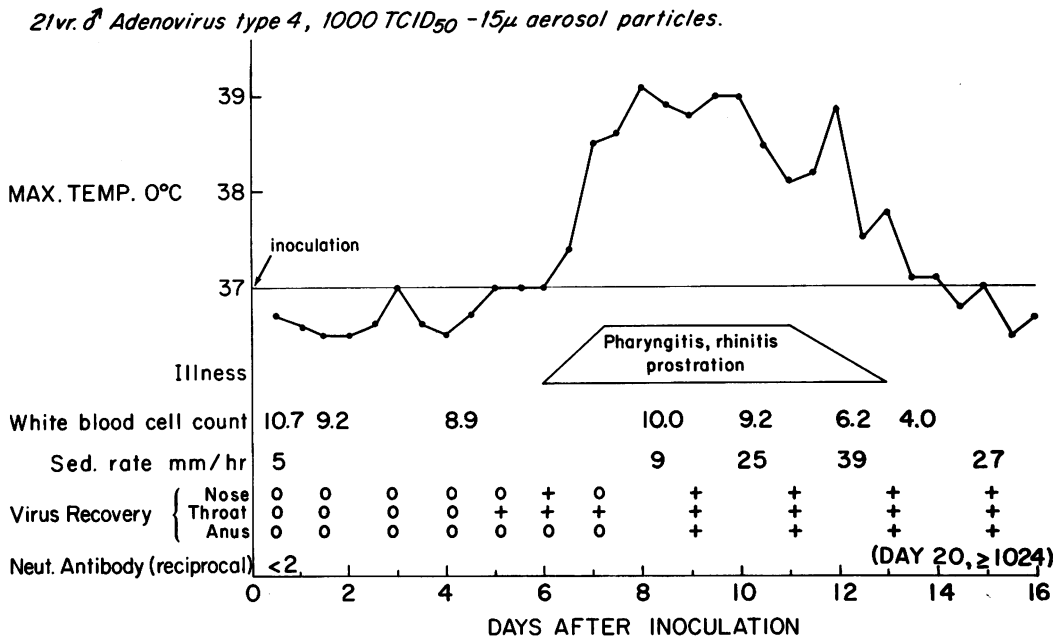

FIG. 3. *Case report of an antibody-free volunteer inoculated with adenovirus type 4 by 15-μ particle aerosol. White blood cell counts are times 10^3 per cm. (Reproduced with the permission of the American Review of Respiratory Diseases.)*

tions into the conjunctival sac resulted in occurrence of conjunctivitis only or pharyngoconjunctival fever, illnesses which rarely occur naturally in type 4 infection, and which were not seen in the present studies (1). These findings suggest that the unique feature of the present inoculations, deposition of virus in the lower respiratory tract, was the major factor accounting for the recruit-type illnesses. This is supported by the fact that small doses of virus given by small-particle aerosol produced illness in all volunteers infected by this method of inoculation, whereas the large dose given by 15-μ particle aerosol caused illness in only three of six infected men. Evidence indicates that most of the 15-μ particles were deposited in the upper respiratory tract, but the possibility exists of deposition in the lower respiratory tract either by direct inhalation or as a result of particle fragmentation (16). It is suggested that in three men this occurred and caused febrile illness.

Three volunteers with pre-existing antibody titers of 1:32 to 1:64 received 6 $TCID_{50}$ by small-particle aerosol, and none became infected or ill.

Inoculations of volunteers with pre-existing antibody into the nasopharynx or conjunctival sac by others have also demonstrated the protective effect of serum antibody (1).

Evidence for Airborne Transmission

Detection of virus in particles produced by coughing, sneezing, and normal expiration. By use of methods (14) for recovery of virus from particles produced by coughs and sneezes, virus titration was carried out on 61 cough collections and 58 sneeze collections from volunteers infected with coxsackievirus A type 21 (Table 9). The collection method involved coughing or sneezing into a collapsed weather balloon through a tight-fitting face mask. The air in the balloon was evacuated through a Shipe impinger to remove airborne particles, and material impacted on the wall of the balloon was collected by rinsing with sterile tissue culture fluid. When the results of both samples were combined, 39% of cough specimens and 50% of sneeze specimens were positive for virus. Thirty per cent of air samples were positive for both events, and the mean quantity present was 30 $TCID_{50}$ and 60 $TCID_{50}$ for cough and sneeze samples, respectively. This close similarity in results is of interest in view of the approximately 20-fold greater number of particles and particle volumes produced by sneezing (14). This finding suggests that the concentration of virus in secretions released in small particles produced by coughing is greater than that produced by sneezing.

Analysis of balloon wall samples revealed a disparity between the two events. Wall samples of sneezes were more frequently positive than the wall samples of coughs, but the mean quantity present was only twofold greater. However, the mean quantity of virus present in the wall samples from sneezes does not include four sneezes in which gross contamination with large quantities of nasal secretion occurred. The wall samples of these sneezes contained 30,000 to 500,000 $TCID_{50}$ of virus. The reasons for the disparity in frequency of detection of virus on the balloon wall for the two events are not known at the present time, since studies have revealed similar particle size distributions for both events (12, 14).

TABLE 9. *Virus recovery from particles in coughs and sneezes produced by volunteers infected with coxsackievirus A type 21*

Phenomenon	No. tested	Per cent positive	Source	Per cent positive	Mean quantity ($TCID_{50}$)
Sneeze....	58	52	Air[a]	30	60
			Wall[b]	45	100
Cough....	61	39	Air[a]	30	30
			Wall[b]	20	50

[a] Assay of Shipe impinger collection of particles suspended in air in balloon.

[b] Assay of 10-ml liquid rinse of balloon wall.

Breathing samples were tested by collecting the entire amount of expired air in Shipe impingers through a closed system for 30-min periods. This testing constituted sampling of air expired for 2 hr per day from four infected volunteers during the period that included occurrence of illness and maximal virus shedding. A volume equivalent to 12 hr of expired air was tested in this way, and all samples were negative for virus.

A number of factors were evaluated to determine the cause for virus release in the process of coughing and sneezing. These evaluations suggested that the presence of nasal obstruction and discharge was the most important determinant for release of virus when infected persons sneeze [with nasal obstruction and discharge, 19 of 24 sneeze samples were positive; without nasal obstruction and discharge, 11 of 34 samples were positive ($P < 0.001$)]. In contrast, positive cough specimens bore a relationship only to the quantity of virus present in respiratory secretions, and this relationship occurred for air samples only [combined nasal and oral secretions, Yates mean score, test, $P = 0.05$ (13)]. Since cough particles would presumably be derived from pharyngeal and lower respiratory secretions, it is suggested that the concentration of virus in these secretions varied proportionately with the secretions tested.

Virus in room air. The contribution to room air contamination by coughing, sneezing, and possibly by other expiratory phenomena of man involves frequency and occurrence of the phemomenon, inactivation of virus, and physical loss of aerosol particles, in addition to quantity of virus released. The significance of these factors in determining environmental contamination was tested by collecting particles present in the air of rooms occupied by volunteers infected with coxsackievirus A type 21 and then assaying the collections for virus.

The large-volume air sampler was used to collect particles from approximately 70% of room air after a period of 2 hr with no ventilation. Shown in Table 10 are the results of testing 30 such samples collected during the acute phase of illness and maximal virus shedding. Of the 30 samples, 14 were positive, and, as cao be seen, the frequency of positive samples increased with increasing quantity of virus present in respiratory

TABLE 10. *Relation of virus quantity in respiratory secretions to virus in room air samples*

Mean (3 vol.) virus quantity in secretions	Air sample		
	No. of tests	No. positive	Mean virus quantitity
10–30[a]	5	1	5[b]
30–100	11	2	160
100–300	5	4	250
300–1,000	6	4	50
1,000–>1,000	3	3	500

[a] Expressed as $TCID_{50}$ per milliliter of secretion.
[b] Expressed as $TCID_{50}$.

secretions [Smirnoff test, $P < 0.01$ (13)]. The mean virus quantity in positive samples is shown in the last column and was sufficiently variable so that no quantitative relationship to virus in respiratory secretions was detected, although it is of interest that the largest quantity present in room air, 28,000 $TCID_{50}$, was in the room with the highest virus concentration in secretions.

Since both positive cough and room air samples were related to quantity of virus in respiratory secretions of infected volunteers, it was suggested that coughing was responsible for contamination of room air with virus. When the results were analyzed by room, it was found that the presence of virus in cough air samples from volunteers occupying a room was significantly related to the recovery of virus from the air of that same room on the same day. [Positive room air samples, 10 of 11 rooms with positive cough air samples; negative room air samples, 2 of 7 rooms with positive cough air sample ($P = 0.03$)]. This further suggested that cough is the important intermediary between virus in secretions and virus in room air. No such relationship was detected for sneezing. These findings are not surprising, since cough as a symptom was recorded as being frequently present in these same volunteers at this time, whereas sneezing was not.

Preliminary report on a transmission experiment. An experiment designed to test whether the occurrence of air contamination with virus is sufficient to produce airborne transmission has been performed (*unpublished data*). Nineteen placebo-inoculated volunteers were exposed to air surrounding infected volunteers by housing the two groups in a converted barracks and separating them with a double-wire barrier. Even distribution of air on both sides of the test building was accomplished by means of large floor fans and was proved by generating an aerosol containing a fluorescein dye on one side and then collecting and analyzing air samples from different locations throughout the building. Coxsackievirus A type 21 infection was produced in 10 volunteers with aerosol inoculation, and all exposed individuals became infected with this virus during the course of the study. A specific separation of results in terms of contact and airborne-acquired infection is not completed, but it is possible to state that airborne transmission unquestionably occurred.

DISCUSSION

The theory that respiratory viruses are transmitted by the airborne route has been popular in the past, primarily because it seemed reasonable to assume that coughing and sneezing, common symptoms of viral respiratory disease, produce aerosols that would accomplish such transmission. Despite this assumption, proof that man produces aerosols that contain virus and that sufficient viral contamination of air occurs to result in this type of transmission, both essential requirements for airborne transmission, has not been obtained (24). The results presented in this report provide this important information. It was shown that individuals infected with respiratory viruses, in this case coxsackievirus A type 21, produce airborne virus in quantities sufficient to infect susceptible individuals. The capacity to produce viral aerosols was tested for three expiratory events. Breathing samples were uniformly negative for virus, whereas cough and sneeze samples were frequently positive. Thus, whereas in man the former event is probably insignificant in producing transmission of the respiratory viruses, it seems likely that it is important in the mouse-influenza system of Shulman and Kilbourne (23) in which airborne transmission has also been conclusively demonstrated. For man, coughing and sneezing appear to be the significant events for producing viral aerosols.

Studies in which virus released by coughing and sneezing was collected in a balloon and separated into an air phase and a wall phase provided quantitative results that correspond roughly to virus involved in airborne transmission and contact transmission, respectively. Virus was recovered more often from the air sample from coughs than from the wall samples, although the wall samples of sneezes were more commonly positive than the air samples. This would suggest that sneezing may be of some significance for that form of transmission involving direct impaction of large particles in the nasopharynx, whereas cough contributes primarily to small-particle

aerosol transmission. Despite the differences in frequency of recovery, the difference in mean quantity of virus in each phase was small and quite similar for each event. These findings are in contrast to the findings of Buckland et al. (5), in which the vast majority of virus released in sneezing was found on the sides of a large sampling bag. However, the different collection methods involved may account for this disparity.

Despite a larger number of particles in sneezes than in coughs, the quantity of virus expelled in the two events was remarkably similar, suggesting that, in these volunteers, the concentration of virus in secretions atomized in coughing was relatively greater than that in secretions atomized in sneezing (12, 14). Inoculation of these volunteers was performed by small-particle aerosol, and, although lower respiratory tract secretions were not quantitated, virus is known to have been deposited at this site and probably induced infection there. Thus, it is possible that the method by which infection was induced may have contributed to the virus recovery results from coughing.

The fact that infected persons are capable of producing airborne virus does not necessarily indicate that virus can be transmitted in this way. Viral aerosols produced by infected persons are subject to dilution in room air, biological decay, and sedimentation. Nevertheless, assuming normal breathing by susceptible volunteers and an infectious dose of about 6 to 30 $TCID_{50}$, assay of air samples from rooms occupied by infected volunteers indicated that transmission would be accomplished in from 5 min to 24 hr. Furthermore, in view of the observed efficiency (11%) of the air-sampling equipment, larger than measured doses of virus were actually available for inhalation (14). In addition, the present data suggest that cough is a most important event in producing viral contamination of air.

The findings described above stimulated the performance of an experiment to test the assumption that airborne transmission is possible, and preliminary results revealed that airborne transmission occurred from infected cases to susceptibles across a wire barrier.

Airborne and contact transmisison was simulated in volunteers by aerosol and nose drop inoculation, respectively. Studies with three different strains of coxsackievirus A type 21 indicated a similar HID_{50} of about 30 $TCID_{50}$ for this virus, when predominant deposition was in the lower respiratory tract (small-particle aerosol), and a lower value when nasal drops were used. Since the latter inoculation method provided deposition only in the nasopharynx, it is suggested that the nasal mucosa exhibited a greater susceptibility to infection with this virus than did the lower respiratory tract. Another picornavirus, rhinovirus NIH 1734, exhibited an even greater difference between the HID_{50} for nasal drop inoculation and for small-particle aerosol inoculation. Thus, the data suggest that, for both of these viruses, the nasal mucosa is the preferred site for infection. Although definitive comparisons are incomplete, present evidence suggests a disparity in infectivity in the opposite direction for adenovirus type 4. This virus exhibits a high degree of infectivity for the lower respiratory tract, but the nasopharynx appears to lack this degree of susceptibility.

The most common illness response to each virus that followed inoculation by nasal drops and small-particle aerosol is shown in Table 11. For comparative purposes, the most common naturally occurring illness response to each virus is also listed. As can be seen for coxsackievirus A type 21, regardless of method of inoculation as well as dose, febrile upper respiratory illness usually results in volunteers, whereas naturally occurring illness is reported to be usually afebrile (2, 18). This disparity may well be explained by the fact that fever in volunteers is usually so brief in duration that, without 24-hr observation, the majority of volunteers would have been designated afebrile. The predominant lower respiratory tract illness that was seen with one

TABLE 11. *Characteristic natural and experimentally induced clinical responses to respiratory viruses*

Virus	Experimental inoculation		Natural inoculation
	Nasopharyngeal	Aerosol, 0.3 to 2.5 μ particles	
Coxsackievirus A type 21	Febrile URI[a]	Febrile URI	Afebrile URI
Rhinovirus NIH 1734	Afebrile URI	Afebrile URI	Afebrile URI
Adenovirus type 4	Afebrile URI	Febrile URI or LRI, or both	Febrile URI or LRI, or both

[a] Upper respiratory tract illness.

inoculum administered by small-particle aerosol appears to have been relatively unique, and was due to properties of the virus in that inoculum that are not usually exhibited by strains of this virus.

For rhinovirus NIH 1734, afebrile upper respiratory tract illness occurs in volunteers regardless of inoculation method and is also the characteristic natural clinical response to this and other rhinoviruses (4, 10). Data thus far available indicate that naturally occurring adenovirus type 4 disease can regularly be reproduced in volunteers only by aerosol inoculation. Nasal inoculation, throat swabbing, and conjunctival inoculation have all failed to reproduce naturally occurring type 4 adenovirus disease (1).

It is therefore suggested that adenovirus type 4 disease is transmitted in natural circumstances primarily by the airborne route. The information available on coxsackievirus A type 21 and rhinovirus NIH 1734 indicates that either airborne or contact transmission would result in the upper respiratory tract illness characteristic of naturally occurring illness. However, the small-particle aerosol inoculation results suggest that airborne transmission would produce a more varied response and account for the lower respiratory tract illness which is sometimes associated with naturally occurring upper respiratory tract disease (4, 10, 18).

Thus, the data presented on production of airborne virus, environmental air contamination with virus, and the demonstration of airborne transmission summarized in the present report indicate that airborne transmission probably occurs naturally. Present information, however, does not indicate whether airborne transmission is the predominant mechanism of natural transmission. At the present time, it seems most reasonable to suggest that both contact and airborne transmission occur in natural circumstances, and that the predominant method of transmission varies with the virus and the opportunity presented in a particular situation. For those viruses and situations in which airborne transmission predominates, it may be possible to devise suitable methods of control of respiratory viral infection.

SUMMARY

Volunteers were inoculated with respiratory viruses by means of nasal instillations and inhalation of aerosols. The former method was used to simulate contact transmission, and the latter to simulate airborne transmission. The HID_{50} for coxsackievirus A type 21 was about 30 $TCID_{50}$ by aerosol and 6 $TCID_{50}$ by nose drops. Similar determinations for rhinovirus NIH 1734 revealed HID_{50} of 0.68 $TCID_{50}$ by aerosol and 0.032 $TCID_{50}$ by nasal drops. The clinical response was characteristically an upper respiratory tract illness for both viruses by both inoculation methods, although coxsackievirus A type 21 illness was usually febrile, and rhinovirus illness usually was not. Incomplete infectivity studies with adenovirus type 4 suggest a disparity in the opposite direction for this infection. Aerosol inoculation revealed an HID_{50} of about 1 $TCID_{50}$ and thus far is the only inoculation method which regularly reproduced naturally occurring ARD.

The suggestion that airborne transmission accounted for some naturally occurring acute respiratory disease was further evaluated by studying the production of airborne virus by coughs and sneezes and the contamination of room air with virus. Coughing and sneezing regularly produced quantities of virus sufficient to infect, whereas breathing did not. Room air samples revealed contamination probably sufficient to infect susceptibles. In addition, preliminary results of a transmission experiment with coxsackievirus A type 21 indicate that airborne transmission unquestionably occurred. It was concluded that both contact and airborne transmission of the respiratory viruses probably occur in natural circumstances, and that the predominant method of transmission may vary with the virus and with the particular environmental situation.

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