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Intracellular Thymidine Triphosphate Concentrations in Wild Type and in Thymine Requiring Mutants of *Escherichia coli* 15 and K12

I. R. Beacham, Kathryn Beacham, A. Zaritsky and R. H. Pritchard

Department of Genetics
University of Leicester, Leicester, England

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The intracellular concentration of thymidine triphosphate has been measured in thymine requiring mutants of *Escherichia coli* K12 and *E. coli* 15, and compared with that in non-mutant strains using thymine, thymidine and a mixture of thymine and deoxyguanosine, in the growth medium. The data show (1) that the thymidine triphosphate concentration rises as the thymine concentration in the growth medium is increased; (2) that the triphosphate concentration in both mutant strains is always less than that in the corresponding non-mutant strains; and (3) that thymidine inhibits its own conversion to thymidine triphosphate and also *de novo* synthesis of the triphosphate in non-mutant strains.

It is known that in thymineless mutants the replication time of the chromosome increases as the concentration of thymine in the medium decreases. The data presented in this paper suggest that the replication velocity is a function of, but not determined directly by, the intracellular thymidine triphosphate concentration.

1. Introduction

Mutants of *Escherichia coli* lacking thymidylate synthetase (*thy*$^-$) differ from non-mutant strains in two respects. They have lost the ability to synthesize thymidylate *de novo* but have simultaneously acquired the ability to utilize thymine in the growth medium as a source of thymidylate. At one level the nature of this pleiotropy has been made clear by work in a number of laboratories (Breitman & Bradford, 1967; Barth, Beacham, Ahmad & Pritchard, 1968; Munch-Petersen, 1968); *thy*$^-$ strains are able to incorporate thymine because, unlike non-mutant strains, they possess a pool of deoxyribose-1-phosphate which is required for the synthesis of thymidine from thymine, the first step in the conversion of thymine to thymidylate. We have previously shown (Beacham & Pritchard, 1971) that the dRib-1-P† is a breakdown product of pyrimidine deoxynucleotides whose concentration is elevated in *thy*$^-$ strains (Munch-Petersen, 1970). We have also suggested (Beacham, Barth & Pritchard, 1968) on the basis of indirect evidence, (a) that the internal concentration of dTTP in *thy*$^-$ strains is determined by the external thymine concentration and the internal dRib-1-P concentration, and rises progressively as the external thymine concentration

† Abbreviation used: dRib-1-P, deoxyribose-1-phosphate.

is raised and (b), that the internal dTTP concentration in *thy*$^-$ strains never reaches the level found in *thy*$^+$ strains, irrespective of the external thymine concentration. In the present paper we have tested postulates (a) and (b) directly by measuring the cellular concentration of dTTP in several strains under a variety of conditions. The results obtained are in accord with both of them.

Apart from providing a direct test of our hypothesis, the significance of these measurements lies in the possibility that under typical growth conditions the dTTP pool in *thy*$^-$ strains is sufficiently low to become the rate limiting step in the polymerization of DNA. With this possibility in mind Pritchard & Zaritsky (1970) determined the replication time of the chromosome of *E. coli* 15T$^-$ (555–7) as a function of thymine concentration. They found a progressive increase in the replication time as the thymine concentration in the medium was decreased. These changes in replication time were not associated with significant changes in growth rate.

Measurements of the dTTP pool at different concentrations of thymine are reported in this paper for both 15T$^-$ (555–7) and K12 (CR34). The values obtained are compared with the replication velocities at the corresponding thymine concentrations using the date of Pritchard & Zaritsky (1970) and Zaritsky & Pritchard (1971). This comparison suggests that the replication velocity is a function of, but not determined directly by, the intracellular dTTP concentration.

2. Materials and Methods

All radiochemicals were obtained from the Radiochemical Centre, Amersham, England.

(a) *Cultural conditions*

Cultures of all strains (listed in Table 1) were grown at 37°C in a New Brunswick gyrotory water bath using M9 synthetic medium (Pritchard & Lark, 1964) with glucose

TABLE 1

List of bacterial strains used

Strain	Genotype	Source
P162 (= CR34)	*thy*$^-$ *thr*$^-$ *leu*$^-$ *thi*$^-$ *dra*$^-$	
P154 (= C600)	*thr*$^-$ *leu*$^-$ *thi*$^-$	
P162-19	*thy*$^-$ *thr*$^-$ *leu*$^-$ *thi*$^-$ *dra*$^-$ *tpp*$^-$	P162†
P162-8	*thy*$^-$ *thr*$^-$ *leu*$^-$ *thi*$^-$ *dra*$^-$ *drm*$^-$	P162†
P162-191	*thr*$^-$ *leu*$^-$ *thi*$^-$ *dra*$^-$ *tpp*$^-$	P162-19, P1 transduction
E. coli 15T$^+$	Prototroph	
P178 (= 15T$^-$ (555–7))	*thy*$^-$ *arg*$^-$ *met*$^-$ *trp*$^-$ *drm*$^-$	
P178-1	*thy*$^-$ *arg*$^-$ *met*$^-$ *trp*$^-$ *tpp*$^-$	P178 (see text)
P178-2	*arg*$^-$ *met*$^-$ *trp*$^-$ *tpp*$^-$	P178-1 (see text)

† Obtained by selection for thymidine resistance (see Beacham, Eisenstark, Barth & Pritchard, 1968). Symbols are those used by Taylor (1970).

(0·4%) as carbon source. The medium was supplemented with L-amino acids (20 μg/ml.) and thiamine (2 μg/ml.) if necessary. Other supplements were supplied at concentrations indicated in each experiment. Cells were harvested in the exponential growth phase when the absorbance at 450 nm was not more than 0·4. An absorbance of 0·4 corresponds

to $1{\cdot}37\times10^8$ particles/ml. in the case of P178 and $9{\cdot}4\times10^7$/ml. for P162 in cultures grown on high concentrations (400 μM) of thymine.

(b) *P1 transduction*

Strain P178 supports growth of phage $P1_{vir}$ (a virulent mutant of P1kc) giving a plaque forming titre of about 30%, that found with K12 strains. It is also transducible by phage grown on *E. coli* K12 strains. Several other strains of *E. coli* 15 were found to be insensitive to phage P1. The technique of Ahmad & Pritchard (1969) was used, with a multiplicity of infection of 0·2. Transductant P178-1 (*drm*$^+$ *tpp*$^-$) was obtained from P178 (*drm*$^-$ *tpp*$^+$) using phage grown on P162-19, and selecting for transductants able to grow on guanosine as sole carbon source (see Ahmad & Pritchard, 1969). The majority of these *drm*$^+$ transductants were also *tpp*$^-$. Transductant P178-2 was obtained from P178-1 using P1 grown on an *E. coli* K12 *thy*$^+$ strain.

(c) *Measurements of pyrimidine nucleotide pools*

Cultures were uniformly labelled at specific activities and concentrations of labelled precursors indicated in the text or Figure legends. Absorbance (450 nm) was determined immediately before extraction. Extracts were prepared by rapidly passing a 5-ml. culture through a Millipore filter and immediately placing the filter in 1·2 ml. of ice-cold 5% trichloroacetic acid. After centrifugation at 8000 rev./min for 10 min, the trichloroacetic acid was extracted ten times with 5 ml. of ether. The extract (100 μl.) was then analysed by thin-layer chromatography on polyethyleneimine-impregnated cellulose (Randerath & Randerath, 1964; Neuhard, Randerath & Randerath, 1965). Two procedures were used: (a) for the measurement of the dTTP pool in *thy*$^-$ strains a relatively non-specific separation was used, since specific isotopically labelled precursors ([2-^{14}C]thymine or [2-^{14}C]thymidine) were available. The extract was applied to a 20 cm × 20 cm chromatogram and developed to 15 cm in the first dimension with distilled water. This removed any labelled thymine (or thymidine) carried over from the medium. The chromatogram was then developed in the second dimension with 1 M-LiCl. In this solvent dTMP, dTDP and dTTP separate with R_f values of 0·80, 0·55 and 0·20, respectively (see Randerath & Randerath, 1964). However, only dTTP and dTDP could be measured since other u.v. absorbing material was found near the dTMP spot. This material appeared to move slightly in distilled water and contained ^{14}C. (b) For the measurement of the dTTP pool in *thy*$^+$ strains [^{14}C]glucose and/or labelled uracil was used. Any residual labelled uracil was removed by development in water as in (a). All eight nucleoside triphosphates were then separated as described by Neuhard *et al.* (1965).

For both procedures (a) and (b) about 20 nmoles of unlabelled marker compounds were co-chromatographed and all spots located by u.v. absorption. The spots were cut out and counted in a liquid-scintillation counter.

(d) *Determination of relative contribution of [^{14}C]glucose and [^{3}H]thymine to DNA-thymine in* thy$^-$ *mutants*

Cultures were uniformly labelled with [^{14}C]glucose (1·5 μCi/μmole; 6·9 mM) and [^{3}H]thymine (5 μCi/μmole), harvested, and washed with Tris–EDTA buffer. DNA was extracted using lysozyme, sodium lauryl sulphate and pronase, and purified by banding it twice in a caesium chloride gradient. The purified DNA was dialysed against water, lyophilysed and hydrolysed with concentrated formic acid in a sealed tube for 30 min at 175°C. The formic acid was evaporated and the hydrolysate redissolved in a solution of unlabelled thymine, uracil and cytosine (200 μg/ml. each). The mixture was chromatographed on a cellulose thin-layer, using distilled water (adjusted to pH 9 with NH_4OH) in the first dimension and *n*-butanol:water (86:14 v/v) in the second. The spots were detected by u.v. absorbtion and each cut into several pieces before counting in a liquid-scintillation counter. The ratio of ^{3}H:^{14}C counts in various pieces of each spot was variable, presumably due to contamination with other ^{14}C-containing material. The pieces with the highest ^{3}H:^{14}C ratio were used. Values were calculated on the basis of (a) the known specific activities of each isotope, and (b) the counting efficiencies, which were determined experimentally.

3. Results

All strains used in these experiments carry at least one additional mutation giving the phenotype Tlr (=thymine low requirement) in addition to the *thy*$^-$ mutation. As first shown by Breitman & Bradford (1967) and later by others (Breitman & Bradford, 1968; Barth *et al*, 1968; Munch-Petersen, 1968), Tlr strains have lesions in the genes specifying either deoxyribomutase (*drm*) or deoxyriboaldolase (*dra*) (see Fig. 1). The effect of these mutations is to block further degradation of dRib-1-P.

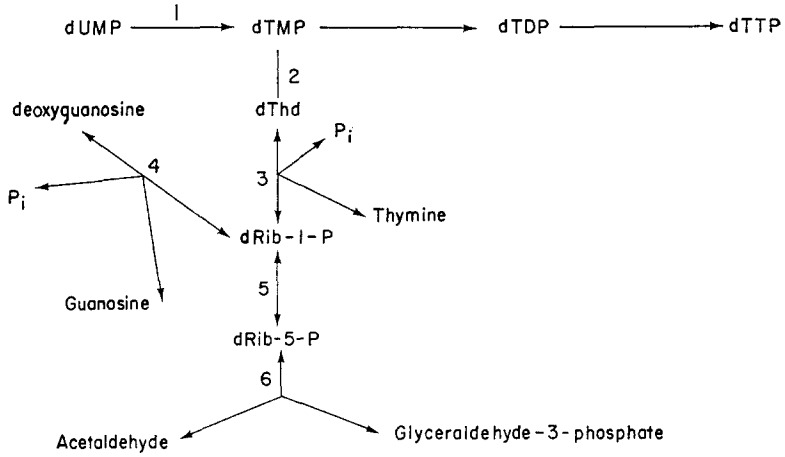

FIG. 1. The synthesis of thymidine triphosphate and the catabolism of deoxynucleosides. The numbers refer to the following enzymes, which are also mentioned in the text: 1, thymidylate synthetase; 2, thymidine kinase; 3, thymidine phosphorylase; 4, purine phosphorylase; 5, phosphodeoxyribomutase; 6, deoxyriboaldolase. Since the enzyme(s) which is responsible for the replication of DNA has not been definitely identified, it is conceivable that dTTP is not a precursor of DNA *in vivo*. However for the purposes of this paper, it will be assumed either that dTTP is the precursor, or that its concentration reflects that of the true precursor.

The higher concentration of this compound so maintained permits strains carrying these mutations to grow on lower concentrations of thymine than corresponding *dra*$^+$ or *drm*$^+$ strains. Strain P178 is *drm*$^-$ (unpublished data) and P162 is *dra*$^-$ (Barth *et al.*, 1968).

In the experiments reported below the minimum thymine concentration used for any strain is that compatible with normal growth, using the criteria for normal growth previously described (Pritchard & Zaritsky, 1970; Zaritsky & Pritchard, 1971). By these criteria the minimum concentration of thymine compatible with normal growth is 2 μM for strain P178 and 20 μM for P162. This tenfold difference is not due to the fact that the former strain is *drm*$^-$ and the latter *dra*$^-$, since isogenic derivatives of P162 which are *drm*$^-$ have a similar thymine requirement to P162.

(a) *Leakiness of* thy$^-$ *mutations in P162 and P178*

Since most of the estimations of the thymine nucleotide pools described in this paper were based on the use of radioactive thymine, it was necessary to determine the relative contributions of the labelled thymine and endogenously synthesized thymine to these pools. For this purpose cultures were uniformly labelled with [^{14}C]glucose

and [^{3}H]thymine and the contribution of [^{14}C]glucose to DNA-thymine was measured (Table 2). The data show that in both strains the contribution of [^{14}C]glucose to DNA-thymine is not more than 5% even when the external concentration of [^{3}H]thymine is the minimum compatible with normal growth. We have made no corrections for this small contribution in subsequent experiments.

TABLE 2

Relative contribution of [^{14}C]glucose to DNA-thymine in thy$^-$ *mutants grown on different thymine concentrations*

Strain	Thymine concn (μM)	Contribution of [^{14}C]glucose (%)
P178	4·0	5·0
P178	40·0	3·0
P162	16·0	3·5
P162	160·0	4·2

For experimental details, see Materials and Methods.

(b) *The dTTP and dTDP concentration as a function of thymine concentration*

The relevant data are shown in Figures 2 and 3. In both strains there is, as predicted (Beacham, Barth & Pritchard, 1968), a progressive rise in the dTTP pool as the thymine concentration in the medium is increased. There are significant differences in the behaviour of the two strains, however. First, at non-saturating thymine concentrations the dTTP pool of P178 is greater than in P162, as might have been expected from the difference in the thymine requirement of the two strains. In addition, however, the maximum dTTP concentration attainable at saturating thymine concentrations is two to threefold greater in P178 than in P162. Also the dTDP:dTTP

TABLE 3

Relative amounts of pyrimidine nucleoside triphosphates derived from [^{14}C]glucose and [^{3}H]uracil

Strain:	15T$^+$			P154		
	Amount of pyrimidine nucleoside triphosphate derived from:					
	[^{14}C]glucose	[^{3}H]uracil	Total	[^{14}C]glucose	[^{3}H]uracil	Total
	(μmoles $\times$ $10^{-4}/A_{450}$)			(μmoles $\times$ $10^{-4}/A_{450}$)		
dTTP	0·54	1·02 (66)	1·54	0·46	1·12 (71)	1·58
dCTP	0·18	0·355 (66)	0·54	0·15	0·38 (72)	0·53
CTP	0·53	0·93 (64)	1·45	0·65	1·09 (63)	1·74
UTP	1·57	3·92 (71)	5·49	1·27	3·33 (72)	4·6

Cells were grown in a 5-ml. culture containing [^{14}C]glucose (uniformly labelled, 3 μCi/μmole; 10 mM) and 6-[^{3}H]uracil (400 μCi/μmole; 200 μM) to a final A_{450} of about 0·64. The figures in brackets in the uracil column are the percentage of total. The percentage contribution by uracil to the total pool is the same for all four triphosphates indicating an absence of radiochemical contamination of the triphosphates by other ^{14}C-containing compounds derived from [^{14}C]-glucose.

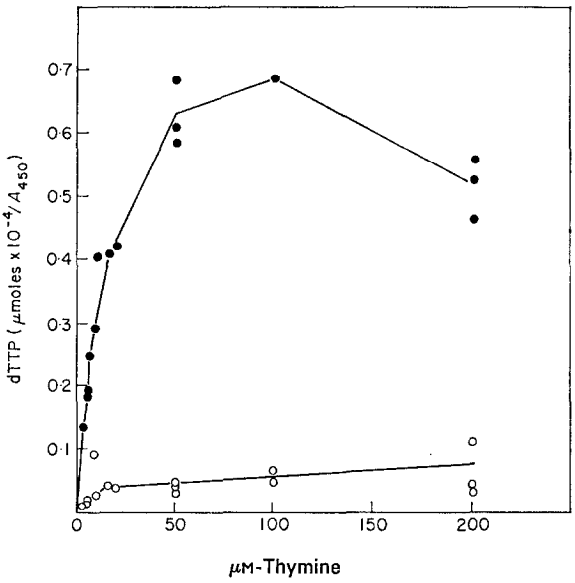

FIG. 2. Intracellular concentrations of dTTP and dTDP in P178 as a function of the thymine concentration. (●) dTTP; (○) dTDP.

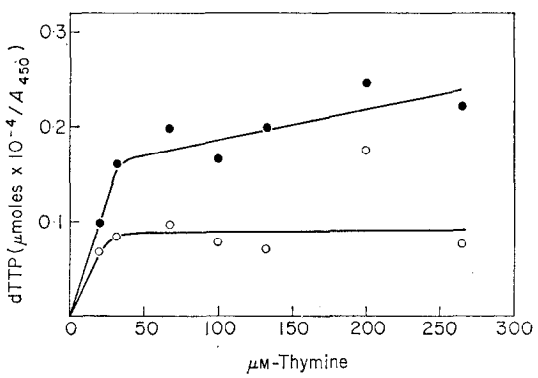

FIG. 3. Intracellular concentrations of dTTP and dTDP in P162 as a function of the thymine concentration. (●) dTTP; (○) dTDP.

ratio is very different in the two strains: in P162 the concentration of dTDP is about half that of the triphosphate, whereas in P178 it is only about 10%. These differences will be discussed later.

To determine whether the dTTP concentration at saturating thymine concentrations reaches the value it has in the corresponding *thy*$^+$ strains, we measured the thymidine triphosphate pool in *E. coli* 15 and P154 (C600) using labelled uracil. In order to use labelled uracil for this purpose it was necessary to determine the relative contributions of external uracil and endogenously synthesized uracil to the pyrimidine nucleotide pool. In the experiment shown in Table 3, [^{14}C]glucose was used to estimate the latter and [^{3}H]uracil the former. It may be seen that 67 to 70% of the pyrimidine triphosphate pools are derived from [^{3}H]uracil. This compares with an 80 to 85%

TABLE 4

Thymidine triphosphate pool in thy$^+$ *strains of* E. coli *15 and K12*

Strain	dTTP†
P154	1·20
15T$^+$	1·49
P162-191	1·11
P178-2	1·15

† μmoles $\times 10^{-4}/A_{450}$.

Cultures were grown in [^{14}C]uracil (20 μCi/μmole; 200 μM) and the dTTP pool determined as in Materials and Methods (procedure (b)). The pool size was calculated assuming a 70% contribution by uracil to the dTTP pool for all strains (see Table 3).

contribution determined by others (Bolton & Reynard, 1954; Dilworth-Cannon & Breitman, 1967*a*,*b*) who measured the relative incorporation of [^{14}C]uracil into the thymine residues of DNA.

From a comparison of the values for the dTTP pool in Table 3 with those in Figures 2 and 3 it is clear that the maximum dTTP pool attainable in P178 and P162 is smaller than that in the two *thy*$^+$ strains. In order to rule out the possibility that this difference might be due to inherited differences in the two strains distinct from the *thy*$^-$ mutation the dTTP pool was also determined in the *thy*$^+$ transductants P178-2 and P162-191 (Table 4). The results show that the dTTP pool in the *thy*$^+$ transductants is very similar to that in the other *thy*$^+$ strains examined.

The important conclusion to be drawn from these determinations is that in all four *thy*$^+$ strains (see Table 4), the dTTP pool is larger than that attained in *thy*$^-$ strains even at saturating thymine concentrations.

(c) *The dTTP pool in* thy$^-$ *strains in the presence of thymine and deoxyguanosine, and in* thy$^-$ tpp$^-$ *strains in the presence of thymidine*

In order to test the possibility that the maximum attainable pool in *thy*$^-$ strains is less than that in *thy*$^+$ strains because the supply of dRib-1-P is limited (such that at high concentrations of thymine essentially all available dRib-1-P is converted to thymidine), the dTTP pool was measured in the presence of thymine and deoxyguanosine. It is known that the addition of a deoxynucleoside promotes the uptake of thymine in *thy*$^+$ strains, presumably by providing a source of dRib-1-P through catabolism of the deoxynucleoside *via* phosphorylases (Boyce & Setlow, 1962; Kammen, 1967; Munch-Petersen, 1967). If dRib-1-P is the limiting factor then the addition of deoxyguanosine should make it possible to raise the dTTP pool to wild-type levels. The results (Figs 4 and 5) show that in P178 and P162-8 the pool is raised at low thymine concentrations by the addition of deoxyguanosine indicating that at these concentrations the pool size of dRib-1-P is limiting the rate of formation of dTTP. In both strains, however, the maximum dTTP pool size in the presence of deoxyguanosine was hardly raised at all above that attainable by high concentrations of thymine alone. In fact, in the case of P178 (Fig. 4), a decrease in the pool size is observed at the higher thymine concentrations used. We conclude that a shortage of dRib-1-P cannot be the only factor limiting the size of the dTTP pool in the *thy*$^-$ strains.

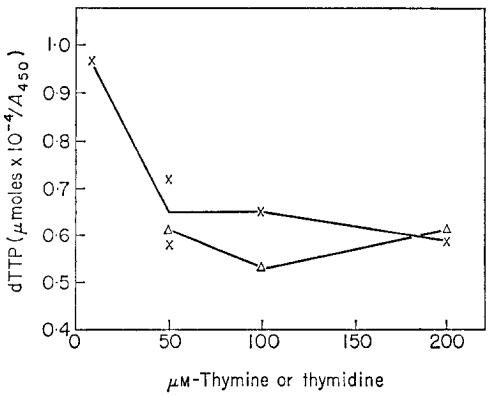

FIG. 4. The intracellular concentration of dTTP in P178 and P178-1 as a function of the thymine or thymidine concentration. –×–×–, P178 with thymine plus deoxyguanosine (1 mM); –△–△–, P178-1 with thymidine.

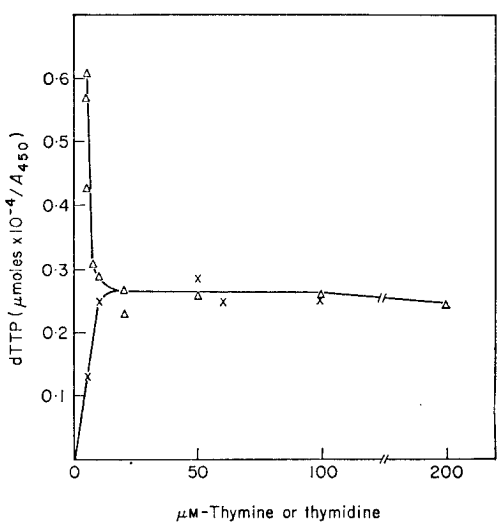

FIG. 5. The intracellular concentration of dTTP in P162 derivatives as a function of the thymidine or thymine (plus deoxyguanosine) concentration. (△) P162-19 with thymidine as a supplement; (×) P162-8 with thymine plus deoxyguanosine (1 mM at all thymine concentrations). P162 (*dra*$^-$) was not used since its growth is inhibited by deoxyguanosine, whereas P162-8 (*dra*$^-$ *drm*$^-$) is resistant.

This conclusion was confirmed by measuring the dTTP pool, in cultures grown on thymidine as a source of thymidylate. In order to use thymidine as a supplement, it was necessary to use a thymidine phosphorylase negative (*tpp*$^-$) mutant since otherwise the thymidine would be rapidly catabolised to thymine (Rachmeler, Gerhart & Rosner, 1961; Fangman & Novick, 1966). The dTTP pool in *thy*$^-$ *tpp*$^-$ mutants, measured using labelled thymidine, is also given in Figures 4 and 5. The results clearly show that wild-type (*thy*$^+$) pool sizes are not obtained. A more striking feature of the results is that in the case of P162-19, a *decrease* in the dTTP pool occurs with increasing thymidine concentration (Fig. 5). This strongly suggests that thymidine inhibits a step in the formation of dTTP from thymidine. This may also be the

explanation for the decrease in dTTP concentration with increasing thymine concentration in the presence of deoxyguanosine found in strain P178 (see Fig. 4).

(d) *The thymidine triphosphate pool in* thy$^+$ tpp$^-$ *strains in the presence and absence of thymidine*

In view of the fact that thymidine may have an inhibitory effect upon dTTP synthesis in P162-19, the effect of thymidine on the *de novo* synthesis of dTTP in *thy*$^+$ transductants of P162-19 and P178-1 was investigated.

In these experiments [^{14}C]uracil was used to estimate the internal contribution to the dTTP pool, and [^{3}H]thymidine to estimate the contribution made by external thymidine *via* thymidine kinase. The results (Table 5) clearly show that in both *thy*$^+$

TABLE 5

Relative contributions of de novo *synthesis and of external thymidine to the dTTP pool in* thy$^+$ tpp$^-$ *strains*

Strain	Thymidine concn (μM)	Amount of pyrimidine triphosphate derived from: *de novo* synthesis				[^{3}H]thymidine
		dTTP	dCTP	CTP	UTP	dTTP
P178-2	Nil	1·20	0·31	1·26	4·54	—
P178-2	100	0·40	0·52	1·82	5·70	0·31
P162-191	Nil	1·11	0·54	1·56	5·43	—
P162-191	4	0·38	0·39	1·69	4·61	0·34
P162-191	100	0·50	0·55	1·87	4·41	0·09

All values are expressed as μmoles $\times 10^{-4}/A_{450}$. Cultures were grown in the presence of [^{14}C]-uracil (20 μCi/mole; 200 μM) and [^{3}H]thymidine (200 μCi/μmole). The *de novo* pathway was estimated from the amount of ^{14}C in dTTP; the amount of dTTP formed from external thymidine was estimated from the tritium content.

transductants, thymidine at a concentration of 100 μM causes a 50 to 60% reduction in the *de novo* synthesis of dTTP; the total dTTP pool was also decreased by 40%. These results are thus consistent with an inhibition of one of the steps between thymidine and dTTP.

The contribution of thymidine to the dTTP pool is 44% in P178-2 and this is consistent with a 50% contribution from [^{14}C]dTMP to DNA-thymine observed by Dilworth-Cannon & Breitman (1967*a*,*b*) in *E. coli* 15; the use of dTMP is equivalent to thymidine since thymidine is an intermediate in the utilization of dTMP (Lichtenstein, Barner & Cohen, 1960; Dilworth-Cannon & Breitman, 1967*a*; Beacham, unpublished results). The contribution of thymidine in P162-191, on the other hand, is about 15%, and this is inconsistent with a 50% contribution to DNA-thymine observed by Fangman (1969) in a *thy*$^+$ *tpp*$^-$ strain of *E. coli* K12. However, this author used only 4 μM-thymidine, and for this reason the contribution to dTTP was measured in P162-191 using this concentration of thymidine. The results (Table 5) show that, in agreement with the observations by Fangman (1969), a 54% contribution to the dTTP pool is obtained when 4 μM-thymidine is used. The *de novo* synthesis of dTTP is still inhibited and the total pool remains below the wild-type level.

It may be noted that there is no significant reduction in the dCTP pool in any of these experiments, suggesting that the inhibition is subsequent to ribonucleotide reductase.

4. Discussion

(a) *Factors determining the intracellular dTTP concentration*

We have previously suggested (Beacham, Barth & Pritchard, 1968) that the reason why *thy*$^-$ strains possess a pool of dRib-1-P is that the block in the *de novo* synthesis of dTTP leads to a reduction in the intracellular concentration of this compound which in turn, by loss of both feedback inhibition and repression of ribonucleotide reductase, causes an accumulation of the other deoxynucleotides which are then degraded to dRib-1-P. On this hypothesis, although the intracellular concentration of dTTP would increase with increasing concentrations of thymine in the growth medium, it could not rise to the wild-type level because if it were to do so, the supply of dRib-1-P necessary for its synthesis would be cut off.

Although the data presented in this paper confirm that there is a smaller dTTP pool in *thy*$^-$ than in *thy*$^+$ strains they do not support our initial hypothesis to account for this difference. Three observations suggest that the size of the dTTP pool in *thy*$^-$ strains is limited by an inhibitory effect of thymidine on one of the subsequent steps in dTTP synthesis. Thus, the presence of deoxyguanosine in the growth medium, to provide an additional source of dRib-1-P, reduces rather than increases the dTTP concentration in strain P178, as the thymine concentration is raised. The addition of thymidine itself has a similar effect in P162-19 and the addition of thymidine to a culture of a *thy*$^+$ strain inhibits the synthesis of dTTP and reduces the intracellular concentration of this compound. An inhibitory effect of thymidine on its own conversion to dTTP has also been observed by J. Cairns (personal communication) in *E. coli* 15T$^-$ (555–7).

Which of the steps in the synthesis of dTTP is inhibited by thymidine is not clear. Since both *de novo* synthesis and synthesis from thymidine are inhibited by thymidine, it seems probable that the inhibited step is common to both routes. Since in P162 and its derivatives, in which the inhibitory effect is greater than in P178, the dTDP concentration was relatively large both in cultures grown on thymine (Fig. 2) and when the medium contained thymidine or thymine plus deoxyguanosine (data not shown), the inhibited step may be the conversion of dTDP to dTTP (see Fig. 1). It might be argued that the difference in the ^3H:^{14}C ratio in dTTP, when different concentrations of thymidine are used (Table 5), indicates a different sensitivity to thymidine of *de novo* synthesis and synthesis *via* thymidine, but an alternative possibility is that since reducing the dTTP pool leads to an increase in the intracellular concentration of dUMP (Munch-Petersen, 1970), which will be the ^{14}C-labelled precursor of dTTP, a greater proportion of the dTTP will be ^{14}C-labelled.

The very low thymine requirement of P178 compared with most Tlr strains (e.g. P162) deserves consideration. As might have been predicted P178 possesses a higher intracelluar dTTP concentration at all thymine concentrations than P162. The greater efficiency with which thymine is converted to dTTP in P178 is probable due in part to a smaller inhibitory effect of thymidine on its synthesis. There is evidence, in addition, that at low thymine concentrations the supply of dRib-1-P is greater in P178 since the concentration of dTTP in P162 is increased by the presence of deoxyguanosine and approximates that for P178 growing on thymine alone (Figs 2 and 5). These differences between P162 (a derivative of *E. coli* K12) and P178 (a derivative of *E. coli* 15) may be strain specific. It seems equally likely, however, that the Tlr mutation in the original *thy*$^-$ mutant of *E. coli* 15 was selected in a medium containing

a very low thymine concentration (see Roepke, 1967) and that this imposed a selective pressure favouring an additional mutation, apart from that in the *drm* gene, which results in a more efficient utilization of thymine. An additional mutation having this effect has been found in an *E. coli* K12 strain (Ahmad & Pritchard, 1971) but in this case present evidence suggests that this mutation is in a regulator gene and leads to constitutive synthesis of enzymes involved in the production of dRib-1-P.

The low dTTP pool in *thy*$^-$ strains, reported here, may not be typical of all *thy*$^-$ strains. Recently published data by Munch-Petersen (1970) show similar dTTP concentrations in a *thy*$^-$ strain growing on high concentrations of thymine, and in its *thy*$^+$ parent. Nevertheless, from a practical point of view the inhibitory effect of thymidine on the synthesis of dTTP means that the reduced replication velocity found in *thy*$^-$ strains growing on thymine (Pritchard & Zaritsky, 1970; Zaritsky & Pritchard, 1971) may not necessarily be completely overcome by addition of a deoxynucleoside to the growth medium.

(b) *Replication velocity as a function of the intracellular dTTP concentration*

Pritchard & Zaritsky (1970) suggested that the change in replication velocity associated with changes in the concentration of thymine in the growth medium was determined either directly by changes in the internal dTTP concentration or by associated changes in the concentration of other deoxynucleotides. In Figure 6 we have plotted the chromosome replication time (C) of P162 and P178 as a function of the intracellular dTTP concentration using the data of Pritchard & Zaritsky (1970) and Zaritsky & Pritchard (1971). It is clear that the replication time changes much more steeply with changes in the dTTP concentration in P162 than it does in P178. Although it is possible that the DNA polymerases in the two strains differ in

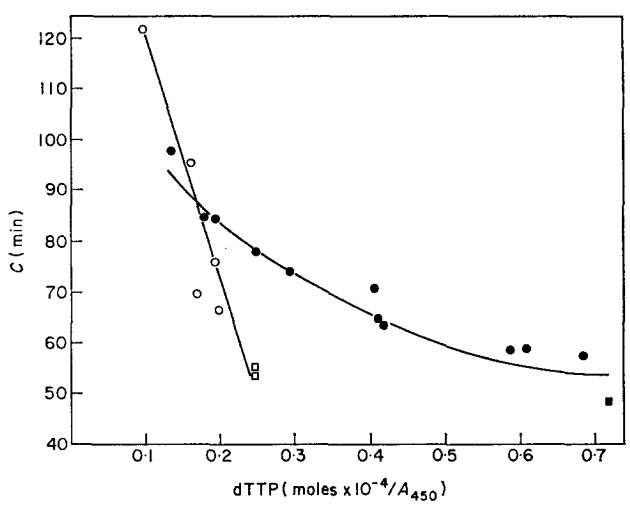

FIG. 6. The replication time (C) as a function of the intracellular dTTP concentration in P162 and P178. Curves were constructed using the experimental values for intracellular dTTP concentrations reported in this paper; the only exception was the dTTP concentration in the presence of 40 μM-thymine and deoxyguanosine for P178 which was obtained from Fig. 4 by interpolation. Corresponding values for C were obtained from the curves in Zaritsky & Pritchard (1971), except for the values for C in the presence of thymine and deoxyguanosine which are from their tabulated data. Values of C were not taken from the extrapolated portions of the curves of Zaritsky & Pritchard (1971). (○) Thymine alone and (□) thymine plus deoxyguanosine for strain P162. (●) Thymine and (■) thymine plus deoxyguanosine for P178.

their response to changes in the concentration of dTTP, we think it more likely that this difference reflects differences in the response of the other deoxynucleotides to changes in the dTTP pool and that, as might be expected, the replication velocity is determined by the relative concentrations of all four deoxynucleotide substrates of the polymerase.

This second possibility is supported by the observation (Munch-Petersen & Neuhard, 1964; Neuhard, 1966) that a reduction in the dTTP pool is associated with changes in the intracellular concentrations of the other deoxynucleotide triphosphates. Characteristically there is an associated increase in the concentrations of dATP and dCTP. but the relative increase in the concentrations of these two deoxynucleotides differs markedly under identical conditions in different strains. Thus, when thymine is removed from the growth medium there is no increase in dCTP in derivatives of *E. coli* 15T$^-$ but an increase of several-fold in a *thy*$^-$ mutant of *E. coli* B (Neuhard, 1966). Such differences may also exist between P162 and P178.

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