Interference Effect of Non-infectious Particles in Infection with Phage f₂

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ABSTRACT

The titre of infectious phage particles in phage lysates stored at +4° C gradually fell. The inactivated particles retained their capacity for adsorption to male cell receptors, however, competing for the latter with infectious particles and thus protecting the cells from infection. The upper limit of the infected cell fraction in a F+ population fell abruptly with aging of the lysate even when the input of p.f.u. was kept constant. F₂ particles inactivated by u.v. radiation behaved similarly to particles inactivated spontaneously during storage of the lysate at +4° C.

In infection of F+ and Hfr Escherichia coli cultures with sex-specific RNA phages, part of the bacterial population, as a rule, survived, even when a high input was used (Cooper & Zinder, 1962; Dettori, Macca
caro & Piccinin, 1961; Paranchych & Graham, 1962). Most of the surviving cells gave colonies which were again sensitive to phage. Some cells may have escaped infection because they were in a state of F⁻ phenocopy at the moment of infection. Some surviving cells contained phage in a sort of carrier state, which did not cause immediate lysis (Hoffmann-Berling & Mazé, 1964; Knolle, 1964; Schindler, personal communication).

MATERIALS AND METHODS

Microorganisms. The strains Escherichia coli K 13, Escherichia coli C and Escherichia coli C, F+ Sm₇ were used. This last strain was prepared by infecting the streptomycin-resistant mutant Escherichia coli C, F⁻ with an F episome in conjugation with the strain K 12 S, F+.

Phage f₂. Lysates were prepared from submersed cultures of Escherichia coli K 13. The cell débris was removed by 20 minutes’ centrifugation at 12,000 r.p.m. and the lysates were stored at +4° C over chloroform. The titre (about 10¹¹ p.f.u./ml.) was stable for several weeks. Anti-f₂ serum was prepared by intravenous immunization of rabbits. K₃₇ = 90.

Medium. 0.5% Bacto Tryptose Difco, 0.5% NaCl, 0.0025 M CaCl₂; pH 7. Tryptose medium was used for cultivation, for washing the cells and for diluting the bacterial culture, lysate and serum.

Phage was assayed by the agar layer technique (Adams 1959). The bottom agar layer contained 1% peptone, 0.5% Bacto Beef Extract Difco, 1% NaCl, 1.5% agar; pH 7. The surface agar layer (0.5%) contained 0.0025 M CaCl₂ and the indicator bacterium C, F+ Sm₇.

The adsorption of the virus was measured in terms of non-adsorbed phage remaining in supernatant of the phage-bacterium mixture after 20 minutes spinning at 6,000 r.p.m. Before spinning, the adsorption mixture was diluted 100-fold in tryptose medium containing 50 μg. chloramphenicol/ml. Infected cells were determined as infective centres after neutralization of free phage by five minutes’ incubation at 37° C in serum diluted in the ratio 1 : 50. The level of free phage not neutralized by antiserum was determined after killing the infected cells with chloroform and was
always less than 1% of all the infective centres resistant to the action of f₂ — antiserum.

Premature lysis. A sample of an infected culture was shaken with a 0.1 volume of chloroform and was incubated one hour with one volume of lytic mixture at 37°C. Lytic mixture: Tris-HCl 0.05 M, EDTA (disodium salt) 0.01 M, lysozyme 100 µg./ml.; pH 8.

U.v. inactivation of f₂ lysate: Five ml. lysate were irradiated 15 minutes on a Petri dish 10 cm. in diameter with a Phillips TUV 30 germicidal lamp from a distance of 24 cm. The lysate titre fell from 10¹¹ to 10⁴ p.f.u./ml.

The bacterial culture was used in the logarithmic phase. It was grown in aerated tubes until it reached a density of 2 × 10⁸ cells/ml. All the experiments were carried out at 37°C.

RESULTS

All the experiments were carried out with Escherichia coli C, F⁺ Smr; which adsorbed f₂ phage efficiently (about 80% in 10 min. with an input of 1) and reproduced it with an eclipse of 27 min. and a latent period of 35 min. (Fig. 1.)

With a low input (0.1) almost every infectious f₂ particle infected a cell; on raising the input, the increment in the infected cell fraction was by no means proportionate and soon reached a definite limit value, which was not exceeded by raising the input further. The uninfected portion of the population survived and the colonies which grew from these cells were usually again phage-sensitive. The same situation was observed in experi-

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**Fig. 1.** Single step growth curve of the phage f₂ in cells of E. coli C, F⁺ Smr. A logarithmic culture (2 × 10⁸ cells/ml.) was infected with an input of 0.05. After 5 minutes' adsorption, non-adsorbed phage was neutralized with antiserum, which was diluted after another 5 minutes by diluting the infected culture to 2 × 10⁸ cells/ml. The number of infective centres was determined by direct plating (1) and extracellular phage was determined after shaking the samples with chloroform. The samples were incubated with chloroform for 60 minutes at 37°C before plating (2). Mature intracellular phage was determined after premature lysis (3).

**Fig. 2.** Relationship of size of infected cells fraction to input in fresh and old lysate. A. Fresh lysate. Adsorption (60 min.) and neutralization performed in presence of chloramphenicol (50 µg./ml.). Bacterial culture 10⁶ cells/ml., B. Fresh lysate. Adsorption 5 min. Bacterial culture 2 × 10⁹ cells/ml., C. Old lysate, whose p.f.u. titre had fallen over a period of several months to 1/5 the original titre. The noninfectious particle titre is taken as being equal to the difference between the original and actual p.f.u. titre. Other details as for B.