

Incomplete Bacteriophage-like Particles in Ultraviolet-irradiated *Haemophilus*

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Incomplete phagelike particles were found in competent and incompetent cells of *Haemophilus influenzae* Rd (transformable) lysed after exposure to ultraviolet radiation.

Stuy (6) studied the effects of ultraviolet (UV) radiation on *Haemophilus influenzae* Rd (transformable) and reported lysis of the irradiated culture.

This report presents electron-microscopic evidence of incomplete phagelike particles in UV-induced lysates of *H. influenzae* Rd. Five strains of other types of *Haemophilus* (transformable and nontransformable) were included for comparative purposes. None of the five showed a drop in turbidity after irradiation. A few incomplete phagelike particles were seen, however, in some cells of two of the five other strains.

Bacteria were grown and exposed to UV radiation (doses allowing 1 to 5% survival) according to the method of Stuy (6). Most experiments were performed on cultures grown with aeration in Levinthal broth to a density of 5×10^8 to 7×10^8 colony-forming units per ml; the competence of transformable strains is very low or absent under these conditions. Competent populations were grown in supplemented Brain-Heart Eugonbroth (1); after irradiation they were diluted fourfold in the growth medium. Irradiated cultures were incubated with aeration at 37 C and examined at 0.5-hr intervals for a decrease in density (optical density measured with a Klett-Summerson colorimeter).

Samples for electron microscopy were fixed in 1% phosphate-buffered glutaraldehyde (pH 6.1) containing 0.2% magnesium chloride and were postfixed in 1% osmium tetroxide. Epon 812 was used for embedding. Thin sections were stained with uranyl acetate and lead tartrate and were examined in an A.E.I. EM6-B electron microscope.

Only *H. influenzae* Rd and Sd (from which Rd was derived) showed a drop in turbidity after

exposure to UV radiation. Other strains examined were: *H. influenzae* types Ra, Rb, Rc, Rf (3) and *H. aegyptius* 15 (4). Rc and Rf are not transformable. The transformability of the other strains is less than that of Rd.

The presence of incomplete phagelike particles in Rd populations which lysed after UV radiation is shown in Fig. 1 to 5. Electron-transparent hexagonal heads (approximately 64 nm in diameter) were devoid of internal structure and were limited by single-layer membranes. The average tail was 110×12 nm. Tail fibers could be resolved in some particles. Tailless heads were the predominant type; particles with tails were very infrequent. Complete phages were not found.

Electron-microscopic examination of the other strains showed a few tailed particles in *H. aegyptius* 15 and tailless particles in *H. influenzae* Rb. Heads of all particles were empty; complete phages were not found. Lack of an indicator strain (10 strains of *Haemophilus* tested) precluded biological titrations of lysates. Acriflavine (5), at a concentration of 0.1 μ g/ml for 30 min, did not affect viability but increased the proportion of Rd cells lysing after irradiation and the number of phagelike particles. The morphology of the particles was not affected by the state of competence or incompetence of Rd populations at the time of irradiation. A strain of Rd more resistant to UV, obtained after repeated exposure of the culture to UV radiation (streak plate method), did not lyse after irradiation but was as highly competent as the parental strain for transformation. Only a few phagelike particles were found.

Incomplete phagelike particles (defective phages, bacteriocins) have been observed in several bacterial species (2) but have not been reported in *Haemophilus*.

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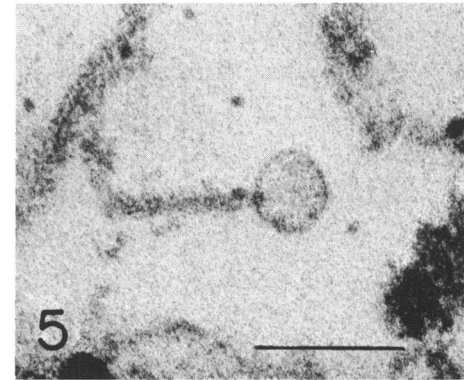
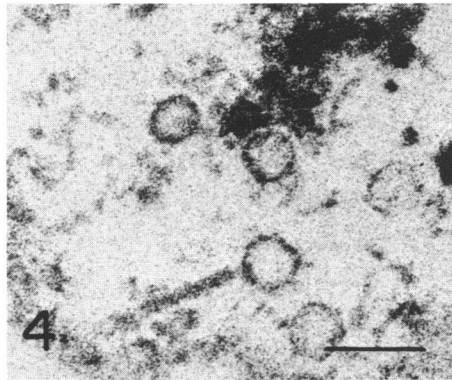
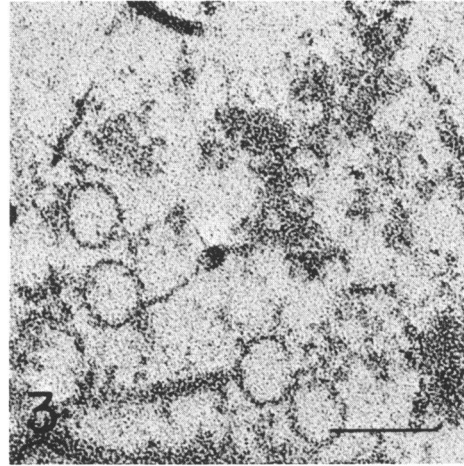
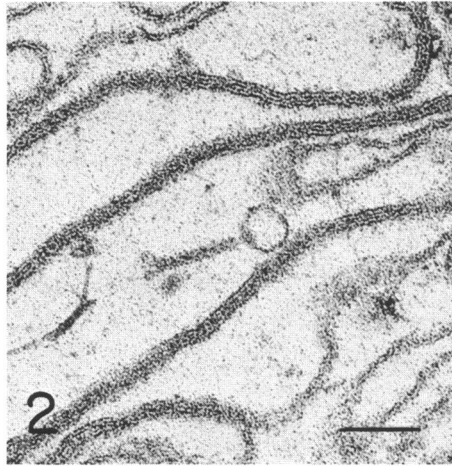
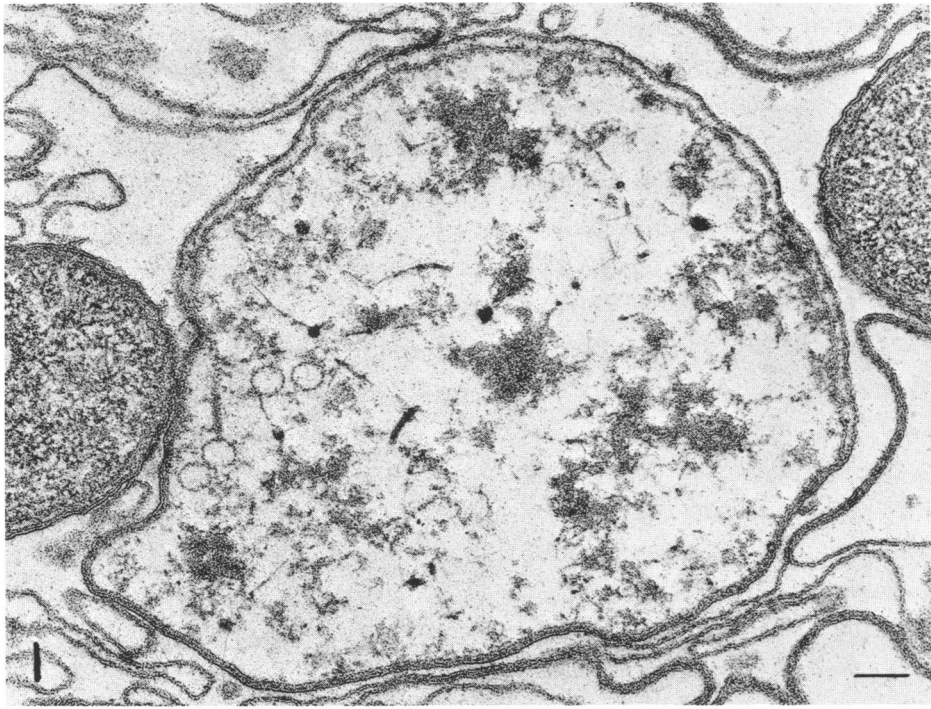


FIG. 1-5. Phage-like particles found in UV-irradiated *H. influenzae* Rd. Specimens fixed at the time of maximum lysis of the culture (2.5 hr after exposure to UV). Fig. 1-3. Competent cells grown and exposed to UV in supplemented Brain-Heart Euginbroth (1). Fig. 1, $\times 75,000$; Fig. 2, $\times 119,000$; Fig. 3, $\times 152,000$. Fig. 4 and 5. Incompetent cells in Levinthal broth, exposed to UV and acriflavine ($0.1 \mu\text{g/ml}$ for 30 min). Fig. 4, $\times 148,000$; Fig. 5, $\times 216,000$. Note electron-dense amorphous material scattered irregularly in disintegrating cells. All marker bars represent $0.1 \mu\text{m}$.

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LITERATURE CITED

1. Barnhart, B. J., and R. M. Herriott. 1963. Penetration of deoxyribonucleic acid into *Hemophilus influenzae*. *Biochim. Biophys. Acta* 76:25-39.
2. Bradley, D. E. 1967. Ultrastructure of bacteriophages and bacteriocins. *Bacteriol. Rev.* 31:230-314.
3. Leidy, G., E. Hahn, and H. E. Alexander. 1956. On the specificity of the desoxyribonucleic acid which induces streptomycin resistance in *Hemophilus*. *J. Exp. Med.* 104:305-320.
4. Leidy, G., E. Hahn, and H. E. Alexander. 1959. Interspecific transformation in *Hemophilus*; a possible index of relationship between *H. influenzae* and *H. aegyptius*. *Proc. Soc. Exp. Biol. Med.* 102:86-88.
5. Lieb, M. 1964. Dark repair of U.V. induction in K12 (λ). *Virology* 23:381-388.
6. Stuy, J. H. 1959. Studies on the radiation inactivation of microorganisms. V. Deoxyribonucleic acid metabolism in ultraviolet-irradiated *Haemophilus influenzae*. *J. Bacteriol.* 78:49-58.