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Control of Expression of the *pyr* Genes in *Salmonella typhimurium*: Effects of Variations in Uridine and Cytidine Nucleotide Pools

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The differential rate of synthesis of five of the pyrimidine biosynthetic enzymes coded for by *pyrB-F*, and the endogenous concentrations of the individual pyrimidine nucleotides were determined in specially constructed mutants of *Salmonella typhimurium*. In the mutants employed the different pyrimidine nucleotide pools may be manipulated individually during exponential growth. The results obtained indicate the following. (i) The expression of *pyrB*, *pyrE*, and *pyrF* is controlled by a uridine nucleotide in a noncoordinate manner. (ii) The expression of *pyrC* and *pyrD* is regulated predominantly by a cytidine nucleotide. Under all conditions investigated, their expression seems to be coordinated, even though the genes are not contiguous on the chromosome. (iii) The low-molecular-weight effectors involved in controlling the expression of the *pyr* genes are neither uridine 5'-monophosphate nor cytidine 5'-monophosphate, but rather the corresponding di- or triphosphates.

The de novo synthesis of the pyrimidine nucleotide 5'-uridylic acid (UMP) in *Salmonella typhimurium* and *Escherichia coli* is catalyzed by the sequential action of the six enzymes: carbamylphosphate synthetase (EC 2.7.2.5), aspartate transcarbamylase (ATCase; EC 2.1.3.2), dihydroorotase (DHOase; EC 3.5.2.3), dihydroorotate dehydrogenase (DHOdehase; EC 1.3.3.1), orotate phosphoribosyltransferase (OMPppase; EC 2.4.2.10), and orotidylic acid decarboxylase (OMPdecase; EC 4.1.1.23) (Fig. 1).

The structural genes *pyrA* through *pyrF* encoding these enzymes have been located on the chromosomes of both *S. typhimurium* and *E. coli* (20, 23). No close linkage has been found between any of the genes (Fig. 2).

Regulation of the first and the second enzyme in the pathway, through feedback inhibition and activation, has been studied in great detail (1, 2, 9, 10), whereas regulation of the pathway at the level of gene expression has been the subject of only a few investigations. Yates and Pardee (25) observed that uracil starvation of pyrimidine auxotrophic mutants of *E. coli* led to an increase in the rate of synthesis of all six enzymes of the pathway. Beckwith et al. (6) determined the specific activities of the six enzymes in *E. coli* starved for pyrimidines. They concluded that: (i) the rate of synthesis of carbamylphosphate synthetase and ATCase was regulated independently of each other and independently of the rest of the enzymes in the pathway; (ii) the rate of synthesis of the last four enzymes, coded for by *pyrC*, *pyrD*, *pyrE*, and *pyrF*, was coordinately controlled. However, our present knowledge of the location of the *pyr* genes on the chromosome (Fig. 2) shows that none of them are so closely linked that they can constitute an operon.

In previous studies (6, 24, 25), pyrimidine nucleotide limitation was accomplished either by uracil starvation of pyrimidine auxotrophic mutants or by the use of the pyrimidine analogue 6-azauracil, which acts by inhibiting OMPdecase (21). Since, however, starvation for UMP leads to starvation for all pyrimidine nucleotides of the cell (Fig. 1), most of these earlier studies did not indicate the nature of the pyrimidine nucleotide effector(s) involved in the regulation of the rate of synthesis of the individual de novo enzymes.

With the isolation of mutants of *S. typhimurium* having a specific cytidine requirement (16), due to mutations in the structural genes for cytidine deaminase (*cdd;* EC 3.5.4.5) and CTP synthetase (*pyrG;* EC 6.3.4.2), it became possible to alter the cytidine nucleotide pools of the cells without, at the same time, interfering with the uridine nucleotide pools (Fig. 1).

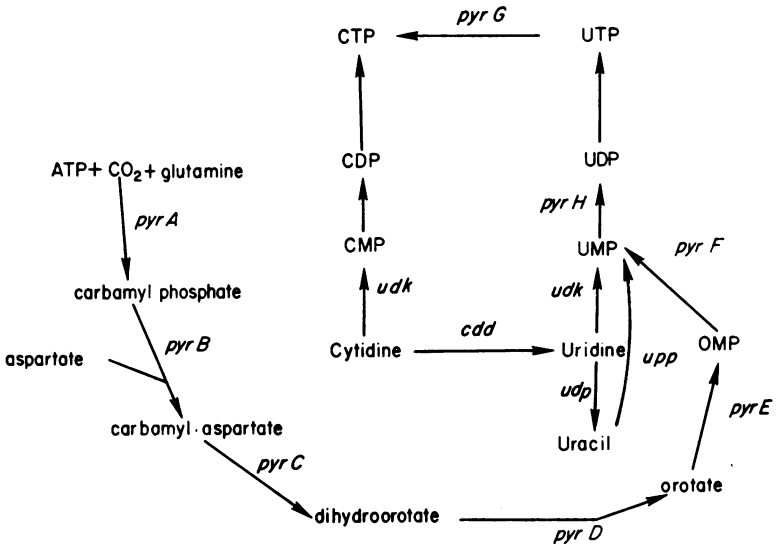

FIG. 1. *Pathway for the biosynthesis of pyrimidine nucleoside triphosphates in S. typhimurium. The enzymes are identified by the corresponding gene designations as follows: cdd, cytidine deaminase (EC 3.5.4.5); pyrA, carbamylphosphate synthetase (EC 2.7.2.5); pyrB, ATCase (EC 2.1.3.2); pyrC, DHOase (EC 3.5.2.3); pyrD, DHOdehase (EC 1.3.3.1); pyrE, OMP pyrophosphorylase (EC 2.4.2.10); pyrF, OMPdecase (EC 4.1.1.23); pyrG, CTP synthetase (EC 6.3.4.2); pyrH, UMP kinase (EC 2.7.4); udk, uridine kinase (EC 2.7.1.48); udp, uridine phosphorylase (EC 2.4.2.3); and upp, UMP pyrophosporylase (EC 2.4.2.9). CDP and UDP, Cytidine and uridine 5'-diphosphate, respectively.*

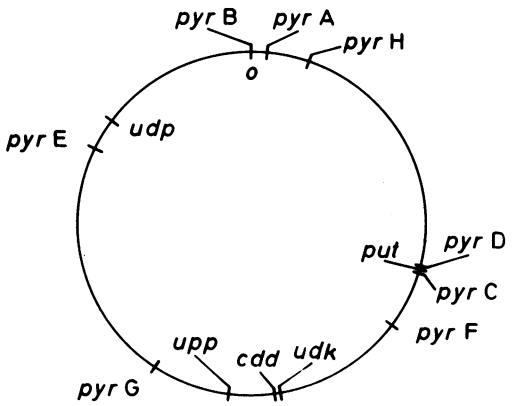

FIG. 2. *Linkage map of the S. typhimurium chromosome showing the location of genes mentioned in the text. Gene designations are explained in the legend to Fig. 1. put, Proline utilization (20).*

Using such double mutants (*cdd*, *pyrG*), Abd-El-Al and Ingraham (1) showed that to repress CPSase synthesis maximally in *S. typhimurium* both cytidine and arginine have to be present in the growth medium. Thus, in addition to arginine, a cytidine nucleotide is involved in regulating the expression of the *pyrA* gene.

In the present paper we attempt to identify the nature of the repressing metabolite(s) involved in control of the rate of synthesis of each of the five other enzymes of the pyrimidine pathway, i.e., those coded for by the genes *pyrB* through *pyrF*. To do this we constructed a series of mutants of *S. typhimurium* that may be grown exponentially under conditions where the composition of their pyrimidine nucleotide pools varies significantly from that of wild-type cells. We compared the specific activity of each of the five pyrimidine enzymes in the mutants under such conditions with the pool sizes of the individual pyrimidine nucleotides.

MATERIALS AND METHODS

Bacterial strains. All strains employed are derivatives of *S. typhimurium* LT2 and are listed in Table 1.

Growth conditions. Bacteria were grown in tris-(hydroxymethyl)aminomethane (Tris)-minimal medium (8) supplemented with 0.15% Norite-treated vitamin-free casein hydrolysate (Casamino Acids). Glucose (0.2%) was used as a carbon source. Supplementary nutrients were added in the following final concentrations: L-cysteine and L-arginine, 50 μg/ml; cytidine, 80 μg/ml; and uracil, 50 μg/ml. Liquid cultures were grown with aeration on a rotary shaker at 37 C. Growth was monitored at 436 nm in an Eppendorf photometer, model 1101M.

Preparation of bacterial extracts. Bacteria were grown exponentially for at least eight generations at 37 C to an optical density at 436 nm of 0.5, filtered on a membrane filter (Millipore Corp.), and washed with

TABLE 1. *Strains used*

Strain	Genotype	Nutritional requirements	Source or reference
KP1217	*cysCD519, cdd-7*	Cysteine	12
JL1219	*cysCD519, cdd-7, upp-22, pyrH1602*	Cysteine	12
JL1269	*cdd-7, pyrH1609*		12
JL1263	*pyrH*[a]		Former HD-58 (18)
KP1300	*cysCD519, cdd-7, upp-22, cmk-1*[b]	Cysteine	5
KP1334	*pyrA81, pyrG1611,*[c] *cdd-7, udp-2*	Arginine, uracil, and cytidine (above 30 C)	Present work

[a] This mutation was formerly (18) designated *pyrR*. However, it has recently been shown to reside in the *pyrH* gene (J. Justesen and J. Neuhard, unpublished data).

[b] New gene designation for the structural gene encoding CMP kinase (5).

[c] This *pyrG* mutation confers heat lability on the enzyme CTP synthetase.

minimal medium. Cells were suspended in cold (4 C) minimal medium and centrifuged.

The cell pellets were kept at −20 C overnight. After thawing, the cells were suspended in 0.1 M Tris-hydrochloride (pH 7.6)-2 mM ethylenediaminetetraacetic acid to an optical density at 436 nm of 10 to 20. The suspensions were sonically treated for 1 min at 0 C in an MSE ultrasonic disintegrator. Extracts were used for enzyme assays without further treatment.

Construction of KP1334. *S. typhimurium* JL1055 (*pyrA81 pyrG1606 cdd-7 udp-2*), which is cytidine requiring at all temperatures due to the *pyrG1606* mutation, was transduced to grow in the absence of cytidine at 30 C by using transducing phage P22 grown on a strain containing the *pyrG1611* allele. The *pyrG1611* mutation confers heat lability to cytidine 5'-triphosphate (CTP) synthetase. KP1334 is still cytidine requiring at 42 C.

Enzyme assays. All assays were performed at 37 C. Specific activities are expressed as units per milligram of protein. One unit of enzyme activity equals 1 nmol of substrate utilized, or product formed, per minute.

ATCase. ATCase was assayed by the method of Gerhart and Pardee (10), except that the assays were performed at 37 C.

DHOase, DHOdehase, OMPppase, and OMPdecase were assayed by the method of Beckwith et al. (6) with the modifications described below.

DHOase. For DHOase, the assay mixture contained in 0.5 ml: 0.1 M Tris-hydrochloride (pH 8.8), 0.2 mM ethylenediaminetetraacetic acid, 2 mM L-dihydroorotate, and bacterial extract. The mixture was kept in ice bath for 5 min and then transferred to 37 C. At 2, 12, and 22 min, 150-μl samples were removed, and the amount of carbamylaspartate formed was determined as in the ATCase assay.

DHOdehase. For DHOdehase, the assay mixture contained in 1 ml: 0.1 M Tris-hydrochloride (pH 8.8), 6 mM $MgCl_2$, and bacterial extract. L-Dihydroorotate (50 μl, 20 mM) was added to start the reaction. The conversion of L-dihydroorotate to orotate was followed continuously at 290 nm in a Zeiss-PMQ III spectrophotometer. An increase in absorbancy of 1.93 (1-cm light path) is equivalent to a change in substrate concentration of 1 mM.

OMPppase. For OMPppase, the assay mixture contained in 1 ml: 0.1 M Tris-hydrochloride (pH 8.8), 6 mM $MgCl_2$, 0.25 mM orotate, and bacterial extract. The reaction was started by addition of 100 μl of 6 mM 5-phosphoribosyl-1-pyrophosphate. The reaction was followed spectrophotometrically at 295 nm. A decrease in absorbancy of 3.67 is equivalent to an increase of OMP concentration of 1 mM.

OMPdecase. The assay mixture contained in 1 ml: 0.1 M Tris-hydrochloride (pH 8.8), 6 mM $MgCl_2$, and bacterial extract. OMP (10 μl, 0.02 M) was added to start the reaction. The reaction was followed at 290 nm. A decrease in absorbancy of 1.38 is equal to a decrease in OMP concentration of 1 mM.

Proteins. Proteins were determined by the method of Lowry et al. (15), with bovine serum albumin as a standard. The crude extracts used for the enzyme assays were spun for 5 min at 6,000 × *g*. The supernatant was used for protein determinations.

Nucleoside triphosphate pools. The nucleoside triphosphate pools were determined in cultures grown for two generations in the presence of [^{32}P]orthophosphate (specific activity, 10 μCi/μmol). Growth conditions in the labeled cultures were exactly the same as in the cultures used for enzyme determinations. Extraction and chromatographic techniques used for determining the individual nucleoside triphosphate pools have been described previously (17). To avoid variation in the calculated pool sizes due to small changes in the concentration of orthophosphate in the medium, all pools are given in percent total ribonucleoside triphosphates.

RESULTS

The availability of *S. typhimurium* mutants blocked in different steps of pyrimidine nucleotide interconversion (12, 19) has made possible the construction of strains in which the different pyrimidine nucleotide pools may be manipulated independently. A study of the effects of such changes in individual pyrimidine nucleotide pools on the levels of the pyrimidine

biosynthetic enzymes should help in identifying the particular pyrimidine nucleotide effector(s) involved in regulating the rate of synthesis of each of the enzymes coded for by the genes *pyrB* through *pyrF*.

The mutants used in the present study display the following unique characteristics. (i) They are capable of growing exponentially under conditions in which their individual pyrimidine nucleotide pools vary from that observed during growth of wild-type cells. (ii) They do not contain mutations in any of the genes *pyrB* through *pyrF* encoding the five enzymes under investigation.

Effect of variation in the uridine nucleotide pools. Mutations in the structural gene for UMP kinase (*pyrH*); rendering this enzyme partially defective result in cells that will grow with reduced uridine 5'-triphosphate (UTP) and uridine 5'-diphosphate pools. The size of the UTP pool, as well as the growth rate of any particular mutant, is dependent on the *pyrH* mutation in question (12).

In Table 2, experiments 1, 4, 5, and 6 give the nucleotide triphosphate pools and the growth rates of $pyrH^+$ strain JL1217 and of three different *pyrH* mutants JL1263, JL1219, JL1269, respectively.

From this table it appears: (i) that the growth rate of the three *pyrH* mutants decreases with a decrease in the relative UTP pools, (ii) the relative CTP pools do not change in parallel with the UTP pools, and (iii) addition of exogenous uracil to the $pyrH^+$ strain results in a definite increase in the UTP pool of the cells (experiment 2). A similar increase is not observed in *pyrH* mutants due to their defective UMP kinase (not shown).

Table 3 lists the specific activities of the five pyrimidine biosynthetic enzymes in strain JL1217 grown in the presence and absence of uracil, and in the three *pyrH* mutants. There is an inverse relationship between the relative activity of each of the five enzymes and the size of the UTP pool, suggesting that a uridine nucleotide may be involved as an effector in the regulation of the rate of synthesis of these enzymes. For three of the enzymes this is illustrated graphically in Fig. 3. The results from Table 3 show also that the rate of synthesis of individual enzymes derepresses to various degrees. Maximal values obtained (ratio of highest to lowest) are 190-fold for ATCase, 14-fold for DHOase and DHOdehase, 32-fold for OMPppase, and 7-fold for OMPdecase.

Effect of variations in the cytidine nucleotide pools. Cytidine added to the medium of wild-type *S. typhimurium* or *E. coli* is quantitatively converted to uridine by the cells. The deamination is catalyzed by the periplasmic enzyme cytidine (deoxycytidine) deaminase (*cdd*) (3, 16, 19; Fig. 1). In contrast, *cdd* mutants convert exogenous cytidine to CTP effectively. All of the mutants employed in the present study contain the mutation *cdd-7* (Table 1).

The effect of exogenous cytidine on the relative nucleoside triphosphate pools of strain JL1217 is shown in Table 2 (experiments 1 and 3). Cytidine causes a significant increase in the CTP pool without affecting the UTP pool. The effect is even more striking in strain JL1269, in which the UTP pool is very low due to the *pyrH* mutation (Table 2, experiments 6 and 7). In this strain, the addition of cytidine results in a CTP pool greater than the adenosine 5'-triphosphate

TABLE 2. *Growth rates and relative pool sizes in different mutants of S. typhimurium*

Expt	Strain	Relevant genotype[a]	Addition to growth medium (μg/ml)	Growth rate doubling/h	Relative pool sizes[b]			
					UTP	CTP	ATP	GTP
1	JL1217		None	2.2	18	13	49	20
2	JL1217		Uracil, 50	2.3	24	16	43	17
3	JL1217		Cytidine, 80	2.3	15	25	43	17
4	JL1263	*pyrH*	None	1.7	7	8	50	35
5	JL1219	*pyrH*	None	1.7	6	15	46	33
6	JL1269	*pyrH*	None	1.0	2	9	55	34
7	JL1269	*pyrH*	Cytidine, 80	1.1	3	45	32	20
8	KP1300	*cmk*	None	1.6	18	10	51	21
9	KP1334	*pyrG*	None	1.2	34	5	42	19

[a] The relevant genotype and pyrimidine addition for each of the experiments are given. For further information see Table 1.

[b] Percent total ribonucleoside triphosphate pool (NTP_{tot}). In the experiments shown, NTP_{tot} was (in micromoles per gram of bacterial dry weight): JL1217, 14; JL1263, 10; JL1219, 9; JL1269, 35; KP1300, 9; KP1334, 29. ATP, Adenosine 5'-triphosphate; GTP, guanosine 5'-triphosphate.

TABLE 3. *Levels of the five pyrimidine biosynthetic enzymes during UTP limitation*

Expt.	Strain	Addition to growth medium (μg/ml)	Pool sizes[a]		Relative sp act[b]				
			UTP	CTP	ATCase *pyrB*	DHOase *pyrC*	DHOdehase *pyrD*	OMPppase *pyrE*	OMPdecase *pyrF*
1	JL1217	Uracil, 50	24	16	0.4	0.9	0.7	0.4	0.5
2	JL1217	None	18	13	1 (48)	1 (50)	1 (50)	1 (55)	1 (45)
3	JL1263	None	7	8	14	4.6	4.2	6.3	1.2
4	JL1219	None	6	15	35	6.2	7.1	8.4	2.3
5	JL1269	None	2	9	75	11.7	11.0	12.8	3.4

[a] Pool sizes are taken from Table 2.

[b] Specific activities are expressed relative to the specific activity found in JL1217 (experiment 2). Values in parenthesis are the absolute specific activities expressed as nanomoles per minute × milligram of protein. For each strain the same extract was used for all the enzyme assays. The figures given are the average of two determinations on the same extract.

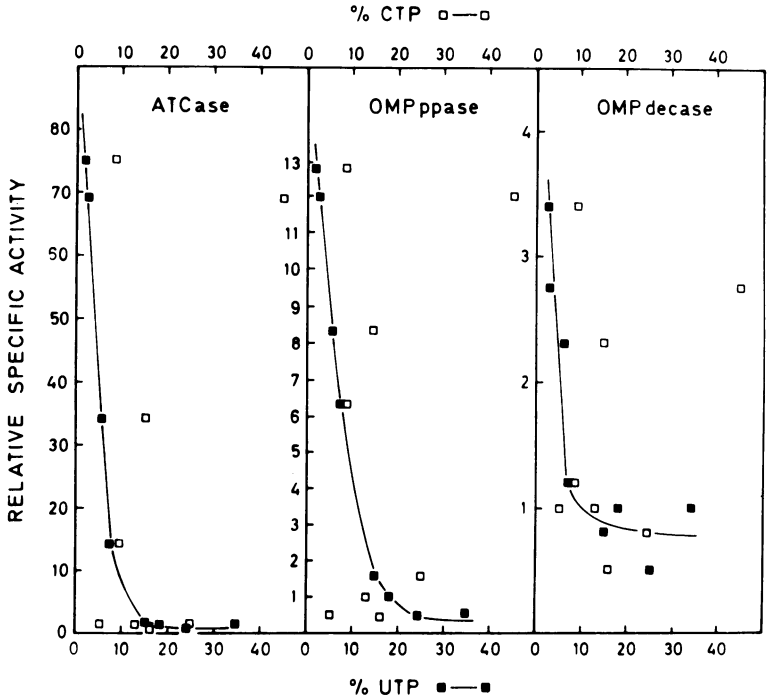

FIG. 3. *Derepression of aspartate carbamyltransferase (ATCase), OMPppase, and OMPdecase in mutants of S. typhimurium growing exponentially with altered UTP and/or CTP pools. The ordinate represents specific activities relative to those found in strain JL1217 growing in absence of exogenous pyrimidines. The abscissa represents the UTP pool (bottom) or CTP pool (top) in percent total ribonucleoside triphosphate pool. Symbols: ■, relative specific activities plotted versus the UTP pools; □, relative specific activities plotted versus the CTP pool. The data are taken from Tables 3 and 4.*

pool without significantly affecting the relative size of UTP.

Table 4 (experiments 1 through 4) gives the specific activities of the five enzymes in strains JL1217 (*pyrH*$^+$) and JL1269 (*pyrH1609*) grown with or without cytidine added to the medium. The data suggest that a high CTP pool is accompanied by significant repression of only two of the enzymes of the pathway (i.e., DHOase and DHOdehase) and that the rate of synthesis of these two enzymes is repressed by the addition of exogenous cytidine both in strains growing with a normal UTP pool (JL1217) and in strains growing with very low UTP pools (JL1269).

To confirm the selective effect of changes in cytidine nucleotide pools on the rate of synthesis of DHOase and DHOdehase, we employed

mutant KP1334, which contains a heat-labile CTP synthetase (*pyrG1611*) (Table 1). At 37 C in the absence of cytidine, the growth of KP1334 is somewhat slower than that of a *pyrG*$^+$ strain. Under these conditions the relative CTP pool of the cells is very low, while the UTP pool is increased (Table 2, experiment 9). From Table 4 (experiment 5) it appears that a low CTP pool is accompanied by an increase in the rate of synthesis of DHOase and DHOdehase.

Nucleoside mono-, di-, or triphosphate? From the results presented in the previous sections it appears that variations in the uridine nucleotide pool or the cytidine nucleotide pool have different effects on the rate of synthesis of each of the five pyrimidine biosynthetic enzymes. We would like to establish the exact nature of the effector nucleotide in each case and thus determine the level of phosphorylation of the effector nucleotide(s). This requires that we modulate the ratio between the pyrimidine nucleoside mono-, di-, and triphosphates in vivo and study the effect of such modulations on the differential rate of synthesis of the individual enzymes. In the present study, uridine nucleotide limitation is accomplished by the use of *pyrH* mutants with high levels of UMP due to their partial defect in UMP kinase. Thus, we conclude that the effector involved in the regulation of the *pyr* genes is not UMP, but rather uridine 5'-diphosphate, UTP, or eventually a uridine sugar nucleotide.

Recently, we isolated and characterized a mutant of *S. typhimurium*, KP1300, lacking cytidine 5'-monophosphate (CMP) kinase activity (5). Mutant KP1300 has a slightly lower growth rate than strain JL1217. Table 2 shows that the UTP and CTP pools of KP1300 are nearly identical to those of the wild type. However, the CMP pool is sixfold higher in the mutant than in the parent strain (5). Since the specific activities of DHOase and DHOdehase are not lower in the mutant than in the parent strain (Table 4), we conclude that the cytidine nucleotide effector involved in repressing the rate of synthesis of DHOase and DHOdehase is not CMP but rather cytidine 5'-diphosphate or CTP.

DISCUSSION

No close linkage is found for any of the pyrimidine genes (*pyrA* through *pyrF*) encoding the six enzymes for the biosynthesis of UMP in *S. typhimurium* and *E. coli* (Fig. 2). The expression of these genes is controlled by pyrimidines; pyrimidine starvation results in derepression of the rate of synthesis of all six enzymes. Previous results obtained with *E. coli* indicate that, whereas the expression of *pyrA* and *pyrB* was regulated independently, the expression of the four remaining genes seemed to be coordinately regulated (6, 25). Recently, however, Dennis and Herman (7) found that partial pyrimidine starvation of a *pyrB* mutant of *E. coli*, created by feeding orotic acid as the sole pyrimidine source, resulted in noncoordinate derepression of the rate of synthesis of DHOdehase (*pyrD*) and OMPppase (*pyrE*).

In all the above-mentioned studies, pyrimidine limitation implied starvation of the cells for UMP, which in turn results in a more or less general starvation for all endogenous pyrimidine compounds. The isolation of mutants in *S. typhimurium* having a growth requirement for cytidine (*pyrG*, *cdd*) allowed the manipulation of the cytidine nucleotide pool without interfering with the uridine nucleotide pool. Abd-El-Al and Ingraham (1) used such mutants to show

TABLE 4. *Levels of five pyrimidine biosynthetic enzymes with variations in the CTP pool*

Expt	Strain	Addition to growth medium (μg/ml)	Pool sizes[a]		Relative sp act[b]				
			UTP	CTP	ATCase *pyrB*	DHOase *pyrC*	DHOdehase *pyrD*	OMPppase *pyrE*	OMPdecase *pyrF*
1	JL1217	Cytidine, 80	15	25	1.4	0.3	0.3	1.6	0.8
2	JL1217	None	18	13	1 (48)	1 (50)	1 (50)	1 (55)	1 (45)
3	JL1269	None	2	9	75	11.7	11.0	12.8	3.4
4	JL1269	Cytidine, 80	3	45	69	0.9	1.1	12.0	2.7
5	KP1334	None	34	5	1.2	5.8	4.6	0.6	1.0
6	KP1300	None	18	10	1.3	1.8	1.9	2.0	1.3

[a] Pool sizes are taken from Table 2.

[b] Specific activities are expressed relative to the specific activity found in JL1217 (experiment 2). Values in parenthesis are the absolute specific activities expressed as nanomoles per minute × milligram protein. For each strain the same extract was used for all the enzyme assays. The figures given are the average of two determinations on the same extract.

that in order to obtain full repression of *pyrA* both arginine and cytidine had to be present in the growth medium. The presence of uracil did not affect the rate of synthesis of carbamylphosphate synthetase significantly.

Using similar mutants (*pyrA*, *pyrG*, *cdd*), Williams and O'Donovan (24) studied the derepression of four other genes *pyrB*, *pyrD*, *pyrE*, and *pyrF*. They determined the specific activities of ATCase, DHOdehase, OMPppase, and OMPdecase after a 1-h starvation period for either cytidine or uracil. From their results it appeared that, whereas starvation for uracil (not for cytidine) resulted in a significant increase in the specific activity of ATCase, only cytidine starvation promoted increased levels of DHOdehase, OMPppase, and OMPdecase. Since, however, their results were obtained with nongrowing cell populations and no simultaneous determinations of the individual intracellular pyrimidine nucleotide pools in the starved cells were performed, it is difficult to make any firm conclusions regarding the differential rate of synthesis of the individual enzymes investigated or the nature of the effector involved (cytidine or uridine nucleotide) in controlling the expression of the genes *pyrB*, *pyrD*, *pyrE*, and *pyrF*.

In the present paper we have likewise studied mutants in which uridine nucleotide and cytidine nucleotide metabolism is partially separated by mutation, i.e., in *cdd* mutants. However, the mutants employed by us contain additional mutations that make it possible to grow these strains exponentially under conditions where the individual pyrimidine nucleotide pools differ significantly from those of the parental strains. This enables us to compare the differential rate of synthesis (i.e., specific activity during exponential growth) of the five enzymes encoded for by the genes *pyrB* through *pyrF* with the intracellular levels of the individual pyrimidine nucleotides. The results obtained (Tables 2 through 4 and Fig. 3) may be summarized as follows.

(i) The rate of synthesis of all five enzymes investigated is increased in cells growing with decreased UTP pools. The extent of derepression varies inversely with the size of the UTP pool (Table 3). However, the extent of derepression observed varies considerably for the individual enzymes. Since the results were obtained with mutants containing partially defective UMP kinases, their UMP pools are high and, thus, UMP may be ruled out as an effector in the control of expression of the corresponding structural genes *pyrB* through *pyrE*.

(ii) The differential rate of synthesis of ATCase, OMPppase, and OMPdecase responds qualitatively in the same way to changes in pyrimidine nucleotide pools. This is illustrated in Fig. 3, which shows the levels of these enzymes in exponentially growing cells of different mutants as functions of the CTP pools or the UTP pools of the mutants. Moreover, the results presented in Fig. 3 suggest that the rates of synthesis of these three enzymes are under control of UTP or a uridine nucleotide closely related metabolically to UTP (e.g. uridine 5'-diphosphate or a uridine 5'-diphosphate sugar) rather than under control of CTP.

(iii) The rate of synthesis of DHOase and DHOdehase seems to respond to variations in both UTP and CTP, since limitations for either of these nucleotides cause derepression (Table 3; Table 4, line 5). However, the finding that high CTP pools are accompanied by full repression of the synthesis of DHOase and DHOdehase independent of the size of the UTP pools (cf. JL1217 and JL1269 with cytidine, Table 4, experiments 2 and 4) suggests that CTP control is "dominant" over UTP control. The results obtained with KP1300, lacking CMP kinase activity (Table 4, experiment 6), indicate that the cytidine nucleotide involved in the regulation is not CMP but rather cytidine 5'-diphosphate or CTP. It should be noted (as illustrated in Fig. 4) that, under all conditions tested, the expression of the *pyrC* and *pyrD* genes seems to be coordinate. Since the two genes are separated by several unrelated genes (e.g., the *put* genes [20]; Fig. 2), they do not comprise an operon. However, it is suggested that they share a common repressor and that their promotor-operator regions are very similar.

Ginther and Ingraham (11) have recently isolated a cold-sensitive mutant of *S. typhimurium*, JL2097, defective in the structural gene coding for nucleoside diphosphokinase (*ndk*; EC 2.4.7.6). Studies of strain JL2097 (11) indicated that cessation of growth of the mutant at the nonpermissive temperature (20 C) results in a 50% decrease in the nucleoside triphosphate pools and no accumulation of nucleoside diphosphates. Under the same condition, the rate of synthesis of ATCase and DHOdehase (no other pyrimidine enzymes were assayed) was repressed. An explanation for these unexpected results may be related to the observation that at the nonpermissive temperature strain JL2097 accumulated significant amounts of ppGpp and pppGpp (11).

Several attempts have been made to isolate mutants showing constitutive synthesis of all

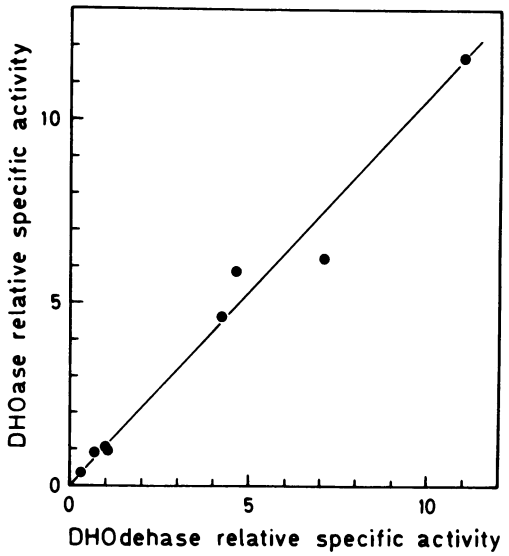

FIG. 4. *Coordinate expression of the pyrC and pyrD genes. The relative specific activities of DHOase are plotted against the relative specific activity of DHOdehase in different mutants of S. typhimurium growing exponentially with altered UTP and CTP pools. The data are taken from Tables 3 and 4.*

the pyrimidine biosynthetic enzymes, either by selecting for resistance to pyrimidine analogues or by selecting for pyrimidine overproducing mutants (18). However, all of the mutants isolated having high levels of the enzymes, when grown in the presence of exogenous uracil (designated *pyrR* [18]), have recently been characterized as mutants defective in the structural gene *pyrH* coding for UMP kinase (J. Justesen and J. Neuhard, unpublished data).

Therefore, no mutants directly affected in the regulation of the expression of all the *pyr* genes have been characterized. This, together with our present results, leads us to believe that at least two repressors are involved in controlling the expression of the *pyr* genes.

Evidence in two biosynthetic systems has demonstrated that the first enzyme in a pathway can play a role in the repression of enzymes in that pathway (13, 14). Such a mechanism does not seem to be operative in the repression of the pyrimidine pathway since it has been shown (22; M. Schwartz, unpublished data) that a deletion mutation that covers the entire *pyrB* locus in *S. typhimurium* does not affect the repressibility or the derepressibility of the residual enzymes of the pathway. However, this need not exclude the following possibilities: (i) that the *pyrB* gene product is involved in its own expression; or (ii) that the gene product of any of the other *pyr* genes could be involved in the regulation of its own synthesis or in the regulation of the synthesis of certain of the other enzymes of the pathway.

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