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Accumulation of Incomplete Particles After UV-Light Induction of *Haemophilus influenzae* Lysogenic for Bacteriophage HP1c1

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Temperate phage HP1c1 produces large quantities of incomplete phage-like particles when grown on *Haemophilus influenzae* BC200, a strain apparently cured of a common defective prophage.

Although the *Haemophilus influenzae* bacteriophage HP1c1 has been utilized extensively in photobiological studies (9), very little is known about the intracellular development of the phage. Such studies are complicated by the presence of a defective prophage in the commonly used strains of *Haemophilus* (11). It has been suggested that the strain BC200 is cured of the defective prophage harbored within the strain Rd (3). This report presents electron microscopic evidence which confirms the absence of defective particles in irradiated nonlysogenic cultures of *H. influenzae* BC200 and describes the development of phage and the accumulation of phage-like products in induced BC200 cultures lysogenic for the bacteriophage HP1c1.

Boling et al. (4) have compared HP1c1 and the defective prophage from Rd by negative staining and have found them to be morphologically similar except for differences in the tail plate region. HP1c1 consists of an icosahedral-appearing head approximately 50 nm in diameter and an elongated contractile tail 120 nm in length. The *H. influenzae* strain Rd was originally isolated by Alexander and Leidy (1). BC200 is a derivative of Rd (3).

Bacteria were grown at 37 C and exposed to UV radiation, as described by Barnhart and Cox (2). A nonlysogenic strain of BC200 was used as a plating indicator. Plating media and dilution buffers have been described previously (2).

Cells were fixed by the addition of 5 ml of culture to 5 ml of 2% OsO_4 in Michaelis buffer, pH 6.0. Casamino Acids were also added to a final concentration of 2% (6). Cells were then embedded in Vestopal W, according to Kellenberger et al. (8). Thin sections were double-

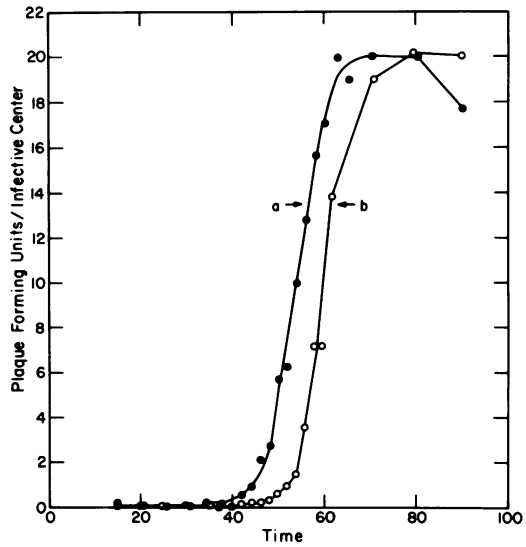
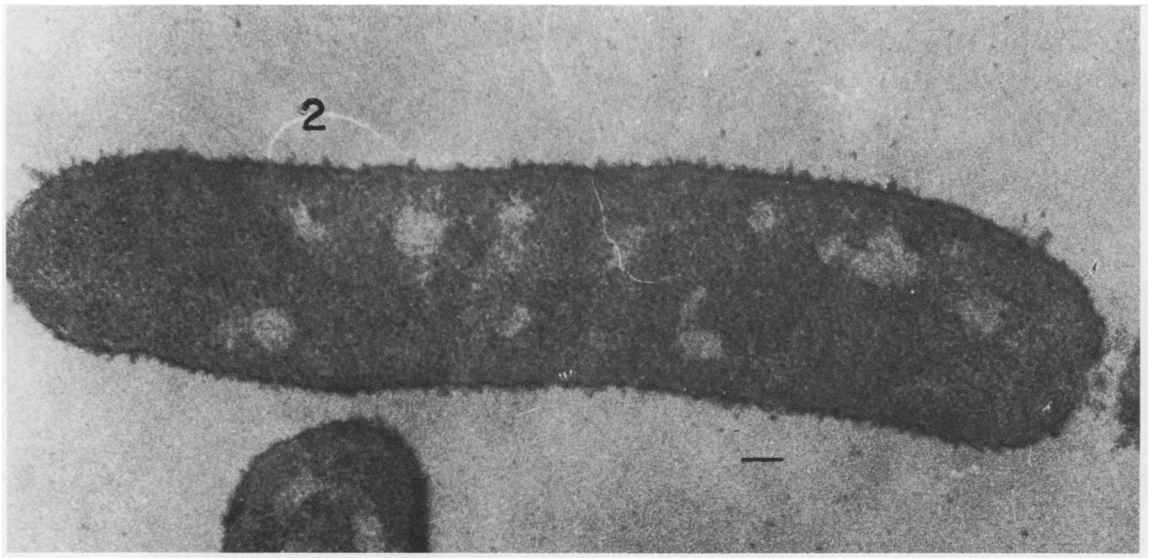


FIG. 1. *Growth of HP1c1 bacteriophage in H. influenzae BC200. (a) Premature lysis. At the times indicated on the abscissa, portions were removed and immediately blown into dilution buffer saturated with chloroform. After proper dilutions were made, these samples were plated for plaque-forming units on nonlysogenic BC200 cultures, as described elsewhere (1). (b) Spontaneous lysis. Samples were removed from the same culture at the times indicated and rapidly diluted and plated on nonlysogenic BC200. The time represents minutes after UV treatment. The burst sizes vary for undetermined reasons from 20, seen in this experiment, to 50.*

FIG. 2-4. *Thin sections of H. influenzae BC200 60 min after UV treatment. Fig. 2 represents a typical control, nonlysogenic cell. Figures 3 and 4 represent cells lysogenic for bacteriophage HP1c1 under conditions where the cell is intact and well preserved (Fig. 3) and where cell lysis is extensive (Fig. 4). All markers represent 100 nm. The arrow in Fig. 3 indicates a complete DNA-filled head.*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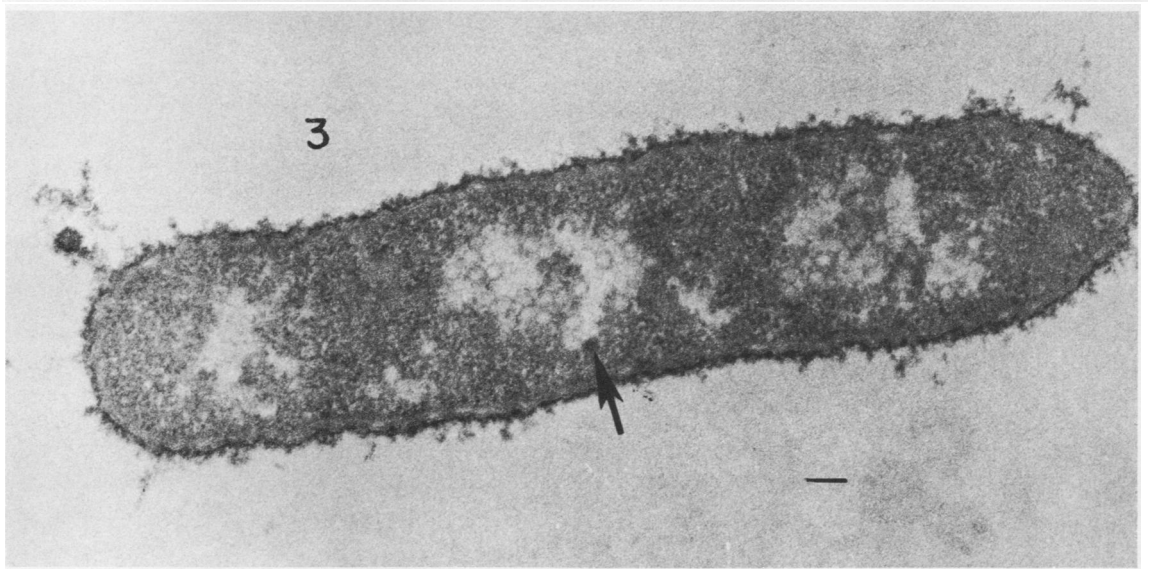
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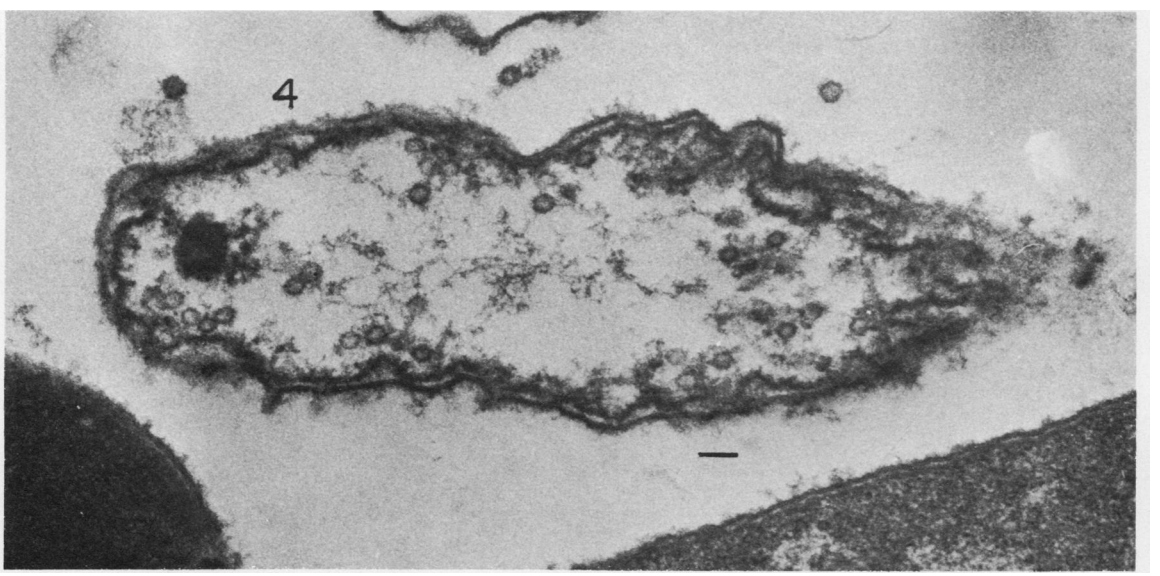
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stained with aqueous uranyl acetate and lead citrate. All samples were examined at 60 to 80 kV by using a 50-μm objective aperture on a Phillips 200 electron microscope.

The intracellular development of HP1c1 phage in *H. influenzae* strain BC200 was initially followed utilizing standard premature lysis techniques (Fig. 1a). This curve indicates that phage production does not reach one per cell until nearly 45 min after UV treatment. Virus particles are produced at a rate of one per min per cell from then on for the next 20 min. The spontaneous lysis curve (Fig. 1b) reveals that the release of phage into the medium begins shortly after the first phage are synthesized.

The accumulation of intracellular phage products was also followed using embedding and thin-sectioning methodology. BC200 cells lysogenic for HP1c1 were fixed at appropriate times after UV induction and later embedded and sectioned. As a control, a nonlysogenic BC200 culture was irradiated, fixed, and embedded at the same times.

Figure 2 illustrates a typical control, nonlysogenic cell at 60 min after UV irradiation. No phage or phage-like products could be seen in 50 sectioned cells of BC200 controls. This observation supports the view that the nonlysogenic strain of BC200 is cured of the defective prophage normally found in Rd strains.

Sections of induced BC200 lysogenic for HP1c1 are shown in Fig. 3 and 4. These micrographs reveal the presence of many phage-like particles which lack the intense, dark-staining appearance of complete phage. Since the evidence presented above shows that the defective prophage is apparently no longer induced in BC200, the presence of "incomplete" phage-like particles in HP1c1-induced cells implies that these particles are either unfinished products of normal maturation or aberrant by-products.

Counts made on the sections of HP1c1-infected cells reveal that the number of complete, intensely staining particles does not reach a level of one per cell section between 45 and 70 min post-UV induction. This may indicate that the final burst of complete phage (about 20 in the experiment shown) is too low to give sufficient quantities of phage in the small volumes represented in the cell sections. Alternatively, it is suggested that phage release and cell lysis may be occurring too rapidly and causes the loss (during preparation for electron microscopy) of cells which have higher burst sizes. This is implied in the growth data which show that cell lysis begins approximately 10 min before phage production is complete (i.e., these data suggest that the lysis process in HP1c1-infected cells occurs too early and may result in the production of "unfinished" products).

The production of "unfilled" heads during the course of "normal" bacteriophage development has been discussed in other systems (5–7, 10). The true relationship between these particles and complete phage is not yet clear.

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