APPLIED MICROBIOLOGY
Vol. 12, No. 5, p. 418-420 September, 1964

Inactivation of Airborne Viruses by Ultraviolet Irradiation

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Received for publication 11 May 1964

ABSTRACT

JENSEN, MARCUS M. (University of California, Los Angeles). Inactivation of airborne viruses by ultraviolet irradiation. Appl. Microbiol. **12**:418-420. 1964.—Aerosolized viruses were passed through a high-intensity ultraviolet (UV) cell. This cell consisted of a long cylindrical aluminum tube [diameter, 7 in. (17.7 cm); length, 36 in. (91.4 cm)] with a highly reflective inner surface and a longitudinally extending helical baffle system which directed airborne particles in close proximity to a centrally located UV lamp. After having been passed through the UV cell, viral aerosols were collected with an Andersen sampler, and viral concentrations were determined by plaque assay methods on tissue cultures. Inactivation rates of greater than 99.9% were obtained for Coxsackie, influenza, Sindbis, and vaccinia viruses, and slightly less for adenovirus (96.8%), when the aerosols passed through the UV cell at 100 ft³/min. At aerosol flow rates of 200 ft³/min, inactivation rates were slightly lower; 91.3 for adenovirus, 97.5 and 96.7 for Coxsackie and Sindbis, respectively, and greater than 99.9% for influenza and vaccinia viruses.

The germicidal effect of ultraviolet (UV) light on an airborne bacterium was first experimentally demonstrated by Wells and Fair (1935). The following year Wells and Brown (1936) successfully demonstrated the recovery of influenza virus from the air 1 hr after aerosolization, and were able to destroy the infectivity of the airborne virus by UV irradiation. Ultraviolet irradiation has been used to a limited extent over the past 30 years in attempts to control airborne spread of microbial infections. Although effective in reducing infectivity rates in many instances, this procedure has not received wide acceptance. The impracticability of applying UV control to all possible airborne routes of microbial spread, plus the injurious effects from direct radiation, have limited its use. This subject has been discussed by Wells (1955) and Riley and O'Grady (1961).

To successfully apply UV irradiation in the control of viral diseases, our understanding of airborne spread of viruses must be increased, and a proper method of sanitary ventilation must be applied. Harper (1961, 1963) presented information on the survival of airborne viruses and demonstrated how their viability is influenced by relative humidity, temperature, and type of suspending fluid. Quantitative evaluation of the effects of UV irradiation on airborne viruses is very limited. A helical baffle UV cell (Helfman, 1963) provided a system in which the inactivation of virus aerosols could be studied. This unit produces a high-intensity UV field and insures effective exposure of airborne particles. The present study evaluates the ability of this cell to inactivate airborne viruses. Five viruses, adenovirus type 2, Coxsackie B1, influenza A, Sindbis, and vaccinia, each representing major viral groups, were tested.

MATERIALS AND METHODS

Aerosol test unit. The aerosol test unit is schematically illustrated in Fig. 1. The unit consisted of the following components.

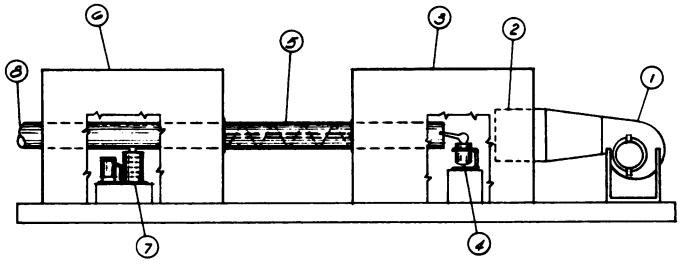

FIG. 1. *Schematic drawing of aerosol test unit. Numbers represent the following: (1) blower, (2) ultrahigh-efficiency filter, (3) aerosol generation chamber [3 by 4 by 3 ft (91.4 by 121.9 by 91.4 cm)], (4) aerosol generator, (5) ultraviolet test cell, (6) sampling chamber, (7) Andersen sampler, (8) outlet to decontamination chambers.*

(*i*) *Aerosol generation chamber.* Air was supplied to this chamber by a blower with a variable output capable of delivering up to 200 ft³/min. The air was first passed through a 0.3-μ Ultra Aire Filter (model 15-85175, Mine Safety Appliances Co., Pittsburgh, Pa.) to prevent external contamination. Six UV lamps were installed inside the chamber for safety and decontamination purposes. Aerosols were produced by an aerosol generator (model 200 A; Schoeffel Instrument Co., Hillsdale, N.J.) which released droplets in the size range of 1 to 4 μ. This self-contained generator dispensed from 0.128 to 0.150 ml of viral suspensions per min, depending on the composition of the fluid, and operated efficiently with 1 to 10 ml of medium. The aerosol was dispensed directly into a tube [diameter, 7 in. (17.7 cm)] which led to the UV test cell.

(*ii*) *Ultraviolet test cell.* The UV cell employed was a basic component of an aseptic air system (Linde-Robbins, Venice, Calif.) and consisted of an aluminum tube [length 36 in. (91.4 cm); diameter, 7 in. (17.7 cm)] with an inner surface highly reflective for UV radiation in the germicidal range. A helical baffle system, extended longitudinally inside the tube, directed the airflow in a cyclonic pattern and brought any airborne particles in close proximity to a

centrally located 2,537 A UV source (General Electric germicidal lamp no. G36T6), with a delivered radiation intensity of not less than 0.03 watt-min/ft^2 (Brodie and Leaney, 1963). The lamp was constructed of a special glass which eliminated most ozone production. Exposure times of about 0.6 and 0.3 sec were obtained at airflow rates of 100 and 200 ft^3/min, respectively.

(*iii*) *Sampling chamber.* The aerosol was passed through the sampling chamber in a tube [diameter, 7 in. [17.7 cm)] that extended directly from the UV cell. An Andersen (1958) sampler was connected to the tube. Special Andersen sampler petri dishes were each filled with 27 ml of 2% agar devoid of additional nutrients. The agar was covered with 0.3 ml of a 20% skim-milk suspension; this formed a thin viscous film over the agar. After the aerosol sample was collected, the skim-milk film was resuspended in 3 ml of Hank's solution which was then assayed for viruses. Six UV lamps were also installed inside this chamber to inactivate any viruses that might have slipped through the sampler. The aerosol was completely inactivated by passage through a series of UV cells leading from the sampling chamber.

Viral suspensions. Adenovirus 2 was grown in FL amnion cells maintained in a medium composed of 20% Tryptose Phosphate Broth (Difco), 0.1% yeast extract (Difco), and 1% agamma calf serum in Hank's balanced salt solution. After 6 days of incubation, the medium was cleared of cellular debris by centrifugation and was used as the aerosolization medium. Coxsackie B1 virus was grown for 3 days in Detroit-6 cells and was suspended in cleared Eagle's medium containing 20% agamma calf serum. Influenza A (WSN strain) and Sindbis viruses were contained in chorioallantoic fluid collected from 12-day-old chick embryos inoculated 2 days previously with the respective viruses. Because of technical difficulties, the 100 and 200 ft^3/min tests for vaccinia virus were run separately. Vaccinia used in 100 ft^3/min tests was in a clarified 10% suspension of infected chorioallantoic membranes from chick embryos; the virus used in tests at 200 ft^3/min was grown in chick embryo tissue culture and suspended in Eagle's medium plus 5% chicken serum. Virus titers and the amount of suspension dispensed during aerosolization are given in Table 1.

Viral assays. All virus titrations were done by tissue culture plaque methods in 30-ml disposable plastic flasks (Falcon Plastic Co., Los Angeles, Calif.). Tissue culture monolayers were inoculated with 0.2 ml of the skim milk-virus suspensions or appropriate dilutions thereof. After 1-hr adsorption at 37 C, the monolayers were covered with an appropriate overlay medium and incubation was continued at 37 C until visible plaques developed. The flasks were then filled with 10% formalin, which fixed the cells and inactivated the virus. The overlay medium and formalin were poured off and cells were stained with crystal violet; this enabled direct counting of the plaques (Holland and McLaren, 1959). Coxsackie B1 virus was assayed in Detroit-6 line of tissue culture; a 0.6% agar-tris(hydroxymethyl)aminomethane (tris) buffer overlay containing 5% agamma calf serum was used, and the cells were fixed and stained after 3 days of incubation. Adenovirus plaques were produced on FL amnion cells. Two tris buffer overlays containing 0.6% agar and double concentrations of Eagle's amino acid and vitamin solutions were used; the first was added after the adsorption period, and the second after 5 days of incubation. Plaques were counted on the 10th to 12th day. Influenza virus plaques were produced on primary chick embryo tissue cultures with a serum-free overlay medium described by Simpson and Hirst (1961); plaques were counted after 5 days of incubation. Sindbis virus was assayed on secondary chick embryo tissue cultures with the use of tris-buffer overlay; plaques were counted after 2 days of incubation. Vaccinia virus was inoculated onto secondary chick embryo tissue culture; no agar overlay was used, and plaques were counted after 36- to 48-hr incubations in nutrient fluid.

Procedure for testing UV cell. Ultraviolet inactivation tests were carried out by first passing the viral aerosol through the lighted UV cell at 100 ft^3/min and then at 200 ft^3/min. Each test lasted 10 min, during which time 10 ft^3 of aerosol were collected by the Andersen sampler. A separate sampler was used for each test. The same procedure was then repeated with the UV cell lamp off. The numbers of viral plaque-forming units (PFU) collected with the lamps on and off were compared, and the percentage of virus inactivated was determined. During the tests, all chambers were sealed, and the chamber UV lamps were on to keep the insides of the chambers from becoming contaminated.

RESULTS

Inactivation rates of 96.88 to greater than 99.99% were obtained for the five virus strains tested when aerosols

TABLE 1. *Inactivation of viral aerosols during passage through a helical baffled UV cell**

Virus	Concn of virus suspension†	Amt of viral suspension dispensed per min	Air-flow rate through UV cell	No. of virus PFU collected per ft^3 of air with		Percentage of virus inactivated by UV light
				UV off	UV on	
		ml	ft^3/min			
Adenovirus	3.4×10^8	0.144	100	29,235	913	96.88
			200	28,016	2,436	91.31
Coxsackie B-1	4.0×10^7	0.143	100	10,755	5	99.95
			200	9,000	225	97.50
Influenza A	1.0×10^7	0.145	100	920	0	>99.90
			200	690	0	>99.86
Sindbis	7.5×10^6	0.150	100	5,644	26	99.53
			200	3,793	124	96.73
Vaccinia	1.0×10^8	0.128	100	27,522	0	>99.99
	2.0×10^7	0.142	200	2,265	0	>99.96

* For description, see Materials and Methods.

† Expressed as number of plaque-forming units (PFU) per milliliter.

were passed through the UV cell at air-flow rates of 100 ft^3/min. Inactivation rates were generally less at air-flow rates of 200 ft^3/min, ranging from 91.31% for adenovirus to >99.99% for vaccinia virus. The number of virus PFU collected in each test and the percentages of viral inactivation are shown in Table 1. The number of virus PFU collected was determined by totaling the amount of virus contained in the 3 ml of Hank's solution-skim milk suspension obtained from each of the six plates of the Andersen sampler. Data for adenovirus, Sindbis, and vaccinia viruses were the average values from two tests each; for Coxsackie and influenza A viruses, values were from single tests.

Some viruses may have slipped through the sampler; from studies with bacteriophage aerosols, slippage was found to be less than 10% of the total. The highest concentrations of virus were found on sampler stages 3 and 4. With Coxsackie virus, for example, the following distribution was obtained on stages 1 through 6: 10,500, 17,500, 21,750, 26,750, 20,250, and 11,250 PFU, respectively. Similar distributions were observed for the other viruses tested. The skim-milk film on agar was about twice as efficient as plain agar or a skim-milk film on a plastic surface for collecting viruses. Standard all-glass impingers were less effective than was the Andersen sampler in this study.

The relative humidity of the air entering the unit was 50% during the adenovirus tests, 66% with Coxsackie, 68% with influenza, 62% with Sindbis, and 65% with vaccinia. The amount of fluid added from aerosolization would increase these values by less than 0.5%. Air temperature was 24 to 25 C.

DISCUSSION

These tests demonstrate the relatively high efficiency of the above-described helical baffle UV cell for inactivating airborne viruses. The conditions of these tests were vigorous. The viruses were aerosolized in the undiluted medium in which they were harvested; this, it was thought, would more closely simulate the materials in which viruses would be aerosolized under most natural conditions. The virus aerosol concentrations were many times greater than one would normally expect to encounter under natural conditions, and the viruses were aerosolized directly into the UV cell with no prefiltration.

Because the viral concentrations and the composition of the aerosolized medium, as well as the sensitivity of the viral assay systems, varied, it cannot be determined whether the slight differences in inactivation rates noted represent variations in susceptibility of the different viruses to UV irradiation or simply reflect variations in the test conditions. The influence of such factors as relative humidity, temperature, and composition of suspending medium on UV inactivation of viruses remains to be determined.

The helical baffle UV cells are designed to work in conjunction with roughing and high-efficiency prefilters. Such filtration removes many of the airborne microorganisms and larger particles that might protect microorganisms from the UV rays, and also prevents accumulation of dust on the UV lamp. The UV cell, when used in conjunction with prefilters and installed in sanitary ventilation systems, should kill virtually all viruses passing through it. Such a system lends itself to areas where affluent or effluent air must be decontaminated or where air recirculation is desired.

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