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# Early Events After Infection of *Escherichia coli* by Bacteriophage T5

## I. Induction of a 5'-Nucleotidase Activity and Excretion of Free Bases[1]

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Thymine-containing compounds, produced during degradation of *Escherichia coli* DNA after infection of the cells with bacteriophage T5, did not accumulate in the cell but were excreted into the medium as the DNA was degraded. The ultimate degradation product was extracellular thymine that was not reutilized when T5 DNA synthesis began. This excretion of thymine may have been due in part to the induction of 5'-nucleotidase activity within 3 min after T5 infection. The level of this activity reached a maximum between 4 to 6 min after infection and then rapidly declined to its preinfection level by 10 to 15 min after infection. Chloramphenicol added before or soon after infection prevented the appearance of the nucleotidase. The induced nucleotidase activity was active not only on dTMP but also on dAMP, dGMP, and dCMP.

One of the consequences of infection of *Escherichia coli* by any of the T-even or T-odd bacteriophages is degradation of the host DNA to acid-soluble products (13). Although the biological significance of this degradation is still not known, it is clear that the processes are very different with the different phages. Of the T-even phages, the T4-induced degradation has been studied in greatest detail. In T4-infected cells, degradation of the host DNA to acid-soluble products is quite slow and is unessential for phage replication, and the degradation products are reutilized for phage DNA synthesis (22). In T7-infected cells, the degradation products are not only reutilized for phage DNA synthesis, but these products or the nucleases producing them also seem to be necessary for phage DNA synthesis (19).

Degradation of host DNA in T5-infected cells is unusually rapid, and the thymine present in host DNA is not reused for phage DNA synthesis (5, 24). Pfefferkorn and Amos found free bases in the medium at 10 min after infection, including at least one-third of the thymine lost from the cells, but no data were presented (15). Furthermore, it is not clear what percentage of the thymine present in the host DNA at the time of infection is eventually lost from the cells after infection. These authors also found bases other than thymine in the medium, but did not indicate which bases or how much. Thus, it appears that products from host DNA degradation after T5 infection are not only not reused for phage DNA synthesis, but some are actually excreted from the cell.

This paper describes experiments designed to discover why the thymine present in host DNA cannot be reutilized for phage DNA synthesis in T5-infected cells.

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### MATERIALS AND METHODS

*E. coli* F and the wild-type T5 stock used for this work have been previously described (4). Infections were performed either in nutrient broth (1) or Fraser's medium (7), both supplemented with 1.0 mM $CaCl_2$.

**Infection of prelabeled cells with T5+.** *E. coli* F was grown to an approximate concentration of $5 \times 10^7$ cells/ml in Fraser's medium at 37 C, at which time 0.50 mg uridine per ml and [$^{14}$C]thymidine (0.015 $\mu$Ci/ml; specific activity, 33 $\mu$Ci/$\mu$mol) were added. After 100 min of continued aeration at 37 C, the cells were centrifuged at 10,000 $\times$ *g* for 10 min and resuspended in Fraser's medium containing 1 mM $CaCl_2$ to an approximate concentration of $5 \times 10^8$ cells/ml. After a 5-min incubation at 37 C with aeration, the cells were cooled to 0 C and infected with T5 at a multiplicity of 5 phage/cell. The infected complexes were incubated without aeration at 0 C for 15 min to permit adsorption, at which time samples were removed to determine surviving cell and unadsorbed phage titers. The infected cells were transferred to the desired temperature and vigorous aeration was begun. The time of transfer was taken as the beginning of infection.

**Analysis of breakdown products by trichloroacetic acid precipitation.** Cells were grown, labeled with

[$^{14}$C]thymidine, and infected with wild-type T5 as described above. At various times after aeration was begun at 25 C, samples were removed for two types of analyses. To analyze total trichloroacetic acid-soluble and -insoluble radioactivity, 0.50-ml portions were removed and added to 0.50 ml of cold 10% trichloroacetic acid. After at least 30 min at 0 C, the samples were centrifuged at 5,000 $\times$ *g* for 15 min, and both the supernatant and the pellet were saved. The pellet was washed twice with 2.0 ml of cold 5% trichloroacetic acid and dissolved in 1.0 ml of cold 5% trichloroacetic acid and dissolved in 1.0 ml of 2 N $NH_4OH$, and 0.40 ml was counted in Bray solution. Portions of the supernatant were also counted in the same manner after extraction of the trichloroacetic acid with ether.

Samples were also analyzed for the presence of trichloroacetic acid-precipitable material in cells and in the medium as follows. Portions (0.50 ml) of infected cells were harvested by mixing with 1.0 ml of cold Fraser's medium and centrifuging at 10,000 $\times$ *g* for 10 min. The cell pellet was resuspended in 1.0 ml of Fraser's medium to which 0.50 ml of cold 10% trichloroacetic acid was added, and the sample was centrifuged at 5,000 $\times$ *g* for 15 min. Both the resulting pellet and supernatant were analyzed for radioactivity as described above. The supernatant from the original cell pellet was also treated with trichloroacetic acid (0.50 ml of cold 10% trichloroacetic acid was added to 0.50 ml of the supernatant), and after the samples were centrifuged at 5,000 $\times$ *g* for 15 min, the pellet was analyzed for radioactivity as described above. The preparation of the various fractions analyzed in this experiment is outlined in Fig. 1.

**Analysis of breakdown products by column chromatography.** Cells were grown, labeled with [$^{14}$C]thymidine, and infected with wild-type T5 as described above. At various times after aeration at 25 C was begun, 2.0-ml samples were harvested on ice and centrifuged for 10 min at 10,000 $\times$ *g*. dTMP, thymidine, and thymine (0.6 $\mu$mol of each per ml) were added to the supernatant, which was then applied to a column of Bio Gel P2 (1.7 by 27.5 cm) and eluted with 0.10 M ammonium formate containing 0.002 M sodium phosphate buffer, pH 6.8. Fractions of 2.0 ml each were collected; 0.50 ml of each fraction was counted on a planchet.

The cell pellet was resuspended in 0.60 ml of 0.10 M Tris-chloride buffer, pH 7.1. These cells were lysed according to a modification of a lysozyme-EDTA procedure described by Warner et al. (23) in which 0.30 ml of the EDTA-lysozyme solution was added to the cell suspension. After a 10-min incubation at 0 C, 0.10 ml of 0.10 M $MgCl_2$ was added to stop the reaction and the sample was freeze-thawed three times. This suspension was centrifuged at 10,000 $\times$ *g* for 10 min, and the supernatants were chromatographed on Bio Gel P2 as described above.

**Appearance of 5′-nucleotidase after infection.** *E. coli* F was grown to a concentration of 4 $\times$ $10^8$/ml in nutrient broth supplemented with 1.0 mM $CaCl_2$. At this density the cells were chilled on ice and divided into 30-ml portions in 250-ml flasks. At 30-s intervals each flask was placed in a 37 C shaking water bath; 4 min later phage were added to each flask at a multiplicity of 5 phage/cell at 30-s intervals. The addition of phage was taken as the zero time point of infection. At appropriate times a flask was removed, the infected cells were poured over ice, and the samples were centrifuged at 10,000 $\times$ *g* for 10 min. In this procedure each time point represents a separate infection. The cell pellet was ether treated according to a modification of the technique described by Vosberg and Hoffmann-Berling (21). The pellet was resuspended in 1.0 ml of cold basic medium (basic medium is 80 mM KCl, 40 mM Tris-hydrochloride [pH 7.4], 7 mM magnesium acetate, 0.4 mM spermidine·3HCl, 2 mM EGTA, and 500 mM sucrose). To this, 1.0 ml of cold ether was added and the suspension was shaken gently by hand for 1.0 min in a capped vial. The non-ether layer was layered on a 4.0-ml cushion of basic medium containing sucrose at a final concentration of 0.80 M and centrifuged at 8,000 $\times$ *g* for 10 min. The pellet was dissolved in 0.40 ml of basic medium and frozen. Each sample was thawed only once. Samples could be thus made from infected cells harvested up to 30 min after infection; after this time, however, lysis began to occur during the ether treatment.

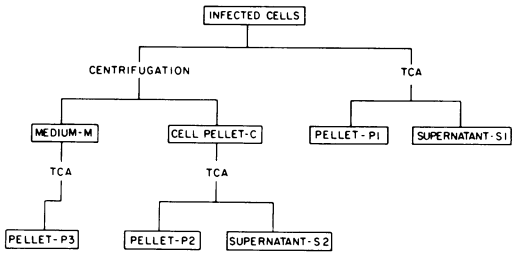


FIG. 1. *Flow chart relating the various fractions analyzed to determine the amount of acid-soluble and -insoluble radioactivity present in various cellular fractions after infection of prelabeled E. coli by wild-type T5 phage. The nomenclature refers to the curves in Fig. 3.*

**Enzyme assays.** The assay mixture for the determination of 5′-nucleotidase activity included in a final volume of 0.07 ml: *N*-2-hydroxyethyl piperazine-N′-2′-ethanesulfonic acid buffer (pH 8.2), 2.0 $\mu$mol; $MgCl_2$, 0.40 $\mu$mol; dithiothreitol, 2.0 $\mu$mol; [$^{14}$C]- or [$^3$H]deoxynucleoside monophosphate, 0.10 $\mu$mol and 15 nCi; and 5 to 20 $\mu$liters of ether-treated cells containing 80 to 150 $\mu$g of protein and 0.01 to 0.10 U of activity. This mixture was incubated at 30 C for 10 min. The reaction was terminated by the addition of 1 ml containing 1.0 $\mu$mol of nonlabeled substrate and placement in a boiling water bath for 2 min. When pyrimidine substrates were assayed, the entire mixture was applied to a column (0.33 by 5.0 cm) of Dowex-1-X8-formate anion exchange resin, and the non-phosphorylated products were eluted with 2.0 ml of water. Portions (1 ml) of the water wash were counted in a Triton X-100-toluene (1:2) scintillation fluid containing 6 g of 2,5-diphenyloxazole per liter. Assay mixtures containing purine substrates were chromatographed on a similar column packed with DEAE-Sephadex-formate and eluted with 2.0 ml of water. One unit of nucleotidase activity is defined as

that amount catalyzing the formation of 1 nmol of non-phosphorylated product per min under the above conditions.

The assay mixture for the determination of thymidine phosphorylase activity contained in a final volume of 0.055 ml: potassium phosphate buffer (pH 7.6), 22 μmol; $MgCl_2$, 0.40 μmol; dithiothreitol, 2.0 μmol; [$^{14}$C]thymidine, 1.0 μmol and 50 nCi; and 5 μliters of ether-treated cells containing 0.01 to 0.10 U of activity. This mixture was incubated at 30 C for 10 min, at which time the reaction was terminated by placement in a boiling water bath for 2 min. A portion of the reaction mixture was chromatographed in the presence of standard thymine and thymidine on silica gel plates (Brinkmann F-254) using a paper chromatography solvent designed by Reeves et al. (18) and containing isobutyric acid, water, 0.10 M EDTA, concentrated ammonia, and toluene (160:22:3:2:20). After visualization under UV light, the thymine and thymidine spots were cut out and counted in a planchet counter. One unit of phosphorylase activity is defined as that amount catalyzing the formation of 1 nmol of product in 1 min under the above conditions.

Deoxynucleoside monophosphate (dNMP) kinase activity was assayed as described previously (4). One unit of activity is defined as that amount catalyzing the formation of 1 nmol of product in 1 min.

## RESULTS

When *E. coli* cells containing DNA labeled with [$^{14}$C]thymidine were infected with T5 phage, the radioactivity was rapidly converted to acid-soluble form at 37 C (Fig. 2). The solubilization began between 2 and 3 min after infection and was nearly 90% complete by 12 min after infection. Because of the rapid solubilization at 37 C, similar experiments were done at lower temperatures to find a more suitable temperature for studying the appearance of acid-soluble products. The rate of degradation decreased with decreasing temperature, and 25 C was chosen for further degradation experiments (Fig. 2).

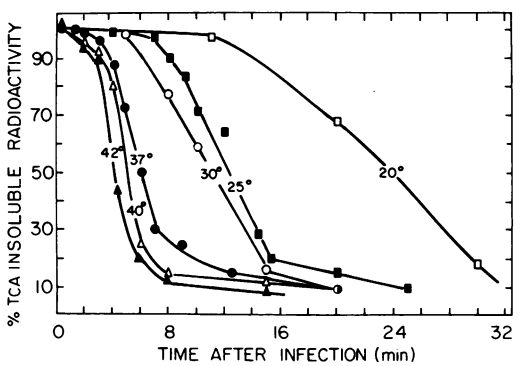


FIG. 2. *Breakdown of host DNA after T5 infection at various temperatures. E. coli F, prelabeled with [$^{14}$C]thymidine, was infected with wild-type T5 phage at the indicated temperatures. Samples were removed at the times indicated by each point and analyzed for trichloroacetic acid-precipitable radioactivity.*

To determine why the acid-soluble degradation products are not reused for phage DNA synthesis, it is necessary to determine what and where these products are. Since phage T5 induces a very active 5'-dNMP kinase after infection of *E. coli* (3), it seems reasonable to assume that the ultimate product is not intracellular 5'-dTMP. Alternative thymine-containing degradation products include intracellular 3'-dTMP, oligodeoxynucleotides, or extracellular products.

To determine where the acid-soluble thymine derivatives accumulate after infection, the host DNA was labeled with radioactive thymidine, and the cells were infected with T5. At various times after infection, equal portions were removed for either precipitation by trichloroacetic acid to determine total precipitable radioactivity or for centrifugation to separate cells from the medium. Equal portions of both resuspended cells and medium were then counted to determine radioactivity present before and after precipitation by trichloroacetic acid. The amount of total acid-precipitable radioactivity in a given portion of culture (designated P1 in Fig. 3A) correlated very well with both the amount of total intracellular radioactivity (designated C) and the amount of acid-precipitable intracellular radioactivity (designated P2). Furthermore, the amount of acid-precipitable material present in the medium (designated P3) was negligible. This would indicate that acid-soluble thymine-containing products do not accumulate inside of cells but are rapidly excreted into the medium.

Ten percent or less of the acid-soluble thymine derivatives were present in the cells (designated S2), and 80 to 100% of the total acid-soluble thymine derivatives could be found in the medium after degradation was completed (designated M) (Fig. 3B). Thus, nearly all of the intracellular radioactivity was acid insoluble and nearly all of the extracellular radioactivity was acid soluble throughout the replication cycle.

To characterize the nature of the intracellular and extracellular radioactivity, equal portions of the medium and extracts of infected cells were chromatographed on Bio Gel P2 gel filtration columns. Thymine, thymidine, and dTMP were added as standards, and the elution patterns of these standards are shown with the 15-min samples in Fig. 4C and 4G. As expected, most of the radioactivity present in the cell ex-

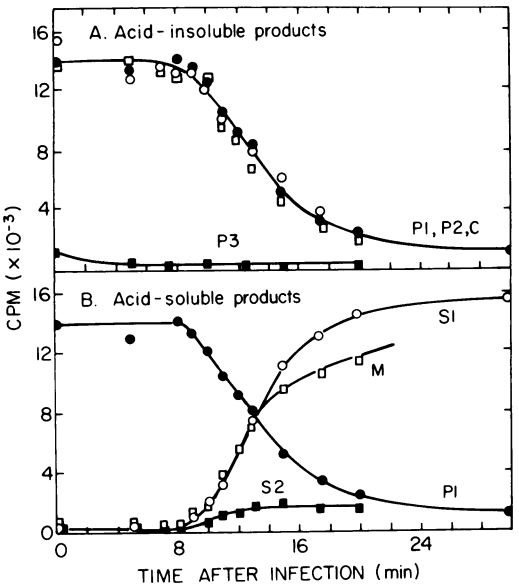


FIG. 3. *Analysis of breakdown products by trichloroacetic acid precipitation. (A) The amount of acid-insoluble material present in the culture (P1, ●) is compared with the total amount of radioactivity present inside cells (C, ○), the amount of acid-insoluble radioactivity inside cells (P2, □), and the amount of acid-insoluble radioactivity in the medium (P3, ■). (B) The amount of acid-insoluble radioactivity in the culture (P1, ●) is compared with the amount of acid-soluble radioactivity in the culture (S1, ○), the amount of acid-soluble radioactivity inside cells (S2, ■), and the total amount of radioactivity in the medium (M, □). The nomenclature for each curve is explained in Fig. 1.*

tracts eluted with the void volume, with some eluting between dTMP and the void volume and a small amount (<5%) eluting as thymine (Fig. 4A-D). In contrast, 100% of the extracellular radioactivity eluted with the thymine standard and presumably was thymine (Fig. 4E-H).

These results confirm the observation of Pfefferkorn and Amos (15) that bases are excreted into the medium, but indicate that 80% or more of the thymine in the host DNA is excreted and thus is unavailable for phage DNA synthesis. Thymine can be taken up by cells only if sufficient deoxyribosyl groups are available (8). Uninfected cells do not take up exogenous thymine unless the deoxyribose-1-phosphate pool has been elevated by mutation or by the addition of a high concentration of a deoxyribosyl donor such as deoxyadenosine or deoxyguanosine to the medium. *E. coli* cells infected with T4 phage incorporate exogenous thymine into DNA considerably better than do uninfected cells, presumably due to an increase in the deoxyribose-1-phosphate pool after infection (9). In contrast, T5-infected cells incorporated exogenous thymine no better than uninfected cells (9).

Although it is not possible to specifically label DNA with radioactive purines, an experiment was done with [$^{14}$C]guanine in place of [$^{14}$C]thymidine. At 15 min after infection at 37 C, the cells were sedimented, and a portion of the medium was chromatographed on a Bio Gel P2 column. The results indicated that two radioactive compounds with elution volumes corresponding to guanine (50%) and deoxyguanosine (50%) were excreted from the cells (data not shown). It is possible that the peaks eluted from the column also contained adenine, deoxyadenosine, guanosine, or adenosine since the adenine and guanine pools equilibrated to some extent. The striking difference between these data and the data in Fig. 4E through H is the complete absence of a nucleoside peak in the latter.

*E. coli* contains both 5'-nucleotidases that can convert dTMP and dGMP to thymidine (14, 20) and deoxyguanosine (14), respectively, and phosphorylases that can convert thymidine and deoxyguanosine to deoxyribose-1-phosphate and thymine (17) and guanine (10), respectively (Fig. 5). The rapid excretion of thymine and guanine into the medium suggests that one or more of these activities may also be induced after T5 infection. To test this possibility, *E. coli* cells were infected with T5 and treated with ether at various times after infection. These nucleotide-permeable cells were then assayed for dTMP, dCMP, dAMP, and dTMP nucleotidase activities. Within 2 min after infection of *E. coli* with phage T5 at 37 C, nucleotidase activities were induced for all of these substrates (Fig. 6). We also assayed 5'-nucleotidase activity in extracts of T5-infected cells. The results were identical to those presented in Fig. 6 using ether-treated cells. All four nucleotides appeared to be equally good substrates. This activity reached and remained at a maximal level from about 4 to 6 min after infection, during which time DNA degration (as measured by conversion to acid-soluble products) proceeded to about half completion (Fig. 2). After 6 min, the nucleotidase activity dropped dramatically and returned to the low level characteristic of host nucleotidases by 20 min, by which time host degradation was completed. Thus the nucleotidase was present and active during the period of active thymine excretion. Figure 6 also indicates the time course of induction of a typical early T5-induced enzyme, dNMP kinase, as compared

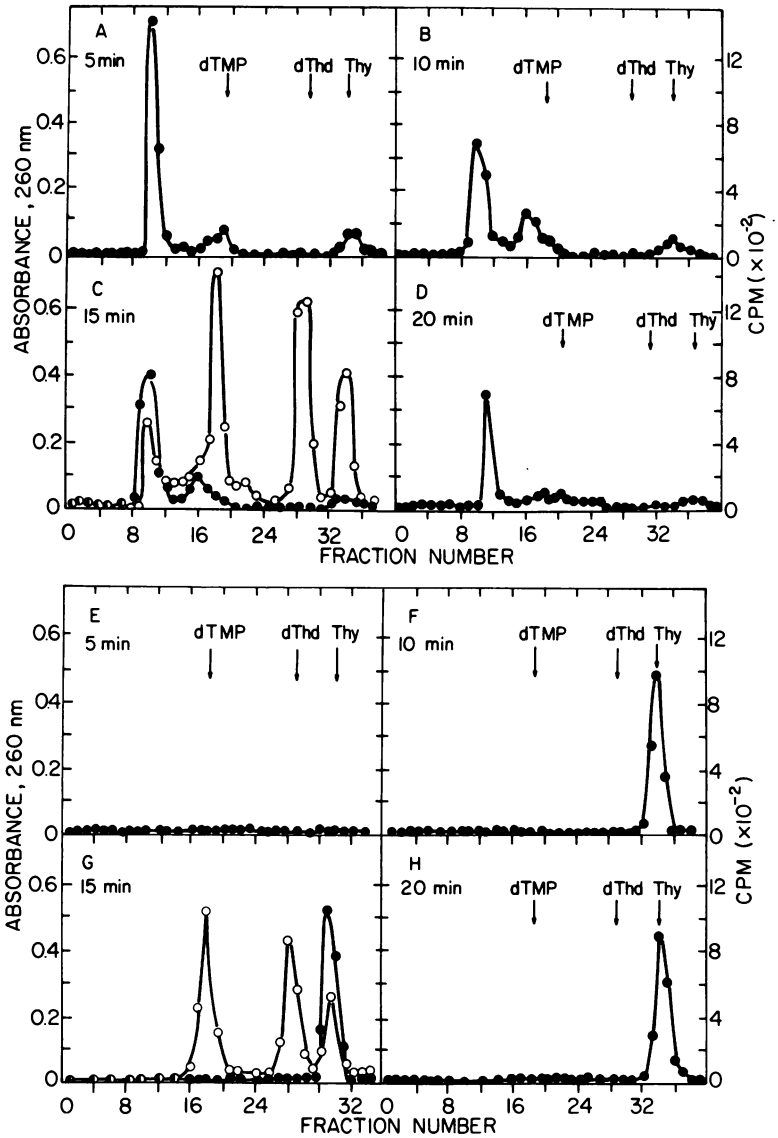


FIG. 4. *Chromatography of intracellular (A-D) and extracellular (E-H) thymine-containing material after infection of prelabeled E. coli with wild-type T5. The profile observed with markers (O and arrows) is compared with that observed with radioactivity-labeled degradation products (●). UV-absorbing material can be observed eluting with the void volume in C. This represents high-molecular-weight host material and was only observed in the chromatography of intracellular products (A-D).*

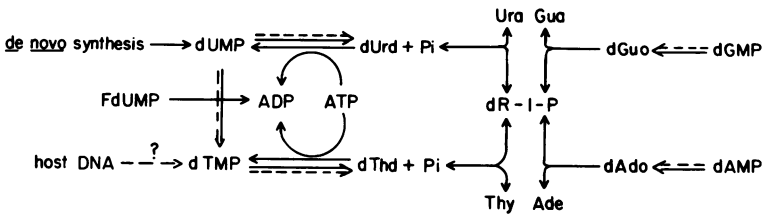


FIG. 5. *Pathways involved in the metabolism of deoxyribonucleotides derived from E. coli DNA after infection with wild-type T5. Both host (——) and phage induced (-----) activities are indicated.*

with that of the nucleotidase. The nucleotidase activity had been fully induced and had returned to uninfected levels before the kinase reached maximal activity.

We also assayed the ether-treated cells prepared after infection for thymidine phosphorylase activity under conditions similar to those used to assay nucleotidase activity. The specific activity in uninfected *E. coli* was about 130 nmol per min per mg of protein, and there was no increase after infection. Since the specific activity of dTMP nucleotidase in infected cells was only 60 nmol per min per mg at its maximal level, the host thymidine phosphorylase activity was about twice that of the nucleotidase activity under these conditions. Thus, the host activity appears to be high enough to rapidly convert any thymidine produced by the phage-induced nucleotidase into thymine.

Chloramphenicol added either 3 min before or 2 min after infection prevented further increase in the nucleotidase activities as determined by measuring the activity with dCMP (Fig. 7). The activity at 2 min in untreated cells was greater than that observed in the cells to which chloramphenicol was added at 2 min in Fig. 7. This was probably the result of some synthesis of the nucleotidase during the harvesting of the untreated sample. Similar experiments measuring dAMP, dGMP, or dTMP nucleotidase gave identical results. This indicates that the appearance of the observed activities requires protein synthesis after infection. Such evidence, however, does not rule out the possibility that

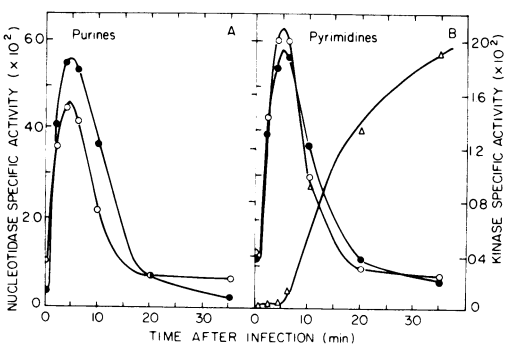


FIG. 6. *Induction of a multifunctional 5'-nucleotidase after infection of E. coli by wild-type T5. The induced nucleotidase is active on the purine deoxyribonucleotides, dAMP (○) and dGMP (●) (panel A), and on the pyrimidine deoxyribonucleotides, dCMP (○) and dTMP (●) (panel B). The time of appearance of an induced early enzyme, deoxynucleoside monophosphate kinase, is shown for comparison (△). For both activities, specific activity is defined as units per microgram of protein.*

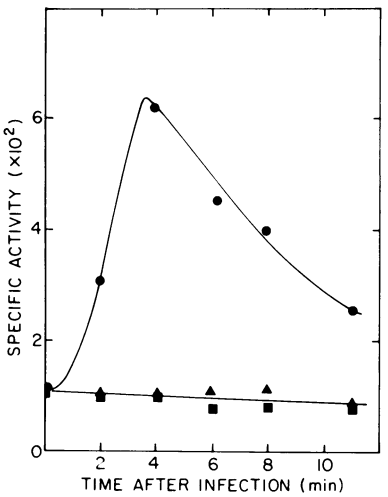


FIG. 7. *Effect of chloramphenicol on the appearance of 5'-nucleotidase after infection of E. coli by wild-type T5. Chloramphenicol was added 3 min before infection (▲), 2 min after infection (■), or not at all (●).*

the phage-induced nucleotidase activities observed result from the induction or modification of an already present host function. It is also not clear from the results presented above whether one enzyme acts on all four substrates or whether four separate nucleotidases are induced.

## DISCUSSION

Our results indicate that most of the thymine present in *E. coli* DNA at the time of infection with T5 phage is excreted into the medium as free thymine after infection. The thymine that is not excreted remains in an intracellular, acid-precipitable form and presumably is undegraded host DNA. Cells labeled with [$^{14}$C]guanine also excrete radioactive bases (guanine and/or adenine) after T5 infection, but they also excrete radioactive nucleosides. This may be due to the difference in the amount of phosphorylase activity for thymidine and deoxyguanosine present in *E. coli*. We obtained a specific activity of 130 nmol per min per mg of protein for thymidine phosphorylase in our ether-treated cells, which agrees well with the value of about 80 obtained by Razzell and Casshyap in extracts of uninduced *E. coli* (16). The induced level is reported to be about 730 (16), which is about sevenfold higher than 105 nmol per min per mg for deoxyguanosine phosphorylase activity reported by Karlström (10) for induced cells. Presumably the uninduced level is considerably lower, indicating that the thymidine phosphorylase activity is probably 5- to 10-fold that for

deoxyguanosine phosphorylase in our *E. coli* cells.

Our results also indicate that phage T5 induces a large increase in 5'-nucleotidase activity after infection of *E. coli* but do not distinguish between the induction of a host enzyme and the induction of a phage-coded enzyme. Protein synthesis is required for the increased activity since chloramphenicol prevents the enzyme induction. If the enzyme is coded by the phage genome, the structural gene is most likely on the FST DNA, since the time course of appearance of the nucleotidase is identical to that reported for two other FST proteins (12).

The induced 5'-nucleotidase provides an explanation for the absence of host DNA degradation products in phage T5 DNA. Phage T5 is unique among the *E. coli* T phages both because of its failure to reincorporate host DNA degradation products into phage DNA and because host DNA degradation and phage DNA do not occur concurrently. This suggests that the high concentration of deoxyribonucleoside 5'-monophosphates that would result from host DNA degradation in the absence of phage DNA synthesis might be toxic to the infected cell; the induced nucleotidase provides a mechanism to eliminate this pool from the cell.

The results also suggest that the activity of the induced nucleotidase is inhibited starting at about 6 min after infection. The nature of this inhibition has not yet been elucidated, but possibilities include the synthesis of an inhibitor, specific proteolysis of the enzyme, or nonspecific turnover of the enzyme. There is precedent for phage-induced inhibitors of host functions, e.g., inhibition of *Bacillus subtilis* dUTPase after infection with phage PBS2 (A. R. Price, Fed. Proc. 33: 1488, 1974), but this inhibition of a phage-induced function appears to us to be novel. Phage T5 may also induce inhibitors to terminate the activity of the nucleases responsible for host DNA degradation, since these nucleases may be unable to distinguish between host DNA and newly replicating phage DNA.

Zweig et al. (24) observed that 5-fluorodeoxyuridine (FUdR) stimulates reincorporation of host degradation products into phage DNA in T5-infected cells and that this reincorporation is inhibited by hydroxyurea. They proposed no mechanism for this reincorporation, but it now seems apparent that this reincorporation is due to the inhibition of thymidylate synthetase by 5-fluoro dUMP with the concomitant accumulation of deoxyribose-1-phosphate from dUMP (Fig. 5). Only after FUdR is added is there sufficient deoxyribose-1-phosphate available to convert the extracellular thymine to thymidine and subsequently dTTP for phage DNA synthesis. Hydroxyurea, an inhibitor of ribonucleotide reductase (11), would prevent synthesis of not only dUMP but also dTTP, dCTP, and dGTP so the reincorporation would not occur. We have obtained similar results with FUdR and also with deoxyadenosine (unpublished data). Deoxyadenosine stimulates uptake of extracellular thymine by supplying a source of deoxyribose-1-phosphate (8) (Fig. 5).

The induction of a 3'-deoxynucleotidase by T-even phages has been reported by Becker and Hurwitz (2). This enzyme is active both on 3'-deoxynucleotides and on the 3'-phosphate termini of DNA but is not active on ribonucleotides or on 5'-deoxynucleotides. The enzyme is induced as early as 4 min, and the activity increases until at least 15 min after infection; no data were given beyond 15 min. No such enzyme was found in T5-infected cells, but the time of infection was not given in this experiment. T-even phage also induce a 5'-phosphatase, but this enzyme is primarily a polynucleotide phosphatase (2). No experiments concerning the induction of a 5'-phosphatase by T5 phage were reported. Purified phage T5 preparations possess some 5'-nucleotidase activity capable of hydrolyzing ATP and ADP, but no results with 5'-deoxynucleotides were reported (6). Since the induction of the T5-induced 5'-nucleotidase was blocked by chloramphenicol (Fig. 7), it is not very likely that the activity observed in our experiments was due to an enzyme already present in the phage.

The substrate specificity of the nucleotidase has not been studied extensively. Although the results indicate that dAMP, dCMP, dGMP, and dTMP are substrates for the induced nucleotidase, it is not clear whether one or more enzymes are induced. By analogy with the dNMP kinase induced by T5 (3), probably only one enzyme is induced. Substrates such as 3'-dNMP, 5'-NMP, 3'-NMP, and nucleic acids have not been assayed yet. The question of how many enzymes are induced and the substrate specificity of each can only be resolved by purification of the induced activities, and such experiments are underway.

## ACKNOWLEDGMENTS

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## ADDENDUM IN PROOF

We have recently become aware of a report by H. J. Rahmsdorf, S. H. Pai, H. Ponta, P. Herrlich, R. Roskoski, Jr., M. Schweiger, and F. W. Studier (Proc. Nat. Acad. Sci. U.S.A. **71**:586–589, 1974) which describes the induction of a protein kinase by bacteriophage T7. The kinetics of appearance and disappearance of this enzyme are very similar to the data shown in Fig. 6 in this paper.

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