NOTES

New Bacteriophage of *Haemophilus influenzae*

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Some properties of a newly isolated *Haemophilus influenzae* bacteriophage are described. The phage is morphologically distinct from previously described *H. influenzae* phages.

Bacteriophages which replicate in *Haemophilus influenzae* have been isolated (1, 4) and although their morphology has not been described in detail, one strain (HP1-C1) is known to have a contractile tail (4) and thus to belong to bacteriophage group A as defined by Bradley (2). By ultraviolet irradiation of 20 strains of *H. influenzae* isolated from clinical specimens, we have recovered three strains of phage of which one, strain N3, could be distinguished from phage HP1-C1, and it is described here.

Phage N3 formed plaques on only 1 of the 20 strains tested (strain 9). The plaques were well defined, but nevertheless were not entirely clear even at the center.

In one-step growth experiments, the latent period was 30 min, and the mean burst size was 20 (Fig. 1).

The rate of adsorption of the virus to *H. influenzae* was measured in the presence of 0.01 M KCN (Fig. 2). Since this rate was slow ($K = 6.3 \times 10^{-12}$ ml/min) and since strain 9 was the only one of 20 tested which transformed (C. T. D. Middleton, unpublished results), it appeared possible that the cells were capable of adsorbing the virus only when they were in a competent state. However, we were unable to detect any decrease in phage titer after adsorption with the standard transforming Rd strain of *H. influenzae* made competent by the method of Goodgal and Herriott (3). Nevertheless, virus deoxyribonucleic acid (DNA) extracted by phenol, using the method of Harm and Rupert (4), produced plaques on competent Rd cells which were mixed with DNA as in the standard transforming procedure of Goodgal and Herriott (3) and which were subsequently treated with deoxyribonuclease and plated in the presence of an excess of strain 9. An

unexpected finding was that the efficiency of plaquing of the phage DNA was four-fold greater on competent cultures of strain 9 than with the standard Rd strain.

FIG. 1. One-step growth experiment. Phage N3 (final concentration 1.5×10^8 plaque-forming units/ml) was added to a log-phase culture of *H. influenzae* strain 9 containing 3×10^8 cells/ml (final concentration). After adsorption for 5 min, the cells were rapidly centrifuged at 37 C, suspended in warm medium, diluted to 10^{-4} times the original concentration, and incubated at 37 C. Samples were assayed at intervals for plaque-forming units.

Negative staining by uranyl acetate showed that phage N3 has a noncontractile tail 200 nm long, which appeared to be hollow (Fig. 3, lower right), and subunits could be resolved occasionally (Fig. 3, arrow). These had a periodicity of 4.0 nm. The head is hexagonal in outline with a diameter of 60

FIG. 2. Rate of phage adsorption. Phage N3 (final concentration 5×10^4 plaques-forming units/ml) was added to a log-phase culture of strain 9 (final concentration 3×10^9 cells/ml) in the presence of 0.01 M KCN. The mixture was shaken at 37 C, and samples were withdrawn at intervals and centrifuged. The supernatant fluid was then assayed for phage.

nm. No base plate is present, and we were unable to demonstrate tail fibers by negative staining.

The morphology of N3 is thus distinct from that of previously isolated *H. influenzae* phages, and it is also distinguishable from a defective phage which has recently been demonstrated by ultraviolet induction of the Rd strain of *H. influenzae* (5). Since the phage from Rd *H. influenzae* has a tail 110 nm long and appears to be similar to phage HP1-C1, we conclude that strain N3 is the first *H. influenzae* phage to be isolated which belongs to the morphological group B (2); since it undergoes one cycle of replication in

FIG. 3. *H. influenzae* phage N3 negatively stained with uranyl acetate. Bar = 100 nm.

the Rd transforming strain of *H. influenzae* and forms plaques on another transforming strain, it may prove to be of some value in genetic studies.

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