Aerosol Stability of Three Acute Respiratory Disease Viruses. (32054)

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(Introduced by H. J. Hearn)
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Knowledge of the aerosol stability of microbial agents inducing disease in man by the respiratory route is essential to an understanding of disease transmission. It has been shown that aerosols of influenza and measles viruses are more stable at low relative humidity (RH) than at high(1,2,3). With poliovirus, on the other hand, greater stability of infectivity has been found at high RH (2).

Aerosol stability information has not been reported for most acute respiratory disease (ARD) viruses. The study reported here was designed to provide data on adenoviruses, types 4 (Adv-4) and 7 (Adv-7), and parainfluenza 3 virus (Para 3). The former viruses are most prominent in acute, febrile undifferentiated respiratory disease of military personnel(4,5,6,7). Para 3 infections commonly occur in infants and children(8,9).

Materials and methods. Viruses. Adv-4, strain 208, FD64 was isolated from an army recruit with acute respiratory disease. The virus employed in the aerosol experiments had been passed 13 or 14 times through HEp-2 tissue cultures containing 2% chicken or fetal bovine sera in Eagle's Basal Medium (BME).* The mean titer of the harvests was, in logarithms$_{10}$, 7.54 tissue culture ED_{50} ($TCED_{50}$) per ml.

Adv-7, strain 1022-2, FD62 was used for aerosol studies after 8 passages in HEp-2 tissue cultures containing 2% fetal bovine serum in BME (log_{10} titer 8.94 $TCED_{50}$/ml).

Para 3 virus was a strain employed in the laboratories of the Walter Reed Army Institute of Research. The virus had been passaged an unknown number of times in monkey kidney and KB cells, followed by 3 passages in human amnion cells and 6 passages in grivet monkey kidney cells (log_{10} titer 6.20 $TCED_{50}$/ml).

Viral assays. Adenovirus titrations were performed in primary human embryonic kidney tissue cultures, utilizing 5 tubes per dilution. Cytopathic effect (CPE) was the indicator of infection. For Adv-4, cultures were observed 21 days; for Adv-7, 15 days. Prior to assay, impinger fluids were passed through 0.8 micron filters† to remove bacterial and fungal contaminants since tests had shown no significant viral loss during filtration. Para 3 virus was assessed in grivet monkey kidney tissue cultures fed with BME and 2% fetal bovine serum again employing 5 tubes per dilution. Samples were not filtered because an apparent 1-log loss of titer was found in a preliminary experiment. The endpoint of assay was hemadsorption on the sixth day with guinea pig erythrocytes(10).

All tissue cultures were incubated at 36 C in stationary tube racks. Titers were computed employing the method of Reed and Muench(11).

Tracer assays. Uranine‡ dye was added to viral suspensions as a physical tracer for aerosols. Samples were collected in distilled water and assayed for fluorescence in a photometer.§

Aerosol procedures. Aerosols were produced in a 500-liter rotating drum(12) with a Collison disseminator(13). Prior to dissemination of viral suspensions, the air within the drum was adjusted to the appropriate temperature (75 F) and relative humidity (20%, 50%, or 80%) by flow of conditioned air through the drum. During the adjustment period, oper-

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* Cell cultures and media purchased from Flow Labs., Rockville, Md. and Microbiological Associates, Bethesda, Md.

† Millipore Corp., Bedford, Mass.

‡ Soluble sodium fluorescein; Fisher Scientific Co., Fairlawn, N. J.

§ Series 540; Photovolt Corp., New York.

ation of a Collison disseminator containing water compensated for the subsequent effect of added moisture that would occur during dissemination of virus into the drum through the same process. With attainment of the desired conditions the disseminator containing virus was operated in place of the water spray for 60 seconds with the air flow continuing through the drum. The drum was then isolated except for conditioned make-up air introduced during sampling.

Aerosol samples were collected with 12.5 l/min All Glass Impingers(14,15) at midpoints of 5, 30, 60 and 180 minutes' cloud age in all trials although viral concentrations at 180 minutes were often below the limit of assay sensitivity. Seperate samplers were employed for viral and dye assays with BME plus Antifoam A‖ as collecting fluid in the former and distilled water in the latter. Sampling times were 1 or 2 minutes for viral collections and 1 minute for dye.

Data processing. It was of interest to examine stability in terms of infectivity loss with time of virus in aerosolized particles and in terms of loss due to physical fallout. The former objective was attained by computing the ratios of virus to dye recoveries at each aerosol age. These values indicated the relative viability of the aerosols. The ratios were then normalized to the ratio of virus to dye in the original suspension before spraying. Conversion of the latter to percentages provided estimates of per cent viability of aerosols at the several cloud ages with respect to the original suspension viability.

The change in aerosol viability with aerosol age was expressed as biological decay rate (per cent per minute) and was based on recoveries through 60 or 180 minutes' cloud age. The decay rates were estimated from a least squares fit of cloud per cent viability with cloud age(16). In addition to biological decay rates (BDR), the physical decay rates of the clouds were estimated. These computations were based on changes in dye tracer concentration with time. The decay rate statistics for each virus were subjected to analyses of variance(16) to test the effect of relative humidity.

‖ Dow Corning, Midland, Mich.

TABLE I. Particle Size Distributions of Viral Aerosols.*

Cloud age (min)	MMD (μ)†	Slope (probits/$\log_{10}$ diam)
5	2.3	4.0
60	2.0	2.6
360	2.0	3.0

* Based on dye recoveries in single stage impactor samplers.
† Mass median diameter.

Result. Preliminary experiments to determine the effect of sodium fluorescein tracer on viral titers showed that one per cent dye would not significantly alter infectivity of suspensions.

Additional preliminary studies were conducted to determine the particle size distribution of aerosols in the drum. Determinations were made with a suspension of adenovirus type 4 containing dye tracer. Single-stage impactor samplers(17) with particle diameter cut-offs of 1 μ, 3 μ, and 5 μ were employed along with a liquid impinger of the Greenburg-Smith type(18) without size selection characteristics for estimation of total aerosol concentration. All estimations were based on dye tracer recoveries. Because of the similarity of the suspending media, it was considered that the distributions obtained with this system would be representative of those for all 3 viruses.

The distributions in terms of mass median diameters (microns) and slopes (probits per log diameter) were estimated at cloud ages of 5, 60, and 360 minutes. Ambient conditions of 75 F and 80% RH were chosen for this test. The data shown in Table I suggest a uniform size range regardless of cloud age.

For Adv-4, eight replicate aerosol trials were completed at each relative humidity level of 20%, 50% and 80%. The average viral and dye recoveries are given in Table II. The means of per cent viability are plotted in Fig. 1 and indicate a much lower rate of infectivity loss at 80% than at 50% or 20% RH. The physical and biological decay rates of adenovirus type 4 were computed from observations at 5, 30, and 60 minutes' cloud age. The means are presented in Table III. There were no significant differences among physical decay rates at the various relative humidities.

TABLE II. Average* Aerosol Concentrations of ARD Viruses and Dye Tracers.

Aerosol age (min)	Relative humidity (%) 20 Virus†	20 Dye‡	50 Virus	50 Dye	80 Virus	80 Dye
			Adenovirus Type 4			
5	138	1.60	35	1.24	315	1.55
30	100	1.47	14	1.36	243	1.44
60	14	1.36	5	1.22	91	1.31
			Adenovirus Type 7			
5	1077	1.60	951	1.24	22,071	1.55
30	255	1.34	121	1.45	16,030	1.42
60	88	1.27	73	1.34	8,261	1.32
180	5	1.14	7	1.14	2,139	1.14
			Parainfluenza 3			
5	35	1.73	51	1.65	13	1.62
30	38	1.61	14	1.53	45	1.47
60	40	1.50	7	1.58	6	1.32

* Each entry is the average of 4 to 8 replications.
† $TCED_{50}$ per liter of aerosol.
‡ Micrograms per liter of aerosol.

There were, however, significant differences among biological decay rates ($P<5\%$). The major difference existed between 80% RH and the means obtained at 50% and 20% RH.

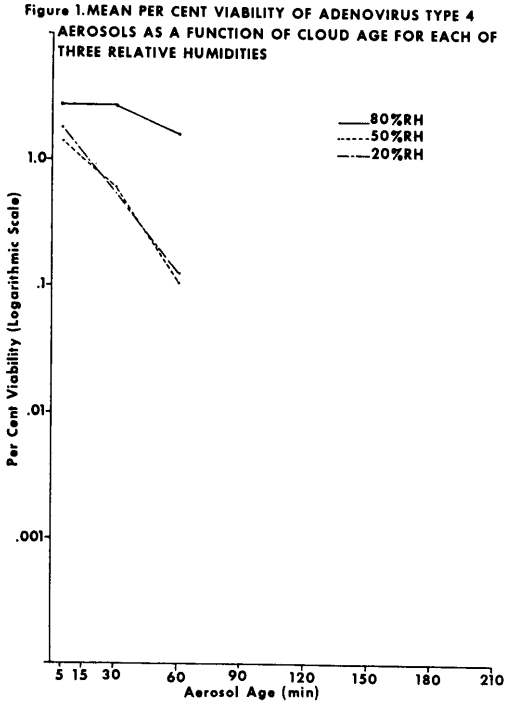

FIG. 1.

Four replicate trials were completed at each RH with Adv-7. Average viral and tracer recoveries are given in Table I. The mean per cent viabilities are plotted in Fig. 2 and decay rate data are presented in Table IV. Because the viral suspension had a 2-log higher titer than did adenovirus 4, more concentrated aerosols were produced and productive sampling was possible through 180 minutes' cloud age. Again, there was no significant effect of relative humidity on physical decay rates. There was, however, an effect of RH on biological inactivation ($P<5\%$) that was similar to that noted with Adv-4. A markedly lower mean decay rate was obtained at 80% RH.

TABLE III. Physical and Biological Decay Rates of Aerosols of Adenovirus Type 4.*

Relative humidity (%)†	Mean decay rate (% per min) Biological‡	Physical§
20	5.0	.16
50	3.6	.16
80	1.5	.14

* Each mean was derived from the data in 8 aerosol trials.
† 75°F.
‡ Standard deviation ±1.86.
§ Standard deviation ± .03.

TABLE IV. Physical and Biological Decay Rates of Aerosols of Adenovirus Type 7.*

Relative humidity (%)†	Mean decay rate (% per min) Biological‡	Physical§
20	2.7	.11
50	2.9	.11
80	.82	.14

* Each mean was derived from the data in 4 aerosol trials.
† 75°F.
‡ Standard deviation ±.58.
§ Standard deviation ±.054.

The aerosol trials conducted with Para 3 (4 replications at each RH) were intended to provide comparative data with virus from the myxovirus group. Recoveries are summarized in Table I. Per cent viability plots are presented in Fig. 3 and decay rate means are given in Table 5. High experimental errors were associated with the trials so that the effects of relative humidity did not obtain significance. However, at 20% RH, consistently

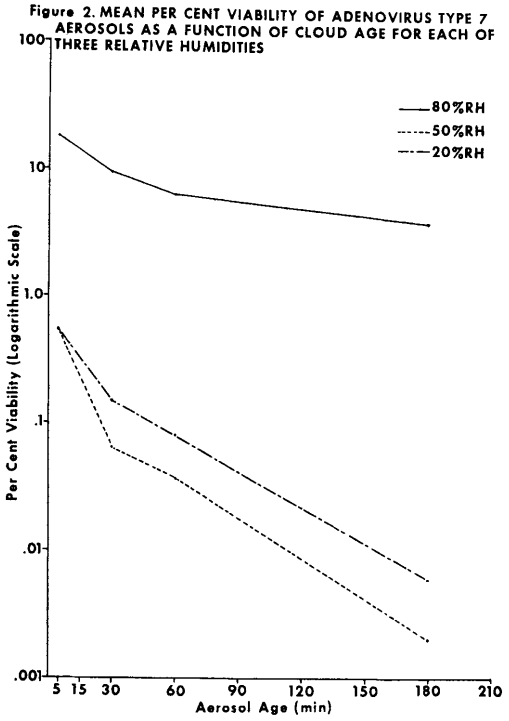

FIG. 2.

lower biological decay rates were obtained than at 50% or 80% RH in each of 4 replicate trials. Further, in tests for airborne virus at 180 minutes after dissemination, an average of 8 $TCED_{50}$ units were recovered per liter of aerosol at 20% RH, but no more than trace amounts were occasionally obtained at 50% and 80% RH.

Discussion. The biological decay rates of airborne Adv-4 and Adv-7, and probably those of Para 3, were affected by the relative humidity condition in the aerosol vessel. Both adenovirus sero-types showed lower decay rates at 80% RH than at 20% or 50%. Tests with 2 strains permit only limited conclusions, but the data suggest a similar response among the adenoviruses as a group. It is interesting to note that aerosols of Para 3 appeared to be affected by relative humidity inversely to the effects observed with adenoviruses in that the lowest decay rate was associated with the lowest RH. Thus, Para 3 resembles other myxoviruses (influenza and rubeola)(1,2,3) in its response to RH. The similarities in behavior of these myxoviruses and their difference from polioviruses(2) and adenoviruses suggest that response to aerosolization is a function of the physical or chemical structure of viruses.

TABLE V. Physical and Biological Decay Rates of Aerosols of Parainfluenza 3 Virus.*

Relative humidity (%)†	Mean decay rate (% per min) Biological‡	Physical§
20	.53	.15
50	3.8	.14
80	3.1	.17

* Each mean was derived from the data in 4 aerosol trials.
† 75°F.
‡ Standard deviation ±2.72.
§ Standard deviation ± .033.

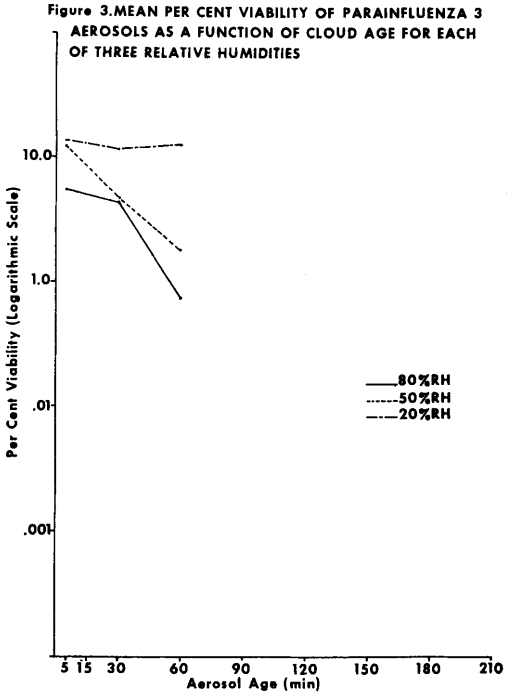

FIG. 3.

There was no major influence of RH on concentrations of airborne virus at the first sampling period (5 minutes) with Adv-4 and Para 3 (Fig. 1 & 3). With Adv-7 about 30 times more virus was airborne at the first sampling period at 80% RH than at 20% or 50% RH. Relatively high passage material was employed in these studies. It should be noted that Hearn *et al* reported an effect of passage on the aerosol characteristics of yellow fever virus(19). It was found that as passage

number increased from 1 to 3 in HeLa cell culture, aerosols of the respective harvests changed from RH insensitive to RH sensitive. The change in aerosol characteristics, however, was in terms of initial aerosol recovery only and was not seen in comparisons of decay rates subsequent to the first sampling period (personal communication). These data suggest different mechanisms of inactivation of virus during or immediately after dissemination and during extended exposure to aerosol conditions. Further, it is suggested that stability of viral aerosols immediately after dissemination can be dependent upon passage history.

The effects of RH on decay of viral aerosols is of major importance in understanding the epidemiology of the associated diseases. For example with Adv-4, the mean BDR at 80% RH was 1.5%/min and the mean at 20% RH was 5.0%/min. Assuming equivalent initial aerosol concentrations, after 3 hours there would be theoretically about 550 times more infective virus airborne at the high RH. After 6 hours, there would be 300,000 times more Adv-4 at 80% than at 20% RH. With parainfluenza virus the BDR means of 3.1%/min and 0.53%/min at 80% RH and 20% RH would result in 100 times more infective virus after 3 hours and 10,400 times more virus after 6 hours at low RH. These relationships are based upon studies of small stable aerosol particles. Whether or not particles of this size are of prime importance in natural disease transmission is not yet known.

Hypotheses attempting to explain the higher incidence of respiratory diseases during the winter season compared with other times of the year have implicated indoor crowding, lack of ventilation and low RH due to heating. The results of the present studies indicate that adenovirus aerosols, unlike aerosols of influenza, parainfluenza and other myxoviruses, have a greater decay rate at low RH than at high. Thus, viruses producing respiratory disease do not all behave the same in aerosols. If low RH plays a major role in the epidemiology of respiratory disease, it may be more important with some agents because of its adverse effects on mucus secretions of the human respiratory tract than on survival of the virus in aerosols.

Summary. Aerosols of adenovirus types 4 and 7 and parainfluenza 3 were tested for biological stability as a function of relative humidity. The levels tested were 20%, 50%, and 80%. Both adenoviruses exhibited maximum stability at 80% RH. Parainfluenza on the other hand exhibited a minimum biological decay rate at 20% RH. Studies were carried out with aerosols having mass median diameters of about 2 μ.

The authors wish to cite the excellent technical assistance of Joseph W. Dominik and John F. Brewer of Fort Detrick and of Martin Schneider of Walter Reed Army Inst. of Research. The statistical analyses by Dr. N. Bohidar of Fort Detrick are also acknowledged.

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Received December 14, 1966. P.S.E.B.M., 1967, v125.

Additional Evidence for the Participation of 5-Hydroxytryptamine in The Intestinal Response to Morphine.* (32055)

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The means by which morphine produces its profound effects on the mammalian gastrointestinal tract has been the subject of a great deal of scientific controversy and even more speculation. Since the early work of Slaughter and Gross(1) on the dog intestine it has generally been thought that cholinergic mechanisms are involved in the pathway mediating the actions of morphine on the small intestine. This concept was supported by the report of Vaughan Williams and Streeten(2) that atropine antagonized the intestinal actions of moderate doses of morphine. Plant and Miller(3), however, found that the effect of morphine on the dog intestine was not modified by atropine and considered that morphine could exert its actions directly on the intestinal smooth muscle. More recently Margolin(4) and Margolin and Plekss(5) have obtained evidence to indicate that in certain species, inhibition of the intestinal propulsive activity is accomplished indirectly *via* the central nervous system.

Burks and Long(6) have presented evidence that some of the effects of morphine on tone of the dog small intestine may be due to local release of 5-hydroxytryptamine (5-HT) by morphine. Since there is evidence which indicates that the intestinal actions of 5-HT may be antagonized by atropine and enhanced by anticholinesterase agents(7), such a hypothesis would be consistent with the finding that eserine may potentiate the action of morphine on the dog intestine(1) and that atropine may antagonize morphine effects (2). Treatment with reserpine has been shown(6) to diminish the spasmogenic effect of morphine on the dog small intestine, but no study of the influence of prior treatment with reserpine on the actions of morphine on propulsive activity of the intestine has been made. The purpose of this investigation therefore was to compare the effects of atropine and physostigmine on morphine and 5-HT actions on propulsive activity of the small intestine of the mouse, to evaluate the effects of reserpine treatment on the response to morphine and to determine whether a 5-HT antagonist, methysergide (UML), would alter intestinal responses to morphine.

Materials and methods. A total of 2470 young adult male Swiss Webster white mice were randomly divided into groups of 10 animals each and the mean values for each group were employed in the analysis of the data. All chemical agents and saline were administered intraperitoneally to the animals in volumes of 0.01 ml/g and their effects on gastrointestinal propulsion assayed by the method of Macht and Barba-Gose(8). Powdered charcoal was suspended in glycerine and administered p.o. to the animals in volumes of 1 ml. At the time of sacrifice by cervical fracture, the entire small intestine from the stomach to the cecum was removed and the distance traversed in cm by the charcoal

* This work was supported in part by USPHS grants B-1396, B-4431 and 1-F1-GM-31, 186-01.

† USPHS Fellow.