Recovery of Airborne Streptococcal L-Forms at Various Relative Humidities

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Aerosolized group A streptococcal L-forms survived better at low than at high relative humidities, and mid-range humidities were the most lethal. Colonial morphology of cells surviving aerosolization was not altered.

Mycoplasmas (13, 14) and L-forms (8) withstand the stresses of aerosolization despite their lack of a rigid cell wall. Sampling of mycoplasma aerosols held at various levels of relative humidity (RH) has shown that survival is best at very low and very high RH and that mid-range RH levels are the most lethal. L-forms, however, have been studied only at low RH (8), and the aerosol survival data are difficult to interpret since only two samples were used to estimate decay patterns.

The present study, in which several replicate samples were taken over long periods of time at various RH levels was designed to determine the effect of RH on aerosolized L-forms of a group A streptococcus and to determine whether these organisms would respond to RH in a manner similar to that reported for mycoplasma.

A nontypable group A, β-hemolytic streptococcus (Richards strain) was supplied by York Crawford, U.S. Naval Medical Research Unit no. 4, Great Lakes, Ill. L-forms were produced on penicillin gradient plates, transferred, and made to revert as previously described (4). Revertant streptococci were identified by Gram stain, production of β-hemolysis on blood-agar, and grouping with antiserum (12). After 40 transfers on agar, the L-form failed to revert to the bacterial form when passed on penicillin-free media. After three passes in liquid media containing 0.05% agar, the stabilized L-forms were easily adapted to grow without agar. The growth medium consisted of Brain Heart Infusion (Difco) broth containing 1.5% NaCl, 10% inactivated horse serum (Grand Island Biological Co.), and 0.5% yeast autolysate (Albini Laboratories, Inc.). When a solid medium was desired, 1.2% agar was added.

After 24 hr of incubation at 37°C, L-form broth cultures which contained between 10⁶ and 10⁸ organisms per ml were aerosolized for 5 min with a modified Wells reflux atomizer into two 500-liter stainless-steel rotating drums (7) installed in a constant-temperature room held at 27 ± 0.2°C. After 2 min of equilibration following aerosolization, the first sample was taken by impingement of air for 1 min on an agar plate contained in a slit sampler operated at 5 liters/min. Thereafter, samples were taken at intervals over a 4-hr period. Physical decay of the aerosol was determined by measuring the relative light scatter of the aerosol with a forward-angle light-scatter photometer. RH inside the drums was obtained by mixing measured amounts of wet and dry air in the secondary air stream, and the RH was ascertained with a wet-and dry-bulb thermometer.

Figure 1 shows the effect of RH on the survival of airborne L-forms. Initial air samples indicated an immediate and varied sensitivity of the L-forms to RH. Approximately 100 times as many L-forms were recovered at 80% RH as were found at 40% RH. During the 4-hr holding period, group A streptococcal L-forms were most stable at 20% RH. At 80% RH, one-tenth as many L-forms per liter were recovered after 4 hr than at 20% RH. Less than 10 organisms per liter were found at 60% RH after 2 hr, and at 40% RH no viable L-forms could be detected at 30 min.

Throughout the entire study, both before and after aerosolization, no apparent alterations in streptococcal L-form morphology were observed. Colonies always grew down into the agar, and, when viewed with the colony microscope, the typical “fried egg” appearance (Fig. 2) described by others (6, 9) was evident. Detailed structural elements, observed when L-forms are prepared by the direct agar-fixation technique (1), also remained unaltered. In view of the lack of the group-specific carbohydrate which is a major component of the streptococcal cell wall (10, 11) and by virtue of their inability to revert, the stable...
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from physical streptococcal total however, streptococcal L-forms represent RH altitude. No aerosolization. Streptococcal L-form colonies on agar surface after aerosolization. X 30.

streptococcal L-forms used did not lend themselves to serological identification. They did, however, consistently produce β-hemolysis on supportive agar medium which contained 5% sheep red blood cells.

This study has shown that aerosolized streptococcal L-forms respond to RH in a manner similar to that reported for mycoplasma (13, 14). These similarities, together with those which showed that mid-range RH values also were the most lethal for certain airborne bacteria (3, 5), lend added weight to the hypothesis that the mechanism of death of microorganisms in aerosols is independent of unique morphological features (14) associated with the cell wall.

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LITERATURE CITED

1. Anderson, R. J. 1967. Acid treatment modification for the preparation of permanent slides of L forms and mycoplasmas. J. Bacteriol. 92:493-494.
2. Beard, C. W., and D. P. Anderson. 1967. Aerosol studies with avian mycoplasma. I. Survival in the air. Avian Dis. 11: 54-59.
3. Beebe, J. M. 1959. Stability of disseminated aerosols of Pasteurella tularensis subjected to simulated solar radiation of various humidities. J. Bacteriol. 78:18-24.
4. Crawford, Y. E., P. F. Frank, and B. Sullivan. 1958. Isolation and reversion of L-forms of beta-hemolytic streptococci. J. Infec. Dis. 102:44-52.
5. Dunklin, E. W., and T. T. Puck. 1948. The lethal effect of relative humidity on airborne bacteria. J. Exp. Med. 87: 87-101.
6. Freimer, E. H., R. M. Krause, and M. McCarty. 1959. Studies of L-forms and protoplasts of group A streptococci. J. Exp. Med. 110:853-879.
7. Goldberg, L. J., H. M. S. Watkins, E. E. Boerke, and M. A. Chatigny. 1958. The use of a rotating drum for the study of aerosols over extended periods of time. Amer. J. Hyg. 68:85-93.
8. Kundsin, R. B. 1968. Aerosols of mycoplasmata, L forms, and bacteria: comparison of particle size, viability, and lethality of ultraviolet radiation. Appl. Microbiol. 16:143-146.
9. Mortimer, E. A. 1965. Production of L-forms of group A streptococci in mice. Proc. Soc. Exp. Biol. Med. 119: 159-163.
10. Rotta, J., W. W. Karakawa, and R. M. Krause. 1965. Isolation of L forms from group A streptococci exposed to bacitracin. J. Bacteriol. 89:1581-1585.
11. Sharp, J. T., W. Hijmans, and L. Dienes. 1957. Examination of the L-forms of group A streptococci for the group-specific polysaccharide and M protein. J. Exp. Med. 105: 153-159.
12. Swift, H. F., A. T. Wilson, and R. C. Lancefield. 1943. Typing group A hemolytic streptococci by precipitin reactions in capillary pipettes. J. Exp. Med. 78:127-133.
13. Wright, D. N., G. D. Bailey, and M. T. Hatch. 1968. Survival of airborne mycoplasmas as affected by relative humidity. J. Bacteriol. 95:251-252.
14. Wright, D. N., G. D. Bailey, and M. T. Hatch. 1968. Role of relative humidity in the survival of airborne Mycoplasma pneumoniae. J. Bacteriol. 96:970-974.

FIG. 1. Effect of RH at 27 C on airborne group A streptococcal L-forms. Data shown are corrected for physical loss and represent biological decay. Each point represents the mean of 16 air samples from a total of four separate experiments at each relative humidity. No single value deviated more than 10% from those shown.

FIG. 2.