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Assessment of Aerosol Stability of Yellow Fever Virus by Fluorescent-Cell Counting

CHARLES J. MAYHEW, W. DOUGLAS ZIMMERMAN, AND NICHOLAS HAHON
Aerobiology and Evaluation Laboratory, Fort Detrick, Frederick, Maryland 21701

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The effects of three temperatures [30, 50, and 80 F (−1.11, 10, and 26.67 C)] and three relative humidities (30, 50, and 80%) on biological and physical decay rates of aerosols of yellow fever virus were investigated. Neither temperature nor relative humidity, independently or jointly, significantly affected biological or physical decay rates. The advantages of assaying yellow fever virus by the fluorescent-cell counting technique are discussed.

Both experimental exposure of animals (3, 5) and accidental exposure of humans (1) attest to the airborne infectivity of yellow fever virus, whose natural mode of dissemination is insect transmission (7). Knowledge of the aerosol behavior of the virus is pertinent to the evaluation of experimental infections, as well as to the prevention and control of the laboratory-acquired disease. Information on the stability of aerosols of yellow fever virus is presently limited to a few conditions of relative humidity (3, 5).

Recently, a quantitative assay was developed for infective yellow fever virus particles. It is based on the enumeration of cells containing fluorescent viral antigen 24 hr after infection (2). The assay is highly precise, sensitive, inexpensive, and suitable for performing replicate determinations easily. In view of these advantages, this method was used to characterize more fully the stability of yellow fever virus in aerosols.

This report describes the aerosol stability of yellow fever virus at different combinations of temperature [30, 50, and 80 F (−1.11, 10, and 26.67 C)] and relative humidity (30, 50, and 80%).

MATERIALS AND METHODS

Virus. A plasma suspension of the Asibi strain of yellow fever virus was used throughout this study. Infective plasma was obtained from rhesus monkeys that had been inoculated intraperitoneally with 6×10^5 cell-infecting units (CIU) of virus and bled at the peak of the febrile response. The plasma was divided into 4.5-ml portions, sealed in glass vials, and stored at −60 C. The plasma suspension of virus had a titer of approximately 1.2×10^8 CIU per ml.

Aerobiological procedures. Aerosol tests with the virus were carried out in an insulated, cylindrical chamber with a volume of 4,800 liters. The design and construction of the chamber was similar to that described by Wolfe (8). Humidity of the chamber atmosphere was controlled by varying the moisture content of the secondary air supply with driers or by injection of small amounts of water. Relative humidity (RH) was measured directly in the chamber by sensing elements (Hydristor, Electrical Hygrometer Element, Bendix Aviation Corp., Baltimore, Md.). The temperature of the chamber atmosphere was measured by a Foxboro recorder (Foxboro Co., Foxboro, Mass.) and controlled by a jacket containing circulating ethylene glycol.

The preparation to be atomized consisted of a mixture of 4.0 ml of virus suspension and 1 ml of a solution of uranine (the disodium salt of fluorescein) incorporated as a physical tracer. The final concentration of the tracer in the suspension was 0.5 mg per ml; preliminary tests had indicated that as much as 1 mg per ml was not detrimental to virus viability. In each aerosol test, the virus-tracer mixture was atomized completely within 20 sec by 50 lb/in^2 of nitrogen through the FK-8 atomizer. At designated intervals after dissemination, the concentration of the cloud was determined by sampling for 1 min with AGI-30 all-glass impingers (Ace Glassware Co., Vineland, N.J.). The flow rates of impingers ranged from 11.4 to 12.6 liters per min. A 20-ml amount of impinger fluid, consisting of medium 199 with 5% calf serum and 2 to 3 drops of sterile olive oil as an antifoamite, was used to sample the cloud for virus. Simultaneously with biological sampling, impingers containing distilled water were used to sample the cloud for physical tracer.

Representative samples of aerosols were collected in settling jars for particle size determination (W. C. Day, *unpublished data*). The size-frequency distribution of particles was determined microscopically by visual sizing with the use of a Porton graticule (4). In this report, the size of particles is described as a volume median diameter (VMD), which is that particle size above and below which 50% of the total volume of particles are found. It was derived by plotting the cumulated percentages of particles against diameters. The VMD of the aerosol particles was the diameter at the point where the line inter-

cepted the 50% axis. The VMD of aerosols was approximately 3 to 4 μ.

Assay of physical tracer. Fluorometric analyses of collecting fluids used to sample the cloud for the tracer (uranine) were made with a Turner model 110 Fluorometer (G. K. Turner Associated, Palo Alto, Calif.). A standard curve was established for a particular lot of tracer that was then used to estimate the tracer content of samples.

Virus assay procedure. The virus content of collecting fluid used to sample the cloud was assayed by fluorescent-cell counting. The technique is based on the enumeration of cells containing fluorescent viral antigen 24 hr after infection of cover slip, McCoy cell monolayers. Details of the procedure have been described previously (2). To measure the virus content of impinger samples taken at 60 min of aerosol age, a 0.5-ml volume of inoculum was employed for the assay instead of the customary 0.2-ml volume. Samples were usually assayed in triplicate.

TABLE 1. *Viable recovery of yellow fever virus aerosols at different conditions of temperature and relative humidity*[a]

Temp	Relative humidity	Percentage of recovery at sample time[b]			
		1 min	15 min	30 min	60 min
F	%				
30 (−1.11 C)	30	2.82	1.34	0.93	0.22
	50	2.76	1.21	0.88	0.13
	80	2.60	1.26	0.80	0.17
50 (10 C)	30	2.10	1.45	1.18	0.22
	50	3.06	1.43	1.30	0.14
	80	2.37	1.62	1.11	0.16
80 (26.67 C)	30	3.04	1.48	0.96	0.16
	50	2.70	1.35	0.78	0.07
	80	2.47	1.14	0.85	0.08

[a] Means of four replicate trials.
[b] 1-min sample.

TABLE 2. *Physical recovery of yellow fever virus aerosols at different conditions of temperature and relative humidity*[a]

Temp	Relative humidity	Percentage of recovery at sample time[b]			
		1 min	15 min	30 min	60 min
F	%				
30 (−1.11 C)	30	20.0	10.1	6.7	4.4
	50	19.2	9.0	5.4	3.7
	80	19.2	9.1	6.4	3.7
50 (10 C)	30	26.1	13.7	9.4	5.2
	50	23.8	12.2	8.3	4.6
	80	20.4	10.1	6.7	4.2
80 (26.67 C)	30	29.2	15.8	10.2	6.9
	50	33.3	14.1	9.7	6.7
	80	20.3	13.9	8.0	4.4

[a] Means of four replicate trials.
[b] 1-min sample.

TABLE 3. *Biological recovery of yellow fever virus aerosols at different conditions of temperature and relative humidity*[a]

Temp	Relative humidity	Percentage of recovery at sample time[b]			
		1 min	15 min	30 min	60 min
F	%				
30 (−1.11 C)	30	13.0	12.7	12.7	4.0
	50	13.7	13.9	17.6	3.2
	80	12.6	12.9	11.5	3.8
50 (10 C)	30	9.0	11.3	14.7	5.1
	50	14.4	13.2	16.6	3.0
	80	12.9	17.4	18.3	4.2
80 (26.67 C)	30	10.4	8.8	9.7	2.5
	50	9.6	9.2	7.6	1.4
	80	11.0	7.1	11.2	4.5

[a] Means of four replicate trials.
[b] 1-min sample.

TABLE 4. *Mean biological decay rates of yellow fever virus aerosols*

Temp	Relative humidity		
	30%	50%	80%
F			
30 (−1.11 C)	1.76[a]	2.36	1.84
50 (10 C)	1.48	2.52	2.18
80 (26.67 C)	2.28	2.74	1.50

[a] Decay rate, per cent per min.

Experimental design. Physical limitations of equipment restricted the investigation to three trials per day. Under these conditions, a split plot design was appropriate. Temperature was considered as the whole plot and RH as the subplot. One replicate of the nine treatment combinations was completed in each 3-day block. Four replications were completed on 12 test days.

Analysis. The basic data reported were the total number of CIU sprayed, the number of CIU recovered per milliliter of impinger fluid, the milligrams of fluorescein dye sprayed, and the milligrams of fluorescein recovered per ml of impinger fluid. Percentages of recoveries were computed as follows:

Percentage of viable recovery = (CIU/ml) (impinger volume)/(impinger flow rate in liters per min) × (chamber volume)/(CIU sprayed) × 100.

Percentage of physical recovery = (mg/ml) (impinger volume)/(impinger flow rate in liters per min) × (chamber volume)/(mg sprayed) × 100.

Percentage of biological recovery = viable recovery/physical recovery × 100.

From the exponential decay model, $y_t = y_0 e^{-kt}$, where y_t = percentage of recovery at time t, y_0 =

percentage of recovery at time zero, and k = the exponential decay rate; linear regressions of the logarithms of percentage of recovery on time were computed for both the physical and biological per cent recoveries of each trial. The logarithm of decay rates (per cent per min) was the variable of analysis. The 5% probability level or less was the measure of statistical significance.

RESULTS AND DISCUSSION

Mean viable, physical, and biological per cent recoveries of yellow fever virus aersols at different combinations of temperature and RH are given in Tables 1 through 3. The initial per cent viable recoveries were markedly lower than those obtained by Miller et al. (5). This may be the consequence of several factors that influence, either individually or concertedly, the behavior and sampling of virus aerosols, e.g., test chamber, source of virus suspension, particle size, and impinger fluid. In studies in which egg yolk was incorporated as a stabilizer into impinger fluid, recovery of yellow fever virus in early periods of aerosol aging ranged from 8 to 28% (C. J. Mayhew, *unpublished data*). The use of this collecting fluid was precluded in our study because amorphous particles were deposited onto cell monolayers, thus interfering with the recognition and enumeration of infected cells. An increase in sensitivity of virus to changes in RH after passage in cell cultures (3) indicated that the source of virus may affect the behavior of yellow fever virus aerosols.

Mean biological and physical decay rates are summarized in Tables 4 and 5. Neither temperature nor humidity, jointly or independently, influenced these parameters. The lack of sensitivity of yellow fever virus to humidity changes paralleled the findings of others (5). The biological decay rates of approximately 2% per min indicate that yellow fever virus is comparable in airborne stability to that of the virus of Rift Valley fever (5). From studies on the influence of RH on the survival of airborne viruses, Songer (6) indicated that medium RH levels tended to be unfavorable for virus survival. A slight trend toward higher biological decay of yellow fever virus at 50% RH, although not statistically significant, was noted in this study (Table 3).

TABLE 5. *Mean physical decay rates of yellow fever virus aerosols*

Temp	Relative humidity		
	30%	50%	80%
F			
30 (−1.11 C)	2.45[a]	2.60	2.66
50 (10 C)	2.43	2.62	2.45
80 (26.67 C)	2.36	2.38	2.55

[a] Decay rate, per cent per min.

The feasibility of employing fluorescent-cell counting assays for estimating the concentration of virus aerosols is indicated by this study. The advantages of the procedure were cited earlier, but some aspects are worthy of further consideration. The technique of intracerebral inoculation of mice is as sensitive for assay of yellow fever virus as the method of fluorescent-cell counting (2). By the former, however, the volume of 0.03 ml is the maximum that may be employed. A constant proportionality has been demonstrated between volume of inoculum and infected cells with the latter assay. The opportunity to detect and to measure small amounts of virus, therefore, may be increased by a factor of 33, when 1 ml of inoculum is employed in the fluorescent-cell counting assay. This facet of the assay attains added importance in attempting to estimate the concentration of virus in aerosols aged for prolonged periods; in such cases, it is likely that inocula in excess of 0.03 ml would be needed to demonstrate the presence of residual virus. Additional advantages associated with the fluorescent-cell counting assay of yellow fever virus is its rapidity (24 hr) and ease of performing replicate determinations, which merely involves inoculation of additional cover slip-cell cultures. In comparison, the method of intracerebral inoculation of mice requires an observation period of from 14 to 20 days and extensive animal-holding facilities, if replicate assays are to be made.

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