ASSAY OF INFECTIOUS RNA FROM BACTERIOPHAGE R 17*

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Bacterial protoplasts have been used to demonstrate the infectious activity of a variety of bacteriophage nucleic acids. Spizizen (1957) and Fraser, Mahler, Shug and Thomas (1957) demonstrated that protoplasts could be infected by subviral particles of the coliphage T2. The single-stranded DNA of bacteriophage ØX174 was shown to be infectious by Guthrie and Sinsheimer (1960) and Sekiguchi, Taketo and Takagi (1960). More recently, Fouace and Huppert (1962) and Knolle and Kaudewitz (1962) have reported that protoplasts can be infected by the RNA of the RNA bacteriophages f2, FH_4, FH_5 and ft5.

The RNA of bacteriophage R17, which was isolated in this laboratory (Paranchych and Graham, 1962), also has been found to be infectious to bacterial protoplasts. However, under conditions similar to those described by Fouace *et al.* (1962), the infectivity yield was found to be very low (one infectious RNA unit per 10^8 phage). An investigation was thus carried out to find more favorable conditions for estimating infectious RNA. This communication describes an assay system in which the infectivity yield has been increased to approximately one infectious RNA unit per 10^5 phage.

Extraction of infectious RNA.

The RNA extraction procedure used was similar to that described by Wecker, Hummeler and Goetz (1962). The following mixture was added to a one ml. sample either of purified phage or of phage-infected *E. coli* K12: 0.3 ml. of 0.2 M

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phosphate buffer, pH 7.2; 0.7 ml. of 3 mM ethylenediaminetetraacetate (EDTA), pH 7.2; 0.5 ml. of 5% sodium dodecyl sulfate (SDS). The sample was mixed for one minute at room temperature, then shaken vigorously for eight minutes at 4°C. with 2.5 ml. of redistilled 80% phenol. After centrifuging at 1500 g for 5 minutes, the aqueous phase was removed and extracted a second time with 2.5 ml. of 80% phenol. Following the second extraction, one ml. of the aqueous phase was mixed with 3 ml. of 95% ethanol and placed at -20°C. to allow the phosphate salts and RNA to co-precipitate. The precipitate was then sedimented and redissolved in one ml. of 3 mM EDTA. Subsequent dilutions of the RNA solution were also made in 3 mM EDTA.

Protoplasts.

"Protoplast stocks" were prepared as described by Guthrie and Sinsheimer (1960) with the modification that the final dilution of protoplasts into nutrient broth to reduce the concentration of bovine serum albumin (BSA) was tenfold rather than fourfold, and the nutrient broth contained 25 µg./ml. of protamine sulfate (Smull and Ludwig, 1962). As shown in Table I, the addition of 25 µg./ml. of protamine sulfate to the protoplast stock caused a marked increase in the plaque-forming activity of the phage RNA.

Table I

Effect of protamine sulfate on plaque-forming activity of phage RNA

µg./ml. of protamine sulfate in protoplast stock	plaque-yields
0	24
25	210
50	150
75	108
100	20

Method of assaying infectious RNA.

Protoplasts were usually prepared from the R17 host strain, K12 Hfr_1, although K12 F^- protoplasts also could be used with equal efficiency.

Serial tenfold dilutions of the infectious RNA were made in 3 mM EDTA, and the diluted samples were then incubated for 20 minutes at 37°C. This incubation of the phage RNA at 37°C prior to protoplast infection caused an approximate tenfold increase in the plaque-forming capacity of the infectious RNA (Table II). It is probable that the increased infectivity was due to disaggregation of RNA molecules during the incubation period. Upon completion of the RNA incubation, 0.1 ml. of each RNA sample was added to 1.0 ml. of protoplast stock. The mixture was incubated for 10 minutes at 37°C., seeded with 0.3 ml. of an exponentially growing culture of Hfr_1 (1×10^8 bacteria per ml.) and plated on nutrient agar plates by the usual soft agar layer procedure (Adams, 1950). The final concentration of BSA in the soft agar layer during incubation of the protoplasts on the agar plates was approximately 0.1%. It should be noted that dilutions of the infectious RNA were made before, rather than after, incubation with protoplasts.

Table II

Plaque yields obtained from phage RNA incubated at 37°C. for various time intervals prior to protoplast infection.

Minutes of incubation at 37°C.	Plaque yields
0	36
5	100
10	140
20	290
40	270
60	130

Relationship between concentration of infectious RNA and plaque yield.

To test the linearity of the assay system, successive twofold dilutions of a phage RNA preparation were assayed for infectivity as described in the previous section. It may be seen in Fig. 1 that when the number of infectious centers formed was between 10 and 1000 per plate, a linear relationship

(plotted on $\log_{10}$-$\log_2$ coordinates) was observed between plaque yield and the concentration of infectious RNA.

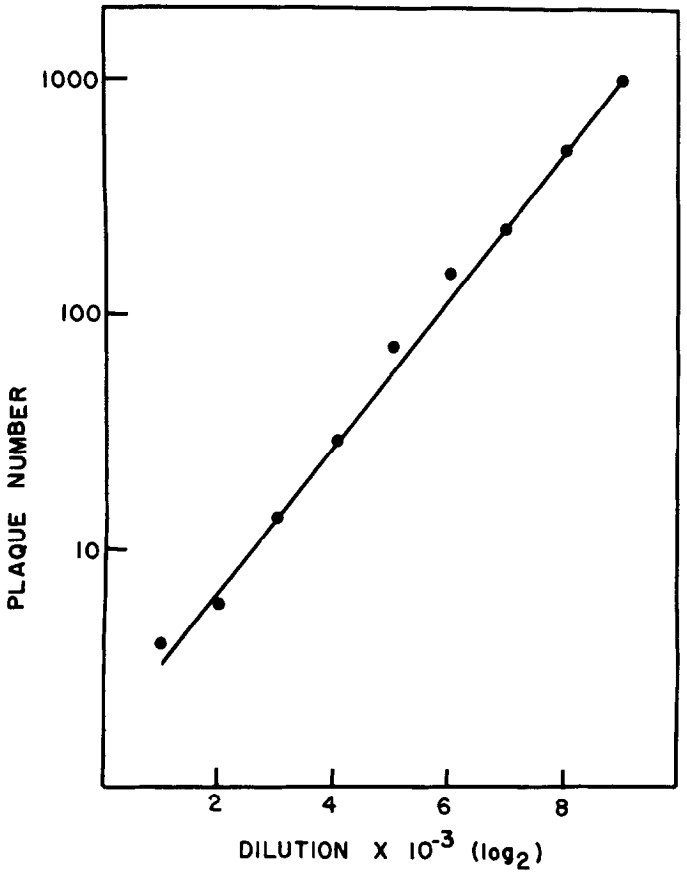

Figure 1.

Relationship between concentration of infectious RNA and plaque yield. Conditions of the experiment are described in the text.

Growth curves.

To compare the formation of infectious RNA with the maturation of phage in R17-infected Hfr_1, the following experiment was performed. Bacteria at a density of 1×10^9 cells per ml. in Tris-maleate medium (Paranchych and Graham, 1962) were infected with phage R17 (multiplicity = 0.1) and incubated for two minutes to allow for the adsorption of phage. The culture was then diluted fivefold and incubated for 70 minutes at 37°C. At various intervals,

one ml. samples were removed, extracted with phenol-SDS, and assayed for infectious RNA as described earlier in this paper. Other samples, removed simultaneously, were lysed by sonic oscillation and assayed for phage as described elsewhere (Paranchych and Graham, 1962). The resulting growth curves are shown in Fig. 2. It can be seen that both phage and infectious RNA increased at identical rates, although the appearance of infectious RNA preceded that of mature phage by about 10 minutes. At 70 minutes after infection, when phage maturation is almost complete under conditions of single infection (Paranchych and Graham, 1962), the phage concentration was 3×10^{10}/ml., while the titer obtained for infectious RNA was 5.8×10^{5}/ml. Thus, an infectivity yield of the order of 1-2 infectious RNA units per 10^{5} phage was obtained. Yields of this order were also obtained with RNA extracts from partially purified phage preparations.

Although the infectivity of RNA preparations is still extremely low as compared to that of mature phage, it should now be possible to successfully apply infectious RNA procedures to the study of a number of problems relating to phage replication.

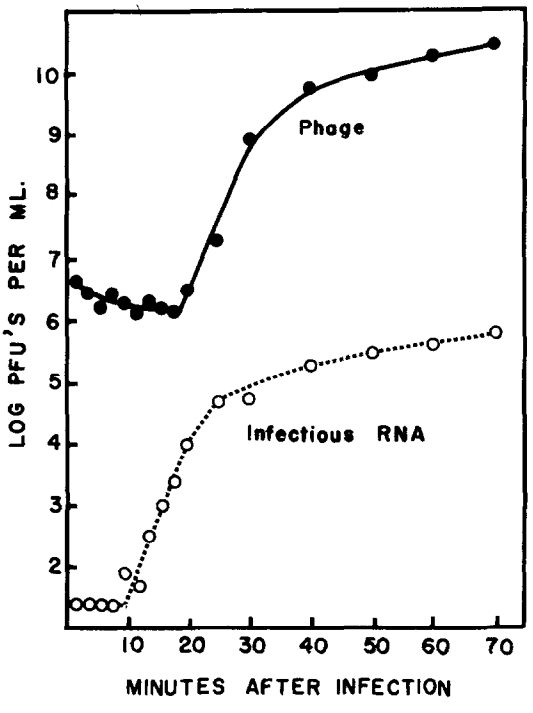

Figure 2.

Growth of infectious RNA and mature phage in R17-infected Hfr$_1$. Conditions of the experiment are described in the text.

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