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# Aerosols of Mycoplasmas, L Forms, and Bacteria: Comparison of Particle Size, Viability, and Lethality of Ultraviolet Radiation[1]

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Aerosols of microorganisms were tested for particle size by use of an Andersen sampler. Mycoplasma aerosols had an average count median diameter (CMD) of 2.1 ± 0.5 μ. *Staphylococcus aureus* L forms gave an average CMD of 4.6 ± 1.7 μ; the diphtheroid L form, a CMD of 3.4 ± 0.3 μ. *Escherichia coli* had a CMD of 5.4 ± 2.5 μ; *Neisseria sicca*, 3.3 ± 0.5 μ; *N. meningitidis*, 3.4 ± 0.2 μ. *S. aureus* ATCC 6538, the parent strain of the L form, yielded a CMD of 3.9 ± 1.2 μ. *Candida albicans* gave an average CMD of 5.9 ± 1.4 μ. All organisms tested survived aerosolizing and could be recovered in viable form for at least 1 hr. Ultraviolet radiation at 2,537 A destroyed the bacteria and mycoplasmas instantaneously, and destroyed 87% of the L forms of *S. aureus*, 69% of the diphtheroid L form, and 98% of the *C. albicans* cells. After irradiation, viable particles of the L form and *C. albicans* aerosols were consistently larger, indicating that clumping led to survival. Submicron size particles were found in aerosols of all species tested except *C. albicans*.

An investigation of aerosols of different genera of microorganisms to establish sizes of viable airborne particles is not of academic interest alone. The epidemiological potential of microorganisms is defined by their particle size and ability to survive aloft for periods of time long enough to extend infectivity in time and space.

Because mycoplasmas and L forms lack cell walls and are encased in a pliable cell membrane, their ability to survive as droplet nuclei is of particular interest. Comparison with bacterial and fungal forms in the formation of aerosols and their suspension in air and relative vulnerability to ultraviolet radiation can determine whether protection is afforded by the cell wall. Understanding the aerodynamics of microorganisms can also suggest methods for control of airborne biological particulates.

The size of particles describes their ultimate fate. Particles larger than 10 μ fall out on horizontal surfaces. Small particles, 10 μ and less, fall out more slowly and tend to remain suspended in confined inhabited space until vented or inhaled. Upon inhalation, these small particles or droplet nuclei are deposited in the respiratory-tract passages according to size. Particles larger than 5 μ in diameter are removed in the upper respiratory passages, whereas most fine particles of 1 μ in diameter are deposited in the alveoli (4). Only microorganisms capable of forming viable airborne particles can be implicated in airborne contagion. The particle size of aerosols of the different genera, therefore, defines their relative importance in airborne infection.

[1] Presented in part at the 67th Annual Meeting of the American Society for Microbiology, New York, N.Y., 30 April–4 May 1967.

## MATERIALS AND METHODS

A sealed plastic cube (2 ft × 2 ft) was used to store the aerosols. Cultures were dispersed with a DeVilbiss no. 40 nebulizer connected to a source of air pressure at 25 psi. Samples were taken with an Andersen six-stage apparatus (1). Organisms were grown in the liquid medium which best supported growth. All *Mycoplasma* species except *M. pneumoniae* were grown in Difco PPLO broth supplemented with 5% rabbit serum and 1,000 units of penicillin per ml. *M. pneumoniae* was grown in Difco PPLO broth supplemented with 20% uninactivated horse serum, 10% yeast extract, and 1,000 units of penicillin per ml. L forms were grown in Trypticase Soy Broth (BBL) with 3% sodium chloride, 10% inactivated horse serum, and 1,000 units of penicillin per ml. *Candida albicans*, *Escherichia coli*, and *Neisseria sicca* were grown in Trypticase Soy Broth;

*N. meningitidis* was grown in Trypticase Soy Broth supplemented with 2% horse blood.

Liquid medium with growth was sprayed into the chamber. The medium used for recovery was the same as the liquid but with agar added.

Ultraviolet radiation emanated from a General Electric Hot Cathode lamp G15T8 emitting 25 $\mu$w per cm$^2$ with 90% of its output at 2,537 A. This lamp is permanently attached to one side of the chamber.

Particle size was determined by counting the colony-forming units on each stage of the Andersen sampler, then plotting the counts on log probit paper. The best line drawn through the points described the following parameters: count median diameter (CMD) was read at 50%, the particle size range of the cloud was read between 2 and 98%, and the percentage of particles in the submicron size could also be estimated (2).

## RESULTS

Three species of *Mycoplasma* aerosols, *M. hominis* II, *M. pharyngis* (Orale I), and *M. pneumoniae* (Bru), have been studied previously (3). *M. hominis* I and *M. pneumonia* (FH) were subsequently investigated. The combined results are shown in Table 1. The average CMD was 2.1 $\pm$ 0.5 $\mu$. This estimate is probably high because of the limited efficiency of the Andersen sampler for particles smaller than 0.9 $\mu$. Stage 6 traps these particles with 50% efficiency. Consequently, 50% of 0.9-$\mu$ particles and more than half of those smaller than 0.9 $\mu$ could escape. However, in all determinations for mycoplasmas the maximal counts were obtained on stage 5 of the sampler. This stage traps particles 2.2 $\mu$ in size with 50% efficiency. Lower counts were obtained on stage 6, indicating a definite fall off of viable particles of smaller size.

Two L forms were studied, the L forms of *S. aureus* ATCC 6538 P and a diphtheroid, both secured from Louis Dienes. The *S. aureus* L form gave an average CMD of 4.6 $\pm$ 1.7 $\mu$; the diphtheroid, 3.4 $\pm$ 0.3 $\mu$. As shown in Table 2, maximal counts were obtained on stage 4, which collects 3.8-$\mu$ particles with 50% efficiency.

The bacterial aerosols studied were *E. coli*, *N. sicca*, and *N. meningitidis* (Table 3). *E. coli* gave a CMD of 5.4 $\pm$ 2.5 $\mu$; *N. sicca*, a CMD of 3.3 $\pm$ 0.5 $\mu$; and *N. meningitidis*, a CMD of 3.4 $\pm$ 0.2 $\mu$. A comparison of the parent strain *S. aureus* ATCC 6538 P with its L form showed that the CMD of the parent-strain aerosol (3.9 $\mu$) did not differ significantly from that of the L-form aerosol (4.6 $\mu$). Ultraviolet radiation destroyed the parent strain more efficiently than the L form. Die-aways of both organisms in air were comparable. An interesting finding was that in the L form only 3% of the cloud in one of six experiments was in the submicron range. No organisms in this range were recovered in the other five experiments. The parent organism, however, yielded particles in the submicron range in five of seven experiments, with an average of 5%. The study of aerosols therefore does not substantiate the presence of more viable submicron particles in the L form than in its parent strain.

The largest particles in the aerosol studies were found with a strain of *C. albicans* isolated from a burned patient's skin (Table 4). The average CMD was 5.9 $\pm$ 1.4 $\mu$.

The smallest particles were formed by *Mycoplasma* aerosols; 19% of the aerosols were less than 1 $\mu$ in size. This represents a significant epidemiological finding, as most air filters are measured for efficiency for 1- to 5-$\mu$ particle retention. A large proportion of mycoplasma

TABLE 1. *Particle size of Mycoplasma aerosols*

| Species | No. of observations | CMD particle size | Relative humidity |
|---|---|---|---|
| | | $\mu$ | % |
| *M. hominis* I (PG 25) | 1 | 1.9 $\pm$ 1.7 | 22 |
| *M. hominis* II (Camp. W) | 1 | 1.6 $\pm$ 1.7 | 46 |
| Campbell (lung)[a] | 1 | 2.6 $\pm$ 2.0 | 49 |
| *M. pharyngis* (Patt) | 7 | 1.9 $\pm$ 0.5 | 21–57 |
| *M. pneumoniae* (Bru, FH) | 4 | 2.5 $\pm$ 0.3 | 24–74 |
| Average | | 2.1 $\pm$ 0.5 | |

[a] Found to be mixture of *M. pharyngis* and *M. pneumoniae*.

TABLE 2. *Particle size of L form aerosols*

| Species | No. of observations | CMD particle size | Relative humidity |
|---|---|---|---|
| | | $\mu$ | % |
| *Staphylococcus aureus* (6538P) L form | 1 | 6.4 $\pm$ 1.9 | 38 |
| | 1 | 3.7 $\pm$ 1.6 | 40 |
| | 1 | 6.8 $\pm$ 1.9 | 37 |
| | 1 | 2.3 $\pm$ 1.5 | 44 |
| | 1 | 3.7 $\pm$ 1.5 | 26 |
| Average | | 4.6 $\pm$ 1.7 | |
| Diphtheroid L form | 7 combined | 3.4 $\pm$ 1.5 | 13–35 |
| | 1 | 2.9 $\pm$ 1.8 | 19 |
| | 1 | 3.6 $\pm$ 1.3 | 32 |
| | 1 | 3.6 $\pm$ 1.3 | 35 |
| Average | | 3.4 $\pm$ 0.3 | |

TABLE 3. *Particle size of bacterial aerosols*

| Species | CMD particle size | Relative humidity |
|---|---|---|
| | μ | % |
| *Escherichia coli* ATCC 11775 | 4.6 ± 2.4 | 22 |
| | 5.2 ± 2.3 | 26 |
| | 2.4 ± 1.6 | 28 |
| | 4.9 ± 3.6 | 17 |
| Average | 4.3 ± 1.2 | |
| *Neisseria* sicca | 3.6 ± 2.3 | 19 |
| | 3.1 ± 1.6 | 45 |
| Average | 3.4 ± 0.3 | |
| *N. meningitidis* | 3.5 ± 3.2 | 19 |
| | 3.6 ± 1.4 | 19 |
| | 3.2 ± 1.8 | 17 |
| Average | 3.4 ± 0.2 | |
| *Staphylococcus* aureus ATCC 6538P | 5.0 ± 1.7 | 25 |
| | 5.0 ± 2.3 | 31 |
| | 3.1 ± 2.1 | 28 |
| | 2.3 ± 1.6 | 26 |
| Average | 3.9 ± 1.2 | |

TABLE 4. *Particle size of aerosols of Candida albicans*[a]

| CMD particle size | Relative humidity |
|---|---|
| μ | % |
| 5.0 ± 1.5 | 19 |
| 6.6 ± 1.7 | 20 |
| 4.8 ± 1.5 | 20 |
| 4.1 ± 1.3 | 20 |
| 5.5 ± 1.7 | 16 |
| 7.5 ± 1.3 | 15 |
| 5.4 ± 1.5 | 14 |
| 4.2 ± 1.3 | 14 |
| 6.6 ± 1.5 | 16 |
| 6.5 ± 1.7 | 16 |
| 6.7 ± 1.8 | 15 |
| 3.7 ± 1.8 | 11 |

[a] Average = 5.6 ± 1.2.

particles, therefore, falls below the size retained and would be efficiently redistributed by a recirculating air-conditioning system.

At no time were particles of less than 1 μ size found with *C. albicans* aerosols. Bacteria and L forms occasionally formed submicron particles. The percentage in this range (occasionally 3 to 15%) was never as high as with the mycoplasmas nor as consistent. The noteworthy findings are that the L-form and bacterial aerosols have the same particle size. The mycoplasma particle size is smaller than both, and this difference is statistically significant ($P = < 0.01$).

TABLE 5. *Die-away of microorganisms in droplet nuclei*

| Species | *K* | Relative humidity | Survival time[a] |
|---|---|---|---|
| | | % | min |
| *Mycoplasma pharyngis* | .01 | 23 | 500 (8.3) |
| *M. pneumoniae* | .05 | 26 | 100 (1.7) |
| | .08 | 77 | 63 (1.1) |
| *Staphylococcus aureus* L form | .03 | 24 | 167 (2.8) |
| Diphtheroid L form | .01 | 35 | 500 (8.3) |
| | .04 | 23 | 125 (2.1) |
| *Escherichia coli* | .01 | 27 | 500 (8.3) |
| | .04 | 20 | 125 (2.1) |
| *Neisseria sicca* | .03 | 45 | 167 (2.8) |
| *N. meningitidis* | .03 | 19 | 167 (2.8) |
| *S. aureus* | .02 | 27 | 250 (4.2) |
| *Candida albicans* | .02 | 20 | 250 (4.2) |
| | .02 | 14 | 250 (4.2) |
| | .02 | 13 | 250 (4.2) |

[a] Numbers in parentheses show survival times in hours.

TABLE 6. *Organisms in aerosols removed by ultraviolet radiation*

| Species | No. of experiments | Per cent removed | Relative humidity |
|---|---|---|---|
| | | | % |
| *Mycoplasma* species... | 5 | 100 | 29–71 |
| *Escherichia coli*..... | 2 | 100 | 19, 25 |
| *Neisseria sicca*..... | 1 | 99 | 42 |
| *N. meningitidis*..... | 1 | 100 | 20 |
| *Staphylococcus aureus*.. | 1 | 100 | 28 |
| *S. aureus* L form..... | 1 | 87 | 41 |
| Diphtheroid L form... | 1 | 69 | 24 |
| *Candida albicans*..... | 2 | 89 | 15, 16 |

Die-away studies were done by sampling immediately after spraying and after a definite time interval in minutes. The die-away constant, $k$, was calculated by using the formula: $K = (\log N_0 - \log N_t)/t$, where $N_0$ = initial number of organisms and $N_t$ = number of organisms at time $t$. Once the die-away constant $k$ had been calculated, the time for survival of a known number of organisms could be determined by substituting in the formula. Calculations of $k$ and the time estimated for the reduction of an initial number of $10^5$ organisms to 1 organism are indicated in Table 5.

The die-away constant $k$ of most forms documents airborne survival of more than 2 hr from an initial aerosol of 100,000 organisms per 5

$ft^3$. *M. pneumoniae*, with a slightly higher die-away, shows a survival potential of 1 hr, starting with an initial aerosol of 100,000 organisms per 5 $ft^3$ at 77% relative humidity. All microorganisms tested can, therefore, be aerially transported and remain viable for 1 hr and usually longer.

Ultraviolet radiation destroyed all microorganisms tested, but the efficiency of this destruction varied. The tests were conducted by spraying without ultraviolet radiation followed by spraying with radiation. From the number of organisms recovered, calculations of the percentage of reduction were made (Table 6).

The average particle size of viable organisms of *C. albicans*, after irradiation, was 7.5 $\mu$. Previous to irradiation, the average particle size was 5.6 $\mu$. According to the *t* test, this difference is significant ($P = <0.05$). One can conclude that small particles of *C. albicans* are vulnerable to ultraviolet irradiation. Larger particles are not as vulnerable owing to some protection afforded by size. This may represent budding forms or adherent organisms. If we assume that one photon destroys one organism, it is logical to theorize that larger particles contain more than one viable unit and require more photons for destruction.

*S. aureus* L forms had a CMD particle size of 7.6 $\mu$ after irradiation. The diphtheroid L forms had a CMD particle size of 4.1 $\mu$. In both these species, the viable particles were larger after irradiation than before. As with *C. albicans*, larger particle size afforded protection against ultraviolet radiation.

## DISCUSSION

Aerosols of mycoplasmas formed the smallest particles tested and differed significantly in size from aerosols of L forms, bacteria, and *Candida*. L forms and bacteria had particles of the same size. The droplet nuclei of *C. albicans* were the largest of the organisms tested.

Aerial survival of all microorganisms was demonstrated. All can survive as airborne droplet nuclei for 1 hr or more at the relative humidities tested (45% and lower), and consequently have the potential for airborne transmission. At 77% relative humidity, *M. pneumoniae* survived for 1 hr as an aerosol. The lack of a cell wall did not preclude formation of viable aerosols by mycoplasmas or L forms.

Ultraviolet radiation destroyed droplet nuclei of bacteria and mycoplasmas instantly with very few survivors. L forms and *C. albicans* were not destroyed as efficiently. When spraying, testing, and irradiation were simultaneous, 13% of *S. aureus* L form, 31% of diphtheroid L form, and 11% of the *C. albicans* droplet nuclei remained. They did not appear as vulnerable as mycoplasma and bacterial forms, probably because of clumping or budding. The *S. aureus* aerosol was destroyed more efficiently by ultraviolet radiation than its L-form aerosol.

Whereas bacteria and L forms did occasionally form submicron particles, 19% of aerosol particles formed by mycoplasmas were submicron in size. This finding has epidemiological implications in airborne infections due to mycoplasmas.

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