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Deoxycytidine Triphosphate Deaminase: Identification and Function in *Salmonella typhimurium*

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The biosynthesis of 2'-deoxyuridine monophosphate (dUMP) has been studied in a cytidine- and uracil-requiring mutant of *Salmonella typhimurium* (DP-55). The dUMP pool and the thymidine monophosphate (dTMP) pool of DP-55, grown in the presence of ^{3}H-uracil and unlabeled cytidine, are found to have the same specific activities. However, only 30% of the dUMP and the dTMP is synthesized from a uridine nucleotide. Seventy per cent is derived directly from a cytosine compound. The identification and partial purification of a Mg^{2+}-dependent 2'-deoxycytidine triphosphate (dCTP) deaminase from *S. typhimurium* suggests that the combined action of dCTP deaminase and 2'-deoxyuridine triphosphate pyrophosphatase accounts for 70% of the dUMP, and therefore the dTMP, synthesized in vivo. The introduction of a thymine requirement (i.e., a block in thymidylate synthetase) into DP-55 results in a 100-fold increase in the size of the dUMP pool. However, the relative contribution of the uridine and cytidine pathways to dUMP synthesis is unaltered. The high dUMP pool is accompanied by extensive catabolism of dUMP to uracil. Partial thymine starvation of the cells results in a significant increase in the dUMP and dCTP pools. Moreover, an increase in the contribution of the dCTP pathway to dUMP synthesis is observed. As a result of these changes the catabolism of dUMP to uracil is augmented.

Bacterial mutants defective in cytidine triphosphate (CTP) synthetase (*pyrG*) require cytidine for growth (19). *PyrG* mutants are readily obtained in both *Salmonella typhimurium* (20) and *Escherichia coli* (Thomassen and Neuhard, *unpublished data*) by penicillin counterselection, provided that the parent strains used for the selections are unable to catabolize cytidine, i.e., are lacking cytidine deaminase (*cdd*). In *S. typhimurium*, *pyrG* maps at approximately 87 min on the *Salmonella* linkage map (C. Beck, *personal communication*) and is, therefore, not linked to any other of the known *pyr* loci of this organism (23).

By introducing a *pyrG* mutation into a strain of *S. typhimurium* already blocked in carbamyl phosphate synthetase (*pyrA*) and cytidine deaminase, a mutant (DP-55) was isolated which, in addition to arginine, had an absolute requirement for uracil or uridine *and* cytidine (19). Thus, the metabolism of cytosine and uracil compounds is completely separated in mutants of the genotype *pyrG*, *cdd*. Owing to this unique property of cytidine-requiring strains, it was possible to show that repression of the synthesis of carbamyl phosphate synthetase is mediated specifically by a cytosine compound (1), and that both a uracil and a cytosine compound are necessary to obtain maximal repression of aspartate transcarbamylase synthesis (20).

Furthermore, labeling studies with DP-55 showed that only about 20% of the thymidine nucleotides of the cells was derived from a uridine nucleotide precursor. Eighty per cent was synthesized directly from a cytidine compound without prior equilibration with the uridine nucleotide pool of the cells (19). It was suggested that the majority of the thymidine triphosphate (dTTP) was synthesized by a pathway not involving uridine diphosphate (UDP) reduction (9, 19).

Selection of thymine-requiring mutants (*thy*) in DP-55 would be expected to yield mutants with a triple requirement for pyrimidines, i.e., uracil, cytidine, and thymine. Such mutants would be ideal for the differential labeling of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). However, it was found that the

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introduction of a *thy* mutation into DP-55 invariably resulted in the phenotypical loss of the uracil requirement.

The present paper describes the properties of thymine-requiring derivatives of *S. typhimurium* DP-55. The study led to the discovery of a new enzyme activity in *S. typhimurium*: 2'-deoxycytidine triphosphate (dCTP) deaminase. The physiological function of dCTP deaminase in thymidine nucleotide metabolism will be discussed. In an accompanying paper by O'Donovan et al. (22), the identification and properties of an *E. coli* mutant lacking dCTP deaminase will be described.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. typhimurium* DP-55 is a cytidine-, uracil-, and arginine-requiring mutant of *S. typhimurium* LT-2 (19). Its isolation and properties were previously described (19, 20). Thymine-requiring mutants (*thy*) of DP-55 were isolated as follows (4). Cells of DP-55 (10^6) were inoculated into 5 ml of glucose minimal medium which, in addition to the required nutrients, contained trimethoprim (8 μg/ml; Burroughs Wellcome & Co., Inc., London), thymine (50 μg/ml) and, in some cases, the mutagen *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (3 μg/ml; NTG; K & K Laboratories Inc., Plainview, N.Y.). The cultures were grown to saturation (10^9 cells/ml; usually 2 to 3 days) with shaking at 37 C. The trimethoprim-resistant mutants were purified on nutrient agar plates containing cytidine and thymine (50 μg/ml). Subsequently single colonies were tested for thymine auxotrophy.

The glucose minimal medium used throughout was previously described (17). Required nutrients were added to the medium in the following concentrations: uracil, 10 μg/ml; cytidine and uridine, 20 μg/ml; arginine, 50 μg/ml. The concentrations of thymine and thymidine used in the different experiments are indicated in the appropriate tables and figures. Cultures were grown at 37 C in a Metabolyte water bath shaker (New Brunswick Scientific Co., New Brunswick, N.J.). Increase of cell mass was observed at 436 nm in an Eppendorf photometer (model 1101 M). One milliliter of bacterial culture with an absorbancy at 436 nm of 1.000 contains approximately 4×10^8 cells or 0.2 mg of dry weight.

Determination of acid-soluble nucleotide pools. Nucleoside triphosphates were determined by previously published procedures (19). The quantitative determination of the nucleoside monophosphate pools was performed by the method of Munch-Petersen (16). All countings were done in a Packard Tri-Carb liquid scintillation spectrometer (model 574) as previously described (18).

dCTP deaminase assay. The reaction mixture contained in 0.5 ml: 25 μmoles of tris(hydroxymethyl)-aminomethane (Tris)-chloride (*p*H 7.1), 1 μmole of $MgCl_2$, 0.5 μmoles of mercaptoethanol, 0.5 μmoles of dCTP, and enzyme. Assays were carried out at 37 C. The reaction was initiated by addition of enzyme. At 3, 7, 12, and 20 min, 0.1 ml of the reaction mixture was added to 0.9 ml of 0.5 M perchloric acid (0 C) and centrifuged. The extinction of the supernatant fluid was measured at 290 nm. Reaction velocities were determined from plots of extinction versus time. The amount of enzyme used in each assay was chosen so that the plots were linear for at least 12 min. Specific activities are expressed as nanomoles of dCTP deaminated per minute per milligram of protein, by using a value of 10.1×10^3 for the decrement in molar extinction coefficients on converting cytidine (deoxycytidine) to uridine (deoxyuridine) compounds. Protein was determined by the method of Lowry et al. (12).

Partial purification of dCTP deaminase. Ten grams (wet weight) of cells of *S. typhimurium* KP-170 was suspended in 20 ml of buffer A [0.05 M Tris-chloride (pH 7.1), 1 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM mercaptoethanol] and sonically oscillated for 60 sec with a Branson sonic oscillator (model S 125). After sonic treatment, the suspension was centrifuged at 0 C for 30 min at 20,000 × g, and the supernatant fluid was treated with one-third volume of 10% streptomycin sulfate. The precipitate was removed by centrifugation. The supernatant fluid from the streptomycin step was subsequently fractionated with ammonium sulfate. The fraction precipitating between 37 and 50% saturation contained the enzyme. This fraction was redissolved in 5 ml of buffer A and dialyzed against 1,000 volumes of 0.05 M Tris-chloride (*p*H 7.1), 2 mM $MgCl_2$, and 1 mM mercaptoethanol. The dialyzed fraction represents a six- to ninefold purification of dCTP deaminase compared to crude extracts.

Radiochemicals. ^{32}P-orthophosphate (carrier free) was obtained from Atomenergikommissionens Forsogsanlaeg (Risø, Denmark). It was delivered in 0.1 M HCl and used directly without neutralization. Uracil-6-^3H and ^{3}H-cytidine were purchased from The Radiochemical Centre (Amersham, England). Although the tritium-labeled cytidine was claimed to be uniformly labeled, it was found that more than 80% of the label is located on the 5-position of the pyrimidine ring. Thus, in this study it is referred to as cytidine-5-^3H.

RESULTS

Isolation and growth characteristics of KP-170. Thymine-requiring mutants were isolated in DP-55 as mutants resistant to the folic acid antagonist trimethoprim in presence of thymine (29). About half of the mutants isolated were spontaneous mutants, whereas the rest were induced by treatment with NTG (4). Thirty independent high thymine-requiring mutants (7) thus obtained were tested for their nutritional requirements. All had an absolute requirement for arginine, cytidine, and thymine (>20 μg/ml). However, in contrast to DP-55, they grew normally in absence of uracil (Fig. 1). Since the arginine and uracil requirements of DP-55 are caused by a single mutation (a deletion in the *pyrA* gene coding for carbamyl phosphate synthetase) and the *thy* mutants of DP-55 all have an absolute requirement for arginine, it may be concluded that the latter are genotypically *pyrA* mutants like the parent. Thus,

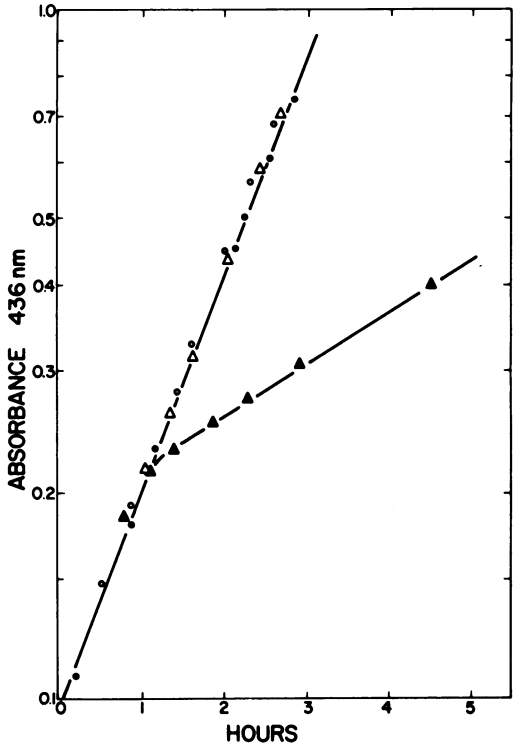

FIG. 1. *Overnight cultures of DP-55 and KP-170 were diluted into glucose minimal medium containing arginine, uracil, cytidine, and, in the case of KP-170, thymine (20 μg/ml) at 37 C. Forty minutes later, half of each culture was filtered, washed free of uracil, and suspended in the same media lacking uracil. Growth was followed spectrophotometrically at 436 nm. Symbols: △, DP-55 plus uracil; ▲, DP-55 minus uracil; ○, KP-170 plus uracil; ●, KP-170 minus uracil.*

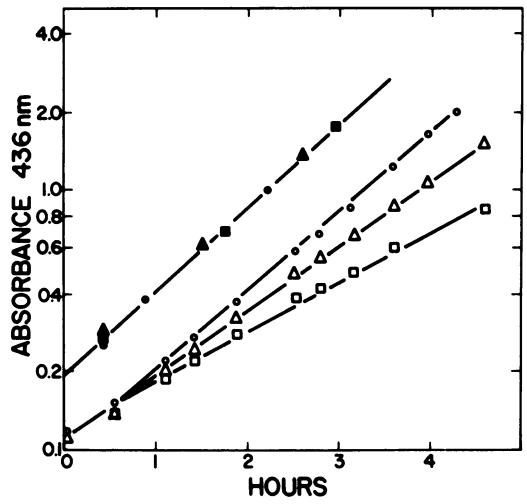

FIG. 2. *An exponential culture of KP-170 was filtered, washed, and suspended in glucose minimal medium containing arginine and cytidine. At 0 min, it was divided into six flasks containing the following additions (final concentrations): ●, thymine (20 μg/ml) + uracil; ○, thymine (20 μg/ml); ▲, thymine (100 μg/ml) + uracil; △, thymine (100 μg/ml); ■, thymidine (50 μg/ml + uracil; □, thymidine (50 μg/ml). Growth was followed spectrophotometrically at 436 nm.*

the mere introduction of a *thy* mutation into DP-55 makes it phenotypically uracil independent (in the presence of cytidine), probably by inducing a new pathway for the conversion of cytidine to uridine nucleotides.

One of the spontaneous *thy* mutants of DP-55, KP-170, was chosen for further studies. Identical results have, however, been obtained with other *thy* mutants.

Figure 2 shows that, in the absence of uracil, a decrease in the growth rate of KP-170 is observed when the concentration of exogenous thymine is increased. If thymine is replaced by thymidine, the growth rate decreases further (Fig. 2). Since uracil addition restores normal growth under all conditions, it may be concluded that high concentrations of thymine or thymidine interfere with the conversion of exogenous cytidine to uridine monophosphate (UMP) in *thy* mutants lacking cytidine deaminase.

Nucleoside triphosphate pools of KP-170. The effect of exogenous thymine on the conversion of cytidine to UMP (Fig. 2) led us to study the nucleotide pools of KP-170 under similar conditions. The pool sizes of the different pyrimidine nucleoside triphosphates are given in Table 1. From Table 1 the following points should be noted. (i) The dTTP pool of KP-170 is dependent on the concentration of exogenous thymine both in the presence and in the absence of uracil. Furthermore, it appears that thymidine is a better precursor of endogenous dTTP than thymine. However, under no conditions does the dTTP pool reach the levels found in the thymine prototrophic parent strain. (ii) In accordance with results previously obtained with a thymine-requiring strain of *E. coli* (21), an inverse relationship between the size of the dTTP pool and that of the dCTP pool exists in KP-170. This relationship is independent of the presence or the absence of exogenous uracil. (iii) In the absence of uracil, the growth-limiting compound seems to be uridine triphosphate (UTP), in accordance with the results shown in Fig. 2.

The data of Fig. 2 and Table 1 indicate that the ability of KP-170 to grow in the absence of exogenous uracil is dependent on a low endogenous dTTP pool, which in turn causes the dCTP pool to increase. [The deoxycytidine diphosphate (dCDP) and deoxycytidine monophosphate (dCMP) pools are probably also increased under

TABLE 1. *Pyrimidine nucleoside triphosphate pools of S. typhimurium DP-55 and KP-170 under different growth conditions*[a]

Strain	Additions (μg/ml)		Doubling time	Pool size[b]			
	Thymine	Uracil		dTTP	dCTP	UTP	CTP
DP-55	0	10	59	0.98	0.56	4.02	3.08
	0	0	240[c]	1.58	0.81	0.37	27.30
	20	10	53	0.14	4.54	2.95	3.12
	100	10	55	0.54	0.62	2.78	2.86
	50[d]	10	54	0.63	0.46	3.02	3.08
KP-170	20	0	55	0.18	3.39	2.43	3.60
	100	0	69	0.63	0.84	1.01	8.46
	50[d]	0	98	0.92	0.56	0.73	16.38

[a] Cells were grown in glucose minimal medium containing arginine, cytidine, ^{32}P-orthophosphate (3 μCi/μmole), and the additions shown above. After two generations of exponential growth, 5-ml samples were harvested, and the pool sizes of the indicated nucleoside triphosphates were determined.

[b] Abbreviations: dTTP, thymidine triphosphate; dCTP, 2'-deoxycytidine triphosphate; UTP, uridine triphosphate; CTP, cytidine triphosphate.

[c] Cells were prelabeled with ^{32}P-orthophosphate for two generations in presence of uracil. Uracil was then removed by filtration, and the cells were incubated for 2 hr in a uracil-free medium with the same specific activity.

[d] Thymine is replaced by thymidine.

these conditions.] In the absence of exogenous uracil, an increase in the dCTP pool is paralleled by an increase in the UTP pool, resulting in stimulation of growth. This suggests that the conversion of cytidine to UMP in *thy, cdd* mutants of *S. typhimurium* involves the deamination of a deoxycytidine nucleotide.

dCTP deaminase of KP-170. In preliminary experiments with toluenized cells (19) as the enzyme source and different cytosine derivatives as substrates, it was observed that neither deoxycytidine nor dCMP was deaminated by *thy* mutants of DP-55. This is in accordance with the fact that these cells lack cytidine deaminase (*cdd*) and that enteric bacteria are devoid of dCMP deaminase activity (10). The only deoxycytidine nucleotide deaminated to any significant extent was dCTP.

The spectrophotometric assay used for measuring dCTP deamination is not very reproducible when applied to toluenized cells or crude sonic extracts, due to interference by the nucleic acids present. Therefore, a partially purified preparation of dCTP deaminase from KP-170 was used for further characterization of this new enzyme activity. Some of the results obtained with this preparation are shown in Table 2. It appears that the enzyme is quite specific for dCTP; CTP is deaminated at a much lower rate. Furthermore, the enzyme has a requirement for Mg^{2+} (Mn^{2+} will replace Mg^{2+}).

In an attempt to identify the product of the enzymatic reaction, a standard assay was run with dCTP as substrate. After completion of the reaction, a portion of the reaction mixture was chromatographed on PEI-cellulose plates (25). The chromatogram revealed that the product of the reaction was a mixture of uracil, deoxyuridine, and deoxyuridine monophosphate (dUMP). No trace of deoxyuridine triphosphate (dUTP), the expected reaction product, was found. Thus, although these results indicate that extracts of KP-170 are capable of converting dCTP to uracil, they do not prove the exact pathway involved in this conversion. However, the finding that

TABLE 2. *Specificity of a partially purified preparation of dCTP deaminase from S. typhimurium KP-170*[a]

Substrate[b]	Specific activity[c]
dCTP	2.80
dCDP	<0.10
dCMP	<0.10
Deoxycytidine	<0.10
CTP	0.63, 0.75[d]
dCTP[e] ($-Mg^{2+}$ + EDTA)	0.22
dCTP[f] ($+Mg^{2+}$ + EDTA)	2.97

[a] Purified as described in the text.

[b] The concentration of substrates in the assays was 1 mM. Abbreviations: dCTP, 2'-deoxycytidine triphosphate; dCDP, deoxycytidine diphosphate; dCMP, deoxycytidine monophosphate; EDTA, ethylenediaminetetraacetic acid.

[c] Expressed as nanomoles converted per minute per milligram of protein. Activities were measured as described for dCTP deaminase.

[d] Obtained with two different enzyme preparations.

[e] Mg^{2+} was removed by dialysis against buffer A, and 1 mM EDTA was present during the assay.

[f] As in footnote *e* except that excess Mg^{2+} (5 mM) was present during the assay.

dCDP, dCMP, and deoxycytidine are not deaminated by the preparation used supports the view that the conversion involves an initial deamination of dCTP to dUTP. The dUTP formed would be expected to be rapidly broken down by dUTP pyrophosphatase (2, 6) to dUMP, which, in turn, may be further degraded to uracil by the sequential action of a phosphatase (2) and thymidine (deoxyuridine) phosphorylase (27; *see* Fig. 3).

In vivo evidence for the "dCTP deaminase-pathway." If the pathway outlined in the previous section is responsible for the ability of *thy* mutants of DP-55 to convert cytidine to UMP, it follows that uracil is a necessary intermediate. Therefore, one would anticipate that the further introduction of a mutation rendering the cells incapable of converting uracil to UMP [i.e., deficient in UMP pyrophosphorylase (23)] would result in the loss of this ability.

Such a mutant, KP-103, was isolated in KP-100 (a thymine-requiring derivative of DP-55 similar to KP-170) after mutagenesis with NTG and penicillin counterselection in the presence of cytidine and thymine. The phenotype of KP-103 is shown in Table 3. KP-103 has, in one step, lost the ability to convert cytidine as well as uracil to UMP (Table 3; *compare* Fig. 3), thereby acquiring an absolute requirement for uridine. Uridine is converted to UMP by uridine kinase (23).

To obtain more direct evidence for the in vivo existence of a pathway capable of converting dCTP via dUMP to uracil, cells were grown in the presence of cytidine-*5-*^{3}H, ^{32}P-orthophosphate, unlabeled uracil, and, in the case of KP-170, unlabeled thymine. After several generations of exponential growth, the specific activities (ratio of ^{3}H counts per min to ^{32}P counts per min) of the different acid-soluble pyrimidine nucleotides were determined. Table 4 gives the results obtained with DP-55 grown in the absence of thymine and with KP-170 grown with two different concentrations of exogenous thymine. Since cytidine monophosphate (CMP) is exclusively synthesized from exogenous tritium-labeled cytidine and UMP is almost entirely derived from unlabeled uracil in the medium, the contribution of a cytidine pathway to the synthesis of dUMP may be calculated from the specific activities of these three compounds. The results are shown in the last column of Table 4. The majority of the dUMP of KP-170 is derived from a cytosine compound without equilibrating with the uridine nucleotide pool (i.e., UMP; Table 4). By increasing the thymine concentration in the medium (i.e., increasing the dTTP pool; *see* Table 1), the contribution from this pathway decreases.

The specific activities of the UMP pools (Table 4) indicate that there is no conversion of cytidine to UMP in DP-55. In KP-170, however, such a conversion does occur, and it seems to be de-

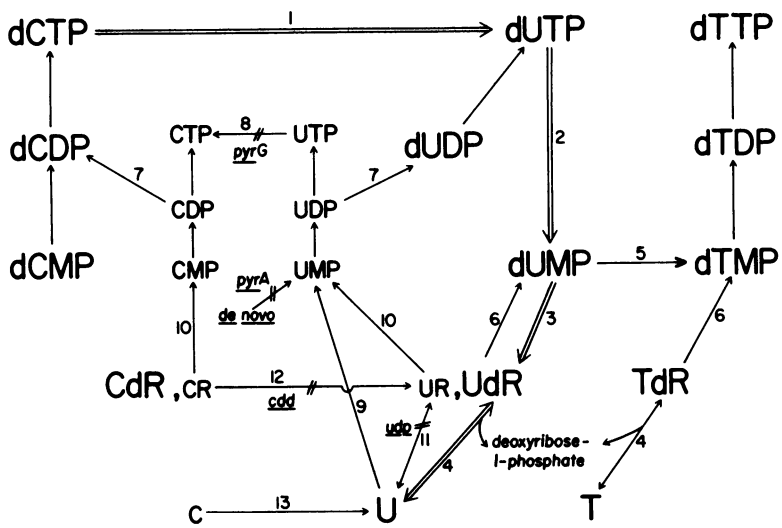

FIG. 3. *Schematic representation of pyrimidine nucleotide metabolism in S. typhimurium. The pathway discussed in the present work is represented by double arrows. The reactions blocked in DP-55 are crossed. U, C, and T stand for uracil, cytosine, and thymine; UR, UdR, CR, CdR, and TdR represent the corresponding ribonucleosides and deoxyribonucleosides.(1) dCTP deaminase, (2) dUTP pyrophosphatase, (3) "phosphatase," (4) thymidine (deoxyuridine) phosphorylase, (5) thymidylate synthetase, (6) thymidine kinase, (7) ribonucleoside diphosphate reductase, (8) CTP synthetase, (9) UMP pyrophosphorylase, (10) uridine kinase, (11) uridine phosphorylase, (12) cytidine (deoxycytidine) deaminase, (13) cytosine deaminase.*

TABLE 3. *Nutritional requirements of S. typhimurium KP-100 and KP-103*[a]

Strain	Nutrients added[b]				
	Thymine, uracil, and cytidine	Uracil and cytidine	Thymine and uracil	Thymine and cytidine	Thymine, uridine, and cytidine
KP-100	+	−	−	+	+
KP-103	−	−	−	−	+

[a] Growth was tested on glucose minimal plates containing arginine in addition to the nutrients shown above. The following concentrations were employed: arginine, 50 μg/ml; thymine, 20 μg/ml; uracil, 10 μg/ml; cytidine and uridine, 20 μg/ml.

[b] Symbols: +, growth; −, no growth. Determinations were made after 48 hr at 37 C.

pendent on the concentration of exogenous thymine. This in agreement with the results of the growth experiments (Fig. 2).

Although dCTP is not converted to uracil and thus to UMP in DP-55, the results shown in Table 4 do not exclude the possibility that dCTP deamination occurs in *thy*$^+$ strains. They may merely indicate that dUMP formed from dCTP is not further broken down to uracil but is utilized for thymidine monophosphate (dTMP) synthesis via thymidylate synthetase (*see* Fig. 3). To test for the synthesis in vivo, DP-55 was grown in the presence of uracil-*6*-^3H, ^{32}P-orthophosphate, and unlabeled cytidine (Cytidine-*5*-^3H cannot be used in this experiment since the label is lost during methylation to thymidine compounds.) The specific activities of the UMP, dUMP, and dTMP pools were then compared. Since the dUMP pools of *thy*$^+$ strains are extremely small compared to those of *thy* mutants (16), and since dUMP and dTMP are difficult to separate in the two-dimensional chromatographic system routinely used for separation of nucleoside monophosphates, the following modifications were introduced. (i) The specific activities of the labeled compounds added to the medium were significantly increased. (ii) The UMP spot and the combined dUMP and dTMP spots from the first chromatogram were eluted and rechromatographed in a different two-dimensional system to ensure complete separation of dUMP and dTMP (*see* legend to Table 5). The results of such an experiment are given in Table 5. As shown, the specific activities of dUMP and dTMP are equal and only 30% of that found in UMP. This indicates that 70% of the supply of dUMP necessary for dTMP synthesis in DP-55 is derived directly from a cytosine precursor, presumably from dCTP, by the sequential action of dCTP deaminase and dUTP pyrophosphatase.

DISCUSSION

This paper describes the identification of a dCTP deaminase in *S. typhimurium*. In an accompanying paper (22), the existence of a dCTP deaminase in *E. coli* and the phenotype of a mutant lacking this enzyme will be reported. No

TABLE 4. *Specific activities of pyrimidine nucleotides in S. typhimurium DP-55 and KP-170 grown with ^3H-cytidine and ^{32}P-orthophosphate*[a]

Strain	Thymine in medium (μg)	Pools[b]	Counts per min in spots[c]		$^3H/^{32}P$	Per cent derived from ^3H-cytidine[d]
			^{32}P	^3H		
DP-55	0	CMP	173	156	0.90	100
		UMP	968	0	0	0
KP-170	100	dCTP	119	38	0.96[e]	98
		CMP	121	119	0.98	100
		UMP	612	36	0.06	6
		dUMP	365	267	0.73	75
KP-170	20					
		dCTP	1159	341	0.89	83
		CMP	296	318	1.07	100
		UMP	1080	202	0.19	18
		dUMP	861	837	0.97	91

[a] Cells were grown in glucose minimal medium containing arginine (50 μg/ml), uracil (10 μg/ml), cytidine-*5*-^3H (10 μg/ml, 100 μCi/μmole), ^{32}P-orthophosphate (5 μCi/μmole), and thymine (concentrations shown above). After two generations of exponential growth, 5-ml samples were harvested and the acid-soluble nucleotides were separated by thin-layer chromatography. The appropriate spots were cut out and counted.

[b] Abbreviations: CMP, cytidine monophosphate; UMP, uridine monophosphate; dCTP, 2'-deoxycytidine triphosphate; dUMP, deoxyuridine monophosphate.

[c] Counts are corrected for background and, in the case of ^3H, for ^{32}P counts in the tritium channel.

[d] The specific activity ($^3H/^{32}P$) of CMP in each experiment is arbitrarily chosen at 100.

[e] The ratios for dCTP are corrected for the presence of three phosphate groups in this compound.

similar enzymatic activity has previously been described in other organisms, except for a phage-specific dCTP deaminase which is induced in *Bacillus subtilis* after infection with phage PBS 1 (30). The identification of a dCTP deaminase in enteric bacteria has led to the elucidation of a previously unreported pathway for dUMP biosynthesis.

The study was initiated after the observation that the introduction of a thymine requirement into a cytidine- and uracil-requiring mutant of *S. typhimurium* (DP-55) made the cells (KP-103 and KP-170) phenotypically prototrophic for uracil (Fig. 1). Studies of the growth rates and the nucleoside triphosphate pools of KP-170, under different conditions of growth, indicated that the cells were able to deaminate a deoxycytidine nucleotide (Fig. 2, Table 1). This was subsequently confirmed in vitro by the finding that crude extracts of KP-170 contained a specific dCTP deaminase (Table 2).

The following observations suggest that dCTP deaminase plays an important role in the biosynthesis of dUMP, the ultimate precursor for thymidine nucleotide biosynthesis. (i) The specific activity of the dUMP pool from DP-55, grown in the presence of uracil-*6*-^3H, ^{32}P-orthophosphate, and unlabeled cytidine, shows that 70% is derived directly from cytidine without equilibrating with the uracil ribonucleotide pool of the cells (Table 5). (ii) The only deoxycytidine compound that is readily deaminated by DP-55 and its derivatives is dCTP (Table 2). (iii) Enteric bacteria contain a very active dUTP pyrophosphatase, catalyzing the hydrolysis of dUTP to dUMP (2, 6; A. Häggmark, Abstr. 4th FEBS Meeting, Oslo, p. 296, 1967). Thus, the sequential action of dCTP deaminase and dUTP pyrophosphatase seems to account for the synthesis of 70% of the dUMP supply in DP-55. Only 30% is derived directly from a uridine nucleotide, presumably via reduction of UDP, catalyzed by ribonucleoside diphosphate reductase (2, 11; Fig. 3).

It was previously reported that 80% of the dTTP of both *S. typhimurium* and *E. coli* is derived directly from a cytosine precursor (9, 19). As a possible explanation for these observations, it was suggested that enteric bacteria, in addition to thymidylate synthetase, contain an alternate pathway for thymidine nucleotide biosynthesis, involving methylation and subsequent deamination of a deoxycytidine nucleotide. (For a more detailed discussion see reference 23.) However, the present finding, the dCTP deamination accounts for 70% of the dUMP synthesized (Table 5), shows that direct methylation of a deoxycytidine nucleotide is not involved in dTTP biosynthesis.

As mentioned above, *thy* mutants of DP-55 acquired an ability to convert exogenous cytidine to UMP, despite the fact that they lack cytidine deaminase. Since uracil appears to be an obligatory intermediate in this conversion (Table 3) and cytidine is neither broken down to cytosine nor deaminated to uridine by these cells (19), it follows that deamination occurs at the deoxycytidine nucleotide level (Fig. 3). Thus, the pathway by which cytidine is converted to uracil in KP-170 seems, likewise, to involve dCTP deamination followed by dUTP hydrolysis, the dUMP so formed being degraded further by the combined action of a phosphatase (2) and thymidine (deoxyuridine) phosphorylase (27; Fig. 3).

Although the relative contribution of the dCTP deaminase pathway to dUMP synthesis is approximately the same in DP-55 and the *thy* mutant KP-170 (i.e., 70 and 75%, respectively; Tables 4 and 5), dUMP is degraded to uracil only in KP-170 (Fig. 1). This difference seems to be related to the fact that a block in thymidylate synthetase invariably results in an extensive accumulation of dUMP (16). (Since the UMP pools of

TABLE 5. *Specific activities of pyrimidine nucleotides in S. typhimurium DP-55 grown with ^{3}H-uracil and ^{32}P-orthophosphate*[a]

Compound[b]	Counts/min in spots[c]		^{3}H/^{32}P	Per cent derived from ^{3}H-uracil[d]
	^{32}P	^{3}H		
UMP	49,782	31,576	0.634	100
dTMP	3,821	736	0.193	30
dUMP	108	22	0.204	32

[a] DP-55 was grown in glucose minimal medium containing arginine, cytidine, uracil-*6*-^3H (4 μg/ml, 700 μCi/μmole), and ^{32}P-orthophosphate (16 μCi/μmole). After three generations of exponential growth, a 15-ml sample was harvested, and the pyrimidine nucleoside monophosphates were separated on poly(ethyleneimine) cellulose plates by the method of Randerath and Randerath (26). The UMP spot and the combined dUMP and dTMP spots were eluted (24) with 1 M pyridine acetate and lyophilyzed. The residues were taken up in water and rechromatographed in two dimensions, using the following solvents: 1st dimension—1 M acetic acid (2 cm above the start line) followed by 1 M acetic acid-3 M LiCl (9:1, v/v) up to 15 cm; 2nd dimension—60 g of ammonium sulfate in 0.1 M potassium phosphate (*p*H 7.0) plus 2 ml of 1-propanol (31), 16 cm. Spots were localized by autoradiography, cut out, and counted.

[b] Abbreviations: UMP, uridine monophosphate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate.

[c] Counts are corrected for background and, in the case of ^{3}H, for ^{32}P counts in the tritium channel (1%). Background counts were: ^{32}P, 31 counts/min; ^{3}H, 11 counts/min.

[d] ^{3}H/^{32}P ratio for each compound in per cent of the ratio for UMP.

DP-55 and KP-170 are approximately equal, a comparison of the ratio of ^{32}P counts per min in dUMP to ^{32}P counts per min in UMP, calculated from the data given in Tables 4 and 5, shows that the dUMP pool of KP-170 is more than 100-fold larger than that of DP-55.) Thus, the high intracellular concentration of dUMP in *thy* mutants may, directly or indirectly, induce the catabolism of dUMP to uracil. In *thy*$^+$ strains, on the other hand, the low dUMP pool will prevent it from being degraded, thereby securing all available dUMP for dTMP synthesis.

Thymine prototrophic cells of enteric bacteria are unable to utilize exogenous thymine for DNA synthesis due to the lack of deoxyribose-1-phosphate necessary for the conversion of thymine to thymidine (8, 15). The mere fact that *thy* mutants are able to utilize thymine shows that a block in thymidylate synthetase results in an increased availability of deoxyribosyl groups. Therefore, the catabolism of dUMP to uracil and deoxyribose-1-phosphate, observed in thymine-requiring mutants, seems to be of significant physiological importance, not as a means of supplying uracil from cytosine compounds, but as a source of deoxyribosyl groups necessary for the utilization of exogenous thymine (16).

If KP-170 is grown in the presence of suboptimal concentrations of exogenous thymine, i.e., with low dTTP pools (Table 1), the cells respond by increasing the flow through the dCTP deaminase pathway. This is supported by the following observations. (i) The dCTP pool increases significantly (Table 1), probably due to a derepressed synthesis of ribonucleoside diphosphate reductase (3); more substrate, therefore, is available for dCTP deamination. (ii) The contribution of the dCTP deaminase pathway to dUMP synthesis goes up from 75 to 91% (Table 4). (iii) The intracellular concentration of dUMP increases further (*compare* with reference 16). (iv) More uracil, and thus deoxyribose-1-phosphate, is generated from exogenous cytidine (Fig. 2, Table 4). A limitation in the endogenous supply of dTTP, therefore, results in an increased production of deoxyribose-1-phosphate which facilitates the conversion of thymine to thymidine and, thus, the synthesis of dTTP from exogenous thymine.

Higher organisms contain dCMP deaminases which in several cases have been shown to be feedback inhibited by dTTP and activated by dCTP (14, 28). The fact that enteric bacteria do not contain a similar enzyme, but possess a dCTP deaminase, emphasizes the physiological importance of a pathway capable of converting deoxycytidine nucleotides to dUMP and dTMP. This is further illustrated by the specific pattern of deoxycytidine nucleotide metabolism observed in T-even phage-infected cells of *E. coli*. To prevent the incorporation of dCTP into phage-specific DNA and, at the same time, to create a ready supply of dCMP for the phage-directed synthesis of 5-hydroxymethyl-dCMP, a specific deoxycytidine triphosphatase (dCTPase) converting dCTP to dCMP is induced after infection (32, 33). Simultaneously, however, a highly regulated dCMP deaminase is induced (5, 13). Thus, although the dCTPase prevents the synthesis of dUMP via the dCTP deaminase pathway, the presence of a dCMP deaminase will compensate for this decrease in the dUMP synthesizing capacity.

The fact that the dCTP deaminase of PBS 1-infected *B. subtilis* (30) and the dCMP deaminases mentioned above are feedback regulated by dTTP suggests that a similar control may be operative with the dCTP deaminase of *S. typhimurium*. Further purification of this enzyme is in progress in our laboratory to allow testing of this assumption.

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ADDENDUM IN PROOF

By using a 100-fold purified preparation of dCTP deaminase from *S. typhimurium*, dUTP has been identified as the exclusive nucleotide product of the enzyme reaction (A. Eisenhardt, *unpublished data*).

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