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Utilization of γ-Aminobutyric Acid as the Sole Carbon and Nitrogen Source by *Escherichia coli* K-12 Mutants

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Wild-type strains of *Escherichia coli* K-12 cannot grow in media with γ-aminobutyrate (GABA) as the sole source of carbon or nitrogen. Mutants were isolated which could utilize GABA as the sole source of nitrogen. These mutants were found to have six- to ninefold higher activities of γ-aminobutyrate-α-ketoglutarate transaminase (EC 2.6.1.19) and succinate semialdehyde dehydrogenase (EC 1.2.1.16) than those of the wild-type parent strains. Secondary mutants derived from these GABA-nitrogen-utilizing strains were able to grow on GABA as the sole source of carbon and nitrogen. They also grew faster on a variety of other carbon and nitrogen sources, and their growth was more strongly inhibited by different metabolic inhibitors than was that of the parent strains. The nature of the two mutations and the possible genes involved are discussed. A scheme of the pathway for GABA breakdown in *E. coli* K-12 is presented.

In the course of studies on the uptake and utilization of glutamate by *Escherichia coli* K-12 conducted in this laboratory (4, 6, 16, 17), attempts were made to grow the bacteria in media in which γ-aminobutyrate (GABA), the decarboxylation product of glutamate, was the major carbon source or nitrogen source, or both. None of several K-12 strains tested was able to grow in these media. However, by mutagenesis and appropriate selection, mutants were quite readily obtained which could utilize GABA as the major source of nitrogen, but all attempts to isolate GABA-carbon-utilizing mutants directly from wild-type strains failed. In contrast to wild-type *E. coli* K-12, GABA-nitrogen-utilizing mutants readily yielded secondary mutants capable of growth on GABA as the source of carbon (M. Marcus, Ph.D. thesis, Hebrew University, Jerusalem, 1968). Those findings suggested that wild-type *E. coli* K-12 was blocked at least in two steps in the utilization of GABA. Other data obtained in that work suggested that the pathway of GABA breakdown in *E. coli* converged with that of glutamate. Earlier work by Noe and Nickerson (19) and by Jakoby and his colleagues (13, 18, 20) with pseudomonads also suggested a link between the catabolism of GABA in these bacteria and that of glutamate. These workers implicated GABA-α-ketoglutarate transaminase in catalyzing the first step in the breakdown of GABA. The second and only other specific step in GABA catabolism is the oxidation of succinyl semialdehyde, the product of the transamination reaction, to succinate, catalyzed by a specific succinyl semialdehyde dehydrogenase. Hardman and Stadtman studied the catabolism of GABA in *Clostridum aminobutyricum* in great detail (8-10). Although the later reactions in the pathway differed entirely from those found in the aerobic pseudomonads, the first step in the breakdown of GABA was the same in the two groups of bacteria, namely, transamination with α-ketoglutarate. Kim and Tchen described a mutant of *E. coli* B which was capable of utilizing putrescine as the sole source of carbon and nitrogen (14). They proposed that the diamine was transformed to succinate as follows: putrescine → γ-aminobutyraldehyde → γ-aminobutyrate → succinyl semialdehyde → succinate; with GABA-α-ketoglutarate transaminase and succinyl semialdehyde dehydrogenase catalyzing the last two steps, respectively. The present paper describes some properties of *E. coli* K-12 primary mutants capable of utilizing GABA as a source of nitrogen and of secondary mutants derived

from the former, which can utilize GABA as a source of both nitrogen and carbon. Our findings directly implicate GABA-α-ketoglutarate transaminase and succinyl semialdehyde dehydrogenase in the catabolism of GABA in *E. coli* K-12. We further show that a general nonspecific permeability factor determines the ability of these bacteria to utilize exogenous GABA as a carbon source at rates sufficient to permit growth.

MATERIALS AND METHODS

Chemicals. L-Aspartic acid and L-glutamic acid were obtained from the British Drug Houses, Ltd., Poole, England. α-Ketoglutaric acid (α-KG), γ-aminobutyric acid (GABA), and α-methyl-DL-glutamic acid (α-MG) were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio. Nicotinamide adenine dinucleotide phosphate (NADP), and reduced NADP (NADPH) and beef liver glutamate dehydrogenase were obtained from Boehringer and Sons, Mannheim, West Germany. Puromycin aminonucleoside, chloramphenicol, and aminooxyacetic acid were products of Sigma Chemical Co., St. Louis, Mo., and penicillin sodium salt was from Rafa Laboratories, Jerusalem. Oxaloacetic acid and ^{14}C-GABA, uniformly labeled, were purchased from Calbiochem, Los Angeles, Calif.; all the other radioactive chemicals were products of the Radiochemical Centre, Amersham, Bucks, England. Succinyl semialdehyde was prepared by the synthesis and hydrolysis of diethylformylsuccinate (12).

Bacterial strains. *E. coli* K-12 Hfr Cavalli (CS 101), a methionine-requiring strain, and glutamate-utilizing and GABA-utilizing mutants, derived from it after ultraviolet mutagenesis, were used. A detailed description of all strains used is given in Table 1.

Media. The minimal salts medium used (23) was of the following composition: NH_4Cl, 0.5%; NH_4NO_3, 0.1%; Na_2SO_4, 0.2%; K_2HPO_4, 0.3%; KH_2PO_4, 0.1%; $MgSO_4$, 0.01%; *p*H was adjusted to 7.1 with KOH. Minimal salts medium without ammonium salts was composed as follows: Na_2SO_4, 0.4%; K_2HPO_4, 0.6%; KH_2PO_4, 0.2%; $MgSO_4$, 0.01%; *p*H was adjusted to 7.1 with KOH. MacConkey agar (Difco) plates were used for counting bacteria. The cultures were maintained on tryptone blood agar base (Difco) slants.

Growth studies. Liquid minimal medium without ammonium salts and with sodium succinate (1%) as the carbon source, supplemented with L-methionine (25 μg/ml), was used in experiments in which the ability of bacteria to utilize a given compound as a nitrogen source was examined. The test compound was added at a concentration of 0.2%. In experiments on the utilization of carbon sources, complete minimal salts medium with L-methionine (25 μg/ml) and the test compound at a concentration of 0.5% (unless otherwise indicated) was used. In either case, the cultures were grown in 20 ml of medium in 100-ml conical flasks provided with side arms to allow direct turbidity measurements (in a Klett-Summerson photoelectric colorimeter, with a no. 42 filter). The flasks were inoculated with cells from fresh, twice-washed cultures from succinate medium, to an initial density of 10^8 cells/ml, except when the carbon and/or nitrogen source was present at concentrations below 0.1%. In the latter case, the initial cell concentration was adjusted to about 10^3 cells/ml, and, instead of periodic turbidity measurements, samples were plated on MacConkey agar. The cultures were incubated with vigorous aeration at 37 C. Generation times were determined graphically from semilogarithmic plots of optical density or number of colonies versus time.

Growth inhibition studies. Bacteria were grown in the appropriate liquid medium and turbidity readings were taken at frequent intervals to determine the growth rate in the absence of inhibitor. When turbidity reached 50 Klett units, the inhibitor was added to the desired concentration, and incubation and turbidity measurements were continued until the culture entered the stationary phase of growth. Per cent inhibition, I, was calculated from the formula $I = (b - a)/b \times 100$, where a is the generation time of the uninhibited culture and b is the generation time in the presence of inhibitor.

Preparation of cell-free extracts. Cultures in the late exponential phase of growth were harvested, washed, and suspended in 0.1 M phosphate buffer, *p*H 7.3, with 0.01% 2-mercaptoethanol, to a cell density corresponding to 5 to 10 mg of protein per ml. The suspension was treated in the cold in a 60-w MSE ultrasonic disintegrator at an output current of

TABLE 1. *Bacterial strains used*

Strain	Relevant phenotype[a]	Origin
Escherichia coli K-12 CS101A	Glut$^-$, GABA-N$^-$, GABA-C$^-$	Wild type
E. coli K-12 CS101B	Glut$^-$, GABA-N$^+$, GABA-C$^-$	UV-induced mutant of CS101A
E. coli K-12 CS101C	Glut$^-$, GABA-N$^+$, GABA-C$^+$	UV-induced mutant of CS101B
E. coli K-12 CS8A	Glut$^+$, GABA-N$^-$, GABA-C$^-$	UV-induced mutant of CS101A
E. coli K-12 CS8B	Glut$^+$, GABA-N$^+$, GABA-C$^-$	UV-induced mutant of CS8A
E. coli K-12 CS8C	Glut$^+$, GABA-N$^+$, GABA-C$^+$	UV-induced mutant of CS8B

[a] Glut$^+$ and Glut$^-$ designate ability and inability, respectively, to grow on L-glutamate as the sole source of carbon; GABA-N$^+$ and GABA-N$^-$ designate ability and inability, respectively, to grow on γ-aminobutyric acid (GABA) as the sole source of nitrogen; GABA-C$^+$ and GABA-C$^-$ designate ability and inability, respectively, to grow on GABA as the sole source of carbon. The concentration of L-glutamate used was 0.5%, and that of GABA was 0.2% when used as nitrogen source and 0.5% when used as carbon source.

1.5 a, for 3 min. Cell debris were removed by centrifugation in the cold at 20,000 × *g* for 20 min. Protein was determined by the method of Lowry et al. (15).

Enzyme determination. Glutamate dehydrogenase (EC 1.4.1.4) and glutamate oxaloacetate transaminase (EC 2.6.1.1) were determined by the methods of Halpern and Lupo (6). Aspartase (EC 4.3.1.1) was determined as described by Marcus and Halpern (17). GABA-α-KG transaminase (EC 2.6.1.19) was determined in the following reaction mixture: phosphate buffer, 0.5 M, *p*H 8.2; GABA, 3 × 10^{-3} M; ^{14}C-α-KG, 2.5 × 10^{-3} M (specific activity, 0.16 mCi/mmole); and bacterial extract equivalent to 200 μg of protein in a total volume of 0.6 ml. The mixture was incubated at 37 C for 20 min, and the reaction stopped by the addition of trichloroacetic acid to a final concentration of 10%. The mixture was loaded onto a 2-ml column of Dowex 50 WX12 H^+ form (400/200 mesh), and the column was washed with 10 ml of water. The bound radioactivity was eluted with 3 ml of 2 M ammonia, into a scintillation vial containing a 1 by 14 cm strip of Whatman no. 1 paper. The vials were heated at 100 C for 20 min, 10 ml of scintillation liquid was added, and the sample was counted in a Packard Tricarb liquid scintillation spectrometer, model 3310. Under these conditions the reaction remains linear with time for about 40 min and with protein concentration up to 500 μg/ml. Enzyme activity is expressed as nanomoles per milligram of protein per minute. Succinyl semialdehyde dehydrogenase (EC 1.2.1.16) was determined as described by Jakoby (12) with the following modifications. Phosphate buffer, 0.1 M, *p*H 9.2, was used; the concentration of NADP was 0.05 mg/ml; and that of protein was 50 to 100 μg/ml. The mixture in a volume of 1 ml was incubated at 40 C, and the reaction was followed in a Gilford spectrophotometer, model 2400. The reaction was linear with time for 20 min and with protein concentration up to 200 μg/ml. Enzyme activity is expressed as nanomoles per milligram of protein per minute.

Uptake of radioactive compounds. Uptake of ^{14}C-labeled compounds was followed as described previously (5, 6) except that the reaction mixture was incubated at 25 C for 4 min.

Analysis of radioactivity in cells incubated with ^{14}C-GABA. The bacteria were grown in minimal medium with 1% succinate, 25 μg of L-methionine per ml, and 50 μg of thymine and uracil per ml, to a density of 180 Klett units (filter no. 42). Chloramphenicol (200 μg/ml) and penicillin (1,000 units/ml) were added, and the cultures were further incubated for 30 min. The cells were diluted in one volume of fresh medium of the same composition, with ^{14}C-GABA (specific radioactivity, 2 mCi/mmole) at a final concentration of 1 × 10^{-4} M and incubated at 25 C for 20 min. The bacteria were filtered on membrane filters (Millipore Corp.; diameter 45 mm, pore size 0.45 μm) and washed with three volumes of medium without GABA kept at 25 C. The filters were dried and counted as described above. A parallel sample was treated with 5% trichloroacetic acid at 4 C for 20 min and then filtered and processed as above. The difference in radioactivity between the acid-treated and untreated samples represents the cold-acid-soluble counts. Another sample was treated with trichloroacetic acid as above, and the filtrate was loaded onto a Dowex 50 WX12 column, H^+ form, 10 by 50 mm. The column was washed with ten volumes of water and then eluted with two volumes of 2 M ammonia. The ammonia eluate was concentrated to 0.4 ml, applied to Whatman 3MM paper, and chromatographed in water-saturated phenol with 0.3% ammonia and traces of KCN. Cold GABA used as a marker gave an R_F of 0.79. The chromatogram was dried, cut into strips 10 mm wide, and counted by liquid scintillation.

RESULTS

Utilization of GABA by E. coli K-12 wild-type and mutant strains. Table 2 shows the growth rates of *E. coli* K-12 wild-type, GABA-nitrogen- and GABA-carbon-utilizing mutants in media with GABA as the carbon or nitrogen source. Wild-type strains, whether capable or not of utilizing glutamate as a source of carbon, grow very poorly, if at all, on GABA as a carbon or nitrogen source (CS101A, CS8A). First-stage mutants (CS101B, CS8B) grow well on GABA as a source of nitrogen, but are unable to grow on it in the absence of a utilizable carbon source. Second-stage mutants (CS101C, CS8C) utilize GABA both as a nitrogen and as a carbon source.

Enzyme levels. Comparison of the activities of glutamate dehydrogenase, glutamate-oxaloacetate transaminase, and aspartase in cell-free extracts did not disclose any significant differences between the wild-type and mutant strains. On the other hand, as shown in Table 3, the first-stage mutants selected for their ability to utilize GABA as a source of nitrogen had six- to ninefold higher activities of both GABA-α-ketoglutarate transaminase and succinyl semialdehyde dehydrogenase than did the wild-type strains. No further increase in the activities of these enzymes accompanied the mutations of GABA-nitrogen-utilizing strains to the GABA-C^+ phenotype.

Since we found no differences in enzymatic

TABLE 2. *Growth rates of wild-type and mutant strains on γ-aminobutyric acid (GABA)*

Strain	Generation time (min)	
	0.2% GABA as nitrogen source	0.5% GABA as carbon source
CS101A	>600	>600
CS101B	210	>600
CS101C	200	210
CS8A	>600	>600
CS8B	170	>600
CS8C	170	220

activity between the GABA-N$^+$ and GAGA-C$^+$ mutants, it was reasonable to look for possible differences in the permeability of the two mutant classes to GABA. The data presented in Fig. 1 and Tables 4 and 5 indeed support the contention that the inability of GABA-N$^+$ mutants to utilize GABA as a source of carbon is due to impaired transport.

Growth of GABA-N$^+$ mutants on high concentrations of GABA as a source of carbon. Figure 1 shows that, although strain CS101B does not grow on GABA as a carbon source at a concentration as high as 0.5%, when the concentration of GABA is raised to 2%, CS101B grows at a rate one-third of that exhibited by the GABA-C$^+$ mutant CS101C.

Growth of GABA-N$^+$ mutants on GABA as the carbon source in the presence of DMSO. Dimethyl sulfoxide (DMSO) was found to enhance polio-ribonucleic acid (RNA) infectivity in tissue cultures by facilitating the

TABLE 3. *Activities of GABA-α-ketoglutarate transaminase (GSST) and succinyl semialdehyde dehydrogenase (SSDH) of wild-type and GABA-utilizing E. coli K-12 mutants*[a]

Strain	GSST activity (nmoles per mg of protein per min)	SSDH activity nmoles/mg of protein/min
CS101A	5.14	13.36
CS101B	46.73	76.82
CS101C	38.21	86.84
CS8A	8.57	17.70
CS8B	46.73	78.82
CS8C	45.00	75.99

[a] Cell-free extracts from cultures grown in succinate (0.5%)-minimal medium to a density of 200 Klett units (filter no. 42) were used. The concentration of bacterial protein was 200 μg/ml in the GSST assay and 50 μg/ml in the SSDH assay. For other experimental details, see text.

TABLE 4. *Effect of dimethylsulfoxide (DMSO) on the growth of mutant CS101B on γ-aminobutyric acid (GABA) as the source of carbon*[a]

Carbon source	Nitrogen source	Generation time (min)	
		Without DMSO	With 0.1 M DMSO
GABA	GABA	>1000	300
GABA[b]	GABA[b]	>1000	265
Sodium succinate	GABA	210	200

[a] Liquid minimal medium with no ammonium salts was used. Where sodium succinate (1%) was used as the major carbon source, 0.2% GABA was added to serve as the nitrogen source; in all the other flasks the concentration of GABA was 1%. Other conditions and procedures were as described in the text.

[b] Culture was grown on 1% GABA as the source of carbon and nitrogen, in the presence of 0.1 M DMSO, to a turbidity of 220 Klett units (filter no. 42). It was then harvested and washed in basal medium devoid of any source of carbon or nitrogen and inoculated into two flasks with minimal medium containing 1% GABA as the source of carbon and nitrogen, to an initial density of 40 Klett units. These secondary cultures, one with and one without 0.1 M DMSO were incubated, and growth was followed as above.

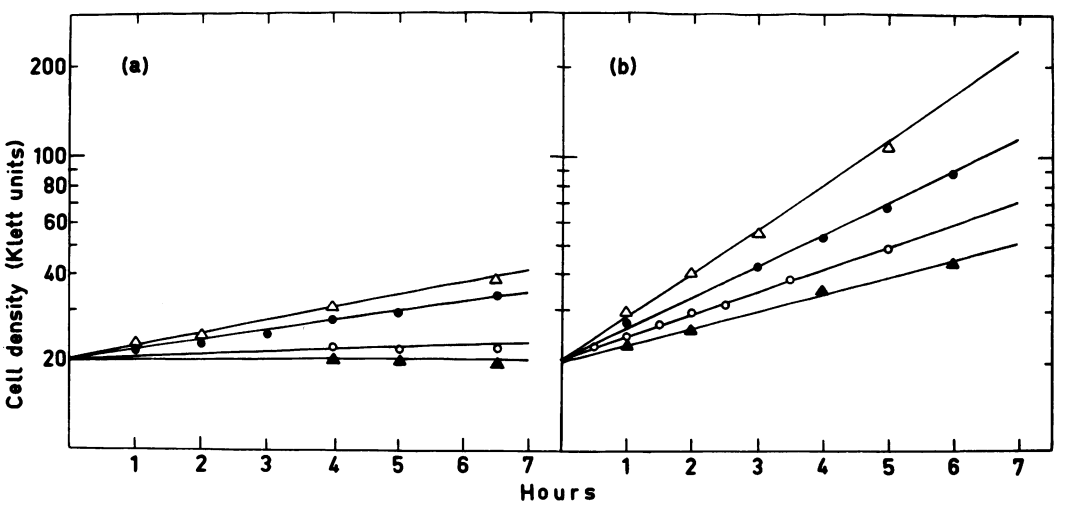

FIG. 1. *Growth of GABA-utilizing mutants on different concentrations of GABA as the source of carbon. (a) Escherichia coli CS101B; (b) E. coli CS101C. Concentrations of GABA were: 0.2%, ▲; 0.5%, ○; 1.0%, ●; 2.0%, Δ For other details, see text.*

TABLE 5. *Uptake of ^{14}C-γ-aminobutyric acid (GABA) by wild-type and mutant strains*

Strain	Total uptake (counts/minute)	Cold-acid-soluble radioactivity (counts/minute)	Cold-acid-soluble radioactivity retained by Dowex-50W, H⁺-form (counts/minute)	Free intracellular GABA	
				Radioactivity (counts/minute)	Concn (10^{-5} M)
CS101A	619	171	154	117	2.48
CS101B	1,261	542	271	114	2.42
CS101C	1,857	651	325	215	4.53

entry of the RNA molecule into the cell (1). Table 4 shows the effect of DMSO on the utilization of GABA at a concentration of 0.5% as the carbon source for growth by strain CS101B. As one can see from the table, there was a dramatic enhancement of growth in the presence of DMSO. To rule out the possibility that the drug acted as a mutagen and that the growth observed was due to selection and overgrowth of GABA-C⁺ mutants, bacteria were pregrown on GABA in the presence of DMSO and then washed and inoculated into GABA medium with and without DMSO. As shown in the table, cells pregrown in the presence of the drug, when transferred to the fresh medium in the absence of DMSO, lost the ability to grown on GABA as a carbon source. Thus, DMSO affects the GABA-C⁻ phenotype of strain CS101B most probably by facilitating the entry of GABA into the cells.

Uptake of radioactive GABA by wild-type and mutant cells. Table 5 presents data on the uptake of ^{14}C-GABA by nonproliferating cultures of CS101A and its GABA-N⁺ and GABA-C⁺ derivatives. When total uptake of radioactivity, or the radioactivity incorporated into the total free pool of metabolic intermediates, or only into its basic fraction, was measured, the GABA-C⁺ mutant was approximately three times as active as the wild-type strain, with the GABA-N⁺ strain occupying an intermediate position. However, when the concentrations of free intracellular GABA were determined, no difference was found between the wild-type and GABA-N⁺ strain, whereas the GABA-C⁺ mutant had a free GABA pool twice as high as those found in the other two strains. It should be pointed out that in none of the strains examined, including the GABA-C⁺ mutant, CS101C, did we find any evidence for the accumulation of GABA against a concentration gradient.

In view of the fact that the enhanced permeability of GABA-C⁺ mutants did not seem to involve an active GABA transport system, we investigated the possibility that the GABA-C⁺ phenotype was due to a mutation of some general permeability factor affecting the permeation of compounds other than GABA as well. This assumption was borne out by experiments

TABLE 6. *Growth rates of CS101 wild-type and GABA-utilizing mutants on various carbon sources*[a]

Carbon source	Generation time (min)		
	CS101A	CS101B	CS101C
Lactose, 0.2%[b]	70	69	60
Fructose, 0.01%[c]	72	75	54
Glycerol, 0.1%[c]	62	66	45
Acetate, 0.01%[c]	150	150	115
Succinate, 0.01%[c]	165	180	81
L-Alanine, 0.1%[c]	95	84	60
L-Aspartate, 0.4%[b]	1,030	1,030	420

[a] Cultures were grown in minimal medium with the indicated carbon sources at concentrations specified in the table. The flasks were incubated in a shaking water bath at 37 C.

[b] Growth was determined turbidimetrically; the initial cell density was approximately 10^8/ml.

[c] Growth was determined by plate counts on MacConkey agar; the initial cell density was approximately 10^3/ml.

TABLE 7. *Growth rates of CS101 wild-type and GABA-utilizing mutants on various nitrogen sources*[a]

Nitrogen source	Generation time (min)		
	CS101A	CS101B	CS101C
NH_4Cl, 0.00083% + NH_4NO_3, 0.00017%[b, e]	156	120	84
L-Glutamate, 0.00001%[c, e]	132	120	60
L-Aspartate, 0.5%[b, f]	105	105	90
GABA, 0.0001%[d, e]	No growth	90	45

[a] Bacteria were grown in minimal medium in which the ammonium salts were substituted by the indicated nitrogen source at the concentration specified. The flasks were incubated in a shaking water bath at 37 C.

[b] Succinate (1%) was the major carbon source.

[c] Glycerol (0.4%) was the major carbon source.

[d] Glucose (0.4%) was the major carbon source.

[e] Growth was determined by plate counts on MacConkey agar; initial cell density was approximately 10^3/ml.

[f] Growth was determined turbidimetrically; initial cell density was approximately 10^8/ml.

TABLE 8. *Growth rates of CS8 wild-type and GABA-utilizing mutants on glutamate*

Carbon source	Nitrogen source	Generation time (min)[c]		
		CS8A	CS8B	CS8C
Glycerol, 0.4%[a]	L-Glutamate, 0.00001%	117	120	90
L-Glutamate, 0.00001%[b]	NH_4Cl, 0.05% + NH_4NO_3, 0.01%	250	220	140

[a] Bacteria were grown overnight in minimal medium from which the ammonium salts were omitted and substituted by 0.01% L-glutamate, in the presence of 0.1% glycerol. Cultures were diluted to approximately 10^3 cells/ml in minimal medium with no ammonium salts, containing 0.00001% L-glutamate as the nitrogen source and 0.4% glycerol as the source of carbon.

[b] Bacteria were grown overnight in minimal medium with 0.1% L-glutamate as the source of carbon. Cultures were diluted to approximately 10^3 cells/ml in minimal medium with 0.00001% L-glutamate as the carbon source.

[c] Growth was determined by plate counts on MacConkey agar.

TABLE 9. *Uptake of ^{14}C-labeled sugars and amino acids by wild-type and GABA-utilizing CS101 mutants*[a]

^{14}C-labeled compound	Uptake (μmoles/g dry wt of bacteria)		
	CS101A	CS101B	CS101C
L-Aspartate, 5×10^{-6} M	2.56×10^{-2}	2.56×10^{-2}	3.25×10^{-2}
L-Glutamate, 1×10^{-5} M	2.06	2.06	3.75
Maltose, 7×10^{-6} M	8.75	6.90	12.50
Sorbitol, 1.25×10^{-5} M	2.80	2.80	3.30

[a] Specific radioactivities employed were: L-aspartate, 1.73 mCi/mmole; L-glutamate, 1 mCi/mmole; maltose, 5.9 mCi/mmole; sorbitol, 8.5 mCi/mmole. For experimental conditions, see text.

summarized in Tables 6 through 10.

Rates of growth of wild-type and GABA-utilizing mutants of E. coli K-12 on various carbon and nitrogen sources. The data given in Table 6 represent the generation times of strains CS101A, CS101B, and CS101C on various carbon sources. Each compound was tested at several (at least three) concentrations; the particular concentration of any given compound indicated in the table is that which gave the best results. One can see that whereas only small and random differences were found between the respective growth rates of strains CS101A and CS101B, growth of the GABA-C^+ mutant was considerably faster than that of the other two strains on any one of the carbon sources tested. Similar results were obtained in other experiments (Table 7) in which the compounds tested served as the source of nitrogen. In Table 8 are given the generation times in glutamate media of three isogenic glutamate-utilizing strains differing from each other only in their ability to utilize GABA. Here too, the GABA-C^+ mutant grew faster on glutamate as the carbon or nitrogen source than did the other two strains.

Uptake of ^{14}C-labeled compounds by nonproliferating wild-type and GABA-utilizing mutant cells. Cultures of *E. coli* K-12 CS101A, CS101B, and CS101C were exposed to ^{14}C-labeled compounds shown in Table 9 in the presence of chloramphenicol (200 μg/ml) for 4 min at 25 C, as described above. As one can see from Table 9, in each case strain CS101C took up more label than did the other two strains.

Effect of metabolic inhibitors on the growth of E. coli K-12 wild-type and GABA-utilizing mutant strains. Table 10 summarizes the results of experiments in which we compared the inhibitory responses of wild-type and GABA-utilizing K-12 mutants to the addition of chloramphenicol, puromycin, aminooxyacetic acid, an inhibitor of transaminases involving α-ketoglutarate (24), and α-methyl-DL-glutamate, a growth inhibitor of glutamate-utilizing *E. coli* strains (6, 7). One sees quite clearly that the growth of GABA-C^+ mutants was more severely inhibited by each of these inhibitors than was the growth of the respective wild-type and GABA-N^+ mutant strains. The only exception was observed with methyl glutamate in the CS101 series, all the members of which were almost fully resistant to the drug. This can be readily explained by the low capacity of the glutamate active transport system in these strains, since, as indicated by our previous studies (4, 7), methyl glutamate is most probably transported into the cell via this system.

TABLE 10. *Effect of metabolic inhibitors on the rate of growth of E. coli K-12 wild-type and GABA-utilizing mutant strains*[a]

Bacterial strain	Chloramphenicol			α-Methyl-DL-glutamate			Puromycin aminonucleoside			Aminooxyacetic acid		
	Generation time (min)		Inhibition (%)	Generation time (min)		Inhibition (%)	Generation time (min)		Inhibition (%)	Generation time (min)		Inhibition (%)
	(a) No inhibitor added	(b) 6 μg/ml	(b − a)/b × 100	(a) No inhibitor added	(b) 100 μg/ml	(b − a)/b × 100	(a) No inhibitor added	(b) 147 μg/ml	(b − a)/b × 100	(a) No inhibitor added	(b) 7.6 μg/ml	(b − a)/b × 100
CS101A	80	170	52.9	108	120	10.0	70	70	0.0	85	132	35.7
CS101B	72	135	46.6	108	111	2.7	60	65	7.7	102	170	40.0
CS101C	80	500	*84.0*	87	96	9.4	60	85	*29.4*	90	273	*67.0*
CS8A	90	225	60.0	84	306	72.5						
CS8B	99	240	58.7	75	204	63.2						
CS8C	100	325	*69.2*	90	No growth	*100.0*						

[a] Cultures were grown in minimal medium with 1% succinate as the source of carbon, except for the puromycin experiment, in which 0.4% glycerol was used. For other experimental details, see text.

DISCUSSION

The experiments described here indicate that the pathway of GABA breakdown in *E. coli* K-12 is the same as that described by Jakoby and his colleagues in *Pseudomonas fluorescens* (13, 18, 20). The first step is transamination between GABA and α-ketoglutarate to yield succinyl semialdehyde and glutamate. This reaction is catalyzed by γ-aminobutyrate-α-ketoglutarate transaminase. Succinyl semialdehyde is then oxidized to succinate by succinyl semialdehyde dehydrogenase. Both these enzymes are very low in wild-type *E. coli* K-12 unable to utilize GABA as a sole carbon or nitrogen source, and a parallel six- to ninefold increase in both activities was found in GABA-utilizing mutants. These findings and our interpretation are in agreement with the conclusions drawn by Kim and Tchen from their studies of putrescine utilization by *E. coli* B (14). The proposed pathway of GABA breakdown also explains an earlier observation from this laboratory that each one of eighteen independently isolated GABA-utilizing mutants, derived from an aspartase-negative, glutamate-nonutilizing *E. coli* K-12 strain, recovered aspartase activity and the ability to grow on glutamate as carbon source (M. Marcus, Ph.D. Thesis, and *personal communication*). As shown previously (17), glutamate is metabolized in *E. coli* K-12 via transamination with oxaloacetate to yield α-ketoglutarate and aspartate, which is then deaminated to fumarate by the action of aspartase. If, as proposed here, transamination with α-ketoglutarate is indeed the first step in the breakdown of GABA by *E. coli* K-12, mutants blocked in either glutamate oxaloacetate transaminase or aspartase, and thus unable to utilize glutamate, should also not be able to grow on GABA. The complete sequence of reactions involved in the utilization of GABA is shown in Fig. 2. As one can see from the scheme shown here, although both GABA-α-KG transaminase and succinyl semialdehyde dehydrogenase are essential for the utilization of GABA as a sole source of carbon, it is not clear whether succinyl semialdehyde dehydrogenase is also required for the utilization of GABA-nitrogen in the presence of another utilizable carbon source. Experiments with revertants of GABA-C^+ mutants now in progress should clarify this point.

The simultaneous increase in the activities of GABA-α-KG transaminase and succinyl semialdehyde dehydrogenase as a result of a single mutational event strongly suggests that a control gene, regulating the synthesis of both enzymes of GABA utilization, is involved. A

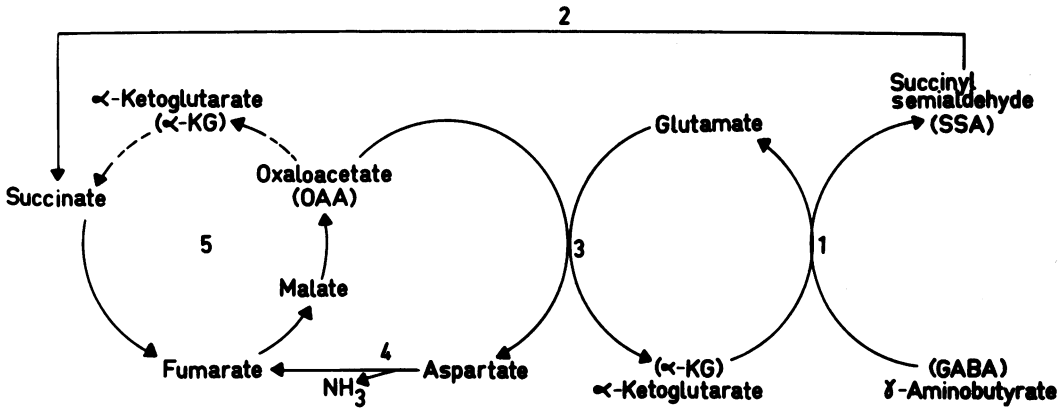

FIG. 2. *Schematic representation of the GABA utilization pathway in Escherichia coli K-12. 1, GABA-α-ketoglutarate transaminase (GSST); 2, succinyl semialdehyde dehydrogenase (SSDH); 3, glutamate oxaloacetate transaminase (GOT); 4, aspartase; 5, tricarboxylic acid cycle.*

similar situation has been described in recent studies on the utilization of histidine by *Salmonella typhimurium* (21, 22). The wild-type LT-2 strain unable to utilize histidine as a single source of carbon or nitrogen, or both, exhibits very low activities of histidase and urocanase, enzymes which catalyze the first two steps in the breakdown of histidine to glutamate. A mutant, PV2, isolated for its ability to utilize histidine as a nitrogen source showed greatly increased levels of these two enzymes. A secondary mutant derived from PV2 was capable of growing on histidine as the sole source of carbon as well as nitrogen. Extracts of this mutant were found to be about three times as active in catalyzing the third and fourth steps of histidine catabolism as were the wild-type and PV2 strains. Both mutations involved the promoter loci of the respective operons. Similar studies are now being undertaken to elucidate the control of the pathway of GABA utilization in *E. coli* K-12.

The experiments described in Fig. 1 and Tables 4 and 5 strongly indicate that the ability of GABA-C$^+$ mutants to grow on GABA as a sole source of carbon is due to enhanced permeability of the cells to GABA. As shown in Tables 6 through 10, the GABA-C$^+$ mutation does not affect the utilization of GABA alone, but also enhances the uptake of a variety of other compounds, as evidenced by the faster growth of these mutants on different carbon and nitrogen sources and by their greater growth-inhibitory responses to various metabolic inhibitors. Similar findings have been recently reported by Crandall and Koch (2). These authors isolated temperature-sensitive (*ts*) mutants of *E. coli* exhibiting decreased growth rates, decreased β-galactoside permease synthesis and activity, decreased generalized transport, and increased permeability, when grown at the restrictive temperature. The pleiotropic effects of these *ts* mutations are ascribed to altered membrane synthesis at high temperature, possibly involving a phospholipid component. A lipid requirement for induction of lactose transport in *E. coli* had been demonstrated earlier by Fox (3). This author concluded that the galactoside permease protein synthesized in the absence of the required fatty acid was not functional, because its incorporation into the membrane depends on new membrane synthesis which did not occur under these conditions. A reduced lipid content was also implicated by Holden et al. in the defective accumulation of amino acids by pantothenate- and biotin-deficient *Lactobacillus plantarum* (11). In view of these findings, it is not unreasonable to assume that the GABA-C$^+$ phenotype described here is a result of a mutation in a gene involved in membrane synthesis.

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