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*EARLY ENZYME SYNTHESIS AND ITS CONTROL IN E. COLI INFECTED WITH SOME AMBER MUTANTS OF BACTERIOPHAGE T4**

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Communicated January 2, 1962

The "amber" (*am*) mutants of bacteriophage T4 are a recently discovered class of mutants that can replicate in certain derivatives of *Escherichia coli* strain K-12 but not in *E. coli* B.[1] The bacterial strains that support growth of the *am* mutants, thus making their isolation and propagation possible, are called the "permissive" hosts. The biochemical basis of this permissiveness has not yet been clarified. With some of the *am* mutants, infection of *E. coli* B leads to a subnormal production of phage DNA; with others, DNA is formed but infection is abortive because of failure of late stages of phage development.[2]

It is known that infection of *E. coli* with a T-even bacteriophage leads to the appearance of several new enzyme activities and to an increase in several other enzyme activities, all related to synthesis of phage DNA ("early enzymes").[3–12] These

changes are detectable 3 to 5 minutes after infection, and the enzyme activities increase until 10 to 15 minutes; then the increase stops. If, however, the infecting phage has been treated with doses of UV light that prevent phage production and synthesis of phage DNA,[3, 13, 14] then the early enzyme synthesis continues for a longer period.[15-18]

The *am* mutants of T4 provide a tool for the study of the genetic control and regulation of the synthesis of the early enzymes. Specifically, it may be possible, on the one hand, to refer specific abnormalities in phage DNA synthesis to specific blocks in the synthesis of early enzymes and, on the other hand, to correlate certain stages in phage development with changes in the kinetics of accumulation of the early enzymes. The present paper reports results with seven of the *am* mutants. For one of these, the failure to induce phage DNA synthesis is explained by specific biochemical blocks; remarkably, two of the early enzyme activities, out of seven tested, are lost. In addition, a comparison of phage mutants reveals a consistent pattern of quantitative relationship between DNA synthesis and the cessation of the increase in the level of the early enzyme activities.

Materials and Methods.‡—The *am* mutants used in this work were derived from phage T4D by treatment with HNO_2, 0.5 *M*, pH 5.0, at 21°C. Stocks of the *am* mutants were grown in the permissive host strain *E. coli* CR63 by standard techniques.[19] T4D, used as the normal control, is a spontaneous revertant from *am* 82 and will be referred to as am^+; stocks of it were also grown in *E. coli* CR63. The phage were purified as follows: bacterial debris was removed by centrifugation for 30 min at 5,000 × *g*, and phage was collected from the supernatant suspension by centrifugation at 0° for one hr at 20,000 × *g*. The pellets were resuspended by covering them with several ml of a buffer solution (containing, per liter, 100 μmoles of sodium phosphate, pH 7.5, 100 μmoles $MgCl_2$, and 10 mg of gelatin); after storage in the cold for several days, homogeneous suspensions were obtained by brief agitation. The medium for phage assay contained, per liter: Bacto agar, 10 gm; Bacto tryptone, 13 gm.; NaCl, 8 gm; sodium citrate, 2 gm; and glucose (autoclaved separately), 1.3 gm. Top agar for phage assay contained, per liter: Bacto agar, 6 gm; Bacto tryptone, 10 gm; NaCl, 8 gm; sodium citrate, 2 gm; and glucose (autoclaved separately), 3 gm.

dCMP, dCTP, and dTMP were purchased from the California Corporation for Biochemical Research. dUMP, ATP, and UDP-glucose were purchased from the Sigma Chemical Company. C^{14}-labeled UDP-glucose was prepared and generously provided by P. W. Robbins. C^{14}-labeled formaldehyde was purchased from New England Nuclear Corporation. The T2-DNA used in the HMC-β glucosyl transferase assay was the gift of P. F. Davison. Tetrahydrofolic acid was prepared by the method of Blakley.[20] The molarity of all Tris buffers refers to the total Tris concentration; that of all ammonium formate buffers refers to the total formic acid-formate concentration.

Preparation of C^{14}-labeled dHMP and unlabeled dHMP: C^{14}-labeled dHMP was prepared enzymatically by a modification of the method of Flaks and Cohen.[21] A 1.2 ml aliquot of an extract of *E. coli* B (harvested 20 min after infection with T2-bacteriophage and prepared as were the concentrated extracts of sets A and B described in the following section) was added to 20 ml of a reaction mixture similar to that previously described for the assay of dCMP hydroxymethylase[15] but modified by doubling the concentrations of formaldehyde and tetrahydrofolate. The mixture was incubated for 3 hr at 37° and then chilled. Then 8 ml of 1 *M* formaldehyde and 1.8 ml of 60% trichloroacetic acid were added. The resulting precipitate was removed by centrifugation and the trichloroacetic acid in the supernatant fluid was removed by extraction three times with two volumes of ether. After removal of residual ether by aeration, the solution was neutralized with 1 *M* NH_4OH and applied to a column of Dowex-1-formate ion exchange resin, 1 × 22 cm. The column was washed with 2 liters of water and elution was begun with 0.015 *M* ammonium formate, pH 3. By this procedure, the dHMP was completely separated from unreacted dCMP and was eluted in the region between 400 and 570 ml of effluent. The effluents containing dHMP were concentrated on a rotary evaporator and the residual water and am-

monium formate were removed *in vacuo* at room temperature. The residue was dissolved in 10 ml of water and was stored at − 20°. The yield of C^{14}-labeled dHMP based on the original dCMP was 41%. Yields as high as 57% have been obtained when additional formaldehyde and tetrahydrofolate were added and the incubation prolonged. Unlabeled dHMP was prepared similarly from unlabeled formaldehyde instead of C^{14}-labeled formaldehyde.

Preparation of extracts from infected bacteria: Two of the three sets of extracts (A and B) were made in identical fashion. Extracts of Set A were prepared from *E. coli* B infected with T4 *am*$^+$, *am* 82, or *am* 122; those of Set B from *E. coli* B infected with *am*$^+$, *am* 81, *am* 116, or *am* 130; those of Set C from *E. coli* B infected with am$^+$, *am* 17, or *am* 90. Cells of *E. coli* B were grown with vigorous aeration at 37° in the glycerol casamino acid medium of Fraser and Jerrel[22] to a concentration of 1.3×10^9 cells per ml. Aeration was then stopped and the culture was stored at room temperature for 30 to 45 min; under these conditions, no further increase in turbidity occurred. Aliquots of 1,300 ml were placed in bottles and warmed to 37°, and L-tryptophan was added to a concentration of 10 mg per liter. Vigorous aeration was begun through sintered glass tubes. After 5 min, 4 phage per bacterium were added, and 4 min later, the same amount of phage was added again. The actual multiplicity of infection 3 min after the first infection was calculated to be 2.4 from measurements of surviving cells.[23] At various times after infection, aliquots of 130 ml were removed and chilled rapidly by admixture with 60 gm of frozen medium. All subsequent steps were carried out at 4°. The cells were collected by centrifugation at $6,000 \times g$ for 10 min, resuspended with a Potter-Elvehjem homogenizer in 0.02 *M* Tris acetate, pH 7.5, and the final volume was adjusted to 4 ml. This suspension was frozen in liquid nitrogen and forced through a Hughes press[24] to break the cells. The supernatant fluid obtained by centrifugation of the extracts for 30 min at $11,000 \times g$ was used for the DNA polymerase assays. For dCTPase assay on extracts in Set B, these extracts were diluted 1 to 8 with 0.02 *M* Tris acetate buffer, pH 7.5. For all other assays, the extracts were diluted 1 to 40 with 0.02 *M* Tris acetate buffer, pH 7.5. Both diluted extracts were centrifuged for 10 min at $11,000 \times g$; the supernatant fluids were used in the enzyme assays.

For the third set (Set C), the infection was performed similarly except that 100 ml of bacterial suspension were used per vessel; the aliquots were removed at various times after infection, pipetted into liquid nitrogen, and forced through a Hughes press. The supernatant fluids obtained by centrifugation of the extracts at $700 \times g$ for 3 min were used for all assays.

Phage production was determined at about 65 min after the initial infection. Aliquots of 0.1 ml of the cultures were diluted into 100 ml of dilution broth (0.8% Difco nutrient broth powder in 0.5% NaCl) previously shaken with 0.3 ml of chloroform. These diluted samples were left at room temperature for at least 30 min, after which aliquots were plated on *E. coli* CR63.

DNA assay: Trichloroacetic acid was added in the cold to aliquots of the diluted extracts to a concentration of 5%. After 10 min, the precipitate was collected by centrifugation at $1,200 \times g$ for 10 min and washed once with cold 5% trichloroacetic acid and twice with cold 95% ethanol. Residual ethanol was removed *in vacuo*. The DNA content of the residue was determined by the Ceriotti procedure[25] with the modification that the indole-HCl reagent was added directly to the residue. The standard was the T2-DNA preparation used as primer in the HMC-β glucosyl transferase assay below.

Enzyme assays: The assay for dCMP hydroxymethylase has been described previously.[15] The DNA polymerase assay is a modification of the procedure of Bollum[26] and will be presented in detail elsewhere. It involves the measurement of the rate of incorporation of label from C^{14}-labeled dHTP into acid-insoluble material by an enzymatic system to which denatured calf thymus DNA[6] is added as primer.

dCTPase assay: This assay is a modification of that of Koerner, Smith, and Buchanan.[27] The reaction mixture contained in 1 ml: enzyme; Tris acetate buffer, pH 9.1, 160 μmoles; magnesium acetate, 5 μmoles; and dCTP, 0.45 μmole. After incubation for 60 min at 37°, the reaction was stopped by addition of 0.2 ml of 0.05 *M* EDTA, pH 8. The reaction mixture was applied to a 3.3 × 30 mm column of Dowex-1-8X-formate ion exchange resin (200–400 mesh). After adsorption, the column was washed with 1 ml of water followed by 3 ml of 0.01 *M* ammonium formate buffer, pH 4.3. Elution of dCMP was then accomplished with 3 ml of 0.06 *M* ammonium formate buffer, pH 2.8. The amount of dCMP was determined by measurement of the absorbancy at 280 mμ.

dTMP synthetase assay: The reaction mixture was identical with that used for the dCMP hydroxymethylase assay, with the exception that dUMP was substituted for dCMP. The mixture contained, in 0.5 ml: enzyme; Tris acetate buffer, pH 8.0, 25 μmoles; EDTA, pH 8.0, 10 μmoles; dUMP, 0.7 μmole; 2-mercaptoethanol, 10 μmoles; tetrahydrofolate, 0.45 μmole; and C^{14}-labeled formaldehyde, 0.5 μmole (total radioactivity, 200,000 cpm). After incubation for 45 min at 37°, the reaction was stopped as follows: the sample was chilled, 1 ml of a cooled solution containing 200 μmoles of formaldehyde and 20 mμmoles of carrier dTMP was immediately added, and without delay the mixture was applied to a 3.3 × 50 mm column of Dowex-1-8X-formate ion-exchange resin (200–400 mesh) in the cold. After adsorption, the column was washed with 5 ml of water at room temperature followed by 4 ml of 0.1 *M* ammonium formate buffer, pH 4.35. Elution of dTMP was accomplished with an additional 8 ml of the same buffer. The entire eluate containing the dTMP was dried by collecting it directly on an aluminum planchet placed on a hot plate, and the radioactivity was measured.

dTMP kinase assay: Enzyme and reagents were incubated under the conditions described by Lehman, Bessman, Simms, and Kornberg.[28] The reaction mixture contained, in 1 ml: enzyme; ATP, 1.4 μmoles; $MgCl_2$, 18 μmoles; Tris acetate buffer, pH 7.5, 40 μmoles; and C^{14}-labeled dTMP, 72 mμmoles (total radioactivity, 10,000 cpm). After incubation for 30 min at 37°, the reaction was stopped by addition of 0.5 ml of 0.072 *M* EDTA, pH 8. The reaction mixture was applied to a 3.3 × 30 mm column of Dowex-1-8X-formate ion-exchange resin (200–400 mesh). After adsorption, the column was washed with 1 ml of water followed by 10 ml of 0.25 *M* ammonium formate, pH 4.35. Elution of dTDP and dTTP was accomplished with 4 ml of 4.0 *M* ammonium formate, pH 4.35. An aliquot was evaporated on a stainless steel planchet and the radioactivity was measured.

dHMP kinase assay: Enzyme and reagents were incubated under essentially the conditions described by Somerville, Ebisuzaki, and Greenberg.[4] The reaction mixture contained, in 0.6 ml: enzyme; ATP, 2 μmoles; $MgCl_2$, 5 μmoles; Tris chloride buffer, pH 8.0, 10 μmoles; and C^{14}-labeled dHMP, 37 mμmoles (total radioactivity, 3,300 cpm). After incubation for 15 min at 37°, the reaction was stopped by the addition of 0.4 ml of 0.03 *M* formic acid. The reaction mixture was applied to a 3.3 × 100 mm column of Dowex-50W-4X ion-exchange resin (hydrogen form, 200–400 mesh), and the effluent was collected directly on an aluminum planchet placed on a hot plate. Then 1.5 ml of 0.01 *M* formic acid was applied to the column, the eluate was collected on the same planchet, and the radioactivity was measured.

HMC-β-glucosyl transferase assay: Enzyme and reagents were incubated as described by Kornberg, Zimmerman, and Kornberg.[8] The reaction mixture contained, in 0.2 ml: enzyme; Tris acetate buffer, pH 7.5, 20 μmoles; $MgCl_2$, 5 μmoles; DNA from bacteriophage T2, 11 mμ moles of DNA-phosphorus, assayed by the method of Chen *et al.*[29]; and C^{14}-labeled UDP-glucose, 10 mμmoles. The rate of incorporation of C^{14}-labeled glucose into DNA was determined by adapting the filter paper technique of Bollum.[26] During the course of the incubation at 37°, aliquots of 25 μl were removed and placed on treated filter paper disks (Schleicher and Schuell Co., No. 595). The treatment involved placing 25 μl of 0.45 *M* EDTA, pH 8, on each disk, which was then dried. EDTA was added to the papers in order to ensure the immediate cessation of the reaction. The dried disks were then treated with trichloroacetic acid and alcohol according to Bollum's procedure and the radioactivity was determined.

Infection of E. coli with T4 and Its Am Mutants.—Phage production and synthesis of DNA: Measurements of phage production by *E. coli* B infected with T4 *am*$^+$

TABLE 1

PHAGE PRODUCTION 65 MINUTES AFTER INFECTION OF *E. coli* B BY T4 *am*$^+$ AND BY *Amber* MUTANTS

Infecting phage	Phage Production per Infected Cell (*E. coli* B) Set A	Set B	Set C
T4 am$^+$	302	240	270
am 82	0.2		
am 122	0.1		
am 81		9	
am 116		87	
am 130		9	
am 17			13
am 90			4

and seven *am* mutants at 65 minutes after infection are given in Table 1. Five of the mutants were selected because they were known to be deficient in DNA synthesis; the other two mutants (*am* 90 and *am* 17) can cause synthesis of phage DNA in *E. coli* B but are defective in some other steps needed for production of infective virus. Table 1 shows that two mutants, *am* 82 and *am* 122, are completely unable to reproduce in *E. coli* B. The other three mutants that are defective in DNA synthesis (*am* 81, *am* 116, and *am* 130) multiply at least to a limited extent. By reference to Figure 1, it may be seen that phage production by these five mutants is roughly related to the level of DNA production in infected cells.

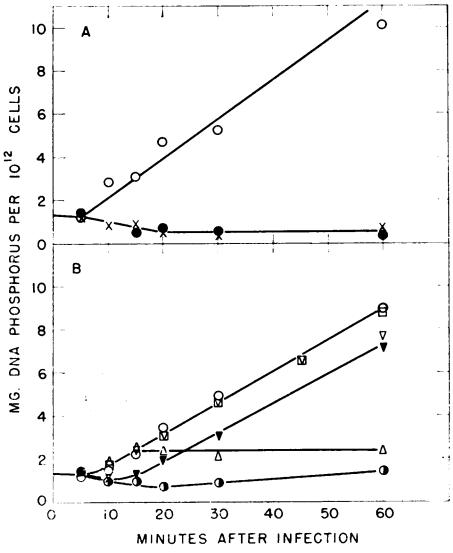

FIG. 1.—Formation of DNA by *E. coli* B after infection by T4 am^+ and *am* mutants. A: ○ = T4 am^+; × = *am* 82; ● = *am* 122. B: □ = *am* 17; ▽ = *am* 90; △ = *am* 130; ▼ = *am* 116; ◑ = *am* 81.

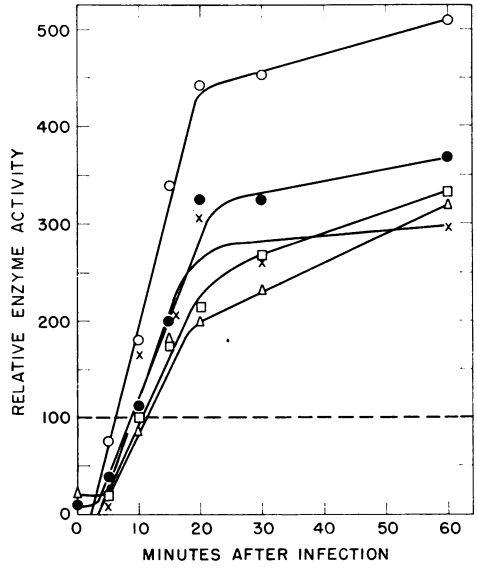

FIG. 2.—Formation of enzymes after infection with *am* 82. ○ = dCMP hydroxymethylase. ● = dTMP synthetase. □ = dHMP kinase. △ = dTMP kinase. × = dCTPase. In this and subsequent figures (3, 4, and 5), the horizontal dotted line represents the level of enzyme activities 15 minutes after infection with T4 am^+.

Infection with mutants *am* 17 and *am* 90 leads to production of very little phage, although DNA production is normal (see Fig. 1B).

The course of DNA synthesis after infection with T4 am^+ and seven *am* mutants is illustrated in Figure 1. The values for DNA formation with *am* 17 and *am* 90 and with T4 am^+ are identical. Two mutants, *am* 82 and *am* 122, are essentially incapable of inducing synthesis of DNA. With *am* 81, DNA synthesis is negligible during the first 20 min, but later there is a slow but significant rate of synthesis. The two remaining mutants, *am* 130 and *am* 116, induce DNA synthesis with kinetics strikingly different from that observed with T4 am^+. In cells infected with *am* 130, DNA synthesis starts at the normal rate but stops abruptly at about 15 minutes after infection. With *am* 116, DNA synthesis is delayed until approximately 20 minutes and then commences at a rate comparable to that observed with cells infected with normal T4 phage.

Formation of early enzymes: Extracts of cells infected either by T4 am^+ or by one of the *am* mutants were made from samples taken at suitable intervals and were examined for the levels of several enzyme activities. Figures 2 through 6 present plots of enzyme activity versus time after infection. Data for dCTPase, dCMP

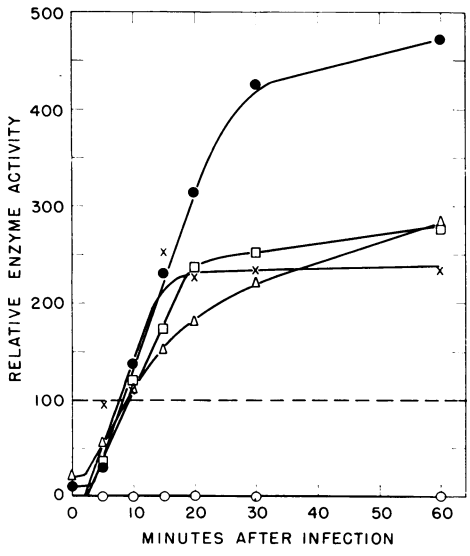

FIG. 3.—Formation of enzymes after infection with *am* 122. ○ = dCMP hydroxymethylase. ● = dTMP synthetase. □ = dHMP kinase. △ = dTMP kinase. × = dCTPase.

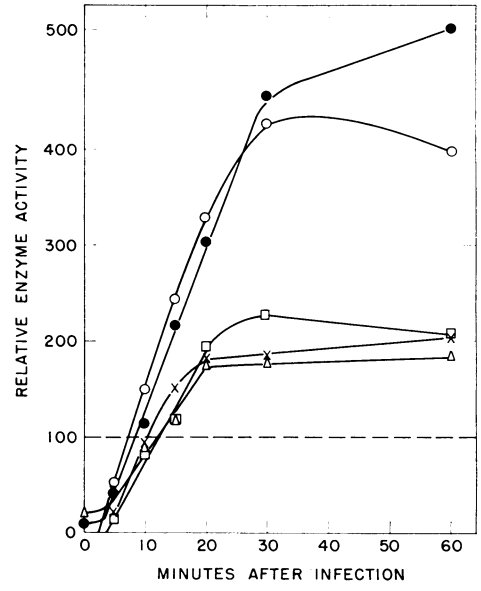

FIG. 4.—Formation of enzymes after infection with *am* 81. ○ = dCMP hydroxymethylase. ● = dTMP synthetase. □ = dHMP kinase. △ = dTMP kinase. × = dCTPase.

hydroxymethylase, dHMP kinase, dTMP kinase, and dTMP synthetase are presented. The values have been normalized by setting the 15-min interpolated value of the respective T4 curves as equal to 100. The values for HMC-β-glucosyl transferase activity at 15 min are given in Table 2 for T4 am^+ and five *am* mutants.

TABLE 2

HMC-β-GLUCOSYLTRANSFERASE ACTIVITY 15 MINUTES AFTER INFECTION

Phage used for infection	Relative activity
T4 am^+	1.0
am 81	4.0
am 82	5.0
am 122	5.0
am 116	2.2
am 130	1.0

The measurements of DNA polymerase activity in extracts of cells infected with am^+, *am* 82, and *am* 122 are shown in Figure 7; this enzyme activity has not yet been measured for the other mutants.

By inspection of Figures 2 to 7 and of Table 2 it may be seen that with four of the mutants, *am* 81, *am* 82, *am* 130, *am* 116, all of the enzyme activities measured are present at levels either equal to or greater than that found after infection with T4 $am.^+$ The case of mutant *am* 122 is unique: neither the dCMP hydroxymethylase nor the phage-induced DNA polymerase activities appear, whereas the other five

enzyme activities are produced. At least in this one instance, the failure of the mutant to induce synthesis of phage DNA is explained by the failure of appearance of two enzyme activities concerned with the synthesis of phage DNA.

The results for mutants *am* 17 and *am* 90 are indistinguishable from those for T4 am^+ (Figs. 6*A* and 6*B*).

Correlation between DNA synthesis and the course of enzyme synthesis: The course of early enzyme synthesis for each of the *am* mutants has been found to be strikingly related to the course of DNA synthesis. Under the conditions of our experiments, there is a customary lag period of approximately 7 to 10 min before DNA synthesis begins in infection with normal T4. In comparing the rates of DNA synthesis in *E. coli* B after infection with the *am* mutants, one may divide the mutants into four categories: (1) *am* 17, *am* 90, and *am* 130, which induce DNA synthesis at

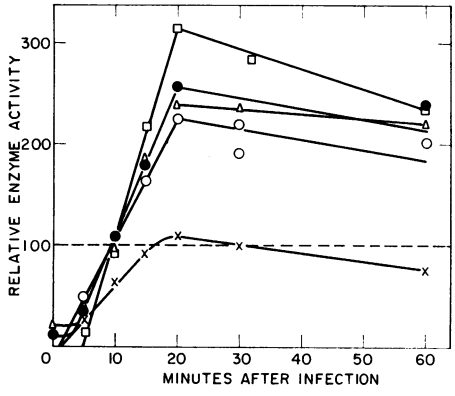

FIG. 5.—Formation of enzymes after infection with *am* 116. ○ = dCMP hydroxymethylase. ● = dTMP synthetase. □ = dHMP kinase. △ = dTMP kinase. × = dCTPase.

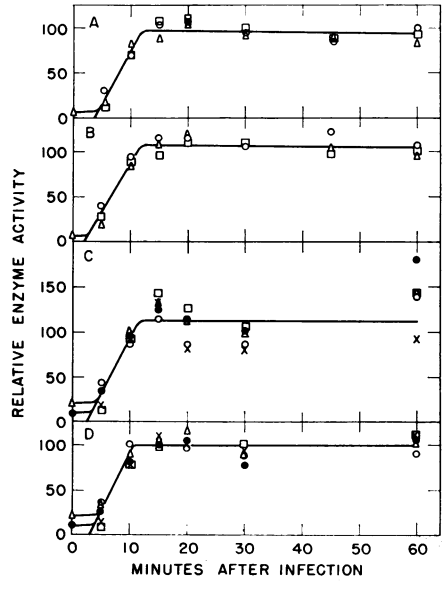

FIG. 6.—Formation of enzymes after infection with several *am* mutants. A: *am* 17. B: *am* 90. C: *am* 130. D: T4 am^+. ○ = dCMP hydroxymethylase. ● = dTMP synthetase. □ = dHMP kinase. △ = dTMP kinase. × = dCTPase.

a normal rate over the whole infection period or at least during the first 15 min; (2) *am* 82 and *am* 122, which do not induce any DNA synthesis; (3) *am* 116, which induces DNA synthesis at a normal rate, but only after an extended lag period; and (4) *am* 81, which after a lag period induces a very limited synthesis of DNA. With mutants of group 1, the course of early enzyme synthesis is essentially that found after infection with normal T4; that is, synthesis of the early enzymes ceases at 10 to 12 min after infection. With mutants belonging to class 2, the curves for enzyme synthesis are comparable to those obtained after infection with irradiated T2 or T4 am^+ bacteriophage,[15, 30] that is, enzyme synthesis continues well beyond 10 min and the amount of enzyme at 60 min may reach levels four to five times greater than after infection with normal T4. In bacteria infected with *am* 116

or *am* 81 (groups 3 and 4), the course of enzyme synthesis is intermediate between those found after infection with mutants in group 1 and group 2: in general, enzyme activities do not reach as high a level as seen in the case of the *am* mutants of group 2, and enzyme synthesis stops at around 20 to 30 min.

Discussion.—In the case of mutant T4 *am* 122, the enzyme studies have provided the explanation for the failure to produce phage DNA and mature phage. In *E. coli* B, this phage is incapable of inducing the appearance of dCMP hydroxymethylase activity. Hence, no dHMP, dHTP, or T4-phage DNA can be made in *E. coli* B. The phage T4 *am* 122 produced in strain CR63 has a DNA that is serologically indistinguishable from that of T4 *am*+, as shown by tests with anti-T4 DNA serum, kindly carried out for us by L. Levine. Yet, the failure of phage production in *E. coli* B cannot be attributed unequivocally to the failure to make dHMP because mutant *am* 122 also fails to induce production of DNA-polymerase. The tie between these two defects can be only a matter of speculation at the present time.

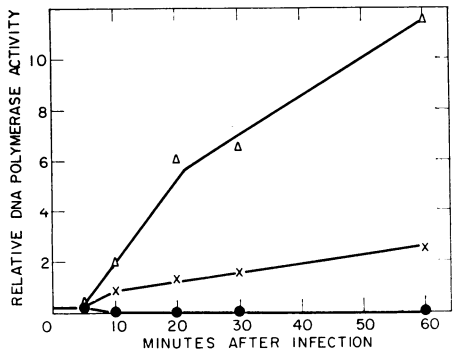

FIG. 7.—DNA polymerase activity after infection of *E. coli* B with T4 *am*+ or with *am* mutants. × = T4 *am*+. △ = *am* 82. ● = *am* 122.

On the one hand, mutant *am* 122 may have undergone two mutational steps. Genetic tests by crossing *am* 122 with other *am* mutants or by backcrossing with wild-type T4D (R. H. Epstein and A. Bolle, unpublished results) indicate that if *am* 122 is a double mutant the two component mutant sites are probably closely linked to one another. On the other hand, the two alterations might result from a single mutation by any one of several mechanisms. For example, the two enzymes may be under the control of a common "operator" gene;[31] or, they may share a common constituent subunit in their molecules; or else, dHMP or dHTP may be needed as inducers of the polymerase. Note, however, that dHMP kinase is fully developed in bacteria infected with T4 *am* 122. As a point of interest, Bessman and Bello[32] have recently reported that one enzyme may be responsible for the phosphorylation of three substrates, dHMP, dTMP, and dGMP.

The finding of a mutant unable to initiate production of two enzyme activities is strong evidence in favor of a direct role of the phage in the determination of the early enzymes. Proof of such a role has been lacking, although circumstantial evidence of it has been produced.[33, 34] Even the present findings do not conclusively prove that the phage has the genes that determine the structure of the proteins of the early enzymes. It is conceivable that these are determined by bacterial genes that become derepressed as a result of phage infection. The latter hypothesis becomes less attractive in view of the findings with T4 *am* 122; the suppression of two enzyme activities out of seven as a result of a phage mutation would require the existence of a set of phage genes, each concerned with the derepression of one or several bacterial genes. Complete proof of the role of phage genes in

determining the structure of the early enzymes will require the finding of phage mutations that lead to the production of specific alterations in the proteins of these enzymes.[35]

The results with the *am* mutants strongly support the hypothesis that phage DNA synthesis is closely related to the regulation of synthesis of early enzymes. With mutants *am* 82 and *am* 122, as with UV-irradiated T2 or T4, failure to synthesize DNA is coupled with extended and exaggerated production of those enzymes that the system can make. With mutants that cause DNA synthesis at a normal rate for at least 15 min, enzyme synthesis terminates at 10 to 12 min as with T4 am^+. With mutant *am* 81, there is a late and slow synthesis of DNA and a synthesis of enzymes that exceeds the T4 am^+ level but that is generally lower than the levels for *am* 82. Finally, with mutant *am* 116 DNA synthesis starts late but at a full rate, and enzyme synthesis continues longer and then ceases as abruptly as with T4 am^+. It will be important to decide whether the controlling parameter in enzyme synthesis is the level of DNA *per se* or some step that only takes place when phage DNA reaches a certain level. Experiments to clarify this point are in progress.

Summary.—A number of amber mutants of bacteriophage T4 that are capable of inducing the production of phage in some strains of *E. coli* K-12 but not in *E. coli* B have been examined for their ability to induce in *E. coli* B the formation of enzyme activities related to the synthesis of DNA. Two of the seven mutants studied had been previously shown to be essentially incapable of inducing DNA synthesis in *E. coli* B, and abnormal kinetics of DNA synthesis were obtained with three others. In one instance (mutant *am* 122), the inability to induce DNA synthesis has been attributed to the lack of appearance of two essential enzymes, deoxycytidylate hydroxymethylase and phage-induced DNA polymerase.

The mutants have proved to be useful tools for examination of the premise that cessation of enzyme formation 10 to 15 min after infection by normal bacteriophage T4 is related to DNA synthesis. After infection with those mutants (*am* 82 and *am* 122) that are incapable of inducing DNA synthesis, enzyme synthesis *continues* for a considerable length of time beyond 10 min. In the case of mutants *am* 130, *am* 116, *am* 17, and *am* 90, cessation of enzyme synthesis could be related to the course of DNA synthesis.

The excellent technical assistance of Edmund Klausner is gratefully noted. We are indebted to Alan H. Cohen[36] for the modification of the dHMP kinase assay used in this study, and to Gerald A. Frank for the modification of the dTMP kinase assay.[37]

* This investigation was supported by research grants from the National Cancer Institute, the National Institutes of Health, U. S. Public Health Service.

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‡ The following abbreviations are used: DNA, deoxyribonucleic acid; dUMP, dTMP, dCMP, and dHMP represent the 5′ monophosphates of deoxyuridine, thymidine, deoxycytidine, and deoxy-5-hydroxymethylcytidine, respectively; dTTP, dCTP, dHTP, and ATP represent the 5′ triphosphates of thymidine, deoxycytidine, deoxy-5-hydroxymethylcytidine, and adenosine, respectively; HMC, 5-hydroxymethylcytosine; dCTPase, deoxycytidine triphosphatase; Tris, tris(hydroxymethyl) aminomethane; EDTA, ethylenediamine tetraacetic acid, sodium salt.

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