rapid exchangeability of apparently incorporated FPA could therefore mean that the proteins containing FPA instead of PA are readily turned over after PA is added; or it could mean that FPA was never really incorporated in the manner of a normal amino acid.

Some later experiments have suggested that the latter is the case. This provides a likely explanation for the competitive inhibition of amino acid incorporation produced by FPA; that is, the inhibition of protein synthesis. According to the suggested very close relationship between protein synthesis and RNA synthesis, it would then explain how an amino acid analog may interfere directly with the synthesis of a specific viral RNA, having shown it unlikely that new enzymes are necessary for the production of WEE RNA in infected cells. Such a direct mode of action of FPA seems in fact to be indicated by our experiments.

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**A BACTERIOPHAGE CONTAINING RNA**

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Although viruses may in general contain either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), there have been no previous reports of a bacteriophage containing RNA. The single-stranded DNA of phage φX-174 is the most similar in structure to RNA. This report describes a bacteriophage which contains RNA but no DNA as its nucleic acid.

Loeb reported the isolation of a number of phages which would grow only on *E. coli* K-12 donor strains (Hfr and F+, males). The inability of the phage to grow on female bacteria is due to its failure to attach to them. By serological criteria, these phage strains fell into three groups, of which there was a single
representative of the first group (f1) and several of the second group (f2). The third group cross-reacts with f2 and might be deemed a subgroup of f2. For a variety of technical reasons, f2 was chosen for detailed study.

Materials and Methods.—Although f2 grows on all tested E. coli K-12 males, its plaques were found to be clearest and to give the highest efficiency of plating on an Hfr strain (which transfers methionine as its first marker) provided by Dr. A. Garen. This strain was used for all of the experiments to be described.

The medium used for growth of bacteria and phage contained in grams per liter: Bacto-tryptone 10, yeast extract 1, glucose 1, and NaCl 8. To this basal medium was added M/500 CaCl₂. In the absence of added calcium, the phage infections are abortive, although the calcium is not needed for attachment. Plaques were obtained by the agar-layer method by the appropriate addition of agar to the medium described above.

One step of the phage purification utilized the technique of gradient centrifugation as described by Meselson et al. DNA was measured by the diphenylamine reaction and the modified diphenylamine reaction as described by Burton. The assay was standardized against both deoxyadenosine and a sample of calf thymus nucleic acid supplied by Dr. Muriel Roger.

RNA was measured with the orcinol reaction as standardized against ribose. It was assumed that only purine bound ribose would be detected and that the purine-to-pyrimidine ratio in the samples was unity.

Phage nucleic acid was prepared as described by Gierer and Schramm. Hydrolysis of phage nucleic acid and chromatography of the products were done according to the methods of Smith and Markham. The sugar present was identified as described by Partridge.

The synthesis of nucleic acid following infection was measured by the Schmidt-Tannhauser procedure as modified by Hershey.

Experimental.—The single cycle growth of f2: Bacteria at a density of 2 × 10⁸ cells per ml were infected with f2. A portion of the culture was assayed for free phage as a function of time, while another portion was lysed by the addition of cyanide to stop growth and chloroform and lysozyme to break open the bacteria. Figure 1 shows intra- and extracellular growth curves of f2 when the bacteria were infected with less than one phage particle per bacterium and diluted to prevent loss of phage by resorption. Figure 1 also shows the intracellular growth of a multiply infected culture maintained at the original density. The most pertinent point to note is the large yield per bacterium amounting to 2,000 to 4,000 in the dilute culture and to better than 9,000 in the dense culture. This latter figure has been as high as 20,000 plaque-forming units (P.F.U.) in some similar experiments, which indicates that the bacteria are probably lysis-inhibited in dense culture.

An electron micrograph of f2: Figure 2 shows an electron micrograph of f2. The phage appears to have about the same size as φX-174. It is not unlikely that the two phages have similar amounts of nucleic acid per particle, about 3 × 10⁻¹² µg (see further evidence below).

The synthesis of nucleic acid following infection: Using large volumes of culture, it was possible to measure the synthesis of nucleic acid following infection. At appropriate time intervals, 10 ml samples were precipitated with trichloracetic acid
(TCA) and subjected to the Schmidt-Tannhauser (S-T) procedure as modified by Hershey to fractionate the nucleic acids. Figure 3 shows the results of one such experiment. We may note that there is a net synthesis of DNA and RNA amounting in both instances to about 2.5 times that originally present. In this experiment, the number of P.F.U. synthesized per bacterium was 12,000. Using our previous

![Graph](image)

**Fig. 1.—The intra- and extracellular growth of f2.**

figure of $3 \times 10^{-12}$ μg of nucleic acid per phage and the figure $6.0 \times 10^{-8}$ μg of DNA present per bacterium, it is apparent that only if almost all of the DNA were in f2 particles could it account for the number of P.F.U.

*An experiment demonstrating the nucleic acid associated with f2:* To ascertain which type of nucleic acid was associated with f2, the following experiment was
Fig. 2.—An electron micrograph of \( \phi 2 \). The phage were negatively stained by embedding in neutral phosphotungstate. This micrograph was kindly taken by Dr. W. Stoeckenius.

![Electron micrograph](image)

Fig. 3.—The synthesis of the nucleic acids following \( \phi 2 \) infection. See text for details.

![Graph](image)
done. A culture of $1.4 \times 10^8$ bacteria per ml was multiply infected and allowed to lyse (chloroform and lysozyme were added to complete lysis). An uninfected culture of equivalent volume with $3.4 \times 10^8$ bacteria per ml was lysed with chloroform and lysozyme. Aliquots of each culture were immediately precipitated with TCA and fractionated by the S-T procedure. Deoxyribonuclease (DNAase) and ribonuclease (RNAase) were added to the rest of both cultures at a concentration of 10 μg per ml each, and the cultures were incubated for one hour. This treatment has no effect on the number of P.F.U. that are present other than occasionally raising it from its initial value. In this instance, there were $1.2 \times 10^{12}$ P.F.U. per ml. At the end of this period, another aliquot was subjected to S-T fractionation. Further aliquots were centrifuged in the Spinco centrifuge at 26,000 g, a force insufficient to sediment any phage. The pellets were dissolved in dilute ammonia and analyzed for pentose and deoxypentose. The supernatants were centrifuged at 60,000 g for one hour. This sedimented not quite half of the phage. The final supernatants were precipitated with TCA. These precipitates and pellets were analyzed for nucleic acid as above. The data are presented in the flow diagrams (Figs. 4 and 5) and are calculated as nucleic acid equivalents per ml of the original lysates.

We may note that in both instances the DNA essentially disappears following the enzyme treatment. The limit was set by the amount of culture used for the assay and the sensitivity of the test, which in this instance was of the order of 5 μg total DNA. In the infected culture, about 20 per cent of the RNA survives treat-

![Flow diagram]

**Fig. 4.—The distribution of the nucleic acids synthesized by a phage-infected culture.** See text for details.
ment while only about 3 per cent survives in the uninfected culture, although there were initially almost equivalent amounts of RNA in both cultures. The RNA-like material which is not lost following enzyme treatment of the uninfected culture appears in the 26,000 g pellet as does an equivalent amount in the infected culture. This may represent pentose in the bacterial debris. RNA appears in both the 60,000 g pellet and the TCA precipitate of the 60,000 g supernatant, while there is none in the pellet and precipitate of the uninfected culture. Phage titers are presented for the supernatants, since sedimenting the phage causes a large loss of viability, amounting to about 90 per cent as determined in many experiments. However, as will be described, there is reason to believe that the particles are still intact although they can no longer form a plaque. The figure of $4 \times 10^{-12}$ µg RNA per P.F.U. calculated from the above data is consistent with the previous estimate.

\begin{center}
\begin{tikzpicture}
  \node (a) {Uninfected bacteria ($3.2 \times 10^8$)};
  \node (b) [below of=a, yshift=-1em] {not treated};
  \node (c) [below of=a, yshift=1em] {enzyme treated};
  \node (d) [below of=b, yshift=-1em] {66 µg RNA};
  \node (e) [below of=b, yshift=-2em] {8 µg DNA};
  \node (f) [below of=c, yshift=-1em] {2 µg RNA};
  \node (g) [below of=c, yshift=-2em] {<0.5 µg DNA};
  \node (h) [below of=f, yshift=-1em] {26,000 g pellet};
  \node (i) [below of=g, yshift=-1em] {2.5 µg RNA};
  \node (j) [below of=g, yshift=-2em] {<0.3 µg DNA};
  \node (k) [below of=h, yshift=-1em] {60,000 g supernatant};
  \node (l) [below of=i, yshift=-1em] {<0.15 µg RNA};
  \node (m) [below of=i, yshift=-2em] {<0.3 µg DNA};
  \node (n) [below of=k, yshift=-1em] {<0.15 µg RNA};
  \node (o) [below of=k, yshift=-2em] {<0.3 µg DNA};
  \node (p) [below of=l, yshift=-1em] {<0.3 µg DNA};
  \node (q) [below of=l, yshift=-2em] {<0.3 µg DNA};

  \draw [->] (a) -- (b);
  \draw [->] (a) -- (c);
  \draw [->] (b) -- (d);
  \draw [->] (b) -- (e);
  \draw [->] (c) -- (f);
  \draw [->] (c) -- (g);
  \draw [->] (h) -- (i);
  \draw [->] (i) -- (j);
  \draw [->] (k) -- (l);
  \draw [->] (l) -- (m);
  \draw [->] (n) -- (p);
  \draw [->] (n) -- (q);
\end{tikzpicture}
\end{center}

**Fig. 5.**—The distribution of the nucleic acids from an uninfected culture that was lysed with chloroform and lysozyme. See text for details.

**The isolation of f2 in large quantities:** The following procedures were empirically developed for the isolation of large amounts of phage. A 15-liter culture (approximately $2 \times 10^8$ bacteria per ml) was infected and allowed to go to lysis. Chloroform and lysozyme were added to complete the lysis. The yield was $7 \times 10^{11}$ P.F.U. per ml. The lysate was made 2 molar with ammonium sulfate and the precipitate collected by centrifugation. This precipitate was suspended in 200 ml of water, brought to pH 8 with NaOH, and then stirred in a blender. DNAase and RNAase were added (each at a final concentration of 2 µg per ml). The solution was centrifuged at 13,000 g and then at 26,000 g, giving a supernatant containing a total of $8 \times 10^{18}$ phage. The supernatant was filtered through a filter candle and the phage again precipitated with ammonium sulfate. The major contaminants
to this point of the purification were constituents of the broth medium that had also been precipitated by the ammonium sulfate. There was only a minor loss of phage viability. The precipitate was resuspended in pH 8 phosphate buffer and the solution centrifuged at 13,000 g. The supernatant was centrifuged at 60,000 g for two hours. Although the phage sedimented almost quantitatively (by the analysis described below), there was a marked loss of viable titer, about 90 per cent. The pellet was resuspended in saline, made to a density of 1.4 with CsCl, and centrifuged in the swinging bucket rotor of the Spinco Model L centrifuge at 125,000 g for 20 hours. A heavy band about 2 mm in width was found in the center of the centrifuge tube and this was carefully removed. This fraction contained all of the infective material and showed no further loss of viable titer. The material was diluted in water and then recentrifuged in the Spinco at 60,000 g. A translucent pellet which was easily resuspended was obtained and was again centrifuged at 12,000 g to remove any further debris. These latter procedures resulted in no further loss of viability. The final solution (of 11 ml) had a viable titer of $5 \times 10^{18}$ per ml of a total of $5.5 \times 10^{14}$ P.F.U. This represents a recovery of about five per cent of viable particles.

The type of nucleic acid present in f2: The preparation described above had an optical density at 260 nm of 55 per ml, which corresponds to about 2.8 mg of nucleic acid per ml. The 260 nm to 280 nm ratio was 1.78, and the 230 nm to 260 nm ratio was 1.05. There was found to be 2.5 mg of orcinol reacting material per ml (presumably RNA) and less than 2 $\mu$g of DNA per ml as determined by the modified diphenylamine reaction.

It is apparent that the quantity of orcinol reacting material corresponds well with the quantity of nucleic acid as determined by optical density. On the other hand, the amount of DNA, assuming the limit of the sensitivity of the analysis to be the true value, divided by the actual infectivity would give a value of about $4 \times 10^{-14}$ $\mu$g of nucleic acid per particle of f2. This value would be equivalent to about $2 \times 10^{-4}$ of a coliphage T2 particle ($2 \times 10^{-10}$ $\mu$g per particle$^{12}$) or about 20,000 M.W.U. However, the above assumption of about $3 \times 10^{-12}$ $\mu$g of nucleic acid per f2 particle would indicate that the final solution had an infectivity of less than 0.1. Thus the quantity of DNA per phage would have to be reduced a further factor of 10. The evidence certainly indicates that f2 contains RNA and no significant amount of DNA.

A value of $3 \times 10^{-12}$ $\mu$g of nucleic acid per P.F.U. gives for the original lysate a value of 30 mg of nucleic acid (by viable titer) before purification. The optical density at 260 nm indicates that virtually all of the nucleic acid was recovered after purification and that the loss of viability in the Spinco still leaves the particles intact.

The base and sugar composition of f2 nucleic acid: A sufficient quantity of f2 to provide approximately 1,400 $\mu$g equivalents of nucleic acid determined by optical density was deproteinized by shaking with aqueous phenol.$^9$ The resulting nucleic acid suspension was suspended in 10 $\mu$l of 1 N HCl and heated for one hour at 100°C in a sealed tube.$^{10}$ The hydrolysate and an appropriate control solution were chromatographed on Whatman #41 filter paper using a mixture of butanol and HCl. Four spots were found having the Rf and absorption spectra of guanine, adenine,
cytidylic acid, and uridylic acid. The molar ratios were $1.17 \pm 0.03$, $1.00$, $1.21 \pm 0.06$, and $1 \pm 0.07$, respectively.

The sugar present was determined by chromatographing the above HCl hydrolysate and a control mixture of sugars on Whatman #1 paper using a mixture of butanol, ethanol, and ammonia. The sugar had an Rf characteristic of ribose.

Summary.—The evidence presented indicates that the bacteriophage f2 contains RNA and not DNA as its nucleic acid. The evidence is based primarily on the analysis of purified material but also on the distribution of the two types of nucleic acid synthesized after infection. Although f2 is an extremely small phage, there is a compensating large yield per bacterium (about 10,000 P.F.U.). Therefore, the synthesis of phage materials can be followed and the phage itself readily purified. In its general features such as adsorption and intracellular growth, f2 resembles the DNA bacteriophages. Further studies on the biology and chemistry of f2 are in progress.

We gratefully acknowledge our indebtedness to Dr. M. Jesaitis for advice and aid in the preparation and analysis of large quantities of f2. We also thank Miss Doris Degen, Mr. S. Cooper, and Mr. M. Estrin for technical aid.

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TRANSCARBOXYLASE, II. PURIFICATION AND PROPERTIES OF METHYLMALONYL-OXALOACETIC TRANSCARBOXYLASE*

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Swick and Wood recently have demonstrated a new type of biochemical reaction in which one compound, a carboxyl donor, is decarboxylated and a second compound, a carboxyl acceptor, is carboxylated. Thus, it is possible to accomplish a direct carboxylation without intervention of CO2 or the expenditure of energy to activate the CO2. The conversion is illustrated in reaction (1).