THE PREPARATION OF COLIPHAGE MS 2 CONTAINING 5-FLUOROURACIL

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The uracil analog 5-fluorouracil (FU) is incorporated into RNA in place of uracil (Horowitz and Chargaff, 1959) and appears to induce base-pairing errors (Naono and Gros, 1960; Champe and Benzer, 1962). In the course of our studies on the effect of FU on protein synthesis we have prepared coliphage MS 2 containing FU (FU phage). In this report we present methods of preparing and isolating FU phage and some interesting properties of these viruses.

Materials and Methods

Escherichia coli C3000, obtained from R. L. Sinsheimer, was grown at 37°C in a medium of the following composition per liter: KH_2PO_4 1.33 gm, K_2HPO_4 13.3 gm, NH_4Cl 2.0 gm, $Fe\,So_4 \cdot 7H_2O$ 3.0 mg, $Mg\,SO_4 \cdot 7H_2O$ 0.40 gm, monosodium glutamate 6.7 gm, glucose 10 gm and thiamine 10 mg. When the cells were infected with MS 2, $CaCl_2$ was added at a final concentration of 0.002 M (Cooper and Zinder, 1963). Exponentially growing cells were infected with 10 plaque-forming units (pfu) per cell and FU (a gift of Dr. R. Duschinsky) was added at various times as indicated in the legends of the figures. Tritiated FU (Nuclear Chicago Corp., Des Plaines, Ill.) was present also in certain experiments, as noted in the legends. Near the end of the virus growth cycle chloroform and lysozyme were added and the phage assayed (Cooper and Zinder, 1963). The phage was purified by the procedure of Loeb and Zinder (1961) with slight modification. For radioactivity measurements yeast RNA was added as carrier and the washed RNA precipitate was counted in a scintillation spectrometer with hyamine and a toluene-based scintillator.

Results and discussion

In the first experiment to be reported the effect on virus yield of FU added at various times during the phage reproductive cycle was determined. When FU at a concentration of 30 µg/ml was present from time 0, 5 or 10 min. after infection no increase in infectious virus was found (figure 1), confirming the results of Cooper and Zinder (1963). However, when FU was added at 15, 20 or 25 min. after infection, active virus particles were formed, though with a reduced yield (figure 1).

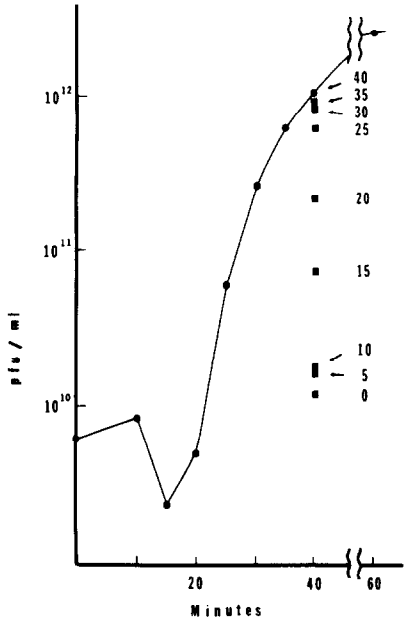

Figure 1. The effect of FU added at various times after infection on the yield of infectious phage. At zero time 1.6×10^{11} pfu of MS 2 was added to 20 ml of culture containing 8×10^{8} bacteria per ml. At the times indicated at the right, aliquots of the culture were transferred to bubbler tubes with sufficient FU to give a final concentration of 30 µg/ml. Phage was assayed in the untreated culture at various times (●) and at 40 minutes in the tubes containing FU (■).

The next experiment shows that FU added at 10 minutes after infection does not enter progeny phage whereas FU added at 15, 20 or 25 minutes does (figure 2). The yield of radioactive FU phage after addition of the analog at each of the latter times was approximately the same when corrected for

the different recoveries of carrier phage: 15 min., 42.3 cpm/O.D. unit; 20 min., 50.2; 25 min., 46.2. (The 10 minute result was < 0.7 cpm/O.D. unit and the result with doubled FU concentration at 25 min. was 72.5.) Our present interpretation of these data is that after addition of 30 μg/ml of FU 15 minutes or more following infection, phage particles continue to be made but the rise in virus titer is due largely to the incorporation into phage of the RNA made prior to FU addition (see results of FU phage infectivity below). When added at or before 10 minutes, a time which corresponds to the onset of phage RNA synthesis in a related coliphage system (Homma et al., 1963), the analog blocks the formation of phage particles. The mechanism of this effect is being investigated.

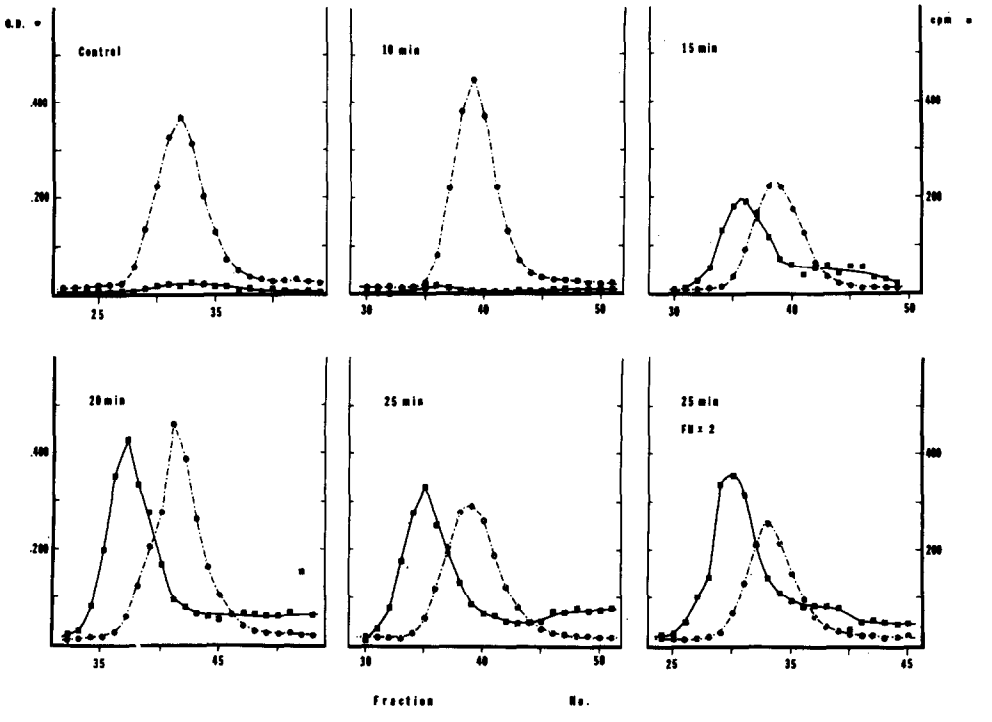

Figure 2. Density gradient centrifugation of MS 2 grown in the presence of H^3-FU. Conditions were similar to those given in the legend of figure 1, except that H^3-FU was used (2.4×10^6 cpm/μ Mole). To 5 ml of each lysed culture 50 O.D. units of purified carrier phage and excess unlabelled FU were added and the phage re-isolated in the usual way. One drop fractions of the CsCl run were collected, and the O.D. at 260 mμ and radioactivity determined. O.D. was measured at a dilution of 1:500. In the control culture H^3-FU was added immediately after lysis.

In the experiment reported in figure 2 it will be noted that FU phage bands in CsCl at a higher density than normal phage. This density difference allows the separation of FU phage from normal so that its properties can be studied. In figure 3 are the results of an experiment in which phage grown in the presence of tritiated FU was banded in CsCl in the absence of added normal phage, and the optical density, infectivity,

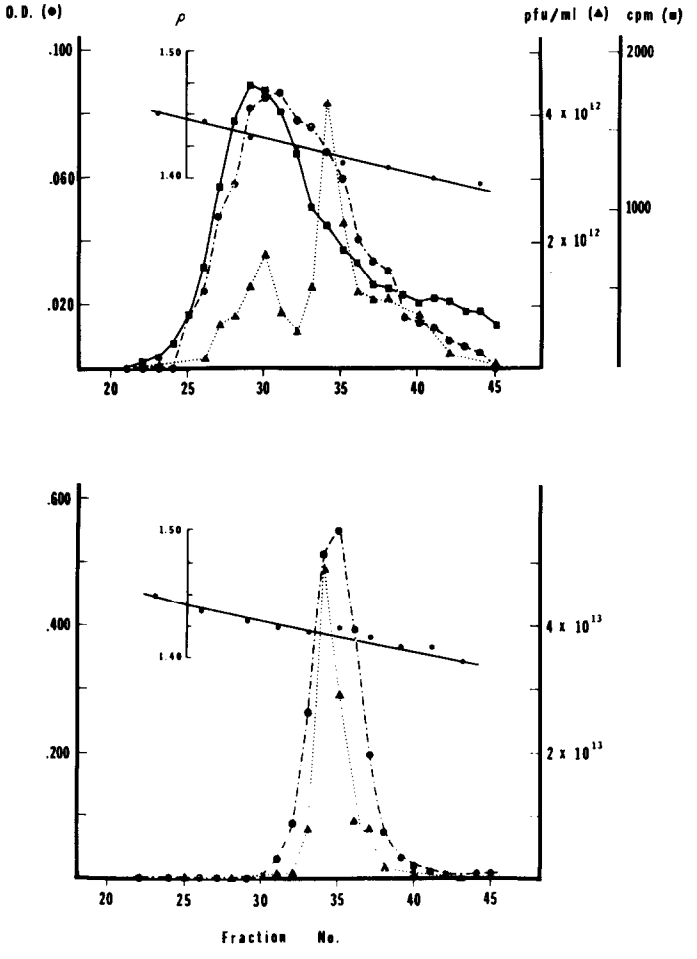

Figure 3. Density gradient centrifugation of FU phage to determine its specific infectivity. Conditions were similar to those given in the legends of figures 1 and 2 except that 25 ml of culture was used, no carrier phage was added, and the specific activity of H^3-FU was 4.7×10^6 cpm/ μ Mole. FU was added at 20 minutes after infection (upper figure). In the lower figure is the result with an untreated culture processed in parallel. O.D. was measured at a dilution of 1:22, and a background of .020 was subtracted. Density was determined by weighing 25 μL of a given fraction in a micropipette at 23°.

and radioactivity of the fractions were compared. As noted in the upper figure there is a broad O.D. peak with a predominance of virus having the density of FU phage. In the experiment presented the density of normal phage was 1.420 and that of FU phage, 1.435. The specific infectivity of the FU phage, determined on the four densest fractions, was less than one-third that of normal phage prepared in parallel (6.8×10^{11} vs. 2.3×10^{12} pfu/O.D. unit). By means of analytical data of Strauss and Sinsheimer (1963), the extent of replacement of uracil by FU in the same fraction of FU phage was estimated as about 20 per cent on the basis of its radioactivity per O.D. unit. Assuming that the loss in infectivity during isolation is the same in normal and FU phage, we conclude that this degree of substitution of uracil by FU in the phage RNA leads to a high percentage of inactive particles. More detailed studies of the physical and biological properties of FU phage are now being carried out.

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