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Glutamate Transport in Wild-Type and Mutant Strains of *Escherichia coli*

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ABSTRACT

HALPERN, YEHESKEL S. (Hebrew University-Hadassah Medical School, Jerusalem, Israel), AND MEIR LUPO. Glutamate transport in wild-type and mutant strains of *Escherichia coli*. J. Bacteriol. **90**:1288–1295. 1965.—Mutants of *Escherichia coli* able to grow on glutamate as their source of carbon showed glutamate dehydrogenase and glutamate-oxaloacetate transaminase activities similar to those possessed by the parent strain. The mutants took up glutamate at a much faster rate and showed a several-fold greater capacity for concentrating the amino acid than did the corresponding parent strains. Curvilinear double reciprocal plots of velocity of uptake versus glutamate concentration were obtained with the *E. coli* H strains. A break in the curve of glutamate uptake was observed with the *E. coli* K-12 strains when incubated in a glucose medium. It is suggested that these findings may be due to allosteric activation of glutamate permease by its substrate.

In a previous paper (Halpern and Umbarger, 1961), evidence was presented implicating a permeability barrier as the factor responsible for the inability of wild-type *Escherichia coli* W to grow on glutamate as the sole source of carbon. It has been suggested that mutations enabling growth on glutamate involve changes in a permeation mechanism, rather than in a later metabolic step. That glutamate dehydrogenase (GDH) was not likely to be involved in these mutations also followed from our earlier results (Halpern and Umbarger, 1960), which indicated that the physiological function of this enzyme in *E. coli* is primarily a biosynthetic rather than a catabolic one. This contention was further supported by Sanwal (1961) and Sanwal and Lata (1961, 1962), in studies on the GDH of *Neurospora* and *Fusarium*. In addition, Vender and Rickenberg (1964) recently showed that a mutant of *E. coli* capable of utilizing glutamate as its sole source of carbon was devoid of GDH activity, and that in other glutamate-utilizing mutants the synthesis of nicotinamide adenine dinucleotide phosphate (NADP)-linked GDH was repressed in the presence of glutamate (Rickenberg and Vender, 1963).

Although our earlier studies on the glutamate-utilizing mutants of *E. coli* W strongly indicated changes in permeability, a better understanding of the glutamate transport systems operating in these bacteria was required. It also seemed worthwhile to extend these studies to other strains of *E. coli*, in particular to strains which are amenable to genetic analysis. Experiments with wild-type and mutant strains of *E. coli* K-12 and H are described in this paper.

MATERIALS AND METHODS

Organisms. The following organisms were used: a methionine-requiring Hfr strain *E. coli* K-12 CS101, unable to grow on glutamate as the source of carbon (supplemented with 50 μg/ml of DL-methionine), hereafter referred to as CS; *E. coli* H, a strain which grew very slowly on glutamate (with a generation time of more than 9 hr); two mutants of CS, CS1, and CS5, and two mutants of H, Hng1, and Hng4, all of which were capable of utilizing glutamate for growth (the CS mutants only when supplemented with methionine).

Selection of mutants. The CS mutants were obtained from cultures irradiated with ultraviolet light in the logarithmic phase of growth, to a survival of 10^{-3}. Samples from the irradiated cultures were inoculated into minimal medium (supplemented with 50 μg/ml of DL-methionine) with glutamate as the major source of carbon, and were incubated in a shaking water bath at 37 C. Single-colony isolates on solid glutamate medium were made from flasks in which turbidity developed. The mutants were purified by streaking on solid glutamate medium. Mutants CS1 and CS5 originated from different flasks, thus representing two independent mutational events.

The H mutants were obtained by the use of *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (NG). Logarithmic cultures were washed and exposed to the action of 600 μg/ml of NG in 0.2 M acetate buffer

(*p*H 5.0) for 30 min at 37 C. The treated cultures were washed, and were incubated overnight in nutrient broth; samples were then plated on minimal-glutamate medium. Single colonies were picked and purified by restreaking on solid glutamate medium. The two mutants used in this work came from separate overnight broth cultures.

Growth media. The minimal medium of Davis and Mingioli (1950), from which citrate was omitted, was used throughout this work. Glucose (0.5%), sodium succinate (1%), or sodium glutamate (0.5%) was used as the source of carbon, as required.

Growth experiments. Bacteria were inoculated into media containing limited amounts of carbon source (0.04% glucose or 0.1% succinate) in flasks provided with side arms to allow direct turbidity measurements, and were incubated overnight in a shaking water bath at 37 C (120 strokes per min). Additional quantities of the appropriate carbon sources were then added, and logarithmic growth was resumed after a very short lag (5 to 15 min). Growth was followed by measuring the changes in optical density in a Klett-Summerson photoelectric colorimeter with a no. 42 filter. Growth rates were graphically determined from semilogarithmic plots of optical density versus times of incubation. In growth-inhibition experiments with α-methyl-DL-glutamic acid, the inhibitor was added after logarithmic growth had been established. The inhibitory effect on growth was determined from the formula: $(k_c - k_e)/k_c \times 100$, where k_c and k_e are reciprocals of the mass-doubling-time of the control and inhibited cultures, respectively.

Preparation of cell-free extracts. Logarithmically growing cultures were harvested, washed, and suspended in one-twentieth the original volume of 0.001 M phosphate buffer (*p*H 7.5); the cells were then treated for 2 min in a 60-w MSE ultrasonic disintegrator. The preparation was centrifuged in the cold at 20,000 $\times$ *g* for 10 min to remove cell debris, and the clear extract was dialyzed overnight in the cold against 10 liters of tap water. The concentration of protein in the dialyzed extract was determined by the method of Lowry et al. (1951).

Assay of GDH activity. The GDH activity of dialyzed cell-free extracts was determined spectrophotometrically by measuring the rate of decrease in the optical density of reduced NADP ($NADPH_2$) at 340 mμ at room temperature in the following reaction mixture (in a total volume of 3 ml): bacterial extract, 30 to 120 μg of protein; α-ketoglutarate (*p*H 8.0), 100 μmoles; NH_4Cl (*p*H 8.0), 100 μmoles; $NADPH_2$ in 0.05 M carbonate-bicarbonate buffer (*p*H 10), 0.3 μmoles; and phosphate buffer (*p*H 8.7), 120 μmoles. Initial rates were constant for at least 3 min, and were used to calculate enzyme activity expressed in micromoles per milligram of protein per hour.

Assay of glutamate-oxaloacetate transaminase (GOT) activity. The GOT activity of dialyzed cell-free extracts was determined by coupling the reaction with glutamate dehydrogenase and measuring the rate of decrease in optical density of $NADPH_2$ at 340 mμ. The reaction mixture contained: bacterial extract, 25 to 50 μg of protein, L-glutamic acid adjusted to *p*H 8.7 with NaOH, 40 μmoles; oxaloacetate, 20 μmoles; NH_4Cl (*p*H, 8.7), 50 μmoles, GDH from liver (Sigma Chemical Co., St. Louis, Mo.), 1.5 International Units; $NADPH_2$ in 0.05 M carbonate-bicarbonate buffer (*p*H 10), 0.24 μmoles; and phosphate buffer (*p*H 8.7), 300 μmoles; in a total volume of 3 ml. Under these conditions, the reaction rate was linear with concentrations of bacterial protein between 10 and 90 μg. Controls from which oxaloacetate was omitted showed no decrease in optical density during the entire experiment (3 to 6 min).

Reduction of triphenyltetrazolium chloride. The capacity of whole-cell suspensions and bacteria disintegrated by sonic oscillation to reduce triphenyltetrazolium chloride in the presence of glutamate was tested as described earlier (Halpern and Umbarger, 1961), with the following modifications. The concentration of whole cells was equivalent to 0.82 mg and that of disintegrated cells, 9.09 mg (dry weight); the incubation time for the latter was 30 min. The concentration of phosphate buffer (*p*H 7.5) was 198 μmoles. The formazan formed was extracted with 3.5 ml of isobutyl alcohol.

Experiments on the uptake of glutamate. Glutamate uptake was studied by two different approaches.

In the first, incorporation of L-C^{14}-glutamate (uniformly labeled; The Radiochemical Centre, Amersham, Bucks, England) into the proteins of logarithmically growing cultures was determined according to the method of Ames (1964) modified as follows. A logarithmic culture was diluted into fresh medium to about 2×10^7 cells per milliliter and was further incubated for 90 min. The concentration of bacteria was determined by plating, and 8-ml samples were added to 2 ml of prewarmed radioactive glutamate at the desired concentration. The incorporation mixture was incubated for 3 min, at which time 1 ml of 55% trichloroacetic acid was added and the tubes were transferred to a boiling water bath for 10 min. The samples were then filtered through Millipore filters (HAWP 02500, 0.45 μ), which had been previously soaked in nonradioactive glutamate (0.001 M). The filters were washed with about 20 ml of 5% trichloroacetic acid, dried, and placed in vials to which a scintillation fluid was added. Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer. Rates are expressed as micromoles per milligram (dry weight) per hour, as calculated from the specific activity of glutamate and data relating cell count to dry weight (2.95×10^8 cells are equivalent to 100 μg, dry weight). The change in substrate concentration during the experiment did not exceed 7% at the lowest concentrations used.

The second approach involved measurement of the accumulation of C^{14}-glutamate in nongrowing

cells. This was done by the method of Kessel and Lubin (1965). Chloramphenicol (200 μg/ml) was added to cultures growing logarithmically in minimal medium with glucose or succinate, when cell density reached about 150 Klett units; incubation was then continued for 30 min. Samples (1 ml) were then taken and incubated with aeration for 2 min at 37 C in the presence of prewarmed radioactive glutamate. (Accumulation was very rapid, reaching a maximum after 2 min, and decreased subsequently by some 20% between 4 and 10 min of incubation.) The incubation mixtures were rapidly filtered through Millipore filters (see above) and washed with 20 ml of growth medium containing 200 μg/ml of chloramphenicol at 37 C. The filters were dried and counted as above. Preliminary experiments have shown that 98.5% of the radioactivity could be removed by cold trichloroacetic acid treatment; the residual radioactivity after hot trichloroacetic acid treatment was slightly below 1%. Intracellular concentrations are expressed as micromoles per milliliter of intracellular water, as calculated from measurements of the specific activity of glutamate and data relating optical density to dry and wet weight of cells (146 Klett units are equivalent to 234 μg (dry weight) per ml or 0.001 ml of intracellular water).

Determination of radioactive glutamate in the internal pool. A 20-ml amount of a suspension containing 11 mg of cells (wet weight) in a chloramphenicol-succinate (CM-S) medium was incubated for 3 min at 37 C in the presence of 2.5 $\times 10^{-5}$ M L-C^{14}-glutamate, filtered on a Millipore filter, and washed with 20 ml of CM-S medium. The bacteria were suspended in 5 ml of double-distilled water and heated for 20 min in a boiling-water bath. Cell debris was removed by centrifugation at 13,500 × *g* for 10 min, and was washed in 2 ml of water; the combined supernatant fluid was passed through a column (1.2 by 15 cm) of Dowex 50 (H^+ form). The column was washed with 20 ml of double-distilled water, and the absorbed amino acids were eluted with 30 ml of 2 M ammonia. The eluate was evaporated to dryness, and the residue was dissolved in 0.5 ml of water. Samples were put on Whatman 3 MM paper, and the chromatogram was developed for 15 hr in water-saturated phenol containing 0.1% ammonia. The glutamate spot was cut out and placed in a vial with scintillation fluid, and radioactivity was determined as above. Between 70 and 75% of the total radioactivity in the pool was recovered as glutamate.

RESULTS

Growth studies. Table 1 gives the growth rates of *E. coli* K-12 CS101, and of the mutants derived from this strain, in minimal medium in which glucose, succinate, or glutamate served as the source of carbon. The table shows that the acquisition of the ability to utilize glutamate was not accompanied by any considerable change in the capacity of the mutant cell to grow on glucose or succinate. It is of interest that among the numerous glutamate-utilizing mutants isolated in the course of these experiments (not shown in the table) there was a considerable variation in rates of growth on glutamate, ranging from a generation time of 1.50 hr to one of 8 hr.

TABLE 1. *Growth of Escherichia coli K-12 CS101 and its mutants on different carbon sources**

Strain	Growth rate (generations per hr)		
	Glucose, 0.5%	Sodium succinate, 1.0%	L-Glutamic acid, 0.5%
E. coli K-12 CS101, wild type (CS)	0.53	0.33	0
E. coli K-12 CS101/glut. 1 (CS1)	0.55	0.31	0.40
E. coli K-12 CS101/glut. 5 (CS5)	0.62	0.38	0.42

* For experimental conditions see Materials and Methods.

TABLE 2. *Glutamate dehydrogenase (GDH) in extracts of Escherichia coli K-12 CS101 and its glutamate-utilizing mutants**

Carbon source in growth medium	GDH activity (μmoles per mg of protein per hr)		
	E. coli K-12 CS101, wild type (CS)	*E. coli* K-12 CS101/glut. 1 (CS1)	*E. coli* K-12 CS101/glut. 5 (CS5)
Glucose, 0.5%	42.6	39.5	37.5
Sodium succinate, 1.0%	23.5	25.0	25.0
L-Glutamic acid, 0.5%	—	13.0	10.0

* For experimental conditions see Materials and Methods.

GDH activity. The GDH activity of extracts from *E. coli* K-12 CS101 and its mutants grown on various carbon sources was compared. In agreement with our earlier findings with *E. coli* W (Halpern and Umbarger, 1960), the highest activity was found with extracts from glucose-grown cells. Glutamate, when serving as the sole source of carbon for the mutant strains, repressed the formation of GDH by more than 65%. No differences were observed in the GDH activities of the parent and mutant strains grown under similar conditions (Table 2).

GOT activity. An alternative mechanism enabling the utilization of glutamate for growth could be an increase in the activity of trans-

TABLE 3. *Glutamate-oxaloacetate transaminase (GOT) activity in extracts of Escherichia coli K-12 CS101 and its glutamate-utilizing mutants**

Strain	GOT activity (μmoles per mg of protein per hr)
E. coli K-12 CS101, wild type (CS)	35.0
E. coli K-12 CS101/glut. 1 (CS1)	35.2
E. coli K-12 CS101/glut. 5 (CS5)	34.5

* The extracts were prepared from cultures grown in a succinate medium. For other experimental conditions, see Materials and Methods.

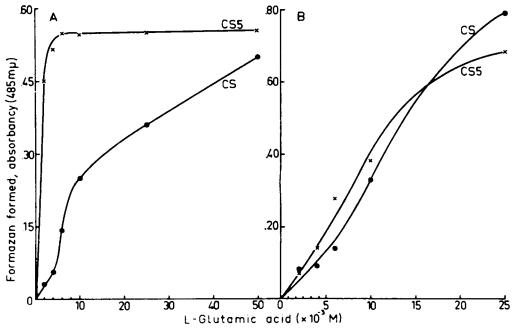

FIG. 1. *Reduction of triphenyltetrazolium chloride by whole cells and disrupted cell preparations of Escherichia coli K-12 CS101 (CS) and its glutamate-utilizing mutant (CS5) in the presence of glutamate. (A) Whole-cell suspensions. (B) Disrupted cells. For experimental conditions, see Materials and Methods.*

aminases for which glutamate is a substrate. To check this possibility, we examined the activity of GOT in extracts of the parent and mutant strains grown in a succinate medium. No differences in activity were found between the mutant and wild-type organisms (Table 3).

Reduction of triphenyltetrazolium chloride. To investigate the possibility that some metabolic block is present in the wild-type organism beyond GDH, and that this lesion is repaired in the mutant, we compared the capacity of the two strains to reduce phenyltetrazolium chloride in the presence of glutamate (Fig. 1). As in the case of *E. coli* W (Halpern and Umbarger, 1961), broken-cell suspensions of the parent strain CS and the glutamate-utilizing mutant CS5 exhibited similar capacities for utilizing glutamate as a hydrogen donor for the reduction of the dye. These results seem to rule out the possibility of a "late" metabolic block interfering with the utilization of glutamate by the parent strain. However, when the reduction of the tetrazolium salt by whole-cell suspensions in the presence of increasing concentrations of glutamate was examined, the mutant strain reached a plateau at a relatively low external concentration of the substrate, 6×10^{-3} M, whereas the parent strain was not yet saturated at a glutamate concentration as high as 5×10^{-2} M. These results indicate differences in the permeability to glutamate of the two strains.

TABLE 4. *Inhibition of growth of Escherichia coli K-12 CS101 and its mutants by α-methyl-DL-glutamic acid*

Strain	Inhibition (%)* in the presence of α-methyl-DL-glutamic acid monohydrate	
	0.02% (w/v)	0.15% (w/v)
E. coli K-12 CS101, wild type (CS)	0	0
E. coli K-12 CS101/glut. 1 (CS1)	41.4	86.7
E. coli K-12 CS101/glut. 5 (CS5)	32.0	98.6

* Inhibition of growth was calculated as $(k_c - k_e)/k_c \times 100$, where k_c and k_e are reciprocals of mass doubling time (hours) in the absence and in the presence of inhibitor, respectively. For experimental conditions, see Materials and Methods.

Effect of α-methyl-DL-glutamic acid on growth. The glutamate analogue, α-methyl-DL-glutamate, has been found to inhibit growth of lactic acid bacteria presumably by interfering with the synthesis of glutamine (Ayengar and Roberts, 1952). In our earlier studies (Halpern and Umbarger, 1961), a good correlation was found between the susceptibility of a number of *E. coli* strains to the inhibitory effect of this analogue and their ability to utilize glutamate as the sole source of carbon for growth. These and other findings were interpreted in terms of a common permeation mechanism responsible for the transport of the two substances into the cell. A similar picture was obtained in this study when we examined the effect of the analogue on the rate of growth of strain CS, and of its mutants CS1 and CS5, in minimal media with succinate or glucose as the carbon source. Table 4 demonstrates that the parent strain CS was entirely resistant to the growth-inhibitory effect of methyl-glutamate, whereas the growth of its glutamate-utilizing mutants was very strongly inhibited by the analogue.

Uptake of glutamate. The experiments described so far disclosed no differences between the parent and mutant strains in the activity of

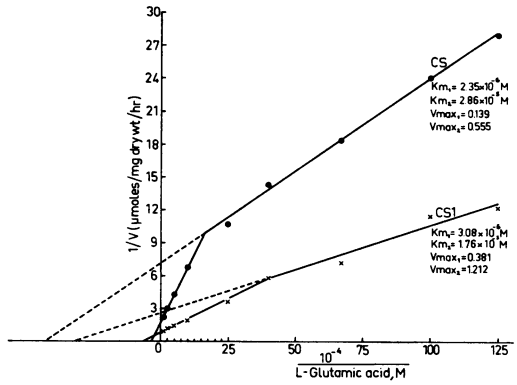

FIG. 2. *Double reciprocal plot for glutamate uptake by incorporation into the proteins of wild-type (CS) and mutant (CS1) strains of Escherichia coli K-12 in a glucose medium. For experimental conditions, see Materials and Methods.*

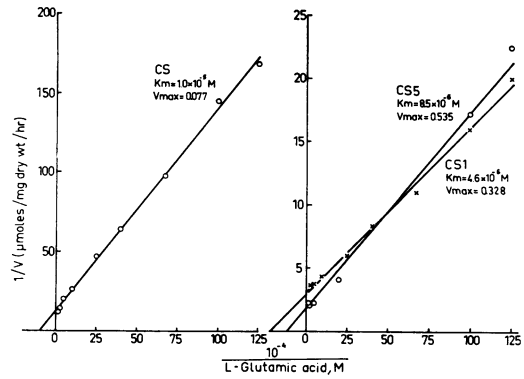

FIG. 3. *Double reciprocal plot for glutamate uptake by incorporation into the proteins of wild-type (CS) and mutant (CS1, CS5) strains of Escherichia coli K-12 in a succinate medium. For experimental conditions, see Materials and Methods.*

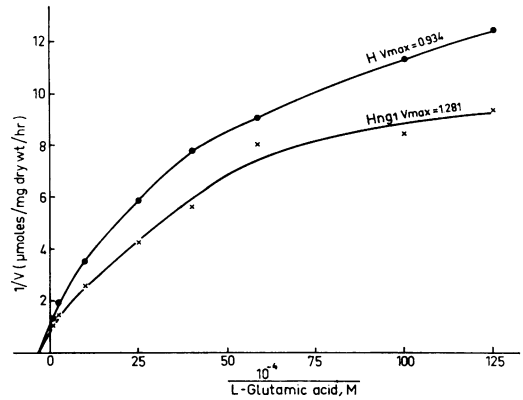

FIG. 4. *Double reciprocal plot for glutamate uptake by incorporation into the proteins of wild-type (H) and mutant (Hng1) strains of Escherichia coli H in a glucose medium. For experimental conditions, see Materials and Methods.*

enzymes concerned with the metabolism of glutamate, nor did they indicate any impairment of the capacity of the wild-type strain to metabolize glutamate, once the permeability barrier has been removed. Moreover, some of the results obtained indicated quite strongly that a permeation mechanism was probably involved in these mutations. However, more direct evidence demonstrating the permeability differences between the wild-type and mutant strains was required.

Studies on glutamate transport are complicated by the great number of metabolic reactions in which this compound normally participates in the cell. We therefore approached this problem in two different ways: (i) measurement of the incorporation of radioactive glutamate into protein under conditions where the uptake of the labeled amino acid was the limiting step (Ames, 1964), and (ii) accumulation of free radioactive glutamate by cells from the logarithmic phase of growth in which protein synthesis had been arrested by the addition of chloramphenicol (Kessel and Lubin, 1965).

Figure 2 presents reciprocal plots for glutamate uptake by incorporation into the proteins of strains CS and CS1 growing logarithmically on glucose. Two things should be pointed out. (i) The curves have a sharp break, as if each were composed of two straight lines which by extrapolation give two different sets of kinetic parameters. (ii) The respective K_m values for the two strains are similar, but the mutant shows V_{max} values twice as high as those obtained with the wild-type cells. (The results obtained with mutant CS5 were similar to those obtained with CS1.) These differences in the rate of glutamate uptake, although significant and reproducible, did not seem large enough to explain the "all or none" growth response to glutamate of the two strains. However, when the experiments were repeated with cultures growing on succinate (Fig. 3), the mutants showed five- to sevenfold higher V_{max} values than the parent strain. Another interesting feature of the succinate series of experiments was their normal rectilinear kinetics. Similar experiments were performed with strain H and its mutants Hng1 and Hng4. Here again, the rates of incorporation were higher with the mutants than with the parent strain, the differences between mutants and wild-type being smaller in glucose medium (Fig. 4) than in cells growing on succinate (Fig. 5).

However, the ratios of mutant to wild-type activity obtained in either medium were much smaller than the corresponding ratios obtained with the K-12 strains (1.4 versus 2.2 in glucose medium and 1.7 to 2.3 versus 4.3 to 7 in succinate medium). The curves obtained with the organisms of the H group had a downward bend in the direction of the higher concentrations of glutamate.

The kinetics of glutamate accumulation by strains CS and CS1 in a chloramphenicol-glucose medium in the presence and in the absence of α-methylglutamate are shown in Fig. 6. Parallel experiments carried out with strains H and Hng1 are illustrated in Fig. 7. All of the strains examined, wild-type and mutant alike, accumulated

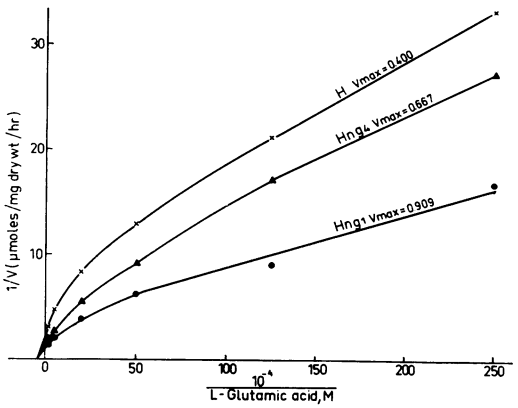

FIG. 5. *Double reciprocal plot for glutamate uptake by incorporation into the proteins of wild-type (H) and mutant (Hng1, Hng4) strains of Escherichia coli H in a succinate medium. For experimental conditions, see Materials and Methods.*

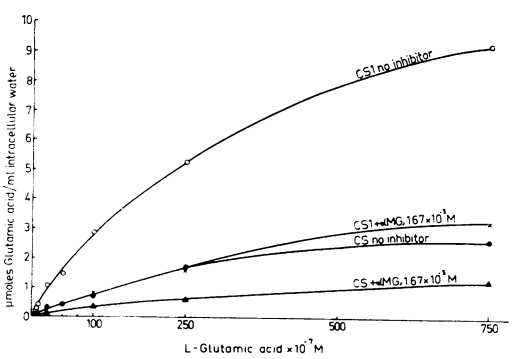

FIG. 6. *Accumulation of glutamate by wild-type (CS) and mutant (CS1) strains of Escherichia coli K-12 CS101 in a chloramphenicol-glucose medium. For experimental conditions, see Materials and Methods.*

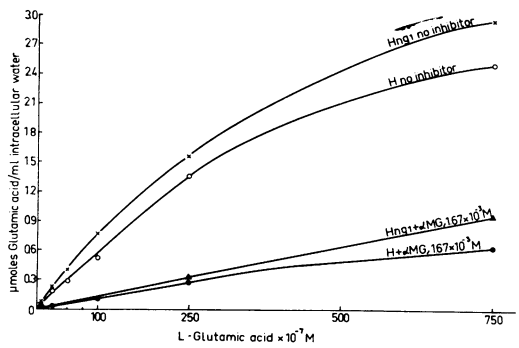

FIG. 7. *Accumulation of glutamate by wild-type (H) and mutant (Hng1) strains of Escherichia coli H in a chloramphenicol-glucose medium. For experimental conditions, see Materials and Methods*

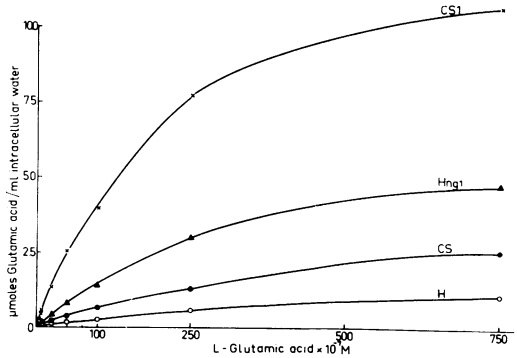

FIG. 8. *Accumulation of glutamate by wild-type (CS, H) and mutant (CS1, Hng1) strains of Escherichia coli in a chloramphenicol-succinate medium. For experimental conditions, see Materials and Methods.*

glutamate against a concentration gradient, but the mutants did it more efficiently than the corresponding wild-type organisms. Thus, at low concentratoins of C^{14}-glutamate in the medium (up to 2.5 $\times$ 10^{-6} M), the intracellular concentration of the amino acid in strain CS1 was 400 times higher than in the medium, whereas in strain CS the acid was concentrated only by a factor of about 120. As in the incorporation experiments described above, the difference between mutant and wild type were much more pronounced in the K-12 group than among the H strains. In experiments in which succinate served as the major carbon source (Fig. 8), the mutants showed a five- to sevenfold greater accumulation of C^{14}-glutamate from the medium than did the corresponding wild-type organisms. Methylglutamate, when added together with glutamate, inhibited the accumulation of the

latter by mutant and wild-type bacteria to a similar extent (Fig. 6 and 7).

DISCUSSION

The mechanism by which the mutants acquired the ability to grow on glutamate as the sole source of carbon might be either a change in glutamate metabolism or a more efficient transport of glutamate into the cell. Since the acquisition of the ability to utilize glutamate was not accompanied by any other changes in the metabolic capacity of the cell, only an early step in glutamate metabolism could be involved in these mutations. GDH and GOT both control early reactions in the glutamate pathway, and therefore we examined the activity of these two enzymes in the different strains. No increase in dehydrogenase or transaminase activity was found in the mutants as compared with the parent strain.

Further evidence against the possible involvement of a metabolic step was obtained from experiments on the reduction of triphenyltetrazolium chloride in the presence of glutamate. Disintegrated cell preparations of wild-type and mutant cultures showed similar capacities for utilizing glutamate as the hydrogen donor. However, with whole-cell suspensions, the mutant reached a plateau of maximal activity at a much lower concentration of substrate than did the parent strain. These results indicated that the mutant possessed a more efficient concentrating mechanism for glutamate than the parent strain.

Direct evidence that the mutations involved changes in glutamate permeation was obtained from experiments on glutamate uptake. Incorporation and accumulation experiments with succinate-grown cultures clearly demonstrated that both the rate of uptake and the capacity to concentrate glutamate were several-fold higher in the mutants as compared with the corresponding parent strains. The ratios of mutant to parent activity were somewhat higher among the K-12 CS strains than among the H-strains. In this connection, it is perhaps of interest that, unlike the wild-type CS strain, the parental strain H is not entirely unable to utilize glutamate but grows on it very slowly (generation time >9 hr).

Another point of interest is the shape of the incorporation curves obtained with the CS strains in glucose medium and with the H strains in both glucose and succinate medium. The sharp break in the reciprocal plot of glutamate uptake by the CS strains in glucose medium observed at a glutamate concentration of 4×10^{-6} M could be interpreted in terms of two glutamate permeases functioning under these conditions. However, this explanation is very unlikely in view of the fact that the mutant showed higher activity than the parent strain in both parts of the curve, and it would be difficult to explain simultaneous changes in two permeases due to a single mutation, unless one assumed that the two permeases represented different aggregations of identical subunits produced under the control of the same gene. On the other hand, both the break in the uptake curve obtained with the CS strains and the curvilinear kinetics of uptake observed with the H strains could be explained by allosteric activation of glutamate permease by its substrate [activation due to binding of glutamate at a site distinct from the active site (Monod, Changeux, and Jacob, 1963)]. Allosteric activation of enzymes by their own substrates and coenzymes has been reported by other investigators (Frieden, 1959; Scrutton, Keech, and Utter, 1965; Sanwal, Stachow, and Cook, 1965).

The greater efficiency of glutamate transport in the glutamate-utilizing mutants may also explain the susceptibility of these strains to the growth-inhibitory effect of α-methylglutamate, if one assumes that the same permeation mechanism is responsible for the transport of the two compounds into the cell. However, this is not to say that any mutant capable of utilizing glutamate must also become sensitive to the analogue; it is quite conceivable that in some cases mutation may increase the permeability of the cell to glutamate without appreciably affecting the transport of the analogue, and vice versa. This problem is further pursued as part of the genetic analysis of glutamate permeation in *E. coli* K-12 now in progress in this laboratory.

ACKNOWLEDGMENT

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