Control of Deoxynucleotide Biosynthesis in *Escherichia coli*. I. Decrease of Pyrimidine Deoxynucleotide Biosynthesis *in Vivo* in the Presence of Deoxythymidylate*

W. Dilworth Cannon, Jr., and T. R. Breitman

ABSTRACT: In *Escherichia coli* 15, 50% of the thymine incorporated into deoxyribonucleic acid (DNA) was derived from exogenous deoxythymidine 5′-monophosphate (dTMP). In addition, cells grown in the presence of 10^{-4} M dTMP have (a) a 50% increase of the total thymine deoxynucleotide pool, (b) approximately a 35% reduction of the contribution *de novo* to this pool, (c) approximately 30% reductions of the uracil and cytosine deoxynucleotide pools, and (d) approximately 30% increases of the uracil and cytosine ribonucleotide pools. These results and the finding of only a small excretion into the medium of thymine compounds derived from synthesis *de novo* indicate regulation *in vivo* of pyrimidine deoxynucleotide biosynthesis at the ribonucleotide reductase level.

Preferential incorporation into bacterial nucleic acid of exogenous nucleic acid bases or their nucleoside derivatives has been shown to result from decreased biosynthesis *de novo* of the corresponding nucleotide. The poor incorporation of thymine and dThd[1] into wild-type *Escherichia coli* DNA is an exception to an otherwise general rule. Because of its rapid degradation to thymine, dThd is incorporated efficiently for only a relatively short time interval (Rachmeler *et al.*, 1961). However, values of 50 and 100% of newly synthesized DNA-thymine have been derived from exogenous dTMP[2] (Siminovitch and Graham, 1955; Lichtenstein *et al.*, 1960). These findings suggested two possibilities: (a) that thymine deoxynucleotide synthesis *de novo* was regulated by thymidylate synthetase, ribonucleotide reductase, or another enzyme in the pyrimidine nucleotide biosynthetic pathway, or (b) an absence of regulation of biosynthesis *de novo* and dilution of thymine deoxynucleotides synthesized *de novo* by those derived from the exogenous source, as was shown in human leukocytes (Cooper *et al.*, 1966).

In the present study, a distinction was made between the above two possibilities by comparing the steady-state level of pyrimidine nucleotide pools in *E. coli* grown with and without exogenous dTMP. The results indicate that dTMP synthesis *de novo* is controlled at the ribonucleotide reductase level.

Experimental Section

Chemicals. Nonradioactive pyrimidine bases, nucleosides, nucleotides, and *p*-nitrophenyl phosphate were purchased from Calbiochem. [2-^{14}C]Uracil was obtained from New England Nuclear Corp. [^{3}H]-dTMP (labeled in the base) was obtained from Schwarz Bio-Research. Chromatographically pure *E. coli* alkaline phosphatase was purchased from Worthington. AG1-X8 (Dowex-1 chloride), 200–400 mesh, was purchased from Bio-Rad.

Paper Chromatography. Descending chromatography was carried out on Whatman No. 1 paper with the following solvents: solvent I, ethyl acetate–formic acid–water, 60:5:35 (Fink *et al.*, 1956); solvent II, 1-butanol–water, 86:14 (Markham and Smith, 1949); solvent III, isopropyl alcohol–hydrochloric acid–water, 170:41:39 (Wyatt, 1951); and solvent IV, isobutyric acid–ammonium hydroxide–water, 66:1:33 (Magasanik *et al.*, 1950).

Counting of Radioactive Compounds. Radioactive pyrimidine compounds were located on paper chromatograms with ultraviolet light or by autoradiography. In either case, to determine radiochemical purity, the chromatograms were cut into strips and counted in toluene phosphor solution in a liquid scintillation spectrometer. Pyrimidine compounds were eluted from the paper with water; ultraviolet spectra at pH 2 were obtained using blank paper eluates. The aqueous solutions were counted either in Bray's (1960) solution or in a solution composed of 600 ml of toluene,

* From the Laboratory of Physiology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received October 14, 1966.*

[1] Abbreviations used: U, uracil; Urd, uridine; UMP, UDP, and UTP, uridine 5′-mono-, 5′-di-, and 5′-triphosphates; Cyd, cytidine; CMP, CDP, and CTP, cytidine 5′-mono-, 5′-di-, and 5′-triphosphates; dUrd, deoxyuridine; dUMP and dUDP, deoxyuridine 5′-mono- and 5′-diphosphates; dCyd, deoxycytidine; dCMP, dCDP, and dCTP, deoxycytidine 5′-mono-, 5′-di-, and 5′-triphosphates; T, thymine; dThd, deoxythymidine; dTMP, dTDP, and dTTP, deoxythymidine 5′-mono-, 5′-di-, and 5′-triphosphates.

[2] Exogenous nucleotides are utilized in *E. coli* after dephosphorylation to nucleosides (Bolton, 1954; Lesley and Graham, 1956; Lichtenstein *et al.*, 1960).

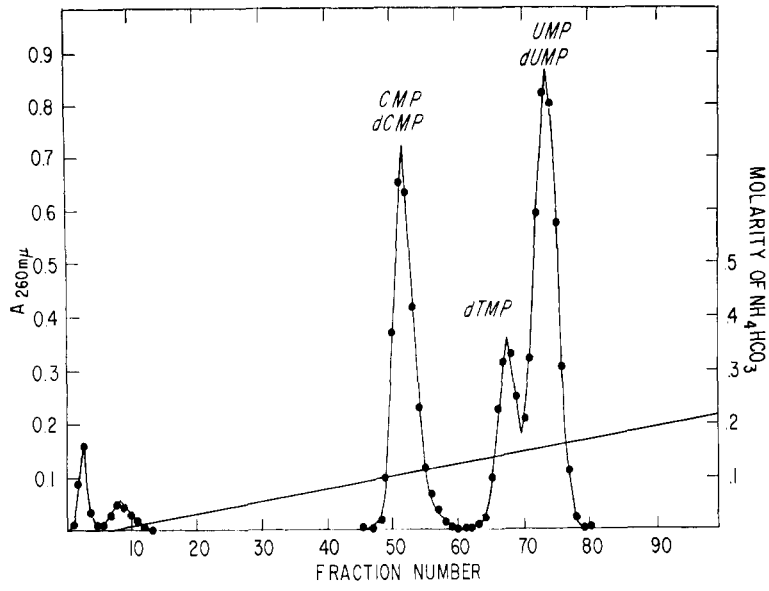

FIGURE 1: Separation of 5′-CMP and 5′-dCMP from 5′-dTMP, 5′-UMP, and 5′-dUMP on a 10 × 1 cm Dowex-1 carbonate column. Elution was carried out with a gradient of NH_4HCO_3 at pH 8.6. 2′- and 3′-CMP were eluted in fractions 63–79, and 2′- and 3′-UMP were eluted in fractions 90–100. Free bases were eluted in the first 15 fractions. Each fraction contained 10 ml.

400 ml of ethylene glycol monomethyl ether, 80 g of naphthalene, 4 g of 2,5-diphenyloxazole, and 0.05 g of 1,4-bis-2-(5-phenyloxazolyl)benzene. Because the latter solution gave better separation of ^{14}C from ^{3}H as well as higher counting efficiencies, it was used for counting all aqueous solutions of double-labeled compounds.

Growth of Cells. An experimental and a control culture were each composed of 50 ml of minimal medium (Davis and Mingioli, 1950) supplemented with 0.2% glucose and containing 196 μM [^{14}C]uracil (16,200 dpm/mμmole). In addition, the experimental culture contained 100 μM [^{3}H]dTMP (145,000 dpm/mμmole). *E. coli* 15 (ATCC 9723) from an exponentially growing culture was inoculated to give a concentration of 1.4×10^7 cells/ml in each culture. The cultures were aerated at 37° by vigorous shaking. Growth was followed turbidimetrically and was terminated after six generations (approximately 60% of maximal turbidity and 7.9×10^8 cells/ml[3]) by rapid cooling. The generation time for each culture was 45 min. Viability was determined by the spread plating technique. DNA was determined by the diphenylamine procedure of Burton (1956).

Analysis of Pyrimidine Nucleotide Pools. Cells were harvested by centrifugation at 20,000*g* for 7 min and washed twice with 40 ml of minimal medium. Each pellet was extracted with 3 ml of ice-cold 0.5 N perchloric acid for 30 min. CMP, dCMP, UMP, dUMP, and dTMP (2 μmoles each) were added as carrier to the acid-soluble extracts of each culture. After heating at 100° for 45 min, the acid-soluble extracts were adjusted to pH 8 with KOH; potassium perchlorate was removed by centrifugation.

The extracts were added to 10 × 1 cm Dowex-1 carbonate columns. The pyrimidine nucleotides were eluted with a linear gradient of 1 l. of 0.5 M NH_4HCO_3, pH 8.6, in the reservoir, and 1 l. of water in the mixing chamber (Figure 1). This gradient permitted complete separation of nucleoside 5′-monophosphates from their corresponding nucleoside 2′- and 3′-monophosphates (from RNA breakdown).

The eluates from each column were pooled into two fractions, one containing CMP and dCMP, and the other containing dTMP, UMP, and dUMP; the fractions were evaporated to dryness. Each residue was dissolved in 2 ml of 0.1 M NH_4HCO_3, pH 8.0, and 43 μg of alkaline phosphatase was added. Incubation was at 23° for 150 min. In a control tube containing 6 μmoles of dTMP, 0.09 μmole of *p*-nitrophenyl phosphate, and 43 μg of alkaline phosphatase in 2 ml of 0.1 M NH_4HCO_3, pH 8.0, liberation of nitrophenol was complete within 20 min. After evaporation, dThd, Urd, and dUrd were separated by two-dimensional chromatography with solvents I and then II. Cyd and dCyd were separated by two-dimensional chromatography with solvents II and then III.

Corrections were made for incomplete recoveries of carrier compounds, contributions from endogenously synthesized compounds, and 7% hydrolysis of dTMP during the hot acid treatment (W. D. Cannon, Jr., and T. R. Breitman, 1966, unpublished data). An analysis of the washes from the culture containing dTMP indicated that the contamination from [^{3}H]-dTMP from the medium was less than 0.02% of the

[3] Cells (1×10^9) corresponded to 0.5 mg dry weight.

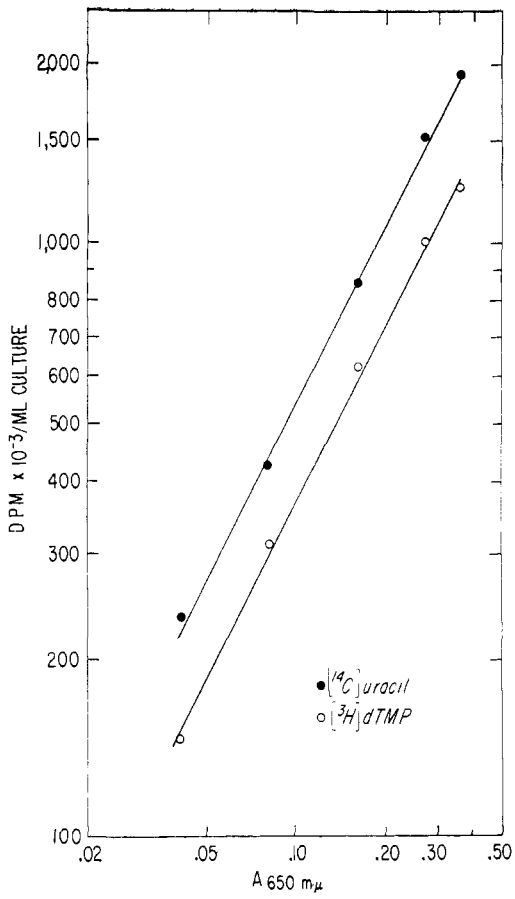

FIGURE 2: Incorporation of radioactivity from [^{14}C]-uracil and [^{3}H]dTMP into nucleic acids in exponentially growing *E. coli* 15. Aliquots were added to cold 5% trichloroacetic acid and passed through a 25-mm Millipore filter (0.45 μ). The filters were washed twice with cold 5% trichloroacetic acid, dried, and counted in toluene phosphor.

intracellular [^{3}H]dTMP pool. Disintegrations per minute were converted to millimicromoles using the experimentally determined specific activities of the RNA and DNA pyrimidines. Recoveries of carrier were between 61 and 78%.

Determination of Specific Activities of Pyrimidine Bases in RNA and DNA. RNA and DNA were extracted from the acid-insoluble pellets by a modified Schmidt–Thannhauser (1945) procedure. The acid precipitates were incubated with 0.3 N KOH; after 17 hr, the solutions were cooled in an ice bath and neutralized with 0.5 N perchloric acid. The DNA and protein were precipitated by addition of trichloroacetic acid to a final concentration of 5%. The RNA nucleotides in the supernatant fraction were separated by two-dimensional chromatography with solvents III and then IV. The DNA was extracted at 90° for 45 min in 5% trichloroacetic acid. After centrifugation the supernatant fraction was dried by evaporation. The residue was hydrolyzed to free bases in 0.3 ml of 60% perchloric acid at 100° for 1 hr. Perchlorate ions were removed by passage through a 4 × 1 cm Dowex-1 formate column. The free bases in the eluate were concentrated by evaporation and separated by two-dimensional chromatography with solvents III and then II.

Examination of the Media. Aliquots of the medium were taken during growth of the culture containing dTMP and filtered; the filtrates were analyzed for exogenous dTMP breakdown. After the addition of thymine, dThd, and dTMP as carrier, the filtrates were chromatographed in two dimensions with solvents II and then I.

After the cells had been harvested from the two cultures, the media were analyzed for nucleotides and free bases. The media were filtered and then acidified with 2 ml of 1.0 N perchloric acid. Norit A (60 mg) was added to each filtrate, collected on filters, and washed with water. Radioactive compounds were eluted with 10% pyridine–50% ethanol. After evaporation and addition of carrier compounds, the residues were chromatographed in two dimensions with solvents II and then I. The nucleotides which remained at the origin with solvent II were rechromatographed with solvent III. Yields were based on the recovery of a known quantity of dTMP in nonradioactive spent medium.

Results

Relative Incorporation of ^{14}C and ^{3}H from the Medium. Incorporation of ^{3}H from exogenous dTMP was proportional to incorporation of ^{14}C from uracil[4] during growth of the experimental culture (Figure 2). Analysis of the medium also indicated that dephosphorylation of dTMP to dThd was proportional to ^{3}H incorporation. During the six generations of growth, a total of 22.3 mμmoles/ml of [^{3}H]dTMP was dephosphorylated to [^{3}H]dThd. Of this amount, only 5.5 mμmoles/ml was utilized for DNA-thymine synthesis, 0.2 mμmole/ml was utilized for maintaining the steady-state level of the [^{3}H]thymine deoxynucleotide pool, and the remainder was degraded to thymine which was excreted into the medium. Total DNA-thymine, estimated by Burton's method, was 10.6 mμmoles/ml in each culture.

Pyrimidine Nucleotide Pools. As shown in Table I, cells from the culture containing dTMP, compared to the control culture, had (a) a 50% increase of the total thymine deoxynucleotide pool; (b) approximately a 35% reduction of the [^{14}C]thymine deoxynucleotide pool; (c) approximately 30% reductions of the uracil and cytosine deoxynucleotide pools; and (d) approximately 30% increases of the uracil and cytosine ribonucleotide pools. No 5-methylcytosine deoxynucleotide pool was found.

[^{14}C]Uracil was used in this study because of its

[4] During the six generations of growth, 61% of the exogenous [^{14}C]uracil was incorporated.

TABLE I: Effect of Exogenous dTMP on the Pyrimidine Nucleotide Pools of *E. coli*.

Compound[a]	Pyrimidine Nucleotide Pools ($\mu\mu$moles/10^9 cells) Exptl Culture[b] (E)	Control Culture[b] (C)	E:C	(Mean E:C ± Std Dev[c])
dCMP	94	130	0.72	(0.69 ± 0.076
dUMP	3.5	4.9	0.71	(0.58 ± 0.13)
[^{14}C]dTMP[d]	170	270	0.63	(0.66 ± 0.024)
[^{3}H]dTMP[e]	240	—	—	—
dTMP (total)	410	270	1.5	(1.4 ± 0.082)
UMP	6400	5400	1.2	(1.0 ± 0.14)
CMP	1500	1100	1.4	(1.1 ± 0.22)

[a] Each compound represents total nucleoside mono-, di-, and triphosphates, and sugar derivatives. [b] An experimental and a control culture contained 196 μM [^{14}C]uracil; in addition, the experimental culture contained 100 μM [^{3}H]dTMP. [c] In this column, the pertinent E:C ratios from two recent experiments (for dUMP, one experiment) designed to measure purine and pyrimidine nucleoside diphosphate derivatives as well as the total nucleotide pool sizes (to be published) have been averaged with those from the present study. [d] Synthesized from [^{14}C]uracil. [e] Synthesized from exogenous [^{3}H]dTMP.

preferential incorporation into the acid-soluble pool (McCarthy and Britten, 1962) and nucleic acid (Roberts *et al.*, 1957; see also Table II) of *E. coli*.[5] To be certain that the use of uracil did not mask an inhibitory effect of a thymine deoxynucleotide on pyrimidine biosynthesis preceding ribonucleotide reduction, cells were grown in the presence of L-[^{14}C]aspartic acid with and without exogenous dTMP. In the culture containing dTMP, expansions of the thymine deoxynucleotide pool and uracil and cytosine ribonucleotide pools were observed (W. D. Cannon, Jr., and T. R. Breitman, 1965, unpublished data). In addition, as shown in the present study, an expansion of the thymine deoxynucleotide pool did not decrease the contribution of synthesis *de novo* to the pyrimidine ribonucleotide pools.

Specific Activities of the RNA and DNA Bases. The specific activities of the nucleic acid pyrimidine bases in the control culture showed less than 12% isotope dilution (Table II). With the exception of thymine, they did not differ significantly between the experimental and control cultures. Thus, expansion of the thymine deoxynucleotide pool in the culture containing dTMP did not appear to significantly influence synthesis *de novo* of UMP and CMP. The specific activity of DNA-[^{3}H]thymine was 64,000 dpm/mμmole, representing a 16% reduction of that expected from the 52% derivation of DNA-thymine from exogenous [^{3}H]dTMP (see preceding section).

Minor Nucleic Acid Components. The DNA from the control culture contained 1.4% of the total cytosine as 5-methylcytosine. Since contaminating RNA represented 3.3% of the total DNA fraction, RNA-5-methylcytosine was 9–17%[6] of the total 5-methylcytosine. Among the minor RNA components, pseudouridylic acid was 0.52% and ribothymidylic acid was 0.26% of the total RNA nucleotides. No tritium was detected in the latter compound.

Decrease of Pyrimidine Compounds Synthesized

TABLE II: Specific Activities of DNA Bases and RNA Nucleotides.[a]

	Specific Activity (dpm/mμmole) Exptl Culture[b] (E)	Control Culture[b] (C)	E:C
DNA			
[^{14}C]Thymine	6,910	14,400	0.480
[^{3}H]Thymine	64,000	—	—
Cytosine	14,500	14,900	0.973
RNA			
UMP	16,200	16,100	1.01
CMP	14,500	14,200	1.02

[a] Specific activities of the [^{14}C]uracil and [^{3}H]dTMP in the media were 16,200 and 145,000 dpm/mμmole, respectively. [b] An experimental and a control culture contained 196 μM [^{14}C]uracil; in addition, the experimental culture contained 100 μM [^{3}H]dTMP.

[5] Compared to labeled uracil, labeled aspartic and orotic acids were poorly incorporated into *E. coli* (W. D. Cannon, Jr., and T. R. Breitman, 1965, unpublished data).

[6] Amos and Korn (1958) reported that 5-methylcytosine was 1–2% of the total RNA in *E. coli*.

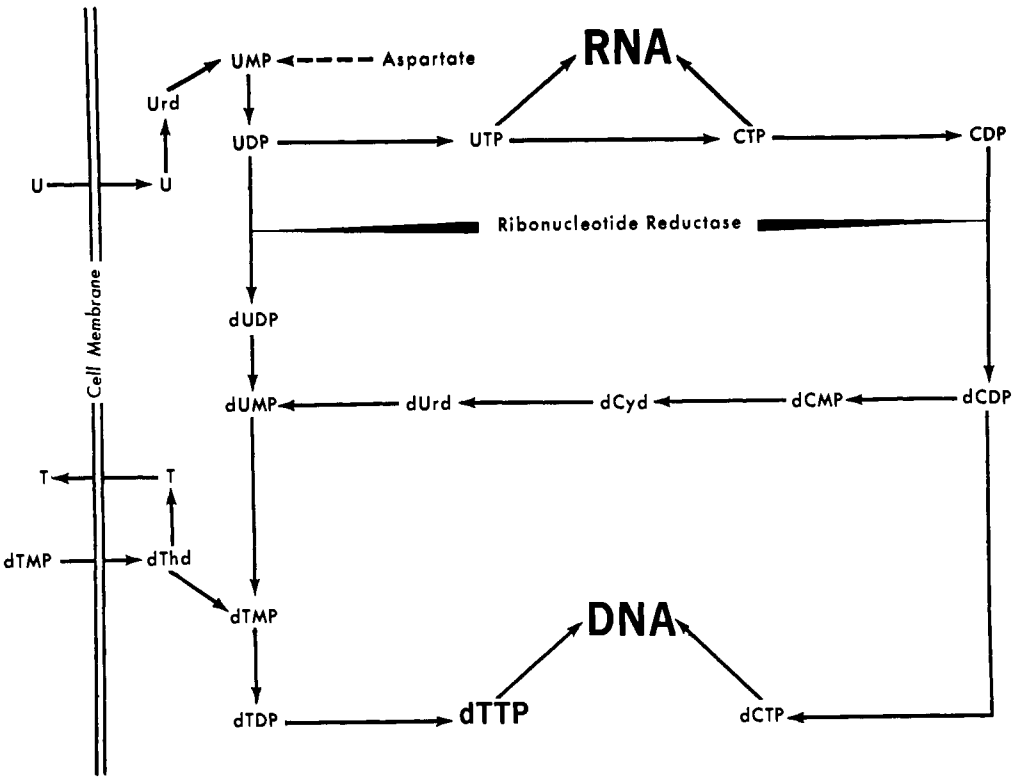

FIGURE 3: Pathways of pyrimidine nucleotide biosynthesis. Arrows indicate direction of flow. See text for discussion.

de Novo. In the culture containing dTMP, 5.5 mμmoles of DNA-thymine, or 52% of the total DNA-thymine synthesized, was derived from exogenous [^{3}H]dTMP. If dTMP synthesis *de novo* was not correspondingly decreased, 5.5 mμmoles/ml of ^{14}C-labeled thymine compounds should be accounted for either intracellularly or in the medium. In Table III are listed the [^{14}C]pyrimidine compounds found in the media. In the control culture, the value for the total [^{14}C]-thymine compounds synthesized per milliliter was 11.5 mμmoles, and represents the sum of 10.6 mμmoles/ml of DNA-thymine (see above), 0.7 mμmole/ml of [^{14}C]thymine compounds from the medium (Table III), and 0.2 mμmole/ml[7] of intracellular [^{14}C]thymine deoxynucleotides. In the culture containing dTMP, this sum was 6.2 mμmoles/ml. The difference of 5.3 mμmoles in the total [^{14}C]thymine compounds synthesized in the two cultures is a further indication of regulation of dTMP synthesis *de novo*.

TABLE III: Compounds Excreted into the Media.

Compound[a]	Excretion into Media (μμmoles/ml of medium) Exptl Culture[c] (E)	Control Culture[c] (C)	E:C
Thymine[b]	840	500	1.7
dTMP	130	190	0.68
UMP	1600	1700	0.94
CMP	170	200	0.85

[a] Synthesized from [^{14}C]uracil. [b] Less than 0.1% of thymine was present as dThd. [c] An experimental and a control culture contained 196 μM [^{14}C]uracil; in addition, the experimental culture contained 100 μM [^{3}H]dTMP.

The presence of extracellular thymine, dTMP, UMP, and CMP indicates lack of economy in utilization of intracellular nucleotides (Table III). The control culture excreted 0.69 mμmole/ml of [^{14}C]-thymine compounds, or 6% of the total 11.5 mμmoles/ml of thymine compounds synthesized. In the culture containing dTMP, this value was 0.84 mμmole/ml, or 14% of the total 6.2 mμmoles/ml synthesized.

Discussion

Control of Deoxynucleotide Biosynthesis. Figure 3 depicts the pyrimidine biosynthetic pathway in *E. coli*. ^{14}C from exogenous uracil was incorporated into every nucleotide shown in Figure 3, whereas ^{3}H from ex-

[7] This value was obtained by converting the 270 μμmoles/10^9 cells for the thymine deoxynucleotide pool (Table I) to 0.2 mμmole/ml (viability count was 7.9×10^8 cells/ml).

ogenous dTMP was incorporated only into thymine compounds (Table I). The finding of 52% of DNA-thymine derived from [^{3}H]dTMP (Table II) might be explained by (a) no biosynthetic regulation and dilution of dTMP synthesized *de novo* with consequent excretion of excess [^{14}C]thymine compounds into the medium; or (b) decreased [^{14}C]dTMP biosynthesis effected by decreased thymidylate synthetase activity resulting in an accumulation of dUMP and its precursors, or decreased ribonucleotide reductase activity resulting in decreased uracil and cytosine deoxynucleotides and an accumulation of ribonucleotides.

The finding of only a small excretion of [^{14}C]thymine compounds in the culture containing dTMP (Table III) eliminates the first possibility of no biosynthetic regulation. However, the results show that associated with the expansion of the thymine deoxynucleotide pool in the culture containing dTMP were reductions in *both* the cytosine and uracil deoxynucleotide pools, a reduction in the contribution of synthesis *de novo* to the thymine deoxynucleotide pool, and increases of the ribonucleotide pools (Table I). These results are consistent with regulation of pyrimidine deoxynucleotide biosynthesis at the ribonucleotide reductase level and not at the thymidylate synthetase step. A distinction, however, cannot be made between feedback control or enzyme repression. While there have been reports of derepression of ribonucleotide reductase (Biswas *et al.*, 1965) there has been no report of repression of this enzyme. It has been suggested by Biswas *et al.* (1965) that the level of ribonucleotide reductase would be inversely related to the intracellular concentration of dTTP. Thus, a low intracellular dTTP concentration should result in derepression, and a high concentration should result in repression. Our results are consistent with this hypothesis and are in agreement with results in mammalian systems implicating dTTP as an end-product inhibitor of CDP reduction (Reichard *et al.*, 1961; Morris *et al.*, 1963; Moore, 1965). In contrast, in human leukemic leukocytes (Cooper *et al.*, 1966) a fivefold increase of the thymine deoxynucleotide polyphosphate pool did not affect deoxythymidylate synthesis *de novo*. On the other hand, dTTP has recently been shown to be an activator, and deoxyadenosine triphosphate an inhibitor, of purified *E. coli* ribonucleotide reductase (Holmgren *et al.*, 1965; Larsson and Reichard, 1966a,b,c). The net effect on the activity and level of this enzyme appears to be a complex interaction between nucleoside triphosphate activators and inhibitors. Experiments to measure the effect of increased levels of intracellular thymine deoxynucleotides on the purine deoxynucleotide pools and on the activity of ribonucleotide reductase are in progress.

Utilization of [^{3}H]dTMP. The amount of dThd made available to the cells by exogenous dTMP breakdown, presumably by 5′-nucleotidase (Neu and Heppel, 1965), represented twice the DNA-thymine requirements of the cells. Failure to incorporate a higher percentage of this dThd into DNA might be explained by (a) degradation of dThd to thymine by thymidine phosphorylase (Rachmeler *et al.*, 1961), and (b) a decrease in thymidine kinase activity effected by an increase in its inhibitor, dTTP (Breitman, 1963; Ives *et al.*, 1963), and a decrease in its activator, dCTP (Okazaki and Kornberg, 1964). Our results show both an increase of thymine deoxynucleotides and a decrease of cytosine deoxynucleotides (Table I).

Pyrimidine Nucleotide Pools. The size of the thymine deoxynucleotide pool in the control culture was 270 $\mu\mu$moles/10^9 cells (a 52-sec supply under our growth conditions). This value compares favorably with the 210 $\mu\mu$moles/10^9 cells (T. R. Breitman and R. M. Bradford, 1964, unpublished data) in *E. coli* 15 and 230 $\mu\mu$moles/10^9 cells obtained by Neuhard and Munch-Petersen (1966) in *E. coli* 15 T$^-$A$^-$U$^-$. It differs significantly from O'Donnell *et al.* (1958), who reported a pool size for dTMP alone in *E. coli* B approximately four times these values, and from Goldstein *et al.* (1960) who found only a trace of dTTP in *E. coli* K_{12}. Differences in nucleotide pool sizes have been shown to be due to differences in bacterial strains, growth conditions, and methods of extraction (Lark, 1961; Smith and Maaløe, 1964; Smith-Kielland, 1964).

In the present study, nucleoside diphosphates, including the diphospho sugar derivatives, and triphosphates were converted to monophosphates. Changes in size of the total nucleotide pool were postulated to reflect parallel changes in the individual components. Excluding nucleoside diphospho sugar compounds, which have been reported to constitute a significant portion of several nucleotide pools (O'Donnell *et al.*, 1958; Okazaki *et al.*, 1960; Goldstein *et al.*, 1960; Franzen and Binkley, 1961), nucleoside triphosphates have been found to represent the major nucleotide pool component by several investigators (Goldstein *et al.*, 1960; Smith and Maaløe, 1964). Neuhard and Munch-Petersen (1966) reported that dTTP is 75% of the total thymine deoxynucleotide pool in *E. coli* 15 T$^-$A$^-$U$^-$.

We are unaware of any previous estimate of the uracil deoxynucleotide pool in *E. coli*. Although it was the smallest pyrimidine deoxynucleotide pool (3.8% of the cytosine and 1.8% of the thymine pools), it was, however, radiochemically pure after chromatography in four solvent systems, and totaled 875 cpm in the culture containing dTMP and 1280 cpm in the control culture. No more than 8% of this dUMP arose from dCMP deamination during acid hydrolysis because only 0.3% of dCMP hydrolyzed under the experimental conditions was deaminated to dUMP (W. D. Cannon, Jr., and T. R. Breitman, 1966, unpublished data). Furthermore, Schein (1966) reported the absence of uracil after perchloric acid hydrolysis of 5′-dCMP under conditions much more drastic than those used in this study.

References

Amos, H., and Korn, M. (1958), *Biochim. Biophys. Acta 29*, 444.

Biswas, C., Hardy, J., and Beck, W. S. (1965), *J. Biol. Chem. 240*, 3631.
Bolton, E. T. (1954), *Proc. Natl. Acad. Sci. U. S. 40*, 764.
Bray, G. A. (1960), *Anal. Biochem. 1*, 279.
Breitman, T. R. (1963), *Biochim. Biophys. Acta 67*, 153.
Burton, K. (1956), *Biochem. J. 62*, 315.
Cooper, R. A., Perry, S. and Breitman, T. R. (1966), *Cancer Res. 26*, 2267.
Davis, B. D., and Mingioli, E. S. (1950), *J. Bacteriol. 60*, 17.
Fink, K., Cline, R. E., Henderson, R. B., and Fink, R. M. (1956), *J. Biol. Chem. 221*, 425.
Franzen, J. S., and Binkley, S. B. (1961), *J. Biol. Chem. 236*, 515.
Goldstein, D. B., Brown, B. J., and Goldstein, A. (1960), *Biochim. Biophys. Acta 43*, 55.
Holmgren, A., Reichard, P., and Thelander, L. (1965), *Proc. Natl. Acad. Sci. U. S. 54*, 830.
Ives, D. H., Morse, P. A., Jr., and Potter, V. R. (1963), *J. Biol. Chem. 238*, 1467.
Lark, K. G. (1961), *Biochim. Biophys. Acta 51*, 107.
Larsson, A., and Reichard, P. (1966a), *Biochim. Biophys. Acta 113*, 407.
Larsson, A., and Reichard, P. (1966b), *J. Biol. Chem. 241*, 2533.
Larsson, A., and Reichard, P. (1966c), *J. Biol. Chem. 241*, 2540.
Lesley, S. M., and Graham, A. F. (1956), *Can. J. Microbiol. 2*, 17.
Lichtenstein, J., Barner, H. D., and Cohen, S. S. (1960), *J. Biol. Chem. 235*, 457.
Magasanik, B., Vischer, E., Doniger, R., Elson, D., and Chargaff, E. (1950), *J. Biol. Chem. 186*, 37.
Markham, R., and Smith, J. D. (1949), *Biochem. J. 45*, 294.
McCarthy, B. J., and Britten, R. J. (1962), *Biophys. J. 2*, 35.
Moore, E. C. (1965), *Proc. Am. Assoc. Cancer Res. 6*, 46.
Morris, N. R., Reichard, P., and Fischer, G. A. (1963), *Biochim. Biophys. Acta 68*, 93.
Neu, H. C., and Heppel, L. A. (1965), *J. Biol. Chem. 240*, 3685.
Neuhard, J., and Munch-Petersen, A. (1966), *Biochim. Biophys. Acta 114*, 61.
O'Donnell, J. F., Mackal, R. P., and Evans, E. A., Jr. (1958), *J. Biol. Chem. 233*, 1523.
Okazaki, R., and Kornberg, A. (1964), *J. Biol. Chem. 239*, 275.
Okazaki, R., Okazaki, T., and Kuriki, Y. (1960), *Biochim. Biophys. Acta 38*, 384.
Rachmeler, M., Gerhart, J., and Rosner, J. (1961), *Biochim. Biophys. Acta 49*, 222.
Reichard, P., Canellakis, Z. N., and Canellakis, E. S. (1961), *J. Biol. Chem. 236*, 2514.
Roberts, R. B., Abelson, P. H., Cowie, D. B., Bolton, E. T., and Britten, R. J. (1957), *in* Studies of Biosynthesis in *Escherichia coli*, Washington, D. C., Carnegie Institution of Washington, Publication 607, p 109.
Schein, A. H. (1966), *Biochem. J. 98*, 311.
Schmidt, G., and Thannhauser, S. J. (1945), *J. Biol. Chem. 161*, 83.
Siminovitch, L., and Graham, A. F. (1955), *Can. J. Microbiol. 1*, 721.
Smith, R. C., and Maaløe, O. (1964), *Biochim. Biophys. Acta 86*, 229.
Smith-Kielland, I. (1964), *Acta Chem. Scand. 18*, 967.
Wyatt, G. R. (1951), *Biochem. J. 48*, 584.