

of subculture. These cells, when used as host bacteria in a plating assay, do not respond to phage infection by forming plaques. However, if they are incubated in phage antiserum and washed free of released phage and then plated as single cells, many of the colonies developing from these single cells can give rise to phage-free cultures which are once more sensitive to phage infection in the plaque assay.

When cultures were infected at high multiplicities (from 10–2000) for 10 min and then the unadsorbed phage inactivated by phage antiserum, all the cells gave rise to infective centres which registered as plaques on indicator plates, yet 50–80% of the infected cells could be shown to survive and give rise to colonies. This paradoxical result may indicate strong interference coupled with a variable initiation of phage multiplication, such that many of the infected cells are only singly infected and many of these divide on the plate before phage infection is initiated. Thus while one of the infected cell's progeny lyses and initiates plaque formation on the lawn of sensitive indicator bacteria, the other progeny cell must, in some way, be resistant to further phage infection and thus be able to give rise to colonies in the presence of phage. This observation and the previously mentioned reversible phage insensitivity may be different aspects of some unusual phage–host cell interaction phenomenon. Without implying any mechanism it has been called the "carrier" state, for it resembles a phage–host interaction of that name observed for phage T7 on *Shigella dysenteriae* (Li, Burksdale, and Garmise 1961).

(iii) *Characterization of Purified f_{Can1}*.—The ultraviolet absorption spectrum of the purified phage in 0.01M phosphate buffer, pH 7.4, showed an absorption maximum at 259 m μ and a minimum at 239 m μ . The 260–280 m μ absorbance ratio was 1.85. Based on the extinction coefficients of enolase and nucleic acid (Warburg and Christian 1942) this ratio indicates that phage f_{Can1} contains approximately 34% nucleic acid.

(iv) *Sedimentation Velocity*.—A suspension of purified phage (A₂₆₀ = 1.0) sedimented as a single boundary in the 0.01M Tris–HCl buffer at 20°C with a sedimentation coefficient of 79.5 S.

(v) *Buoyant Density in Caesium Chloride*.—The phage buoyant density was calculated from the radial position of the phage band at equilibrium, recorded on ultraviolet photographs taken at 44,770 r.p.m.; the following equation (modified from Sueoka 1961) was used:

$$\rho = \rho_0 + \frac{1}{2}K \omega^2 r^2 - \frac{1}{2}(r_m^2 + r_b^2),$$

where ρ is the buoyant density of the phage, ρ_0 is the density of the solution, r is the radial position of the band, r_m is the radial position of the meniscus, and r_b the radius of the bottom of the sector cell. K is a concentration-dependent coefficient equal to $d(I\omega^2 r)/dr$, and has the value of 7.35×10^{-10} at 39% (w/w) caesium chloride (Trautman 1960). The buoyant density of the phage f_{Can1} is 1.42 g/cm³.

(vi) *Characterization of f_{Can1} Nucleic Acid*.—The ultraviolet spectrum of the nucleic acid extracted from the phage in 0.01M phosphate buffer, pH 7.4, containing 0.002M MgSO₄, gave a maximum at 258 m μ and a minimum at 231 m μ . The 260–280 m μ absorbance ratio was 2.23 and the 260–230 m μ ratio was 1.84. On the addition of 0.1 ml deoxyribonuclease (1 mg/ml) to 3 ml of the phage nucleic acid in a 1-cm cuvette, the A₂₅₈ increased from 0.735 to 0.740 after incubation for 20 min at room

temperature. With the addition of 0.05 ml ribonuclease (1 mg/ml) the A258 increased to 0.926 in 20 min, an optical density increment of 25.3%. Thus the nucleic acid of phage f_{Can1} is RNA. The Dische diphenylamine test was performed on a 1-ml aliquot of purified phage (A260 = 2.0). No DNA was detected. The quantity of RNA in a purified phage suspension of A260 = 21.4 was assayed by the method outlined by Webb (1956). This suspension contained an equivalent of 2.09×10^{-3} M RNA phosphorus (assuming a purine : pyrimidine ratio of 1). Thus the atomic extinction for RNA phosphorus in intact phage is 10,200.

(vii) *Neutralization of Phages R17 and M2 by f_{Can1} Antiserum.*—Both M2 phage (obtained from Professor G. A. Maccacaro) and R17 phage (obtained from Dr. A. Graham) are neutralized to a similar extent as f_{Can1} by f_{Can1} antiserum. Precise neutralization studies to detect antigenic differences among these phage strains have not been made.

IV. DISCUSSION AND CONCLUSIONS

The inhibition of DNA synthesis with 5-fluorodeoxyuridine in cultures of *E. coli* K-12 Hfr strains inoculated with sewage samples proved to be a good method for the isolation of an RNA phage. The RNA phage characterized here, f_{Can1} , is very similar to those RNA phages already reported. This similarity is further confirmed by the fact that both phage R17 (Paranchych and Graham) 1962 and phage u_2 (Dettori *et al.* 1961) are strongly inactivated by rabbit antiserum prepared against f_{Can1} .

The maintenance of the "carrier" state appears to depend on continuous infection of sensitive cells by phage released into the culture medium by lysis of a small proportion of the phage-infected cells. If this is so, then the phage-free progeny cells of those infected cells in which phage multiplication is delayed must gradually lose their resistance to infection and thereby constantly renew the pool of phage-infectable (sensitive) cells.

The analysis and characterization of the "carrier" state must go much further before the reality of the interpretation suggested here can be assessed.

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