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Isolation, Characterization, and Mapping of *Escherichia coli* Mutants Blocked in the Synthesis of Ornithine Decarboxylase

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Several *Escherichia coli* K-12 mutants blocked in the synthesis of ornithine decarboxylase (OD) were isolated after transduction for *serA*$^+$ in a strain (MA197) blocked in agmatine ureohydrolase (AUH) with a mutagenized phage lysate of P1. The new double-polyamine mutants were characterized by an unconditional polyamine dependence; either putrescine or spermidine was required for normal growth. The mutational block was varified by the demonstration of a virtual absence of OD activity in cellular extracts. The mutation, designated *speC,* was mapped by P1 transduction in several strains and was shown to have a cotransduction frequency of 17.2% with *serA*. Map order was established as *serA speB speC metK*. A derivative of one of the OD mutants having wild-type levels of AUH and blocked in OD was utilized along with an OD AUH mutant and an OD$^+$ AUH strain to explore the phenomenon of "pathway selection" using growth rate as a parameter. Polyamine pool studies were carried out simultaneously. The results presented here support the hypothesis of pathway selection, implying a preferential utilization of exogenous arginine rather than endogenously produced arginine in polyamine biosynthesis.

Escherichia coli has two pathways for the constitutive synthesis of putrescine for which ornithine is the common branch point (17). Ornithine may be directly decarboxylated to putrescine or it may be the indirect precursor of putrescine via arginine and agmatine formation. In the latter case, arginine is first converted to agmatine which is, in turn, stoichiometrically converted to putrescine and urea (Fig. 1). In both cases, spermidine is formed by the transfer of an aminopropyl group from decarboxylated *S*-adenosylmethionine to putrescine (19, 22).

It has been shown (4, 11) that addition of arginine to *E. coli* K-12 produces a depletion of the ornithine pool by feedback inhibition and repression of the arginine biosynthetic pathway. This phenomenon has made possible the isolation of a number of mutants that require putrescine in the presence of arginine but not in its absence (6, 13). These arginine conditional putrescine auxotrophs were shown to be blocked in either arginine decarboxylase (EC 4.1.1.19) or agmatine ureohydrolase (AUH; EC 3.5.311) formation (6, 13). The demonstration that the genes for arginine decarboxylase and AUH, designated *speA* and *speB*, respectively, map close together near *serA* on the *E. coli* linkage map (12) indicates that genes for polyamine biosynthesis may constitute an operon (Fig. 2).

To explore further the possible location of other genes for polyamine synthesis near *serA*, a search for mutants specific to this region of the chromosome was undertaken. Phage lysates of P1 were mutagenized with hydroxylamine and employed in transductions in which the selected marker was *serA*. This method, called localized mutagenesis (7), makes it possible to mutagenize only that segment of the recipient chromosome that is replaced by the transducing phage deoxyribonucleic acid.

Since putrescine is synthesized by two alternate routes, we used an arginine conditional *speB* phage host and recipient so that one of these routes (the one from arginine to putrescine) was already blocked. We looked for, and found, auxotrophs that required either spermidine or putrescine for growth in the absence of arginine. Enzyme assays confirmed our initial assumption that these were ornithine decarboxylase (OD; EC 4.11.17) mutants. The mapping study presented here showed that the gene for OD, designated *speC,* maps between min 56 and 57 on the *E. coli* linkage map (23).

The hydrolysis of agmatine produces equimolar amounts of urea and putrescine in *E. coli*. This is the only route to urea production in this organism (15), so that urea formation can be

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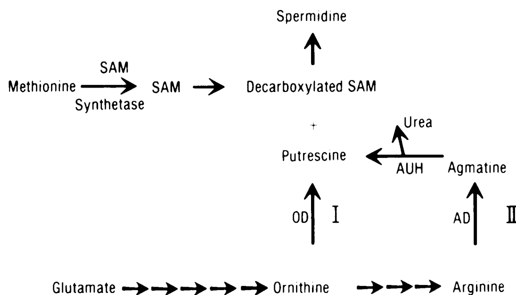

FIG. 1. *Schematic representation of putrescine and spermidine biosynthesis in E. coli. Abbreviations used: SAM, S-adenosylmethionine; OD, ornithine decarboxylase; AD, arginine decarboxylase; AUH, agmatine ureohydrolase. Pathways I and II are indicated.*

used to measure putrescine production via this pathway. Using this index, Morris and Koffron (16) were able to show that urea production and, hence, putrescine production from arginine increase with addition of exogenous arginine. In the absence of arginine supplementation, putrescine was shown to be formed mainly from endogenous ornithine (16). Thus, *E. coli* strains grown in minimal medium synthesize putrescine mainly from ornithine (pathway I), whereas the same strains utilize arginine (pathway II) almost entirely for putrescine production when sufficient arginine is added to the medium.

The shutdown of pathway I from ornithine to putrescine in strains grown in arginine-supplemented medium occurs as a consequence of ornithine depletion after feedback inhibition and repression of ornithine biosynthesis (4, 11). Ornithine is utilized as the substrate for putrescine formation (pathway I) when strains are grown in minimal medium. The level of arginine biosynthetic enzymes is rather constant whether or not arginine is present. Therefore, Morris and Koffron proposed that there might be two functionally separate arginine pools and that the exogenously formed pool might be proportionately more available to arginine decarboxylase. Additional support for this hypothesis has come from the work of Tabor and Tabor (21), who demonstrated an unequal incorporation of L-[^{14}C]citrulline and L-[^{3}H]arginine into putrescine in an ornithine auxotroph. The decarboxylation of exogenously formed arginine was clearly preferential to that of endogenously formed arginine, whereas both labels were equally incorporated into protein.

The isolation of OD mutants enabled us to confirm "pathway selection" by measuring the growth rate of these mutants in the presence and absence of arginine. In addition, we were able to correlate growth rate with polyamine pool size under several experimental conditions.

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MATERIALS AND METHODS

Bacterial strains and media. A summary of the bacterial strains employed is presented in Table 1. Genotype designations follow those published previously (12). The OD mutation is designated *speC*. Other definitions of symbols and map positions can be found in reference 23.

The minimal medium used was medium A of Davis and Mingioli (1), with 0.5% glucose. The solid medium was prepared by the addition of agar to a final concentration of 2%. Enriched medium plates (AA) were prepared by adding an arginine-free amino acid supplement to the minimal medium as described previously (12). L-Arginine (1,000 μg/ml) and L-methionine (100 μg/ml) were added to the enriched plates for the testing of a conditional polyamine requirement (12). Putrescine and spermidine were added at 100 μg/ml. Thiamine was added at 1 μg/ml. The scoring of resistance to L-ethionyl-L-alanine was performed on plates to which 100 μg of the methionine analogue had been added per ml.

Transductions. Transductions employed phage P1 and were performed by the method of Glansdorff (3).

Isolation of mutants. The following procedure was developed in collaboration with R. Celis. A phage stock having a titer of 10^{10} plaque-forming units per ml was prepared on strain MA195 by means of confluent lysis (3). The stock was then amplified as follows. Two 1-liter flasks each containing 100 ml of 869 medium (3) were inoculated with an overnight culture of strain MA195. The cultures

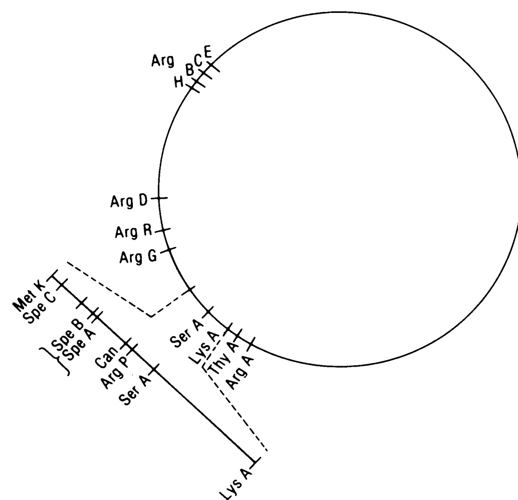

FIG. 2. *Map positions of genes for polyamine biosynthesis in E. coli.*

TABLE 1. *Description of E. coli K-12 strains used*

Strain	Genotype						Source or derivation
MA197[a]	*serA*	*thr*	*leu*	*thi*	*speB*		MA195
MA195	*lysA*	*thr*	*leu*	*thi*	*speB*		MA176
MA176	*serA*	*thr*	*leu*	*thi*	*lysA*		PA260 (F. Jacob)
MA233	*serA*	*thi*	*metK*				E4 (R. Greene)
MA255	*thr*	*leu*	*thi*	*speB*	*speC*		Mutagenesis of MA195
69[a]	*thr*	*leu*	*thi*	*speC*			MA261
4-3, 5-3, 9-3	*thr*	*leu*	*thi*	*speB*	*speC*		Mutagenesis of MA195
197-T[a]	*thr*	*leu*	*thi*	*speB*			MA197
MA261[a]	*serA*	*thr*	*leu*	*thi*	*speB*	*speC*	MA255

[a] Strains were derived by P1 transduction from the strain denoted.

were grown with shaking at 37 C to an absorbancy at 580 nm of 0.1 (Lumetron). $CaCl_2$ was added at 2.5×10^{-2} M with phage at a multiplicity of infection of 1. Flasks were returned to the shaker. After 3.5 h, 5×10^{-3} M trisodium citrate was added. At 4 h, chloroform was added and the cellular material was pelleted (16,300 × *g*, Sorvall RC2-B, 20 min, 0 C). The supernatant was decanted and retained for concentration in an ultracentrifuge (100,000 × *g*, Spinco, 1 h, 0 C). The supernatant was decanted, and the pellet was suspended in 869 medium supplemented with 2.5×10^{-3} M trisodium citrate, 2.5×10^{-3} M $MgSO_4$, and 2.5×10^{-2} M $CaCl_2$. This suspension volume was 0.01 of the original, and the final titer was 10^{12} plaque-forming units per ml.

Mutagenesis was performed as follows. The phage concentrate was diluted 1:10 in 869 medium containing 0.45 M hydroxylamine-hydrochloride (sulfate free, Eastman Kodak), 2.5×10^{-2} M $CaCl_2$, and 2.5×10^{-3} M ethylenediaminetetraacetic acid; the pH was adjusted to 6.0. The lysate was incubated without shaking at 37 C for 50 h, concentrated in an ultracentrifuge as before, and suspended in 869 medium containing $CaCl_2$, $MgSO_4$, and sodium citrate as described previously. The final titer was 10^6 plaque-forming units per ml, and the lysate was stable at 4 C in the presence of chloroform for at least 2 months.

Selection procedure for polyamine auxotrophs. After transduction with mutagenized phage, serial dilutions of the recipient strain (MA197) were plated onto enriched plates containing spermidine but not serine; *serA* was used as the selected marker. *serA*$^+$ transductants were then streaked onto plates from which all polyamines or polyamine precursors had been omitted. After 35 to 40 h, spermidine was added underneath the agar. Colonies that subsequently grew out were picked as putative spermidine requirers.

Assay for OD. Cells were grown in minimal medium containing spermidine, harvested, washed, and broken by sonic treatment. The sonically treated material was centrifuged at 8,590 × *g* for 10 min at 4 C. Enzyme assays were performed as described by Z. Leifer (Ph.D. thesis no. 5411B, New York University, New York, N.Y., 1972) according to the method of Tabor and Tabor (20). The final reaction mixture contained 3.2 μg of pyridoxal phosphate, 0.24 μmol of L-[U-^{14}C]ornithine (0.33 Ci/mol), 1.2 μmol of $MgSO_4$, 40 μmol of tris(hydroxymethyl)-aminomethane-hydrochloride, and 0.1 ml of cellular extract in a final volume of 0.5 ml at a pH of 7.5. Control strains showed a linear increase in counts per minute per milligram of protein for the 10-min assay period. Total counts per minute were proportional to the amount of extract used in the assay. Blanks, which were either distilled water or boiled extract, gave counts equal to that of background. The evolution of radioactive CO_2 was measured.

Assay for AUH. Cells were grown in minimal medium containing trace metals (Z. Leifer, Ph.D. thesis, New York Univ., New York, N.Y., 1972), harvested, washed, and treated with sodium dodecyl sulfate and chloroform. The final reaction mixture consisted of 13 μmol of agmatine sulfate and 90 μmol of tris(hydroxymethyl)aminomethane - hydrochloride to which was added 0.3 ml of cellular extract. The method used was that of Hirshfield et al. (6). Urea was measured by the method of Hunninghake and Grisolia (8).

Protein determination. The method of Lowry et al. (10) was employed for determining protein.

Polyamine starvation. Strains having a block in pathway II (AUH) were depleted of polyamines as follows. Strains were grown in minimal medium plus all supplements required for normal growth to an absorbancy at 490 nm of 0.3 and refrigerated for 4 h since polyamines leak out in the cold. Cells were then harvested, washed in IXA (1), and suspended 1:20 (vol/vol) at a cell density of 2.5×10^7 in IXA with all of the supplements except polyamines and 1,000 μg of L-arginine per ml. Cells were incubated overnight with shaking, harvested, and washed twice prior to inoculation into media for growth studies.

Strains having a block in pathway I were handled similarly except that they were not incubated with arginine, but rather were suspended at a cell density of 2.5×10^7 in IXA with all requirements except polyamines. The strains were refrigerated overnight, harvested, and washed twice prior to use in growth studies.

Growth studies. For liquid growth studies, strains depleted of polyamines were inoculated into minimal medium both with and without polyamines and polyamine precursors. Cultures were incubated at 37 C with shaking, and optical density was monitored with a Lumetron colorimeter. At the end

of each experiment, samples of the cultures were removed and tested for phenotypic characteristics. In addition, samples were removed for polyamine pool assays.

Polyamine pool measurements. The fluorimetric technique used for measurement of the polyamine pool (2) was modified slightly from that of Leifer (Ph.D. thesis).

RESULTS

Isolation and characterization of OD mutants. Among 5,000 colonies tested during the initial search for polyamine mutants, one colony proved to require either putrescine or spermidine for growth in the absence of arginine. This mutant, denoted MA255, required either putrescine or spermidine in both minimal and enriched media. The phenotype of the mutant grown on plates in the presence of 1,000 μg of arginine per ml was like that of the parent MA197; i.e., it was still "sensitive" to arginine. Full growth was obtained only in a medium containing spermidine or putrescine. The growth retardation shown by strain MA255 in a medium not containing arginine was considerably greater than that shown on plates containing arginine, indicating that the OD block was more complete than that of AUH (see Table 6). Enzyme studies (Table 2) showed a virtual absence of OD activity in the mutant.

A second group of mutants was subsequently isolated which had growth requirements identical to those of MA255. In addition, these mutants had a second mutation affecting growth rate closely linked to the OD marker (cotransduction frequency of 64% [unpublished mapping data]). Enzyme data for these mutants are included in Table 2. Moderate growth retardation (++ rather than +++ growth on enriched plates at 18 h) was observed in both minimal and enriched media. The metabolic basis of this phenotype has not been clarified further. Enzyme data for these mutants are also included in Table 2.

TABLE 2. *Specific activity[a] of OD*

Strain	CO_2 produced (nmol/min per mg of protein)
MA197	15.0
MA195	16.0
MA255	0.26
1-2	0.38
2-1	0.19
3-3	0.26
4-3	0.10
5-3	0.08
6-2	0.12
7-2	0.32
8-2	0.18
9-3	0.16

[a] Measured by following growth of strains in minimal medium plus spermidine to an absorbancy at 490 nm of 0.4 (1.87×10^9 cells). Cells were washed twice and sonically treated, and cell supernatants were assayed for evolution of CO_2 after incubation with L-[^{14}C]ornithine.

Map location of speC with respect to serA. A preliminary experiment to establish the cotransduction frequency between the gene for OD, *speC*, and *serA* was undertaken. The recipient in the transduction was MA197; the phage host was MA255. Transductants for *serA*$^+$ were selected in the presence of spermidine. These were then tested for co-inheritance of *speC* by streaking onto plates containing no polyamines or polyamine precursors. The data presented in Table 3 show a cotransduction frequency of 17.2% for *speC* and *serA*. Similar results were obtained for the same pair of markers in transductions in which P1 was grown on three of the other nine OD mutants. The data are summarized for one of these mutants in Table 4. In these experiments, *serA*$^+$ transductants were classified according to size (reflecting presence or absence of the second mutation affecting growth rate) prior to restreaking for the scoring of *speC* entry. Marker entry was then studied in each subgroup of the total population. The cotransduction frequency of the two markers in each subpopulation was then multiplied by the

TABLE 3. *Map Location of speC with respect to serA in MA255: distribution of the unselected marker[a]*

Class	No.	Frequency (%)
serA$^+$	384	100
serA$^+$ *speC*	66	17.2

[a] Data were obtained for transductions in which MA197 was the recipient and MA255 was the phage host. Selection was on AA plates containing spermidine, and *serA*$^+$ transductants were scored on AA plates (12).

TABLE 4. *Map location of speC with respect to serA in strain 4-3: distribution of the unselected marker[a]*

Class	Small colonies		Large colonies	
	No.	Frequency	No.	Frequency
serA$^+$	141	100	151	100
serA$^+$ *speC*	87	61.7	9	5.9

[a] Distribution in the total population (in which of 1,456 colonies counted 310 were small) is: $0.787 \times 5.9 + 0.213 \times 61.7 = 17.8\%$. MA197 was the recipient for a transduction in which the phage host was strain 4-3. The selected marker was *serA*, and transductants were then scored on AA plates (12).

proportion, expressed as a percentage, of each subpopulation to the total population. The sum of the separate proportional distributions is equal to marker frequency within the whole population (12).

Map location of speC with respect to speB. To establish map order for *speC*, *speB*, and *serA*, another recipient, MA176, was used in a transduction in which P1 was grown on MA255. After selection of recombinants for *serA*$^+$ as before, colonies were tested for co-inheritance of *speB* by streaking onto plates containing 1,000 μg of arginine and 100 μg of methionine per ml. (W.K.M. has observed enhancement of the arginine-sensitive phenotype with the addition of methionine to the medium.) A cotransduction frequency of 23.8% for *serA*$^+$ and *speB* was obtained (Table 5), which is in close agreement with published data (12). After this, all *speB serA*$^+$ transductants were streaked onto plates containing no polyamines or polyamine precursors to test for *speC* marker entry. A cotransduction frequency of 47.5% was obtained between *speB* and *speC*.

From this experiment, it was possible to establish the map order of the *spe* genes as being *serA speB speC*. If all of the *serA*$^+$ *speB* transductants had also been *speC*, it would have been necessary to conclude either that *speC* maps between *serA* and *speB* or that the two genes lie too close together for map order to be recognized. If *speC* mapped between *serA* and *speB*, a double crossover would be required to produce *speB speC*$^+$ transductants, and the frequency of this event in this region of the chromosome would be about 0.4% (unpublished data for *serA* and *metK*).

Map location of speC with respect to metK. The *metK* gene governing the formation of *S*-adenosylmethionine synthetase has been mapped both in *Salmonella typhimurium* (9) and *E. coli* (5). In the present study, strain MA233 was used as the recipient in transductions with P1 grown on MA255 in which *serA*$^+$ was the selected marker. The *metK* mutation affects colony size; colonies with this marker grow slowly (12). Transductants were divided by size prior to testing for other markers. A summary of the results obtained by this method is presented in Table 6. The calculated value for cotransduction frequency between *serA* and *metK* was 5.2%. Other values obtained for the cotransduction frequency between *serA* and that for the linkage of *speB* and *speC* were in reasonable agreement with those obtained for the same pair of markers in other experiments. The cotransduction frequency of *metK* and *speC* obtained was 22.9%.

TABLE 5. *Map location of speC with respect to speB in MA255: distribution of the unselected markers*[a]

Class	No.	Frequency
1. *serA*$^+$	432	100
2. *serA*$^+$ *speB*	103	23.8
3. *serA*$^+$ *speB speC*	49	47.5

[a] Data were obtained in transductions in which MA176 was the recipient; P1 was grown on MA255. Transductants for *serA* were then scored on AA plus spermidine, AA plus arginine, and AA. Frequency of class 3 was determined among class 2 transductants.

Pathway selection. We decided to examine a consequence of pathway selection—growth in minimal medium. If pathway selection is a distinct regulatory phenomenon, it should follow that a strain blocked in OD synthesis should grow poorly in minimal medium. A study of this question was initiated in three isogenic strains that were blocked in polyamine biosynthesis in different ways. A *serA*$^+$ transductant of MA197, 197-T, was utilized as the strain blocked only in pathway II, and MA255 was employed as the double mutant for both pathways. In addition, a *speB*$^+$ (AUH$^+$) transductant of MA255 was isolated. This strain, 69, was shown by enzyme assay to have normal levels of AUH and to be blocked in the synthesis of OD. It was possible to recognize this strain by means of its poor growth on plates not supplemented with either arginine or putrescine (Table 7).

The three strains were starved for polyamines and inoculated into minimal medium as described above. The growth of 197-T in minimal medium is shown in Fig. 3, curve A. This strain, which is not blocked in pathway I, also did not show an enhanced growth rate in a medium supplemented with ornithine, spermidine, or putrescine. The inoculation of this strain into a medium supplemented with 1,000 μg of arginine per ml produced a doubling time (130 min) like that shown in curve C (Fig. 3) for MA255 in an identical medium.

Strain 69 has only the OD block. The fact that it could be recognized by its poor growth on plates is evidence for pathway selection. Although arginine is formed endogenously and the pathway between arginine and putrescine is not blocked, the growth rate was restricted in a minimal, unsupplemented medium and showed a doubling time of 140 min. Doubling time in arginine-supplemented medium was 65 min (Fig. 4).

Polyamine pool assay. Measurement of the polyamine pools was undertaken to establish the relationship between changes in growth

TABLE 6. *Map Location of speC with respect to metK: distribution of unselected markers*[a]

Class	Small colonies		Large colonies		Total population
	No.	Frequency	No.	Frequency	Frequency
1. *serA*$^+$	295	100	276	100	100
2. *serA*$^+$ *speB*	71	24	119	43.1	27.8
3. *serA*$^+$ *speB speC*	29	40.8	68	57.1	44.1
4. *serA*$^+$ *speB speC metK*$^+$	1	3.4	67	98.5	22.9

[a] Distribution of markers in the total population was estimated as shown in Table 4. MA233 was the recipient, P1 was grown on MA255, and the selected marker was *serA*. Size distribution was calculated from the colonies after transduction for *serA*; a selected number of each class was then mapped. Of the total population, 79.5% was found to be small and 20.5% was found to be large. L-Ethionyl-L-alanine was added to AA plates (100 μg/ml) for the scoring of class 4 transductants. The frequency of class 3 was determined among class 2 transductants, and the frequency of class 4 was based on class 3 transductants.

TABLE 7. *Growth of polyamine mutants on plates*[a]

Medium	Growth of strain:		
	MA255	197-T	69
AA + Spd	+++	+++	+++
AA	±	+++	+
AA + Arg	+	+	+++
AA + Orn	±	+++	+

[a] Strains were grown in 869 medium and streaked onto plates of the following composition: AA, enriched plate containing amino acid supplement; AA + Spd, AA plus 100 μg of spermidine-hydrochloride per ml; AA + Arg, AA plus 1,000 μg of arginine plus 100 μg of methionine per ml; A + Orn, AA + 100 μg of ornithine per ml. Plates were read at 18 to 20 h. The same results were obtained with minimal medium plates (IXA with B_1, threonine, leucine, and the noted supplements), except that plates were read at 30 to 36 h.

rate observed for the three strains and polyamine pool levels during several experimental conditions.

Strain 69 showed a slight reduction in doubling time during growth in minimal medium supplemented with citrulline (80 min) and a further reduction in doubling time in the ornithine-supplemented medium (100 min) compared with a doubling time of 65 min when strain 69 was grown in minimal medium plus arginine. Lines 1, 4, and 5 of Table 8 give polyamine pool values corresponding to these growth conditions. These data suggest that a putrescine pool of 60 nmol/mg (dry weight) of cells is adequate for normal growth when the spermidine pool is about 20 nmol, but that when this pool falls to less than 10 nmol the growth rate is decreased.

Lines 1 and 11, which give pool values for strains 69 and 197T in nonrestrictive media

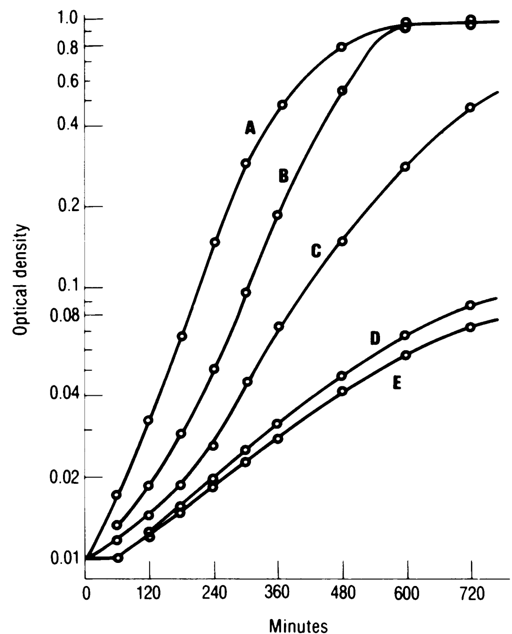

FIG. 3. *Effect of an OD mutation in an AUH-blocked strain. Strains were grown in minimal medium supplemented with thiamine, threonine, and leucine except where otherwise specified. (A) strain 197-T, minimal medium; (B) MA255, minimal medium plus putrescine; (C) MA255, minimal medium plus arginine; (D) MA255; minimal medium; (E) MA255, minimal medium plus ornithine.*

without polyamine supplementation, are in agreement with the data of other workers (22); i.e., that the spermidine pool is one-third the size of the putrescine pool during normal growth in minimal medium. Increasing the putrescine pool by putrescine supplementation resulted in a decrease in the spermidine pool (lines 2, 7, and 9), whereas increasing the sper-

midine pool (data shown for strain 69 in line 3) was accompanied by a decrease in the putrescine pool. Normal growth was observed with either putrescine or spermidine.

The growth of the three strains in restrictive media was accompanied by decreased polyamine pools and the appearance of cadaverine (lines 6, 8, and 10). This polyamine is formed by the decarboxylation of lysine. Lysine decarboxylase can be induced by growing cells in a high concentration of lysine at low pH. In addition, cadaverine may appear as a constituent of the polyamine pool in the absence of lysine supplementation and at the physiological pH after polyamine depletion (2). Cadaverine may substitute for putrescine as an alternative substrate for aminopropyl transfer and does have growth-stimulating effects in these strains. The separation of cadaverine and putrescine was clearly observed by means of prior ultraviolet light visualization of the chromatography plate but was less successfully resolved by the recorder. The data are presented as a summed total in Table 8. The R_f value for dansylated N-3-aminopropyl-1,5-diaminopentane (2) is similar to that of spermidine and might, therefore, mimic an apparent increase in the spermidine pool (lines 8 and 10).

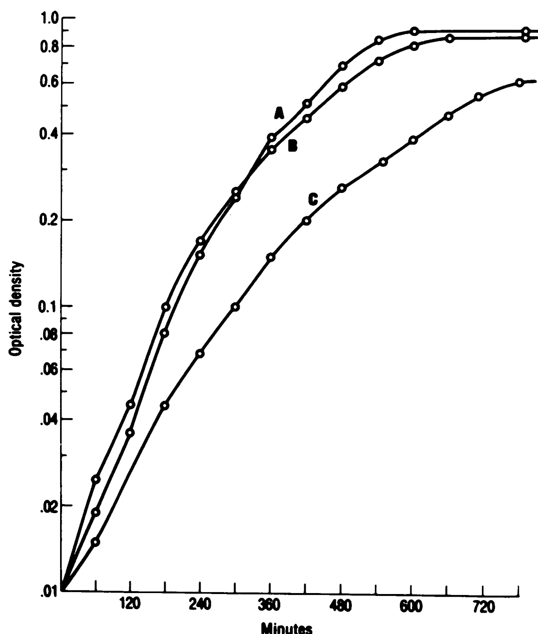

FIG. 4. *Effect of an OD mutation in an AUH^+ strain. Strain 69 was grown in minimal medium supplemented with thiamine, threonine, and leucine except where otherwise specified. Strain 69 in (A) minimal medium plus spermidine, (B) minimal medium plus arginine, and (C) minimal medium.*

DISCUSSION

The isolation of OD mutants has made it possible to locate the OD gene on the *E. coli* chromosome. The placement of this gene further indicates a clustering of genes related to polyamine biosynthesis.

The necessity of putrescine biosynthesis for normally growing *E. coli* cells was demonstrated by the Tabors (20), who showed that even under severe arginine-ornithine restriction an ornithine auxotroph used 5 to 18% of the supplementary arginine or ornithine for polyamine biosynthesis during growth in a chemostat. Similarly, strains deficient for AUH and arginine decarboxylase activity (6, 14) showed distinct retardation of growth when polyamine depletion was accomplished through growth of strains in high concentrations of arginine. The present study demonstrates nonconditional putrescine dependency in *E. coli*. The ten strains described here have a virtually complete block in OD as indicated by enzyme assay. The strains show markedly reduced growth rates in either a minimal or enriched medium that does not contain polyamines or arginine. The retardation of growth rate is reversed immediately by the addition of either putrescine or spermidine to the growth medium. Putrescine supplementation tends to be correlated with a considerably lowered spermidine pool which occurs with no reduction in growth rate until the level falls below 12 nmol/mg (dry weight) of cells. This may indicate that putrescine can partially replace spermidine as a growth requirement. In addition, this may explain the fact that mutants with a requirement for spermidine only have never been isolated despite extensive efforts to obtain them.

The growth studies described here support the hypothesis that there are two functional arginine pools in putrescine biosynthesis (16, 21) and that exogenous arginine is preferentially utilized for polyamine biosynthesis. As shown in this study, the OD mutant 69 is retarded in its growth in minimal medium although there is no block in the arginine synthetic pathway. Moreover, in preliminary studies (S. Cunningham-Rundles, Fed. Proc. 34:666, 1975), it was shown that the arginine pool of this strain is similar to that of 197-T (AUH mutant) when both strains were grown in minimal medium. The growth of strain 197-T is not restricted in this medium. The residual growth of strain 255 is not restricted in this medium. The residual growth of strain 255 in minimal medium that is not supplemented with polyamines may be occurring as a result of cadaverine biosynthesis or it may reflect the

TABLE 8. *Polyamine pools in several strains under different growth conditions*[a]

Strain	Medium[b]	Polyamine pool (nmol/mg of cell dry wt)		Mass doubling (min at A_{490})
		Putrescine	Spermidine	
69	MM + Arg	62.5	23.0	65
69	MM + Put	104.0	TR[c]	80
69	MM + Spd	8.2	78.0	65
69	MM + Cit	58.0	9.5	80
69	MM + Orn	50.0	9.3	100
69	MM	20.0[d]	4.8	140
255	MM + Put	170.0	12.0	65
255	MM	6.5[d]	24.5[e]	275
197-T	MM + Put	150.0	15.0	60
197-T	MM + Arg	1.9[d]	23.0[e]	130
197-T	MM	104.0	37.5	60

[a] Polyamine pool assays were made during the growth curve experiments. Cell samples were removed when cultures reached an absorbancy at 490 nm (A_{490}) of 0.4. Dansylated derivatives of the polyamines were prepared, run with known standards, corrected to nanomoles per milligram (dry weight) of cells by means of a standard curve relating optical density, cell number, and weight for these strains.
[b] MM, Minimal medium; Arg, arginine; Put, putrescine; Spd, spermidine; Cit, citrulline; Orn, ornithine.
[c] TR, Trace.
[d] Actually cadaverine and putrescine together.
[e] May include cadaverine analogue of spermidine.

degree to which polyamines are not directly required for growth. Strain 69 is less retarded by growth in minimal medium than is strain 255. This probably indicates a partial utilization of pathway II in addition to growth stimulation by induction of cadaverine biosynthesis.

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LITERATURE CITED

1. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B[12]. J. Bacteriol. **60**:17–28.
2. Dion, A. S., and S. S. Cohen. 1972. Polyamine stimulation in nucleic acid and synthesis in an uninfected and phage-infected polyamine auxotroph of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **69**:213–217.
3. Glansdorff, N. 1965. Topography of cotransducible arginine mutations in *Escherichia coli* K-12. Genetics **51**:167–179.
4. Gorini, L. W., W. Gundersen, and M. Burger. 1961. Genetics of regulation of enzyme synthesis in the arginine biosynthetic pathway of *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **26**:173–182.
5. Greene, B. C., C. H. Su, and C. T. Holloway. 1970. S-adenosylmethionine synthetase deficient mutants of *Escherichia coli K-12* with impaired control of methionine biosynthesis. Biochem. Biophys. Res. Commun. **38**:1120–1126.
6. Hirshfield, I. N., H. J. Rosenfeld, Z. Leifer, and W. K. Maas. 1970. Isolation and characterization of a mutant of *Escherichia coli* blocked in the synthesis of putrescine. J. Bacteriol. **101**:725–730.
7. Hong, J., and B. N. Ames. 1971. Localized mutagenesis of any specific small region of the bacterial chromosome. Proc. Natl. Acad. Sci. U.S.A. **68**:358–372.
8. Hunninghake, D., and S. Grisolia. 1966. A sensitive and convenient micromethod for estimation of urea, citrulline and carbamyl derivatives. Ann. Biochem. **16**:200–205.
9. Lawrence, D. A., D. A. Smith, and R. J. Rowbury. 1968. Regulation of methionine synthesis in *Salmonella typhimurium* mutants resistant to inhibition by analogues of methionine. Genetics **58**:473–492.
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
11. Maas, W. K. 1961. Studies on repression of arginine biosynthesis in *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **26**:183–191.
12. Maas, W. K. 1974. Mapping of genes involved in the synthesis of spermidine in *Escherichia coli*. Mol. Gen. Genet. **119**:1–9.
13. Morris, D. R., and C. M. Jorstad. 1970. Isolation of conditionally putrescine-deficient mutants of *Escherichia coli*. J. Bacteriol. **101**:731–737.
14. Morris, D. R., and C. M. Jorstad. 1973. Growth and macromolecular composition of a mutant of *Escherichia coli* during polyamine limitation. J. Bacteriol. **113**:271–277.
15. Morris, D. R., and K. L. Koffron. 1967. Urea production and putrescine biosynthesis by *Escherichia coli*. J. Bacteriol. **94**:1516–1519.
16. Morris, D. R., and K. L. Koffron 1969. Putrescine biosynthesis in *Escherichia coli*. Regulation through pathway selection. J. Biol. Chem. **244**:6094–6099.
17. Morris, D. R., and A. B. Pardee. 1966. Multiple pathways of putrescine biosynthesis in *Escherichia coli*. J.

Biol. Chem. 241:3129-3135.

18. Srinivasan, P. R., D. V. Young, and W. K. Maas. 1973. Stable ribonucleic acid synthesis in stringent (*rel*$^+$) and relaxed (*rel*$^-$) polyamine auxotrophs of *Escherichia coli* K-12. J. Bacteriol. 116:648-655.
19. Tabor, H., S. M. Rosenthal, and C. W. Tabor. 1957. The role of putrescine and spermidine in the enzymatic synthesis of spermidine in *Escherichia coli* extracts. J. Am. Chem. Soc. 79:2973-2979.
20. Tabor, H., and C. W. Tabor. 1969. Formation of 1,4-diaminobutane and of spermidine by an ornithine auxotroph of *Escherichia coli* grown on limiting arginine or ornithine. J. Biol. Chem. 244:2286-2292.
21. Tabor, H., and C. W. Tabor. 1969. Partial separation of two pools of arginine in *Escherichia coli*. Preferential use of exogenous rather than endogenous arginine for the biosynthesis of 1,4-diaminobutane. J. Biol. Chem. 244:6383-6387.
22. Tabor, H., and C. W. Tabor. 1972. Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, and spermine and related amines. Adv. Enzymol. 36:203-268.
23. Taylor, A. L. 1973. Linkage map of *Escherichia coli*. Bacteriol. Rev. 36:504-524.