

Effect of Relative Humidity on Dynamic Aerosols of Adenovirus 12

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Dynamic aerosols of adenovirus 12 were generated in the same Henderson apparatus under conditions of high, medium, and low relative humidity. High relative humidities resulted in more recovery of adenovirus 12 from aerosols and lungs of newborn Syrian hamsters. At 89, 51, and 32% relative humidity, the total infectious virus recovered from a 20-min aerosol was $10^{6.7}$, $10^{6.0}$, and $10^{4.3}$ TCD₅₀, respectively. Hamsters exposed to these 20-min aerosols retained measured lung doses of $10^{3.0}$, $10^{2.4}$, and $10^{1.0}$ TCD₅₀, respectively. The measured retained lung doses were compared to calculated inhaled lung doses based on both total virus aerosolized and total virus recovery from the aerosols.

Previous studies of the factors affecting survival of airborne bacteria (6, 7) and viruses (1, 5, 10, 13, 14) have established that the effect of relative humidity is an important and unpredictable factor. Aerosols of influenza, vaccinia, Venezuelan equine encephalomyelitis (5), parainfluenza (10), and Semliki Forest viruses (1) are more stable at a low relative humidity. Yaba virus (14), Rous sarcoma virus (13), and poliovirus (5) are more stable at a high relative humidity.

During studies on the transmission of oncogenic adenovirus 12 to newborn hamsters (Davis, unpublished data), it was necessary to evaluate the effects of relative humidity on dynamic aerosolization of the virus. The only reported relationship between adenovirus aerosols and relative humidity had been obtained by using a static aerosol chamber. Adenoviruses 4 and 7 are more stable in static chamber aerosols at 80% relative humidity than at 50 or 20% (10).

The objectives of this study were (i) to determine the effects of relative humidity on the recovery of infectious adenovirus 12 after dynamic aerosolization in a Henderson apparatus and (ii) to determine the relationship between relative humidity of the aerosol and recovery of infectious virus from newborn Syrian hamster lungs.

MATERIALS AND METHODS

Hamsters. Sixty-five newborn, random-bred, Syrian hamsters (*Mesocricetus auratus*) were obtained from our closed hamster colony. Twelve to 18 hr after birth, the hamsters were placed in nylon mesh exposure cups suspended in the center of a stainless-steel exposure box (12.5 by 15.0 by 47.5 cm) of the Henderson apparatus (8). At 0, 10, 30, and 60 min after exposure for

20 min to an aerosol of adenovirus 12, groups of five animals were sacrificed and lungs were collected for assay of virus infectivity.

Virus inoculum. The same stock of adenovirus 12 served for all aerosol exposures. The methods for production, concentration, and assay of this stock have been previously reported (3, 12). It was produced and assayed on monolayer cultures of human embryonic kidney cells. Before being aerosolized, the virus suspension was frozen and thawed three times at -90 C and 37 C, respectively, and sonically treated for 3 min at 60 counts/sec in a Raytheon sonicator. The total protein concentration of the virus suspension was 900 mg/100 ml (2). Immediately before aerosolization, 1% antifoam (Antifoam A, Dow Corning Co., Midland, Mich.) was added to the virus suspension. Preliminary experiments showed that the addition of 1% antifoam to spray or impinger bottles had no measurable effect on viral infectivity.

Aerosol exposure. The three aerosols of adenovirus 12 were generated from the same Collison atomizer of a Henderson apparatus (8). The virus was aerosolized into the animal exposure chamber for 20 min at the rate of 0.305 or 0.310 ml per min and air dilutions of 12.5 or 28.4 liters per min (Table 1). The average life time of the aerosol in the exposure chamber was 43 and 19 sec, respectively. At 0 to 1, 10 to 15, and 19 to 20 min during each exposure, 12.5 liters of air per min leaving the exposure chamber was diverted through low-point, all-glass impingers (AGI) containing 20 ml of Hanks balanced salt solution (HBSS) and 1% antifoam. Those samples were assayed to determine the 50% tissue culture dose (TCD₅₀) per minute of aerosol. The temperature during each exposure remained at 28 ± 0 C or 29.5 ± 0.5 C (Table 1). Adenovirus 12 is highly stable at these temperatures. All work was conducted within a freon-tight biological safety cabinet system.

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TABLE 1. Exposure data on aerosolization of adenovirus 12 in a Henderson apparatus at different relative humidities

Relative humidity (%)	Aerosol temp (C)	Aerosolization rate (ml/min)	Air dilution factor (liter/min)	Collision bottle titer ^a	
				0 min	20 min
89 ± 4	28 ± 0	0.305	12.5	10 ^{7.2} ± 0.2	
51 ± 2	29.5 ± 0.5	0.310	28.4	10 ^{7.2} ± 0.2	10 ^{7.78} ± .08
32 ± 1	29.5 ± 0.5	0.305	28.4	10 ^{7.2} ± 0.2	10 ^{7.2} ± 1.6

^a TCD₅₀/ml as titered in human embryonic kidney cells.

humidity was produced in the exposure chamber by aerosolizing 0.31 ml of virus suspension in 28.4 liters of air per min (Table 1). Relative humidity, measured by a psychrometer interposed in the exhaust air stream, varied between 49 to 53% during the 20-min exposure period. The temperature of the air leaving the exposure chamber varied between 29 and 30 C.

For the high relative humidity experiment, the relative humidity of the make-up air was increased by reducing the air flow to 12.5 liters per min and sparging the air through a water-soaked natural sponge placed in the bottom of a 3-liter flask. The relative humidity varied between 85 and 93% during the last 17 min of exposure after an initial 3-min adjustment period of 72 ± 13% required because of the decreased air flow. The temperature of the exhaust air remained constant at 28 C.

Low relative humidity was produced by initially cooling then warming 20.5 liters of make-up air per min. Moisture was removed from the make-up air by passing the air through cold copper tubing immersed in an acetone-dry ice bath and allowing water to freeze on the inner surface of the tubing. The air was then warmed by passing it through a similar copper coil and water bath at 70 C. The relative humidity of warmed make-up air was as low as 10%. After mixing with the 8 liters of air per min flowing through the spray bottle, the total flow of 28.5 liters per min varied in relative humidity between 31 and 39% and in temperature between 29 and 30 C.

Assay procedures. Spray bottle and 1- or 5-min impinger bottle samples were assayed in secondary monolayer cultures of human embryonic kidney cells grown in Leighton tubes (12). Tenfold dilutions of the virus samples were made in HBSS. Confluent monolayer cultures grown in Leighton tubes were washed once with phosphate-buffered saline at pH 7.2, inoculated, and incubated for 1 hr at 37 C. Maintenance medium was then added and changed every fourth day. Cultures were examined for the characteristic adenovirus cytopathic effect. The TCD₅₀ was calculated by the method of Reed and Muench 21 days after inoculation (11).

The TCD₅₀ of virus retained per hamster lung represents the mean amount of infectious virus retained in five hamster lungs collected and pooled 10 min after exposure. This time was used because preliminary exposures at the medium relative humidity resulted in similar titers at 10, 30, and 60 min postexposure of 10^{2.16}, 10^{1.97}, and 10^{2.10} TCD₅₀, respectively. The hearts and large pulmonary and aortic vessels were

TABLE 2. Relationships between relative humidity and recovery of adenovirus 12 from dynamic aerosols and newborn hamster lungs

Relative humidity (%)	Total virus aerosolized (TCD ₅₀)	Virus recovery from aerosols		Virus recovery from lungs	
		TCD ₅₀	Per cent	TCD ₅₀	Per cent ^a
89 ± 4	10 ^{8.0}	10 ^{6.7}	4.6	10 ^{3.0}	12.6
51 ± 2	10 ^{8.0}	10 ^{6.0}	1.05	10 ^{2.4}	8.0
32 ± 1	10 ^{8.0}	10 ^{4.3}	0.02	10 ^{1.0}	0.32

^a Per cent of theoretical maximal inhaled dose.

removed and the surfaces of the lungs were washed with HBSS. Each group of five lungs was then pooled, weighed, made up to a 20% suspension in cell culture medium without serum, and frozen at -90 C until titrated. Lungs from hamsters aerosolized with uninfected cell culture suspensions at medium relative humidity served as controls.

The calculations used for determining the total virus aerosolized, total and per cent virus recovery from aerosols and lungs, theoretical maximum inhaled dose, and TCD₅₀ per milliliter of aerosol are as follows: total virus aerosolized = spray bottle titer × volume of virus aerosolized; total virus recovery from aerosols (impinger titers were multiplied by 2.28 in the medium and low relative humidity exposures because only 12.5 liters per min of the total 28.5 liters per min were diverted through the impinger) = AGI titer × AGI diluent volume × (length of exposure/length of AGI collection); per cent virus recovery from aerosols = (total virus recovery from aerosol/total virus aerosolized) × 100; per cent virus recovery from lungs = (total virus recovery from lungs/theoretical maximum inhaled dose) × 100; theoretical maximum inhaled dose = TCD₅₀ per milliliter of aerosol × minute respiratory volume of hamster (13) × length of exposure; TCD₅₀ per milliliter of aerosol = total virus aerosolized/total air dilution.

RESULTS

Virus recovery from aerosols. There was marked variation in recovery of infectious adenovirus 12 when it was aerosolized in a Henderson apparatus at different levels of relative humidity (Fig. 1).

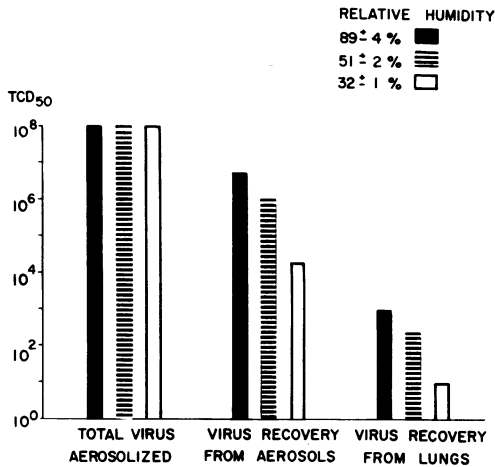


FIG. 1. Effect of relative humidity on the recovery of adenovirus 12 from dynamic aerosols and newborn hamster lungs.

Maximum virus survival occurred at a high relative humidity. At $89 \pm 4\%$ relative humidity, $10^{5.4}$ TCD₅₀ per min or a total of $10^{6.7}$ TCD₅₀ of adenovirus 12 was recovered from the 20-min dynamic aerosol exposure (Fig. 1). Only 20% ($10^{6.0}$ TCD₅₀) as much infectious virus was present in aerosols at $51 \pm 2\%$ relative humidity and 0.4% ($10^{4.3}$ TCD₅₀) at $32 \pm 1\%$ relative humidity (Fig. 1). The total virus recovery, however, was relatively low at each relative humidity (Table 2).

Virus recovery from lungs. The total adenovirus 12 retained in the lung also varied after 20-min aerosol exposures at the three different levels of relative humidity (Fig. 1). The highest pulmonary retention of adenovirus 12 occurred in newborn hamsters exposed to the high relative humidity aerosols. At $89 \pm 4\%$ relative humidity, there was a total recovered lung dose of $10^{3.0}$ TCD₅₀. Only 25% as much virus was recovered from lungs exposed to medium relative humidity ($10^{2.4}$ TCD₅₀) and 1% after the low relative humidity exposure ($10^{1.0}$ TCD₅₀). Since there was blood in the pulmonary vessels, blood was assayed separately but virus could not be re-isolated. No virus was detected in the washings from the pleural surface. The parallel assay of lungs from control littermate hamsters produced no cytopathic effects in the human embryonic kidney cultures.

The per cent recovery of infectious virus from the lungs after aerosol exposure was greater in the high than medium or low relative humidity experiments (Table 2). The theoretical maximum inhaled doses were $10^{3.9}$ TCD₅₀ at high relative humidity and $10^{3.5}$ TCD₅₀ at medium and low

relative humidities. At high relative humidity, 12.6% of the theoretical maximum inhaled dose was recovered. Percentages of only 8.0 and 0.32 were recovered after the medium and low relative humidity exposures, respectively.

The maximum inhaled dose was also calculated from the total virus recovery in the aerosols rather than total virus aerosolized. This comparison of the virus recovery from aerosols and lungs revealed that the measured lung dose at each relative humidity was higher than the calculated inhaled dose. The latter doses were $10^{2.6}$, $10^{1.6}$, and $10^{-1.4}$ TCD₅₀, respectively, at the high, medium, and low relative humidities.

DISCUSSION

The relative humidity of dynamic aerosols is an important factor in the recovery of adenovirus 12 from dynamic aerosols. The most infectious virus was recovered from high humidity aerosols, whereas only 20.0 and 0.4% as much virus were present in the medium and low relative humidity aerosols. Similar results have been reported for static aerosols of adenovirus 4 and 7. Miller et al. (10) observed a much lower rate of infectivity loss at 80% than at 50 or 20% relative humidity. Only 3.3% as much adenovirus 7 was present in aerosols at 20 or 50% relative humidity as at 80% relative humidity. Furthermore, a major effect of relative humidity on adenovirus 7 was observed at the first sampling period (5 min). Therefore, the effect of relative humidity on adenoviruses in both dynamic and static aerosols appears to be rapid.

Although relative humidity influenced the recovery of infectious virus from dynamic aerosols, all three exposures still resulted in a relatively low recovery of infectious virus. Similar losses across the Henderson exposure chamber occurred after aerosolization of Yaba (14) and Rauscher murine leukemia virus (9). Many factors, such as sonic treatment, electrostatic precipitation, physical decay, and impingement, could have influenced the survival of the virus. The limited studies on survival of airborne viruses have shown that factors such as extraneous protein concentration, salt concentration (1), and the suspension medium (13) markedly affect survival of airborne viruses. These factors remained constant during our experiments and were not evaluated individually.

The primary effect of relative humidity on recovery of virus from the lungs was an indirect one. There was less available viable virus recovered from aerosols at the lower relative humidities. Therefore, it was not surprising to observe

a decreased total detectable lung dose of virus in the animals exposed at the low relative humidities. Comparison of virus recovery from aerosols and lungs suggests a good correlation between the two observations, although a slightly higher percentage of the available virus was retained at the higher relative humidities.

Interpretation of differences between calculated and measured lung doses was restricted because of the inability to measure the small respiratory volume of the hamster. Based on Guyton's formula (4), a 2-g newborn hamster has a minute respiratory volume of 1 ml per min. All of the measured lung doses (Table 2) were between the calculated doses based on aerosolized and impinged virus. Based on total aerosolized virus, the calculated inhaled doses were $10^{3.9}$, $10^{3.5}$, and $10^{3.5}$ TCD₅₀ at high, medium, and low relative humidities. When the total virus titers recovered from aerosols (Table 2) were used, the inhaled doses were $10^{3.6}$, $10^{1.6}$, and $10^{-1.4}$ TCD₅₀. Correlation between these doses is further restricted since neither the per cent retention of the inhaled dose nor the relation between particle size and infectivity was determined.

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