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Characterization of *Mycoplasma* Aerosols as to Viability, Particle Size, and Lethality of Ultraviolet Irradiation[1]

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ABSTRACT

KUNDSIN, RUTH B. (Harvard Medical School, Boston, Mass.). Characterization of *Mycoplasma* aerosols as to viability, particle size, and lethality of ultraviolet irradiation. J. BACTERIOL. 91:942–944. 1966.—Viable aerosols of four strains of *Mycoplasma*: *M. hominis* II, *M. pharyngis*, *M. pneumoniae*, and an undetermined strain recovered from a lung at autopsy were dispersed, and the particle size was determined. The median diameter of the droplet nuclei ranged from 1.5 to 3.1 μ. *M. pharyngis* had a dieaway constant k of 0.008, indicating a survival potential of 6 hr from 10,000 colony-forming units at 23% relative humidity. Ultraviolet irradiation destroyed over 99% of the aerosols of all four strains concurrently with spraying. The particle size and viability of the droplet nuclei carrying *Mycoplasma* were consistent with the theory of airborne transmission of lower respiratory-tract infection.

Discovery of a new agent of human infectious disease initiates speculation as to its mode of transmission. Although *Mycoplasma* species have been recognized as agents of respiratory-tract infection in birds and animals for decades, it is only since 1962 that *M. pneumoniae* has been definitively implicated in acute disease of the lower respiratory tract in humans (2).

It seemed appropriate to investigate the *Mycoplasma* to determine whether they fulfill the criteria for airborne agents of respiratory-tract infection as established by Wells (6). Wells demonstrated that the infective agent in pulmonary tuberculosis can be airborne. Tubercles in the lung and death of rabbits resulted when organisms were inhaled as fine particles or droplet nuclei. The same number of organisms failed to initiate fatal tuberculosis in rabbits when inhaled as coarse particles, and only a rare tubercle was found upon killing the animals. Parallel results were obtained with influenza virus. There were no survivors among mice exposed to fine droplet nuclei, whereas there were survivors among mice exposed to coarse droplet nuclei, as well as survivors among mice that were given the virus by nasal instillation. Fine particles which carried to the lower respiratory tract were most efficient in inducing bacterial as well as viral infection. An industrial hygienist who has studied the inhalation of particulate matter observed that particles over 10 μ in size are almost completely screened out in the upper respiratory passages; 80% of 5-μ particles are also removed. Smaller particles are inhaled and preferentially deposited in the lung (3).

The four problems therefore, that seem to warrant investigation with *Mycoplasma* are: (i) Can viable droplet nuclei be formed experimentally? (ii) Do droplet nuclei remain viable long enough to be epidemiologically significant? (iii) Are the particles small enough to reach the alveoli? (iv) Can *Mycoplasma* aerosols be destroyed?

MATERIALS AND METHODS

A cube of Plexiglas with a volume of 8 ft^3 (0.2 cubic meter) was used. A Devilbiss no. 40 nebulizer, capable of producing fine particles, generated the aerosols, with air pressure at 25 psi. The confined air was sampled with the Andersen apparatus (1). This device permits determination of particle size as air is drawn through six successive sieve samplers at increasing velocity, with the result that larger particles impinge on the agar below the larger holes. Smaller particles, de-

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pending on their size and inertia, impinge beneath the successively smaller and smaller sieves.

The plates were counted under a microscope (× 10 to 30) after 1 week of incubation. The colonies were visualized by spraying the surface of the agar with a 1:50 dilution of Dienes' stain. The number of colonies counted on each stage was plotted as a cumulative per cent on logarithmic probability paper, and the count median particle diameter was determined by reading directly from the graph (4).

The organisms used were Campo W (*M. hominis* II) secured from Harry Morton; *M. pharyngis* (Patt strain) and *M. pneumoniae* (Bru strain), both secured from Wallace Clyde; and Campbell (an undertermined isolate recovered at autopsy from a patient who died of fulminating pneumonitis of unknown etiology).

Campo W, *M. pharyngis*, and Campbell strains were grown for 3 to 4 days in PPLO broth (Difco) fortified with 5% rabbit serum and penicillin, but without yeast extract.

M. pneumoniae (Bru) was grown for 14 days in biphasic medium. A thin layer of tryptic digest agar at the bottom of an Erlenmeyer flask was covered with PPLO broth fortified with 20% uninactivated horse serum, 10% yeast extract, and 1,000 units per ml of penicillin. A 1-ml amount was sprayed into the chamber for each test. Samples for all except *M. pneumoniae* were taken with the Andersen apparatus, with use of PPLO agar (Difco) with 5% rabbit serum and penicillin. *M. pneumoniae* was recovered on PPLO agar fortified with horse serum, yeast extract, and penicillin.

The ultraviolet (UV) radiation emanated from a General Electric Hot Cathode lamp G 15 T8 emitting 25 μw per cm^2 at 1 meter with 90% of its output at 2,537 A. This lamp was attached to one side of the interior of the chamber.

RESULTS

Particle size distribution of Campo W aerosol is shown in Fig. 1. The cumulative percentage of colony-forming units (CFU) recovered on each stage of the air sampler was plotted against the particle size collected with 50% efficiency by that stage. The best straight line was drawn through the points. The size at 50% was read as the count median diameter (CMD). Campo W aerosol had a CMD of 1.5 μ, with a cloud size ranging from 0.4 to 5 μ. Experimental findings for other strains were graphically analyzed in the same manner.

Campbell strain had a CMD of 2.6 μ with a cloud size ranging from 0.6 to 10 μ. *M. pharyngis* aerosols in four experiments conducted on the same day showed a range of from 1.6 to 1.7 μ CMD. The average of seven experiments was 1.9 μ CMD. Two experiments with *M. pneumoniae* showed a CMD of 2.6 and 3.1 μ. The results are shown in Table 1.

These experiments answered two questions.

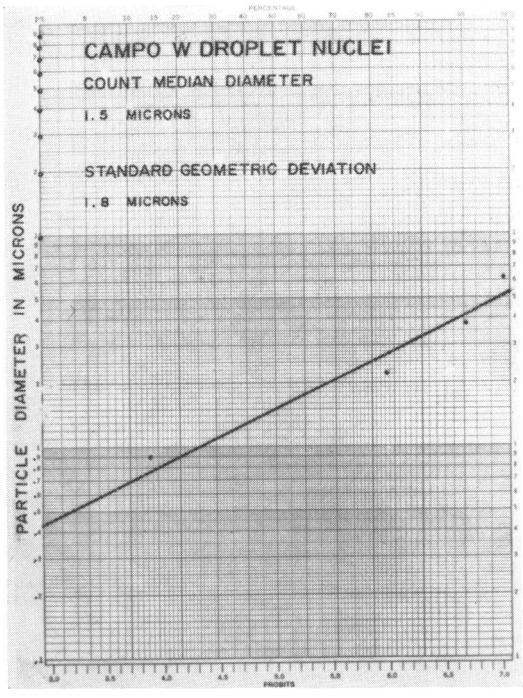

FIG. 1. *Particle size distribution of Campo W aerosol.*

Viable droplet nuclei could be formed and recovered experimentally. They were of a size to be hygienically significant, since 50% of the particles were 3 μ or less in size.

The dieaway of *M. pharyngis* aerosol at a relative humidity of 23% was slow. Starting with an aerosol of 39,000 CFU per 5 ft^3, 7,100 CFU were recovered at the end of 45 min. Thus, there was not even one logarithm reduction in 45 min.

Calculation of the dieaway constant by the standard first-order reaction formula yielded a k of 0.008. By extrapolation, at this rate of decay, it would take over 6 hr for 10,000 CFU of *M. pharyngis* to dieaway in the plastic cube described at a relative humidity of 23%.

M. pneumoniae was recovered 30 min after atomization at relative humidities of 26 and 34%. Longer survival times were not tested.

The effect of UV radiation was tested by spraying a culture for 2 min with no irradiation and by sampling the air simultaneously for a total of 5 min. This was repeated with irradiation. The results are shown in Table 2.

DISCUSSION

Warren (5) reported that three strains of *Mycoplasma* of animal origin, several strains of L forms, and *Streptobacillus moniliformis* could

TABLE 1. *Count median diameter and standard geometric deviation of Mycoplasma aerosols*

Mycoplasma strain	Date of expt	Series	Count median diam	Standard geometric deviation	Relative humidity
			μ	μ	%
Campo W	18 August 1964		1.5	1.8	46
Campbell	1 September 1964		2.6	2.0	49
M. pharyngis	9 October 1964		3.1	2.1	44
M. pharyngis	9 November 1964	A	1.7	2.8	40
		B	2.1	2.1	57
M. pharyngis	15 December 1964	A	1.5	2.3	26
		B	1.7	2.0	25
		C	1.6	1.8	22
		D	1.7	1.6	21
M. pneumoniae	15 April 1965	A	2.6	3.2	24
		B	3.1	1.8	26

TABLE 2. *Effect of ultraviolet irradiation on Mycoplasma aerosols*

Mycoplasma strain	Colony-forming units		Reduction	Relative humidity
	UV off	UV on		
			%	%
Campo W	3,050	0	100.00	54
Campbell (lung)	9,270	7	99.92	69
M. pharyngis	2,580	4	99.84	64
M. pharyngis	64,900	2	99.997	71
M. pneumoniae	1,130	0	100.00	29

withstand 1 to 2 hr of exposure to UV radiation on an agar plate. Similar observations have been made with vegetative bacterial cells. Aerosols are far more vulnerable than organisms suspended in liquid or on an agar surface.

The minimal distance traveled by a viable particle from the orifice of the nebulizer in the interior of the cube through glass tubing to the first or upper stage of the Andersen sampler was 3 ft (0.9 meter). Since viable airborne organisms were recovered 45 min after spraying, the distance they traveled in the interior of the cube is unknown. In unconfined space, the distance traversed would be determined by air currents and ventilation.

These experiments show that the lethal effect of UV radiation on airborne *Mycoplasma* aerosols is instantaneous and extraordinarily efficient (99.9%).

Wells defined a droplet nucleus as a particle of less than 10 μ in size. The size is dependent upon the solids content of the fluid from which the droplet nuclei originate, as well as the relative humidity of the atmosphere in which they are suspended. The broth culture in which the organisms were grown was used to generate the aerosols. Whether the solids content of the culture medium approximates the solids content of particles from human expiratory processes cannot be determined until *Mycoplasma* species from human contamination are actually recovered from the air.

These experimental findings show that *Mycoplasma* species can survive being aerosolized, and can be recovered again in viable form.

ACKNOWLEDGMENT

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