Effect of Relative Humidity on Experimental Transmission of Sendai Virus in Mice (36691)

J. van der Veen, Yvonne Poort, and D. J. Birchfield
Department of Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands

The role of relative humidity (RH) in the survival of airborne respiratory agents has been the subject of several studies (1–5). The studies were carried out with aerosols. Because of the complexity of conditions determining the significance of environmental factors in respiratory infections in man extrapolation of laboratory findings with aerosols to natural transmission may not be valid. Studies on experimental animal models might be better suited to elucidate the role of different variables. A model for experimental airborne transmission of Sendai virus in mice was developed in this laboratory (6). This system was used to study the effect of RH and ventilation rate and to compare the transmissibility of 2 strains of Sendai virus.

Materials and Methods. Four-week-old male Swiss mice (Rockefeller Institute strain by origin) from a specific pathogen free colony were used. The colony was negative for antibodies against Sendai virus (7). Sendai virus was passaged 13 times in mouse lung. The identity of the virus was confirmed in complement fixation (CF) and hemagglutination inhibition tests using guinea pig antiserum. Transmission cages and methods of transmission have been described in detail elsewhere (6). Mice to be used as transmitters were inoculated intranasally with 100 plaque-forming units (PFU) of virus. On the day of transmission the mice were tranferred to transmission cages in a separate room. The cages, made of transparent plastic, were divided into two compartments by means of a double-wire barrier. For direct contact experiments, transmitter mice and uninfected contact mice were housed together in the same compartment. For airborne transmission experiments, transmitter mice were placed in one of the two compartments of a cage and uninfected mice in the other. The distance between mice in adjacent compartments was 1–4 in. "Open" cages of wire mesh along all sides were used for airborne transmission over longer distances as described under Results.

Transmission experiments were carried out on days 6 and 7 after inoculation of transmitter mice. Uninfected mice were exposed to infected transmitters for 24 hr, after which the contact animals were removed and a new group of uninfected mice were exposed to the same transmitters for 24 hr. Transmitters and contact mice were transferred to cages (1 mouse/cage) and kept in "quarantine" for an additional period of 2 weeks. Subsequently, they were bled. Development of CF antibody was used as the criterion for infection. Control experiments with uninfected mice not exposed to infection and kept under the same conditions of "quarantine" never showed evidence of cross-infection by infected contacts. In one experiment exposure was also on days 5 and 8 after inoculation of transmitter mice.

All transmission experiments were carried out in incubators under controlled environmental conditions. Unless otherwise stated, the incubators were kept at 20° and 60–70% RH, and were supplied with 10 air exchanges/hr.

Results. Relative humidity. To study the effect of relative humidity on transmission over a short distance, infected transmitter mice were placed in transmission cages, 1 mouse/cage. Six uninfected mice were added to each cage, 2 mice in the compartment housing the transmitter and 4 in the other compartment. Cages were placed in incubators (4 cages/incubator). In each experiment, 2 incubators were kept at 40–45% RH

TABLE I. Effect of Relative Humidity on Transmission of Sendai Virus Infection in Mice.

Type of contact	Relative humidity (%)	Contacts infected	
		No.	%
Direct	40–45	55/111	50[a]
	60–70	68/112	61
Air, 1–4 in.	40–45	43/288	15[b]
	60–70	63/287	22
5–6 ft	30–35	7/96	7[c]
	60–70	31/96	32

[a] Difference between lower and higher RH not significant.

[b] $p < .05$, compared to 60–70% RH.

[c] $p < .0005$, compared to 60–70% RH.

and 2 other incubators at 60–70% RH. The combined results of 5 experiments are summarized in Table I (upper and middle section). The rate of transmission by aerial route appeared to be significantly increased at higher RH.

It is possible that only the larger respiratory droplets carrying virus were responsible for the transmitted infection in transmission cages. To determine the effect of RH under conditions in which true airborne transmission by droplet nuclei occurred, subsequent experiments were carried out permitting separation between mice over a longer distance. Four transmitter mice were placed in an "open" cage of wire mesh inside an incubator, and 8 uninfected mice in each of 2 "open" cages inside another incubator. The outlet and inlet of the incubators were interconnected through a pipe, the distance between mice in the 2 incubators being 5–6 ft. Conditions of ventilation were applied so that air flowed from the incubator housing the transmitters to the other incubator. Table I (lower section) summarizes the combined results of 3 comparative experiments at 30–35% and 60–70% RH. Again, it was found that the rate of transmission was increased at higher RH.

Ventilation rate. It is to be expected that airborne transmission involving droplet nuclei would be influenced by ventilation (8). Comparative transmission experiments were conducted, therefore, at 3 different rates of ventilation. Four transmitter mice were placed in an "open" cage inside an incubator. Twenty-four uninfected mice were added to this incubator, 8 to the cage housing the transmitters and 16 to another "open" cage. The cages were separated by 2 in. All mice exposed to infection by physical contact became infected. Transmission by the airborne route was less effective (Table II). Ventilation rate affected airborne transmission; a lower frequency of transmitted infection was obtained at the highest ventilation rate.

Serial airborne transmission. In previous studies in this laboratory Sendai virus was serially transmitted by the airborne route from infected contacts to new contacts in successive groups of mice (6). To study whether these passages selected a more readily transmissible strain of virus, nasal washings were collected from mice infected at transmission 8. The transmissibility of this viral strain was compared with that of the lung-adapted strain that had been employed for the transmission experiments. Mice were inoculated intranasally with 100 PFU of one of these strains and then served as transmitters. Only the ability to transmit infection by the airborne route was studied, transmission cages being utilized. Four groups each of 6 uninfected mice were exposed to the same transmitter mouse on days 5, 6, 7 and 8 after inoculation of the transmitter. The combined results of 4 experiments are summarized in Table III, which shows no significant difference in transmissibility between the 2 viral strains.

Discussion. The present data indicate that mice inoculated with Sendai virus are able to

TABLE II. Effect of Ventilation Rate on Transmission of Sendai Virus Infection in Mice.

Air exchanges/hr	Contacts infected[a]	
	No.	%
5	16/32	50[b]
10	32/64	50
20	16/62	26

[a] Distance between transmitters and contact mice was 2–5 in.

[b] $p < .05$, compared to 20 air exchanges/hr.

TABLE III. Transmissibility of Two Strains of Sendai Virus.

Viral strain[a]	Contacts infected[b] No.	%
Airborne passage[c]	95/380	25
Lung adapted	93/382	24

[a] Transmitter mice were infected with 100 PFU of virus.

[b] Distance between transmitter and contact mice was 1–4 in.

[c] Nasal washings recovered at eighth transmission.

transmit virus by the airborne route to uninfected animals. About one third of uninfected mice separated from infected transmitter mice by a distance of 5–6 ft became infected after a 24-hr exposure period. The effect of increased ventilation in decreasing the frequency of transmitted infection provides further evidence of airborne transmission.

We hoped that it would be possible to study the effect of relative humidity at strictly controlled levels between 20 and 70%. With the system used, however, the lowest humidity levels attainable were about 40% for short distance transmission and about 30% for long distance transmission. In addition, RH varied during the 24-hr exposure period at ranges of 5–10%. Despite these limitations, a significant effect of RH on transmission could be demonstrated. The rate of transmission appeared to be consistently increased at high RH.

These findings were surprising in view of the results of other studies. Schulman and Kilbourne (9) studying experimental transmission of influenza virus infection in mice found greater rates of transmission at 47% RH than at 70%. Other investigators (1–2) showed that influenza virus aerosols survived better at RH levels of 40% or lower. Studies with aerosols of parainfluenza 3 virus demonstrated that at 20% RH lower biological decay rates were obtained than at 50 or 80% RH (4). On the base of these studies with aerosols it has been suggested that vulnerability of airborne viruses to high RH might play an important role in transmission of respiratory infection under natural conditions. Assuming that the response of Sendai virus to RH resembles that of parainfluenza 3 and influenza viruses, this suggestion fails to explain the results of the present studies. It should be noted, however, that there may be considerable difference in size and composition between experimentally produced airborne particles and naturally expelled droplets and droplet nuclei. Further, many variables may affect survival of viruses in air and effects attributed to RH may be largely due to other factors (10–11). In this respect it is noteworthy that, although both adenovirus infection and influenza show a prevalence during the cold season (12) when the indoor RH is low, adenovirus aerosols, in contrast to influenza virus aerosols, have a greater decay rate at low RH than at high (4).

A possible explanation for the results of the present studies is that low RH has an adverse effect on nasal mucus secretions. The discrepancy in the effect of RH on transmission of Sendai virus and influenza virus infections (9) might be explained by assuming that infectious droplets produced by mice infected with these viruses originated in different portions of the respiratory tract. Previous studies in this laboratory indicated that the time of maximal transmissibility of Sendai virus infection was correlated with the occurrence of high virus titers in the nasal passages (6). Transmissibility of influenza virus infection was not related to availability of virus in the nose (13). Thus, if low RH has an adverse effect on nasal secretions, its role may be more important with Sendai virus infection.

Summary. The effect of relative humidity on airborne transmission of Sendai virus infection in mice was studied. Greater rates of transmission were observed at higher RH than at a lower one. The rate of transmission was reduced by increased ventilation. A viral strain recovered after serial airborne transmissions showed no evidence of increased transmissibility.

1. Hemmes, J. H., Winkler, K. C., and Kool, S. M., Nature (London) 188, 430 (1960).

2. Harper, G. J., J. Hyg. 59, 479 (1961).

3. de Jong, J. G., and Winkler, K. C., Nature (London) **201**, 1054 (1964).

4. Miller, W. S., and Artenstein, M. S., Proc. Soc. Exp. Biol. Med. **125**, 222 (1967).

5. Wright, D. N., Bailey, G. D., and Hatch, M. T., J. Bacteriol. **96**, 970 (1968).

6. van der Veen, J., Poort, Y., and Birchfield, D. J., Arch. Gesamte Virusforsch. **31**, 237 (1970).

7. van Nunen, M. C. J., and van der Veen, J., Arch. Gesamte Virusforsch. **22**, 388 (1967).

8. Riley, R. L., and O'Grady, F., "Airborne Infection, Transmission and Control." Macmillan Co., New York (1961).

9. Schulman, J. L., and Kilbourne, E. D., Nature (London) **195**, 1129 (1962).

10. Harper, G. J., Arch. Gesamte Virusforsch. **13**, 64 (1963).

11. Anderson, J. D., and Cox, C. S., "Airborne Microbes" (Symp. Soc. Gen. Microbiol., 17th) p. 203. Cambridge Univ. Press, London (1967).

12. van der Veen, J., Oei, K. G., and Abarbanel, M. F. W., J. Hyg. **67**, 255 (1969).

13. Schulman, J. L., and Kilbourne, E. D., J. Exp. Med. **118**, 257 (1963).

Received April 20, 1972. P.S.E.B.M., 1972, Vol. 140.