Biochemistry of Deoxyribonucleic Acid-defective Amber Mutants of Bacteriophage T4

III. NUCLEOTIDE POOLS*

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SUMMARY

Nucleotide pools were analyzed under different conditions of infection by bacteriophage T4, for three reasons: (a) as an approach to identifying the defective functions associated with conditional lethal phage mutants bearing uncharacterized lesions in DNA replication; (b) to gain information about the control of nucleotide synthesis in phage-infected Escherichia coli; and (c) as an aid to interpretation of earlier data (Matthews, C. K. (1968) J. Biol. Chem. 243, 5610) which suggested that the rate of total RNA synthesis decreases when DNA replication is blocked. In infection by wild type T4D there are no significant changes in ribo- or deoxyribonucleoside triphosphate pool sizes, except for replacement of dCTP by 5-hydroxymethyl-dCUT. By contrast, infection by DNA-negative mutants causes up to 30-fold pool expansions of dATP and dTTP, with hydroxymethyl-dCUT accumulating to similar extents. The dGTP pool, however, does not expand significantly, suggesting that the synthesis of this nucleotide is regulated more closely than those of the others. Ribonucleoside triphosphate pools do not expand dramatically in infection by mutants in genes 42, 44, or 62, but up to 5-fold pool expansion is seen in infection by mutants in genes 41 and 45. These studies indicate that the products of genes 41, 44, 45, and 62 do not directly affect the synthesis of DNA precursors. Moreover, comparative studies on the rates of labeling of RNA precursors late in infection support the earlier reported conclusion that the rate of RNA synthesis decreases considerably when DNA synthesis is blocked. Finally, the data allow calculation of the adenylate energy charge and of approximate concentrations of nucleotides in exponentially growing E. coli. The energy charge is about 0.9, in agreement with published work, and this value does not change significantly under the conditions of infection examined. The intracellular concentrations of nucleoside triphosphates range from 0.1 mM for dGTP to 2.7 mM for ATP. These values allow one to ask which of various enzyme feedback effects observed in vitro might reasonably be assumed to be operating in vivo.

The genome of bacteriophage T4 contains some 30 genes known to be involved, either directly or indirectly, in viral DNA synthesis (1-3). Some of these genes code for enzymes of DNA precursor synthesis, such as thymidylate synthetase or ribonucleoside diphosphate reductase. Others code for enzymes of DNA metabolism at the macromolecular level, such as DNA ligase or 5-hydroxymethylcytosine-glucosyltransferases. At least one gene, gene 32, codes for a nonenzymatic protein essential to DNA replication and recombination (4). Interest in this laboratory (5, 6) is focused upon those genes, particularly 41, 44, 45, and 62, whose products have not been identified either in terms of reaction catalyzed or specific function in DNA replication. Amber mutants in genes 44 or 45 are classified as DNA-negative, or DO, because phage DNA synthesis is virtually undetectable under nonpermissive conditions of infection (2, 5). Mutants in genes 41 and 62 have been classified by Warner and Hobbs as "D8" (2) because a small amount of DNA synthesis does occur after infection. None of the products of these four genes have been characterized, although it has been suggested that the gene 44 product is involved in initiation of replication (6), and that the gene 41 product participates along with DNA ligase (gene 30) in the conversion of nascent DNA fragments to high molecular weight DNA (7, 8).

By way of asking whether genes 41, 44, 45, or 62 control hitherto undiscovered steps in DNA precursor synthesis, I have determined nucleotide pool sizes following infection of Escherichia coli B with T4D (wild type) and appropriate conditional lethal mutants, as reported in this paper. Previous workers, notably Warner and Hobbs (9) and Elam and Koerner (10), have studied nucleotide pools in T-even phage-infected cells. However, Elam and Koerner studied only the nucleoside monophosphate pools and they confined their studies to wild type T2 phage, while Warner and Hobbs converted all nucleoside polyphosphates to monophosphates prior to separation and analysis. Thus, although they did observe deoxynucleotide pool changes in infection by various T4 mutants, they could not tell whether the changes were occurring primarily in pools of mono-, di-, or triphosphates. Moreover, because they used [3H]uracil to label nucleotides being analyzed, their data provided information only about the pyrimidine nucleotides. In the present study I have used 32P to label all nucleotides and have used thin layer chromatography on PEI-cellulose (11), to obtain data primarily on nucleoside triphosphate pools, al-
earlier conclusion was correct. Data in the present paper indicate that it was.

...though some data are presented as well on ribonucleoside mono- and diphosphate pools.

Two additional reasons for our interest in nucleotide pool analyses are as follows: (a) Several phage-coded enzymes of nucleotide metabolism, notably ribonucleoside diphosphate reductase (12, 13), deoxycytidylate deaminase (14, 15), and possibly thymidine kinase (16), exhibit complex feedback activation and inhibition effects in vitro in the presence of various nucleotides. By learning the intracellular concentrations of these nucleotides, and their fluctuations under various conditions of infection, one might ask whether effects observed in vitro might actually be physiologically significant; (b) Several years ago, on the basis of pulse-labeling data with [3H]uracil, I reported that the rate of RNA synthesis decreases at least 10-fold in infection by DO mutants when compared with infection by wild type T4. However, these data could be explained just as easily by proposing that blockage of DNA synthesis leads to accumulation of both deoxyribonucleotides and ribonucleotides (see Fig. 1). Thus, in a pulse-labeling experiment the intracellular RNA precursor pools would be of lower specific activity in a DO infection than under normal conditions, and radioactive label incorporated into RNA would be decreased even if the actual rates of RNA synthesis were identical. Since the putative RNA-DNA coupling is a phenomenon of some interest, it seemed desirable to determine unambiguously whether my earlier conclusion was correct. Data in the present paper indicate that it was.

**EXPERIMENTAL PROCEDURE**

*Escherichia coli* CR63 was used as a permissive host for propagation and assay of amber mutants of T4, and *E. coli* B was used as nonpermissive host for all experiments described in this paper. T4D and all phage mutants used came originally from the laboratory of Dr. R. S. Edgar. Table I lists all phage strains used, both by strain number and mutant type. For example, a strain of mutant type am44 refers to a phage bearing an amber mutation in gene 44, while ts42 refers to a phage bearing a temperature-sensitive mutation in gene 42, and so forth. This nomenclature will be used throughout the paper to identify strains used in each experiment both by mutant gene and type of mutation. All strains used were purified genetically in this laboratory by backcrossing at least twice against T4D.

The culture medium used for all experiments was Medium A, a low-phosphate Tris-glycerol casamino acids medium described by Kemper and Magasanik (17). The only phosphorus in this medium is the phosphate present in the casamino acids. The actual phosphate concentration of each batch of Medium A was determined from its phosphate concentration and its radioactivity, determined under acid-soluble pool. The actual specific activity of the medium was usually about 0.1 mM. Unless otherwise specified, cells were grown and infected at 37°C, under which conditions *E. coli* B has a mass doubling time of about 50 min. Phage lysates were prepared by growth and infection of cells in phosphate-buffered glycerol-casamino acids medium (Medium B) (19). Phage were purified by differential centrifugation and treatment with DNase and RNase. The final phage pellets were resuspended in Medium A.

All nucleotides used as chromatographic markers were purchased from P-L Biochemicals, except for dHTP, which was generously supplied by Dr. A. Kornberg. H332P04, [2-3H]uracil, and [5-3H]uracil were purchased from New England Nuclear. Thin layer chromatography was carried out on 20 × 20 cm sheets of PEI-cellose, 0.1-mm layers backed by heavy aluminum foil, supplied by Brinkmann Instruments.

Labeling and extraction of nucleotides was carried out as follows. Bacterial cultures were grown with forced aeration for at least 2.5 generations, to about 3 × 108 ml⁻¹, in Medium A, containing 32P in 10 μCi per ml. The specific activity of phosphorus in the medium was about 10 μCi per μmole, a level previously shown (20), and confirmed in these studies, not to affect the growth rate of the bacteria. The reason for the relatively low growth period in radioactive medium is the fact that a considerable fraction of the deoxyribonucleotide pool in phage-infected cells is derived from the breakdown of bacterial DNA (10), and it was necessary to bring the specific activity of this DNA as nearly as possible to the corresponding value for the acid-soluble pool. The actual specific activity of the medium in each experiment was determined from its phosphate concentration and its radioactivity, determined under counting conditions identical to those used for radioactive nucleotides.

Cells were sampled for viable count, then infected with phage at an average multiplicity of five to six, and at the indicated time intervals 5.0 ml aliquots were removed for extraction of nucleotides as follows. Each sample was passed rapidly through a 0.45-μm Millipore filter, which was immediately placed in 2.0 ml of 0.4 M ammonium formate buffer, pH 3.0, in an ice bath.

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**Table I**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mutant type</th>
<th>Mutant gene product</th>
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<tbody>
<tr>
<td>T4D</td>
<td>Wild type</td>
<td>Unknown</td>
</tr>
<tr>
<td>N81</td>
<td>am41</td>
<td>Unknown</td>
</tr>
<tr>
<td>N122</td>
<td>am42</td>
<td>Deoxycytidylate hydroxymethylase</td>
</tr>
<tr>
<td>L13</td>
<td>ts42</td>
<td>Deoxycytidylate hydroxymethylase</td>
</tr>
<tr>
<td>N82</td>
<td>am44</td>
<td>Unknown</td>
</tr>
<tr>
<td>E10</td>
<td>am45</td>
<td>Unknown</td>
</tr>
<tr>
<td>E1140</td>
<td>am62</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

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1 The abbreviations used are: dHMP, dDHP, and dHTP, 5'-deoxyhydroxymethyldeoxycytidine, 5'-dihydroxymethyl, and diphosphates, respectively; rNMP, rNDP, and rNTP, ribonucleoside mono-, di-, and triphosphate, respectively; dNTP, deoxyribonucleoside triphosphate; HMC, 5'-hydroxymethylcytosine.
The cell layer was scraped from the filter and resuspended with a flat-ended spatula, and each mixture was allowed to sit at ice temperature, with occasional gentle agitation, for 1 hour, following which it was centrifuged at 4°C for 10 min at 3000 x g and the precipitate discarded.

In some early experiments the ammonium formate buffer used for extraction was at pH 3.4. While this work was in progress Bagnara and Finch (21) reported that the extractability of nucleotides from E. coli by sodium formate buffers decreases sharply above pH 3.0. I found no significant differences in nucleoside triphosphate recoveries between ammonium formate buffers at pH 3.0 or pH 3.4. However, both ammonium formate extraction techniques gave considerably higher recoveries, in my hands, than extraction with perchloric acid, either by the recommended technique of Bagnara and Finch (21) or by the method of Neuhard and Thomassen (22).

Nucleotides in each extract were separated by two-dimensional thin layer chromatography on PEI-cellulose sheets (11). Aliquots of each extract were mixed with aqueous solutions of marker nucleotides at 0.01 M each. Each mixture was applied as a small spot at a location 2 cm in from each edge, near a corner, such that the final application was 20 μl of extract and 0.05 μmole of each marker. The sheets were washed in methanol (11) and dried. For chromatography of di- and triphosphates a Whatman No. 3MM filter paper wick was stapled to one edge of each sheet, and the solvent for the first dimension, 1.0 M LiCl saturated with boric acid and adjusted to pH 7.0, was allowed to run up the sheet and about 3 cm onto the wick. The sheets were removed and dried, each wick was cut off and discarded, and the sheets were washed in methanol and dried again. Chromatography in the second dimension employed the ammonium sulfate system of Randerath and Randerath (11), or, when it was desired to quantitate diphosphates as well as triphosphates, the stepwise formate buffer elution system (11). Nucleoside monophosphates were resolved by Procedure 2 of Randerath and Randerath (11). In general GMP and GDP were poorly resolved in the solvent systems used, and data for these nucleotides are not shown.

After chromatography, spots of marker nucleotides were located under ultraviolet light. Correspondence between radioactive material and ultraviolet light-absorbing material was verified by radioautography. Each chromatogram was placed against 3-M type R x-ray film for at least 16 hours. Provided that radioactive material is about 20% higher when radioactive spots were cut out than when the adsorbent containing radioactive material was scraped off the surface of a sheet and placed in a counting vial.

From the radioactivity of each nucleotide, the specific activity of phosphorus in the medium, and the cell concentration at the time of infection, it was possible to calculate each nucleotide pool size as picomoles per 10^6 cells. For presentation in this paper, these values have been converted to molecules of nucleotide per cell.

**RESULTS**

**Triphosphate Pools**—Fig. 2 shows the ribo- and deoxyribonucleoside triphosphate pools as a function of time after infection by T4D and mutants N82 (am44) and N122 (am42). Except for the replacement of dCTP by dHTP, there are no significant changes in triphosphate pool sizes after T4D infection. The failure of deoxyribonucleoside triphosphate pools to expand is of interest in light of the much higher rate of DNA synthesis in phage-infected, as compared with uninfected, cells (5, 23). Presumably the dNTP pools are maintained constant after infection by a much higher rate of turnover, although this has not yet been
accumulating. Accumulate might result from increased turnover rather than not yet been ruled out for infected cells. That failure of dGTP to change. Thus, it seems that in both infected and uninfected cells, the synthesis of dGTP is regulated more considerably, but that of dGTP does not change. Infection by N122 no detectable dHTP is formed, as expected from the mutation in gene 42, which leads to failure of these cells to form active dCMP hydroxymethylase. However, in other regards the pattern seen is similar to that observed with N82.

From the experimentally determined pool sizes, the number of nucleotides per T4 DNA molecule (approximately 4 × 109), and the nucleotide composition of T4 DNA, one can estimate the amount of each dNTP accumulating, in terms of phage-equivalent units per cell. For the 21-min sample in the N82 experiment of Fig. 1, these values are, for dATP, dTTP, dHTP, and dGTP, respectively, 27, 18, 50, and 1. Since N82 synthesizes far less than 1 phage-equivalent unit per cell of DNA (27, 5, 27, 32), it is apparent that the failure of dGTP to accumulate is not directly responsible for the absence of detectable DNA replication. Further insight into this point can be gained if we consider pool sizes in terms of millimolar concentrations. Freedman and Krish (29) have presented data from a Coulter counter which indicate that the mean cell volume of E. coli B/r, grown under conditions similar to those of this study, is about 0.0 μm3. If we assume that cells of E. coli B are of similar size and that triphosphates are not compartmented in the cell, then we can calculate approximate intracellular concentrations, as shown in Table II. Note that the least abundant nucleotide, namely dGTP, is present at approximately 0.1 mM. It is probable that this value is within a range of optimal concentrations for the T4 DNA replication machinery. For example, Goulain et al. (30) used a routine assay for T4 DNA polymerase (possibly the true “replicase”) in which each triphosphate was present at 0.033 mM, presumably within the range of optimal concentrations. Thus, the low level of dGTP following DO infections does not appear to contribute directly to the limitation of DNA synthesis.

In comparing the values determined in Table II with previously published data on triphosphate pools in E. coli, one is struck by the fact that most workers have reported pool sizes in terms which cannot be directly compared with our units of mass per cell, terms such as mass per unit of dry weight of cells (22, 25).

### Table II

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Pool size</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>1.47</td>
<td>2.7</td>
</tr>
<tr>
<td>GTP</td>
<td>0.57</td>
<td>1.1</td>
</tr>
<tr>
<td>CTP</td>
<td>0.37</td>
<td>0.7</td>
</tr>
<tr>
<td>UTP</td>
<td>0.75</td>
<td>1.4</td>
</tr>
<tr>
<td>dATP</td>
<td>0.10</td>
<td>0.3</td>
</tr>
<tr>
<td>dGTP</td>
<td>0.07</td>
<td>0.1</td>
</tr>
<tr>
<td>dCTP</td>
<td>0.11</td>
<td>0.2</td>
</tr>
<tr>
<td>dTTP</td>
<td>0.12</td>
<td>0.2</td>
</tr>
</tbody>
</table>

These values were obtained as the average of the values for the three zero time samples in the experiments of Fig. 1.

Dinitrouracil uptake—One could reasonably argue that pulse-labeling data do not reflect true rates of RNA synthesis even in the absence of ribonucleotide pool expansion because of the possible development of a barrier to the uptake of labeled uracil into cells. If such a barrier developed in DO infections but not in normal infection, then pulse-labeling data late in infection would underestimate the rate of RNA synthesis in DO infections. Accordingly, I measured rates of [2-3H]uracil uptake into cells early and late after infection by T4D, N82 (am44), and E10 (am45). As shown in Table III, a barrier to the uptake of uracil does not develop late in infection by the DNA-negative mutants.

### Rates of Labeling of RNA Precursors

A direct demonstration of the validity of pulse-labeling data can be obtained if we can...
show that immediate RNA precursors, namely the ribonucleoside triphosphates, become labeled during a pulse at equal rates in normal and DO infections. As many workers have pointed out (cf. References 35–37), the relationship between rate of incorporation of isotope into RNA and the rate of RNA synthesis depends critically upon the specific activity of labeled RNA precursors. Our earlier experiments were carried out under conditions where cells were grown before infection at a sufficiently high uracil concentration to repress endogenous pyrimidine synthesis. Hence, the infected cells were dependent upon exogenous uracil for synthesis of all pyrimidine nucleotides. Therefore, in order to conclude directly that pulse-labeling with uracil provides a valid index of rate of RNA synthesis, we must show that the rates of labeling of CTP and UTP pools are equal when one compares normal and DO infections late in infection, when the difference in rates of RNA labeling is greatest.

An experiment demonstrating the above point is shown in Fig. 4. In this experiment cells were labeled continuously with $^{32}$P, as described for the experiments in which absolute pool sizes were measured, and unlabeled uracil was present continuously as in our pulse-labeling experiments. At 25 min after infection by T4D or N82 (am44), [5-3H]uracil was added, and the cultures were sampled at 30 and 60 s after labeling. Under these conditions the $^3$H:$^{32}$P ratio provides a valid index of specific activity,
since $^{32}$P radioactivity is directly related to total nucleotide concentration. As shown in Fig. 4, the rates of UTP labeling and of CTP labeling are virtually identical between the two cultures.

Riva et al. (37) have performed a very similar experiment to show that the absolute rate of RNA synthesis decreases following a temperature upshift in a $ts^{43}$ T4 mutant. However, as these authors pointed out, their thin layer chromatographic system did not resolve ribonucleotides from deoxyribonucleotides, so that dNTP pool expansion occurring after a temperature upshift would tend to increase the $^{32}$P content of recovered UTP and CTP, and hence to cause underestimation of the true specific activities of pyrimidine rNTPs. However, both my experiment and that of Riva et al. lead unambiguously to the same conclusion that inhibition of T4 DNA synthesis leads to an inhibition of RNA synthesis. Thus, not only are replication and late gene transcription coupled (36), but replication and total transcription are coupled as well.

**Adenosylate Energy Charge**—Knowledge of the AMP, ADP, and ATP pools, as shown in Figs. 2 and 3, allows one to calculate the adenosylate energy charge, defined by Atkinson and coworkers (38) as \( \frac{[\text{ATP}]}{[\text{ATP}] + \frac{1}{2} [\text{ADP}]} \). For uninfected *E. coli* this value is about 0.90, only slightly higher than the value reported by Chapman et al. (38), and this value does not change significantly over the 21-min time period covered in these experiments.

**Other DO Mutants**—In Fig. 5 we see that extending the period of infection with N82 to 30 min allows one to detect a limited rNTP pool expansion. Considerably greater rNTP accumulation, however, up to 5-fold, is seen in infection with E10 (am45). This is interesting in light of our earlier observation (5) that uracil incorporation into RNA is even more severely depressed in infection by E10 than in infection by other DO mutants. It would appear that this extra inhibition is due to this rNTP pool expansion rather than an even more severely depressed rate of RNA synthesis, although I have not yet tested this directly by an experiment of the type shown in Fig. 4.

The expansion of ribonucleotide pools following E10 infection occurs primarily among the triphosphates, although, as shown in Fig. 6, limited accumulations of mono- and diphosphates can be seen.

Fig. 7 presents the triphosphate pool patterns for N81 (am41) and E1140 (am62). Both show the same type of dNTP pattern as previously described. Regarding the rNTP patterns, E1140 resembles N122 and N82 in that no dramatic changes occur after infection, while N81 shows large rNTP accumulations, just as seen with E10.

**On "Dual Function" of dCMP Hydroxymethylase**—Chiu and Greenberg (39) have suggested that the T4 gene 42 product, deoxycytidylate hydroxymethylase, plays two roles in the synthesis of phage DNA, first, the already established role of forming 5-hydroxymethyldeoxycytidylate from deoxycytidylate and deoxycytidylate hydroxymethylase, plays two roles in the synthesis of phage DNA, first, the already established role of forming 5-hydroxymethyldeoxycytidylate from deoxycytidylate and

![Fig. 5. Nucleoside triphosphate pools following infection of *Escherichia coli* B by T4D (left-hand panels), N82 (am44; center panels), and E10 (am45; right-hand panels).](http://www.jbc.org/)

![Fig. 6. Ribonucleoside mono- and diphosphate pools following infection of *Escherichia coli* B by N82 (am44: left-hand panels) or E10 (am45; right-hand panels).](http://www.jbc.org/)
El140 dNTP's $25

N81 rNTP's $50

R140 rNTP's

.-----•UTP

FIG. 7. Nucleoside triphosphate pools following infection of Escherichia coli B by N81 (am41; left-hand panels) or El140 (am62; right-hand panels). Note the difference in scales for the N81 and El140 data.

hence generating a pool of hydroxymethylcytosine nucleotides, and second, an as yet uncharacterized role as an element of the "DNA replication complex." This suggestion stemmed from the observation that an active dCMP hydroxymethylating activity seemed to be present following infection by T4 tsL13, a ts gene 42 mutant, at a nonpermissive temperature, but that no DNA is synthesized. Chiu and Greenberg concluded from this that the replication complex function is more heat-labile in the mutant gene 42 protein than is the hydroxymethylase activity. However, they did not verify their conclusion that the mutant hydroxymethylase is active in vivo by demonstrating that a pool of hydroxymethylcytosine nucleotides is formed at 42°. Presumably the HMC nucleotide accumulating would be dHTP, since an active gene 1 protein (deoxynucleotide kinase) is present. I have asked whether any dHTP is detectable following infection by L13 at 42°. As shown in Fig. 8, no detectable dHTP is formed, even though the dATP and dTTP pools expand significantly. This suggests that the mutant gene 42 protein is not active as a dCMP hydroxymethylase in vivo at 42°. In turn this makes somewhat less compelling the postulate that this protein plays a dual role, since the failure to generate HMC nucleotides can alone account for the inability of ts42 mutants to synthesize DNA at restrictive temperatures.

DISCUSSION

Three major questions emerge from the present work. (a) What is the nature of replication-total transcription coupling? (b) Why do ribonucleoside triphosphates accumulate in infection by some mutants (am41, am45) but not others (am42, am44, am62)? (c) Why do dGTP pools not expand concomitantly with those of dATP, dTTP, and dHTP?

Regarding the relationship between DNA synthesis and total RNA synthesis, Riva et al. (37, 40) have argued plausibly that replication-late transcription coupling is related to the formation of single-strand interruptions as a consequence of discontinuous replication of DNA. Such gaps or nicks, presumably, must be present for binding of RNA polymerase and initiation of transcription or both. In nonreplicating DNA nicks are repaired by DNA ligase essentially as rapidly as they are formed, such that initiation of transcription cannot occur. In this context it would be of interest to examine the integrity of parental DNA strands late in infection by DNA-negative mutants. If coupling is causally related to the presence of nicks, we would expect these strands to be relatively intact, whereas a considerable amount of short-stranded phage DNA can be detected at late times in normal infection (41). Of course, interpretation of the results of such an experiment might be complicated by repair or recombination effects.

A question which must be asked about the different patterns of rNTP accumulations is whether the observed phenomena are gene-specific or mutant-specific. So far all of our pool studies have been carried out with only one amber mutant in each gene. If we are to conclude that rNTP accumulation or nonaccumulation is related to the function of a particular gene product, we must be sure that all nonleaky amber mutants in a given gene show the same pattern, in other words, that the pattern observed does not result from some peculiarity of one mutant within a given gene. If the effects are found to be gene-specific, then we would want to ask whether rNTP accumulation under certain conditions is due to increased synthesis or decreased turnover. If it is the latter, then this would suggest that total RNA synthesis rates are even more severely blocked in those mutants showing accumulation than in those in which the rNTP pools do not expand, and this may ultimately provide a clue to the mechanism of coupling, particularly in conjunction with information on DNA strand integrity.

Presently available data shed little light on the question of why dGTP pools do not expand to the same extent seen with other dNTPs. In uninfected cells the failure of dGTP to accumulate when DNA synthesis is blocked results from decreased synthesis rather than increased turnover (22), and it seems reasonable to
assume that the same is true in infected cells. One might have expected that ribonucleotide reductase would be the key step at which deoxyribonucleotide synthesis is regulated. However, (a) the extensive accumulations of dATP, dTTP, and dHTP which occur suggest that feedback inhibition of this enzyme is quite ineffective in vivo; and (b) on the basis of in vitro studies with this enzyme, no conditions have yet been found in which ADP reduction is stimulated while GDP reduction is inhibited. Therefore, the critical step in regulating the rate of synthesis of dGTP probably lies elsewhere, and its ultimate identification will require a thorough analysis of all purine ribo- and deoxyribo-nucleotide pools.

Finally, some cautionary remarks should be made in light of the present work, about the widespread use of pulse labeling with radioactive thymine or thymidine to estimate rates of DNA synthesis in T-even phage-infected cells. As shown in Fig. 9, there are three metabolic routes for formation of thymidylate in the phage-infected cell; the action of either the host or phage-coded thymidylate synthetase and the breakdown of host DNA (27). In order for pulse labeling data to accurately reflect rates of DNA synthesis, one must show that the relative exogenous contribution of thymine nucleotides to DNA is the same under all conditions studied. This is almost certainly not the case when one compares infected with uninfected cells, for in uninfected cells two of the three endogenous pathways are not present. Moreover, the activity of these pathways changes with time after infection, with the viral synthetase beginning to be formed at about 5 min and thymine nucleotide release from host DNA beginning at 10 min (27). Thus, it seems quite unlikely that the exogenous contribution would remain constant during infection.

 Especially vulnerable to misinterpretation is the use of thymidine labeling to compare rates of DNA synthesis before and after temperature shiftup in infection by a tsDNA-defective mutant (cf. 37, 39, 42). This procedure is quantitatively inaccurate, for, as shown in this paper, the blockage of DNA resulting from a shiftup expands the dTTP pool, with a resultant decrease in its specific activity, and hence of the exogenous contribution. In addition, since several steps of thymidylate metabolism are feedback-regulated by dTTP (see reactions marked with asterisk in Fig. 9), there are additional, and unpredictable, changes in the relative activities of the various pathways which would result from dTTP pool expansion. To be sure, the quantitative errors introduced by these phenomena may be relatively small in experiments of short duration; I have not yet measured the precise kinetics of dNTP pool expansion after a shiftup. However, since all three endogenous routes to dTMP can be blocked by appropriate mutations (27, 43), with resultant total dependence upon exogenous thymine or thymidine for DNA synthesis, it is suggested that investigations on rates of DNA synthesis use appropriate mutant plagues and bacterial strains.

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Biochemistry of Deoxyribonucleic Acid-defective Amber Mutants of Bacteriophage T4: III. NUCLEOTIDE POOLS
Christopher K. Mathews


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