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Isolation and Partial Characterization of an *argR* Mutant of *Salmonella typhimurium*

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An arginine regulatory mutant (i.e., mutated in the *argR* gene) has been isolated from a strain of *Salmonella typhimurium* LT2. The *argR* mutant was found to excrete arginine into the growth medium with glycerol but not glucose as carbon source. Constitutive synthesis of arginine biosynthetic enzymes was observed. Whereas previous results (A. T. Abd-El-Al and J. L. Ingraham, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, K169, p. 175) have shown constitutive synthesis of carbamyl phosphate synthetase in the *argR* mutant, the regulation of the synthesis of the last five enzymes of the pyrimidine pathway was unaffected. However, in *pyrH* mutants, known to exhibit derepressed synthesis of the pyrimidine enzymes, a 10-fold derepression of ornithine transcarbamylase was observed.

The arginine biosynthetic system of *Escherichia coli* has received extensive study for 20 years (for a recent review, see reference 22). However, arginine metabolism in the closely related bacterium *Salmonella typhimurium* has not been as intensively investigated. In both of these genera, a single carbamyl phosphate synthetase (CPSase) (encoded by the gene *pyrA*) catalyzes the synthesis of carbamyl phosphate (CP) (18, 23). CP is considered the first intermediate of pyrimidine biosynthesis and is also required at the sixth step of arginine biosynthesis (Fig. 1).

To date, no true regulatory mutants in which the pyrimidine pathway is affected have been isolated, but mutants are available that mimic constitutive synthesis of all six pyrimidine enzymes. These mutants do not have a regulatory dysfunction per se, but rather contain a partial impairment of the enzyme uridine monophosphate kinase, encoded by the gene *pyrH* (9).

Regulatory mutants (mutated in the *argR* gene) altered in control of the arginine pathway of *E. coli* were isolated in 1961 by Maas (15), and a brief report of a method for the isolation of arginine regulatory mutants of *S. typhimurium* has been published (10). A variety of methods have been established for the isolation of arginine regulatory mutants of *E. coli*, such as selection for canavanine resistance (11, 15), *N*-acetyl amino acid utilization in the presence of arginine by various amino acid auxotrophs (3), and phenotypic suppression of proline auxotrophy in the presence of arginine (10).

Auxotrophs for certain amino acids are capable of utilizing the *N*-acetyl derivative of the required amino acid; such utilization is precluded when arginine is added to the medium because the added arginine represses the synthesis of the enzyme that deacylates the *N*-acetyl amino acid, namely *N*-acetylornithinase (NAOase). Thus, mutants selected for the ability to use the *N*-acetyl amino acid in the presence of arginine are, generally, *argR* mutants.

Phenotypic revertants of *proA*, *proB*, or *proAB* deletion mutants can be obtained (4, 10, 14); however, such proline-independent strains do not result from true back-mutation, but rather occur from a mutation in the arginine pathway in the *argD* gene (encoding the enzyme *N*-acetylornithine-δ-transaminase [AMTase]) of *E. coli* (21); the homologous locus in *S. typhimurium* is *argG* (19). The biochemical basis for the phenotypic suppression of proline auxotrophy, termed indirect suppression (7, 14), is outlined in Fig. 2. These double mutants are able to grow in the absence of arginine because a nonspecific transaminase partially substitutes for the missing enzyme (10). When arginine is added, arginine biosynthesis is repressed (22) and the indirectly suppressed mutants become proline requiring. Arginine regulatory mutants can be derived from indirectly suppressed proline auxotrophs by selecting for strains capable of growth in the presence of arginine without added proline (10).

We undertook our studies on arginine metabolism with the primary aim of exploring the interrelationships of the arginine and pyrimidine pathways of *S. typhimurium*. For such a

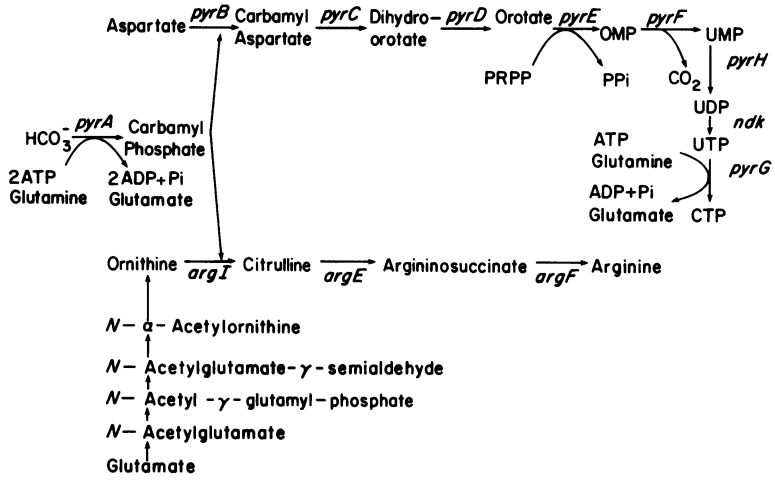

FIG. 1. *Pyrimidine and arginine biosynthetic pathways of Salmonella typhimurium. Genetic symbols for the enzymes are shown in italics. The gene designations are as follows: pyrA, carbamyl phosphate synthetase (EC 2.7.2.5); pyrB, aspartate transcarbamylase (EC 2.1.3.2); pyrC, dihydroorotase (EC 3.5.2.3); pyrD, dihydroorotate dehydrogenase (EC 1.3.3.1); pyrE, orotidine monophosphate (OMP) pyrophosphorylase (EC 2.4.2.10); pyrF, OMP decarboxylase (EC 4.1.1.23); pyrH, uridine monophosphate (UMP) kinase (EC 2.7.4.4); ndk, nucleoside-diphosphokinase (EC 2.7.4.6); pyrG, cytidine triphosphate (CTP) synthetase (EC 6.3.4.2); argI, ornithine transcarbamylase (EC 2.1.3.3); argE, argininosuccinate synthetase (EC 6.3.4.5); and argF, argininosuccinase (EC 4.3.2.1).*

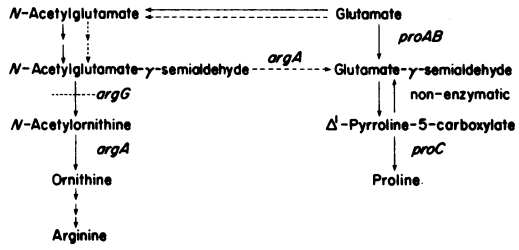

FIG. 2. *Biosynthesis of arginine and proline from glutamate in E. coli and S. typhimurium. In argG mutants of S. typhimurium (equivalent to argD mutants of E. coli), the argA enzyme (argE in E. coli) mediates the deacylation of N-acetylglutamate-γ-semialdehyde to glutamate-γ-semialdehyde. Symbols: →, Normal pathways; - - →, proline-suppressed pathway in proAB argG double mutants. The genetic designations represent the following enzymes: argG, N-acetylornithine-δ-transaminase (EC 2.6.1.11); argA, N-acetylornithinase (EC 3.5.1.16); proAB, glutamate-γ-semialdehyde dehydrogenase; proC, pyrroline carboxylate reductase (EC 1.5.1.2).*

project, it was important to isolate arginine regulatory mutants. The studies described herein report the isolation and partial characterization of an *argR* mutant and the effect of a *pyrH* mutation on the arginine pathway. The *argR* gene has been located at 106 min on the *S. typhimurium* chromosome (12), which is analogous to the position on the *E. coli* K-12 chromosome (11).

MATERIALS AND METHODS

Organisms. Most of the bacterial strains used in this study were derivatives of *S. typhimurium* strain LT2. Strain P110 is derived from *S. typhimurium* strain LT7. Table 1 lists the strains used, their genotypes, and the source. Bacteriophage P22-L4 was used for transductions and was received from K. E. Sanderson.

Media and growth conditions. The composition of the defined medium, AG medium, has been reported (12). Nutritional supplements were added at 100 μg/ml unless stated otherwise. The complex medium was nutrient broth (Difco). Solid media were prepared by the addition of agar to 1.5%. Growth procedures were as previously described (12).

Preparation of cell extracts and enzyme assays. The method of preparing cell extracts and determining protein concentration has been reported (13). Assays for the last five enzymes of the pyrimidine pathway and for ornithine transcarbamylase (OTCase) were conducted according to previously published procedures (13). The assays of NAOase and AMTase were carried out as previously described (5). Specific activities are defined as nanomoles of product formed (or substrate utilized) per minute per milligram of protein.

***N*-acetylhistidine utilization.** The procedure described by Baumberg (3) was used as a possible method of selecting arginine regulatory mutants.

Selection of indirectly suppressed mutants. The basic procedure for the isolation of indirectly suppressed mutants of proline auxotrophs and the subsequent selection of arginine regulatory mutants from indirectly suppressed strains has been described (10). The following is a detailed presentation

TABLE 1. *Bacterial strains*

Strain	Genotype	Source
LT2	Wild type	P. E. Hartman
JL84	HfrA, *metB406 hisD23 gal-50*	J. L. Ingraham
P21	*proAB21*	K. E. Sanderson
B75	*argB75*	K. E. Sanderson
F87	*argF87*	K. E. Sanderson
SU453	*his-1009 trpE2 metA22 xyl-1 strA201*	K. E. Sanderson
P110	*proC110* (LT7)	K. E. Sanderson
TR192	*ser-791 his-644*	J. Roth
JL1269	*pyrH1609 cdd-7*	J. L. Ingraham
KD1272	*pyrH1609 cdd-7 pro-5*	This study
KD1275	*cdd-7*	This study

of the protocol used in this study. Strain P21 was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (2), and samples of the mutagen-treated culture were spread on AG plates. After 72 h of incubation at 37°C, some of the developing colonies were picked and purified on AG medium. These colonies were then tested for the phenotype of being proline requiring in the presence of arginine.

Selection of arginine regulatory mutants. Those indirectly suppressed isolates showing arginine sensitivity were grown to stationary phase in nutrient broth medium. Samples were spread on AG plates containing arginine and incubated. Resulting colonies were picked and purified on AG medium supplemented with arginine and then tested for the ability to grow on unsupplemented AG medium.

Genetic techniques. (i) Conjugation. The method for introducing the *pyrH*$^+$ allele into a mutant *pyrH* strain has been described (13).

(ii) Transduction. The methods used with P22-L4 were as previously published (6). For cotransfer of *argG*$^+$ and *strA*, we used *strA* as the selective marker and screened for the cotransfer of *argG*$^+$ to indirectly suppressed strains as described (14). Selection for transfer of *argG*$^+$ was also performed. Kuo and Stocker (14) have reported that *argG*$^+$ transductants of indirectly suppressed strains are easily recognizable as fast-growing colonies on a background of slow-growing recipient cells when plated on defined medium supplemented with proline. Their basic procedure was followed except that the plates were incubated at 20°C rather than 36°C. The *argG*$^+$ (or *strA argG*$^+$) transductants were then tested for arginine excretion on indicator plates (12) and assayed for AMTase activity.

Syntrophic studies. The testing of various strains for the excretion of arginine or proline was carried out as described by Berg and Rossi (4) with slight modification. The test and indicator strains were grown in nutrient broth medium, and testing was carried out on a medium consisting of the basal salts of AG medium and 0.4% glycerol as carbon source.

Quantitation by bioassay of arginine excreted. A standard curve relating the amount of growth of strain F87 to various limiting concentrations of arginine was prepared. The *argR* mutant test strain was grown in various media to a cell density of 6×10^8/ml. The cells were pelleted by centrifugation, and the supernatants were collected and filter sterilized. A 5-ml volume of a sterile culture supernatant was added to a flask containing 5 ml of AG medium. The flask was inoculated with 0.05 ml of a strain F87 culture (grown in arginine limiting medium). The flasks were incubated and growth was monitored. In the flasks in which no growth occurred within 12 h, a known, limiting concentration of arginine was added. The resulting growth was followed, and the amount of growth was checked against the standard curve to insure that it was consistent with that expected for the particular arginine concentration. This procedure was necessary in order to eliminate the possibility that a lack of growth was due to the accumulation of a toxic product in the culture fluid rather than to an actual deficiency in arginine excretion.

Chemicals. Substrates and reagents for the various enzymes were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals were reagent grade.

RESULTS

***N*-acetylhistidine utilization and arginine sensitivity.** A histidine auxotroph of *S. typhimurium* (strain TR192) was tested for the ability to use *N*-acetylhistidine as histidine source. The strain grew with *N*-acetylhistidine as histidine source, but growth was not inhibited by arginine as occurs with *E. coli* (3). Measurement of the level of NAOase in cells grown in the presence and absence of arginine explained the lack of arginine sensitivity. In the presence of arginine, NAOase was repressed from a specific activity of 18.4 to 8.3, and thus an adequate level of NAOase was still present in the cells to carry out the deacylation of *N*-acetylhistidine. Various intermediates of the arginine pathway were then tested for their repressive effect on the synthesis of NAOase, but none was as effective as arginine (R. A. Kelln, unpublished observations).

Indirectly suppressed proline auxotrophs and arginine-resistant derivatives. After nitrosoguanidine mutagenesis, derivatives of strain P21 were isolated that grew in the absence of proline, although at a slow rate. Eleven selected isolates were then tested for their growth responses on various media. All of the isolates showed excellent growth on medium supplemented with proline and arginine. Nine of the eleven isolates failed to grow with arginine as the sole supplement. These nine isolates were assumed to be *argG* mutants (Fig. 2). A number of the indirectly suppressed isolates showing arginine sensitivity were chosen, and arginine-resistant derivatives of these isolates were selected spontaneously.

Enzymes activities of various mutants. The activities of two enzymes of the arginine pathway (encoded by unlinked genes) were assayed from isolates grown in the presence of both proline and arginine (Table 2). The most significant finding was the high levels of OTCase in extracts of arginine-resistant derivatives of isolate 11.

Growth rates in various media. The growth rates in defined media of the parent strain (P21), an indirectly suppressed isolate, and of an arginine-resistant strain derived from it are given in Table 3. Growth of strain P21 occurred only in the presence of proline. Isolate 11 grew slowly in AG medium or in AG medium plus proline; it did not grow at all when arginine was the sole supplement. In the presence of both amino acids, the growth rate of isolate 11 was comparable with that of strain P21. The arginine-resistant derivative of isolate 11 showed a similar growth pattern to isolate 11 except that it grew in the presence of arginine, but at a slow rate. This result indicated that the supply of glutamate-γ-semialdehyde for the cross-feeding of the proline pathway was limiting.

TABLE 2. *NAOase and OTCase activities in various isolates*[a]

Isolate[b]	Sp act[c]	
	NAOase	OTCase
4	42.8	240
8	29.1	56.4
11	40.3	16.2
4-7	94.8	242
8-4	58.3	268
11-5	175	1138
11-6	138	992
11-9	124	1214
11-10	125	1142
11-11	111	1102
11-12	108	1110

[a] Cells were grown in AG medium supplemented with proline and arginine.

[b] The first number denotes the original indirectly suppressed proline auxotroph derived from strain P21. The number after the hyphen designates the arginine-resistant subisolate.

[c] As defined in the text.

TABLE 3. *Growth rates of various strains in different media*

Strain	Relevant genotype	Generation time (min) in various media[a]			
		No additions	Proline	Arginine	Proline + arginine
P21	*proAB*	–[b]	55	–	52
11	*proAB argG*	185	175	–	52
11-5	*proAB argG argR*	150	150	144	54

[a] The strains were grown in AG medium with supplements as indicated.

[b] –, No growth.

Transductions. Kuo and Stocker (14) have demonstrated the cotransduction of *argG* and *strA* in *S. typhimurium* LT7. A lysate of P22-L4 was prepared on an *strA argG*$^+$ donor strain (strain SU453) and used to infect the recipient strain, isolate 11-5. A second lysate of phage P22-L4 was prepared on wild-type LT2 and used to infect the recipient strain. The *argG*$^+$ *strA* transductants were assayed for AMTase activity. These transductants showed high levels of AMTase, whereas the recipient strain was devoid of measurable activity (Table 4). The *argG*$^+$ transductants were also assayed for AMTase activity, and similar observations were made. The *strA argG*$^+$ and *argG*$^+$ transductants were tested for arginine excretion on suitable indicator plates; both were found to excrete arginine.

Syntrophic studies. Berg and Rossi (4) have reported that *proAB argG* double mutants of *S. typhimurium* LT7 excrete proline. Table 5 presents our results of syntrophic studies with LT2 strains. The wild-type LT2 did not excrete proline or arginine. The *argG proAB* mutant strain excreted proline, and this excretion was independent of the *argR* mutation. Arginine was excreted only by the *argR* mutant strain with the wild-type *argG*$^+$ allele. This finding suggests that in an *argR* mutant the other transaminase(s) synthesizes insufficient *N*-acetylornithine for adequate arginine biosynthesis.

Pyrimidine and arginine enzyme levels in the *argR* mutant. The last five enzymes of the pyrimidine pathway (along with three enzymes of the arginine pathway) were assayed to determine whether any of these pyrimidine biosynthetic enzymes were derepressed in an *argR* mutant (Table 6). With strain P21, repression of the three arginine enzymes occurred when the strain was grown in the presence of arginine. In the *argR* derivative, very high levels of the same three arginine enzymes were observed. Moreover, the levels were unaffected by growth in the presence of arginine. Indeed, a 300-fold derepression of OTCase was found in the *argR* mutant in comparison to the repressed level of strain P21. The other two arginine enzymes assayed in the *argR* mutant were not as markedly derepressed.

Even with this high level of OTCase, the pyrimidine enzyme levels were unaffected. It was anticipated that the marked increase in

OTCase would result in excessive utilization of CP, causing a limitation of CP for pyrimidine biosynthesis. However, NAOase was derepressed to a far lesser degree than OTCase, and the possibility that the synthesis of ornithine may be a limiting factor was considered. The basic experiment was repeated with ornithine added to the medium. The results were unchanged; no effect on the pyrimidine enzyme levels was observed (R. A. Kelln, unpublished observations).

Enzyme levels in *pyrH* and *pyrH*$^+$ strains. In the *argR* mutant, the loss of the arginine regulatory gene product affected the synthesis of only one pyrimidine enzyme, namely CPSase. As mentioned earlier, *pyrH* mutants show derepressed pyrimidine enzyme levels (9, 13, 20), and it was in our interest to observe whether derepression of pyrimidine enzymes affected the enzyme levels of the arginine pathway (Table 7). The *pyrH* mutant had a 10-fold higher OTCase level than the *pyrH*$^+$ strain.

Quantitation of excreted arginine. The *argR* mutant strain was grown under four different conditions to the same cell density. The amount of arginine present in the culture fluid was determined by bioassay (Table 8). The parent strain excreted a small amount of arginine only with glycerol as carbon source and ornithine added to the medium. The *argR* mutant likewise excreted an appreciable amount of arginine (7.5 μg/ml) with glycerol as carbon source, and ornithine supplementation increased the amount of arginine excreted. With glucose as the carbon source, the *argR* mutant failed to excrete arginine even when ornithine was added to the medium.

TABLE 4. *Activity of AMTase of various strains*[a]

Strain[b]	Relevant genotype	Sp act
11-5	*proAB argG argR*	0
11-5 G_1		93
11-5 G_2	*proAB argR strA*	111
11-5 G_3		105
11-5 G_4		107

[a] Cells were grown in AG medium plus proline.
[b] Strains 11-5 G_1 through 11-5 G_4 are *strA argG*$^+$ transductants of strain 11-5.

TABLE 5. *Syntrophic properties of different strains*

Relevant genotype of test strain	Growth response[a] of indicator strain		
	proAB[b]	*proC*	*argF*
Wild type (LT2)	−	−	−
proAB argG	+	−	−
proAB argG argR	+	+	−
proAB argR	ND	ND	+

[a] Symbols: −, No growth; +, growth; ND, not done.
[b] Denotes the relevant genotype of the indicator strain.

DISCUSSION

Our initial attempt to isolate an arginine regulatory mutant of *S. typhimurium* LT2 by the conventional canavanine resistance method (15) was unsuccessful. *S. typhimurium* was found to be naturally resistant to canavanine (12) even when AF medium (16) was used. The next approach was based on the selection of mutants from histidine-requiring strains that are capable of utilizing *N*-acetylhistidine as histidine source in the presence of arginine (3). This approach also proved inappropriate, because arginine supplementation resulted in only a twofold repression of the deacylating enzyme, NAOase. Various attempts to increase the repression of this enzyme were unsuccessful.

Consequently, a third method was used, an approach that had apparently already been successful with *S. typhimurium* LT2 (10). As our starting strain, we chose a multisite deletion mutant in the *proAB* region to eliminate any possibility of true back-mutation when selecting for proline-independent derivatives. A number of proline-independent strains were isolated. Those isolates showing arginine sensi-

TABLE 6. *Comparison of enzyme levels in an argR*$^+$ *strain and an argR mutant*

Strain[a]	Relevant genotype	Additions to medium[b]	Sp act of enzymes of the arginine and pyrimidine pathways[c]							
			ATCase	DHOase	DHO-dehase	OMP-ppase	OMP-decase	AMTase	NAOase	OTCase
P21	*proAB*	None	14.7	130	13.5	46	14.6	17.4	18.7	63.6
		Arginine	11.6	129	11.8	45	14.6	7.5	8.2	4.8
KD2111	*proAB argR*	None	11.8	148	13.0	46	12.1	95.8	191	1393
		Arginine	13.7	132	13.1	46	11.0	110	216	1423

[a] Strain KD2111 is an *arg*$^+$ transductant of isolate 11-5 (*proAB21 argG11 argR5*).
[b] The medium was AG medium with added proline.
[c] Enzyme abbreviations: ATCase, aspartate transcarbamylase; DHOase, dihydroorotase; DHOdehase, dihydroorotate dehydrogenase; OMPppase, orotidine monophosphate pyrophosphorylase; OMPdecase, orotidine monophosphate decarboxylase; AMTase, *N*-acetylornithine-δ-transaminase; NAOase, *N*-acetylornithinase; OTCase, ornithine transcarbamylase.

TABLE 7. *Comparison of enzyme levels in a pyrH⁺ strain and a pyrH mutant*[a]

Strain	Relevant genotype	Sp act		
		ATCase	DHOdehase	OTCase
JL1269	*pyrH cdd*	606	34.5	476
KD1275	*cdd*	10.2	10.8	50.4

[a] The strains were grown in AG medium.

TABLE 8. *Quantitation of arginine overproduction: amount of arginine excreted by an argR⁺ strain and an argR mutant under various growth conditions*[a]

Strain	Relevant genotype	Amt of arginine excreted[b]			
		AG	AG + ornithine	A-glycerol	A-glycerol + ornithine
P21	*proAB*	<1[b]	<1	<1	2
KD2111	*proAB argR*	<1	<1	7.5	11

[a] The cells were grown in AG medium or in modified AG medium where glycerol was the carbon source instead of glucose. The quantitation of arginine in the culture supernatants was carried out as described in Materials and Methods.

[b] Values are expressed as micrograms of arginine per milliliter of culture filtrate.

tivity were assumed to be *argG* mutants and were used for the selection of arginine regulatory mutants.

Though all of the arginine enzymes were not assayed, those enzymes chosen for study were encoded by unlinked genes, thus effectively eliminating operator mutations. Only one isolate, isolate 11, yielded arginine-resistant mutants of the desired phenotype. These mutants had highly elevated enzyme levels when grown in the presence of arginine. Kadner and Maas (11) have reported that selection of *argR* mutants of *E. coli* using canavanine resistance gives rise to at least three classes of *argR* mutants. Class I mutants show fully derepressed enzyme synthesis under all growth conditions. Class II mutants are fully derepressed in the absence of arginine, but when arginine is present they are partially or fully repressed. Class III mutants show partial derepression that remains constant under all growth conditions. Comparisons of our mutants to these various classes were not carried out, but from later experiments it would seem reasonable that the arginine regulatory mutant of isolate 11 is representative of class I.

Indirectly suppressed proline auxotrophs have been described as having slow growth rates on minimal medium or on proline-supplemented medium, as well as being sensitive to arginine (10, 14). With isolate 11, selection for the *argR* mutation completely overcame the sensitivity to arginine, but the growth rate in the presence of arginine alone was still very slow (Table 3). Thus, even in the *argR* mutant, when grown in the presence of arginine alone, *N*-acetylglutamate-γ-semialdehyde synthesis appeared impaired and consequently partially prevented the cross-feeding of the proline pathway. With the constitutive synthesis of the arginine enzymes, it would appear that the reason for impaired *N*-acetylglutamate-γ-semialdehyde synthesis was a consequence of feedback inhibition of the first enzyme of the arginine pathway. It should be possible, therefore, to isolate mutants that are insensitive to feedback inhibition by selecting derivatives that grow rapidly in the presence of arginine.

The regulation of proline biosynthesis is a subject of controversy at present (4). Evidence has been provided for regulation at both the first and last steps and, in addition, a pleiotropic effect on proline regulation resulting from the introduction of an *argD* mutation in *E. coli* has been demonstrated. Our results lend further support to the previous observation (4) that indirectly suppressed proline auxotrophs of *S. typhimurium* excrete proline. Thus, the accumulated evidence has shown that a bypass of a regulatory point in proline biosynthesis occurs in indirectly suppressed proline auxotrophs.

CPSase has been designated as the first enzyme of pyrimidine biosynthesis in *E. coli* (21) and *S. typhimurium* (19). However, when an *argR* mutation was introduced into *S. typhimurium*, this enzyme was found to be constitutively synthesized (A.T. Abd-El-Al and J.L. Ingraham, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, K169, p. 175). In an *E. coli* K-12 strain harboring an *argR* mutation, the repression of CPSase by arginine and/or uracil was markedly reduced, but some repression did occur (17). Pierard et al. (17) made the suggestion (based on the decreased repressibility of CPSase) that repression of the enzyme may be exerted by a complex repressor molecule, composed of the *argR* gene product and another gene product related to the pyrimidine pathway. In the *S. typhimurium* mutant, evidence points to an involvement of only the *argR* gene product, which presumably forms a complex with arginine, and some pyrimidine compound that alone results in repression of CPSase. An interaction of the arginine repressor and a pyrimidine compound is supported by the finding that OTCase was repressed to a greater extent by the addition of both pyrimidines and arginine than by the addition of arginine alone (13). The differences observed in CPSase regulation

in *E. coli* K-12 and *S. typhimurium* LT2 may be a function of the nature of the *argR* mutation (either class I, II, or III). However, inherent differences in CPSase regulation in these two genera of bacteria may also account for the change.

The common feature of the two transcarbamylases ATCase and OTCase is the requirement of CP as a substrate. When OTCase was constitutively synthesized in the *argR* mutant, the ATCase level was unaffected; when ATCase was derepressed in the *pyrH* mutant, OTCase was derepressed as well. ATCase may preferentially use CP and may create a partial arginine starvation that should result in derepression of OTCase and the other enzymes of the arginine pathway. In support of this statement is the isolation of a *pyrA* mutant (1) having an arginine requirement but not a pyrimidine requirement. It seems pertinent to point out that the genes encoding OTCase and ATCase are located adjacent to each other on the *E. coli* (21) and *S. typhimurium* (19) chromosomes and that these two enzymes exhibit the greatest degree of derepression of the enzymes of the two pathways.

Determination of the amount of arginine excreted produced two interesting results. First, glucose-grown cells did not excrete arginine even when supplied with ornithine. Gorini and Gundersen (8) have reported a form of catabolite repression for glucose-grown cells of *E. coli* B; the arginine enzyme levels are much lower in glucose-grown cells than in glycerol-grown cells. Perhaps even in the absence of the *argR* product, the level of derepression in glucose-grown cells of *S. typhimurium* is markedly lower than that in glycerol-grown cells. In view of such a glucose effect, a study on enzyme levels in glycerol-grown cells appears warranted. Second, the amount of arginine excreted with glycerol-grown cells could be increased when ornithine was added to the medium, showing that in the *argR* mutant ornithine biosynthesis was a limiting factor in arginine overproduction. Even in the parent strain, arginine was excreted when ornithine was added. Thus, in a strain with the wild-type *argR* allele, it appears that the rate of ornithine synthesis plays an important role in the maintenance of balanced arginine synthesis.

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