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Glutamate Transport in *Escherichia coli* K-12: Nonidentity of Carriers Mediating Entry and Exit

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The exit of glutamate from *Escherichia coli* K-12 cells preloaded with the radioactive amino acid and its relation to the reaction of entry were studied. Experiments with cells preloaded to different intracellular concentrations of radioactive glutamate confirmed our earlier conclusion that glutamate exit was a first-order reaction. L-Glutamate, competitive inhibitors of glutamate uptake (D-glutamate and L-glutamate-γ-methyl ester), noncompetitive inhibitors of glutamate uptake (L-serine and L-alanine), and the energy poison NaN_3 all accelerated glutamate exit 2.8-fold. No additive effect was observed in the presence of NaN_3 together with L-glutamate. Preloading with cold L-glutamate did not increase the rate of uptake of radioactive glutamate. It is concluded that the acceleration of glutamate exit in the presence of L-glutamate in the medium is not due to exchange diffusion and that L-glutamate and azide affect exit indirectly by preventing recapture. Sucrose, 25%, slowed down glutamate exit by a factor of about 4.7 and increased the steady-state level of glutamate accumulation to about the same extent. Increasing the intracellular K^+ concentration enhanced glutamate uptake but did not affect the half-time of exit. It is concluded that separate carriers are most probably involved in mediating the entry and exit reactions.

Experiments presented in an earlier communication from this laboratory described the rapid exit of glutamate from *Escherichia coli* K-12 cells which accumulated the radioactive amino acid (6). Glutamate exit behaved as a first-order reaction with a high temperature coefficient, suggesting a mechanism of facilitated diffusion, as postulated for the efflux of sugar molecules from bacterial cells (9, 12, 13, 16). Glutamate-utilizing mutants which exhibited several-fold higher rates of glutamate uptake than the parent strain showed half-times of exit similar to wild type. Furthermore, growth in the presence of glutamate enhanced the cells' capacity for glutamate uptake, but did not affect the rate constant of the exit reaction. These findings were taken as an indication that the exit and entry reactions are mediated by separate carriers (or by different components of a common carrier system). The present paper provides further support for this contention. The data reported here also suggest that the energy-requiring step in the active transport of glutamate in *E. coli* K-12 is a component of the entry process.

MATERIALS AND METHODS

Microorganism. *E. coli* K-12 CS7, a methionine auxotroph capable of utilizing glutamate as the major carbon source, was used (19).

Growth media. Except where otherwise indicated, the bacteria were grown in the medium of Davis and Mingioli (3), from which citrate was omitted and to which 50 μg of DL-methionine/ml was added. Glycerol or sodium succinate, as indicated, served as the major source of carbon.

Cultivation. The bacteria were grown overnight in the appropriate medium, diluted in fresh medium of the same composition to a density of about 30 Klett units (filter no. 42), and further incubated until a turbidity of 150 to 160 Klett units (late logarithmic phase) was reached. The cultures were incubated with aeration at 37 C.

^{14}C-L-glutamate uptake. Glutamate uptake was determined according to Kessel and Lubin (14) as described earlier (8).

Measurement of glutamate exit. Cells were preloaded with radioactive glutamate and diluted

2,000-fold in fresh medium without the radioactive amino acid. Samples were taken at frequent time intervals, and the residual radioactivity in the cells was determined. The details of procedure were as described earlier (6).

RESULTS

Effect of intracellular glutamate concentration on exit rate. Earlier studies on the rate of loss of radioactive glutamate by preloaded cells of *E. coli* K-12 indicated that the exit process was a strongly temperature-dependent first-order reaction, most probably facilitated diffusion (6). To demonstrate unequivocally that the rate constant of the exit reaction is not concentration dependent, the experiment shown in Fig. 1 was performed. Cells were exposed to different concentrations of ^{14}C-labeled glutamate for 10 min, at which time a steady-state level of accumulated intracellular radioactivity was well established. Within the concentration range of glutamate used (10^{-5} to 5×10^{-5} M), the intracellular

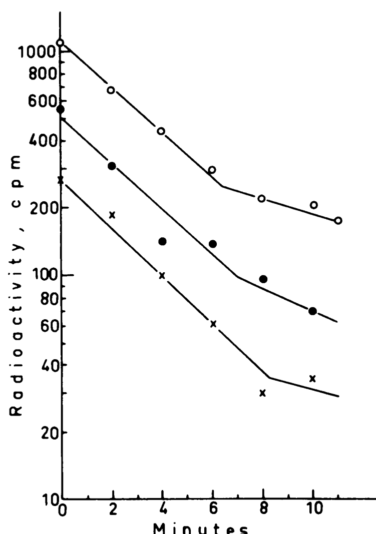

FIG. 1. *Effect of intracellular glutamate concentration on the rate of exit. Escherichia coli CS7 was grown in Davis-glycerol (0.5%) medium at 37 C. ^{14}C-L-glutamate accumulation at external concentrations of radioactive glutamate (15 μCi/μmole) of: 10^{-5} M (×), 2×10^{-5} M (●), and 5×10^{-5} M (O), at a bacterial density of 160 Klett units (filter no. 42), was carried out in Davis medium with glycerol and sodium succinate, 0.5% each, as the energy source, in a shaking water bath at 30 C for 10 min. Exit was started by diluting 0.1 ml of the uptake mixture in 200 ml of fresh medium without glutamate, containing NaN_3, 0.01 M, prewarmed to 30 C. Samples (20 ml) taken at 2-min intervals were filtered, washed, and further treated as described (6).*

radioactivity increased upon increasing the concentration of substrate in the medium. The preloaded cells were diluted in fresh medium without glutamate to which NaN_3, 0.01 M, was added, and residual radioactivity in the cells was determined at frequent time intervals. One can see that the reaction indeed showed a first-order dependence on the concentration of intracellular glutamate until about 80% of the initial radioactivity disappeared from the cells. It is quite evident that the half-time of the exit reaction did not depend on the initial intracellular concentration of glutamate, giving a value of 3 min at each of the three concentrations used. The rate constant of the exit process can be readily calculated: $k = \ln 2/\tau_{1/2}$ (where $\tau_{1/2}$ is the half-time of the reaction). For the temperature employed (30 C), k was 0.23 min^{-1}.

Effect of glutamate and inhibitors of glutamate uptake on the rate of exit. We previously reported that the rate of glutamate exit from the cells is greatly accelerated in the presence of L-glutamate or compounds which inhibit glutamate uptake (6, 7). The kinetics of the exit reaction in the presence of L-glutamate, in the presence of the competitive inhibitors of glutamate uptake D-glutamate and L-glutamate-γ-methyl ester, and in the presence of the noncompetitive inhibitors of glutamate uptake L-serine and L-alanine are shown in detail in Fig. 2. L-Glutamate as well as competitive and noncompetitive inhibitors of glutamate uptake all increased the rate of exit almost threefold. The finding that noncompetitive inhibitors of glutamate uptake were as effective as glutamate itself in accelerating exit of glutamate from the cells supports our contention that the effect of exogenous glutamate is due to prevention of recapture of radioactive glutamate by the cells rather than to exchange diffusion (6, 11, 18). That exchange diffusion is not the only factor involved is also indicated by the finding presented in Fig. 3 that preloading with cold glutamate does not increase the rate of uptake of ^{14}C-glutamate by cells subjected to such pretreatment (see also 20).

Effect of NaN_3 on the rate of ^{14}C-glutamate exit in the presence of exogenous glutamate. Blocking the energy supply by the addition of NaN_3, 0.01 M, greatly enhances the rate of glutamate exit (6). Energy supply may be required to slow down exit by reducing the affinity of the carrier for intracellular glutamate (11, 16). Alternatively, the energy requirement may be connected with the recapture process, and prevention of the latter will,

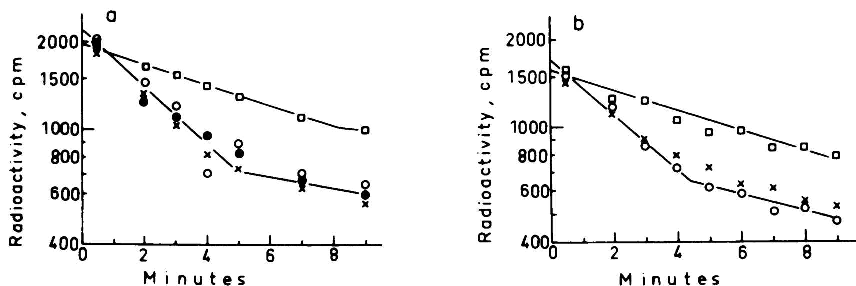

FIG. 2. *Effect of L-glutamate and inhibitors of glutamate uptake on the exit reaction. The culture was grown in Davis-succinate (0.5%) medium at 37 C. The cells were loaded with ^{14}C-L-glutamate (15 μCi/μmole) at an external concentration of 5×10^{-5} M and bacterial density of 150 Klett units. After 10 min of uptake at 30 C, 0.1 ml of the mixture was diluted in 200 ml of fresh medium with the following additions. (a) No additions, □; L-alanine, 2.5×10^{-2} M, O; L-glutamate-γ-methylester, 5×10^{-3} M, ●; L-glutamate, 5×10^{-3} M, ×. (b) No additions, □; L-serine, 2.5×10^{-2} M, ×; D-glutamate, 5×10^{-3} M, O.*

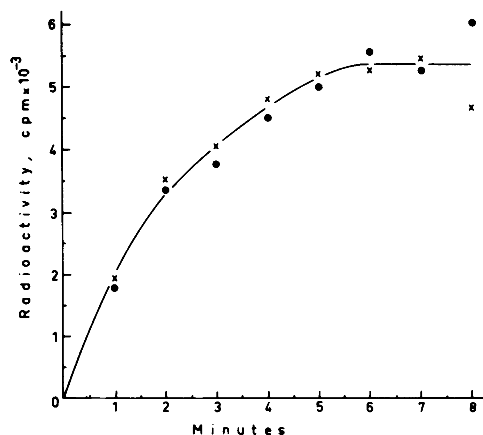

FIG. 3. *Effect of preloading with nonradioactive glutamate on the rate of uptake. The bacteria were grown in the basal medium (B7) of Frank and Hopkins (5) with potassium succinate, 0.04 M, and NaCl, 0.015 M, with shaking at 37 C. When the culture reached a density of 130 Klett units, chloramphenicol, 200 μg/ml, was added, and incubation was continued for 30 min. The cells were centrifuged, suspended in fresh medium without methionine and with chloramphenicol to a density of 85 Klett units, and divided in two 5-ml portions. Nonradioactive glutamate, 5×10^{-5} M, was added to one portion (×), and both were incubated for 10 min at 30 C. ^{14}C-L-glutamate was then added to each mixture to a final concentration of 5×10^{-5} M and a specific activity of 1 μCi/μmole, and incubation was continued. Samples (0.5 ml) were taken at frequent intervals, filtered, washed, and counted as described (8).*

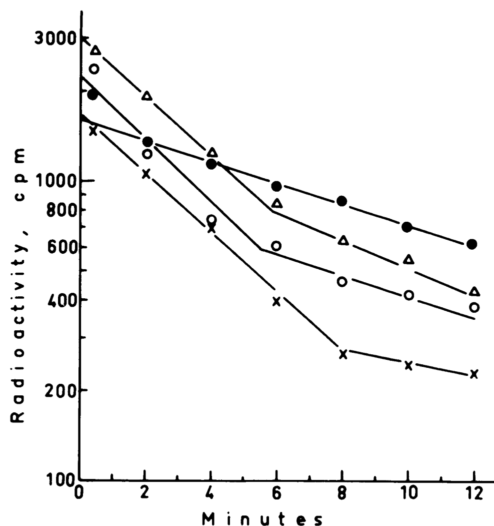

FIG. 4. *Effect of glutamate and NaN_3 on the rate of exit. The bacteria were grown and loaded as in Fig. 1. Exit of glutamate was measured in the presence of the following additions: no additions, ●; NaN_3, 10^{-2} M, ×; L-glutamate, 5×10^{-3} M, O; NaN_3, 10^{-2} M, plus L-glutamate, 5×10^{-3} M, △.*

of course, increase the apparent rate of exit (6). The experiment described in Fig. 4 supports the latter alternative. One can see that although glutamate or NaN_3 each results in an almost threefold enhancement of ^{14}C-glutamate exit, when present simultaneously they show no additive effect whatsoever. This would seem to indicate that the two compounds act in the same process, that is, by preventing recapture.

Effect of sucrose on the rate of glutamate exit. In the course of these studies, we have observed that increasing the osmotic activity of the uptake medium with NaCl or sucrose greatly increases the steady-state level of glutamate accumulation by the cells. Similar findings have been reported concerning the accumulation of K^+ ions by *E. coli* after hypertonic shock (4) and concerning proline uptake by *Salmonella oranienburg* when the a_w of the medium was reduced to 0.97 (2). In both cases,

the authors concluded that the increased accumulation was due to acceleration of influx rather than to reduced efflux. In Fig. 5, data are presented on the effect of sucrose, 25%, on the rate of glutamate exit in the presence of NaN_3, 0.01 M, from cells preloaded to different levels with the radioactive amino acid. One can see that, as in Fig. 1 (exit in the absence of sucrose), the rate constant does not depend on the intracellular concentration of glutamate; that is, sucrose does not alter the order of the exit reaction. However, in the presence of sucrose, exit is much slower than in its absence, with a k value of 0.049 min^{-1} as compared with that of 0.23 min^{-1} calculated from Fig. 1. Furthermore, the results of kinetic studies on glutamate uptake in the presence and in the absence of sucrose shown in Fig. 6 and 7 strongly indicate that the entry reaction is not affected by sucrose. Figure 6 represents Lineweaver-Burk plots of glutamate uptake upon incubation for 6 min at 30 C. The data show that the affinity for glutamate did not change in the presence of sucrose. Time curves of glutamate uptake at 30 C in the presence and in the absence of sucrose are given in Fig. 7. Two points are immediately apparent: (i) the steady-state level of intracellular radioactivity in the presence of sucrose was 3.63 times as high as that in the control, and (ii) the time required to reach the steady state was approximately five times longer in the presence of sucrose than in its absence. Since cell turbidity increased by ca. 30% upon addition of 25% sucrose, most probably indicating a decrease in cell or protoplast volume (see also 15), the actual difference in glutamate accumulation by the experimental and control suspensions was 4.72-fold. In the steady state, influx equals efflux (9). Therefore, if we assume that glutamate influx *does not* change in the presence of sucrose, and since efflux is a first-order reaction, it follows that the steady-state intracellular glutamate concentration in the presence and in the absence of sucrose should be inversely proportional to the respective rate constants of exit. From the exit data in Fig. 1 and 5, one finds a ratio of 4.69, which is not significantly different from the accumulation ratio of 4.72 calculated from Fig. 7. It seems, therefore, reasonable to conclude that the enhancement of glutamate uptake by sucrose is due only to reduced exit.

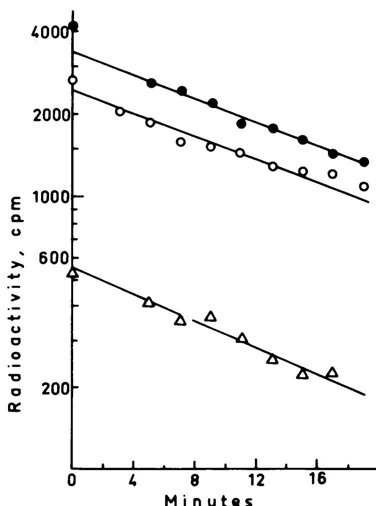

FIG. 5. *Effect of sucrose on glutamate exit. Conditions were the same as in Fig. 1 except that accumulation and exit were performed in the presence of sucrose, 25%, and the concentrations of glutamate in the accumulation phase were: 5 × 10⁻⁵ M, ●; 2 × 10⁻⁵ M, O; 5 × 10⁻⁶ M, Δ.*

Maximal rate of entry. Knowing the rate

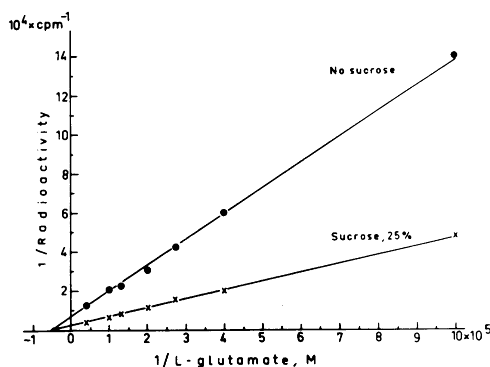

FIG. 6. *Reciprocal plots of glutamate uptake in the presence and in the absence of sucrose. The bacteria were grown in Davis-glycerol (1%) medium at 37 C. Uptake was performed in 2 ml of medium as above supplemented with sodium succinate, 0.5%, chloramphenicol, 200 μg/ml, and sucrose, 25%, where indicated, at a bacterial density of 40 Klett units (64 μg [dry weight]/ml). The mixtures were incubated for 10 min at 30 C, radioactive glutamate (1 μCi/μmole) was added to the concentration indicated, and incubation was continued for 6 min. The entire reaction mixture was filtered, washed three times with 3 ml of the appropriate medium without glutamate, and counted. Accumulation in the absence of sucrose reached equilibrium in less than 4 min, so that the 6-min samples represent true steady-state conditions. In the presence of sucrose, equilibrium was reached only after about 16 min; therefore, the lower curve does not represent steady-state conditions. However, since one gets the same* K_m *values from initial-rate and steady-state data (13), comparison between the two curves as to* K_m *is valid.*

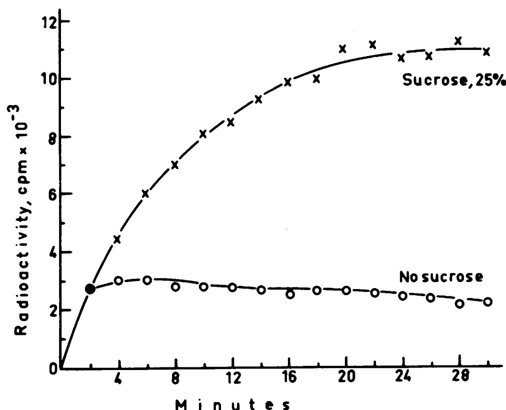

FIG. 7. *Effect of sucrose on steady-state glutamate accumulation. The bacteria were grown in Davis-succinate (0.5%) medium at 37 C. Uptake was carried out at 30 C in the presence of 5×10^{-5} M ^{14}C-L-glutamate (1 μCi/μmole), at a bacterial density of 75 Klett units. Samples (0.5 ml) were taken at frequent intervals, filtered, washed, and counted as in Fig. 6.*

constant of exit, one can calculate the value of the maximal initial rate of entry V_{max}^{en} from steady-state accumulation data like those in the upper curve of Fig. 6 (9). The change in internal substrate concentration is represented as:

$$\frac{d[Si]}{dt} = \frac{V_{max}^{en}[Se]}{[Se] + K_m^{en}} - k[Si]$$

where Si is internal substrate, Se is external substrate, K_m^{en} is the Michaelis constant for entry, and k is the rate constant for exit. At the steady-state level of accumulation,

$$\frac{d[Si]}{dt} = 0$$

or

$$\frac{V_{max}^{en}[Se]}{[Se] + K_m^{en}} = k[Si]$$

or

$$\frac{1}{[Si]} = \frac{k \cdot K_m^{en}}{V_{max}^{en}} \cdot \frac{1}{[Se]} + \frac{k}{V_{max}^{en}}$$

A plot of $1/Si$ versus $1/Se$ (Fig. 6, upper curve) gives a straight line with an intercept of k/V_{max}^{en} and a slope of $k \cdot K_m^{en}/V_{max}^{en}$. Substituting the value of 0.23 min^{-1} calculated from Fig. 1 for k, we obtain V_{max}^{en} = 13.4 nmoles per mg (dry weight) per min or 2.75×10^6 molecules per cell per min.

Effect of NaN_3 and sucrose on leucine transport. To see whether our findings on glutamate transport reflect some common features of amino acid transport in this organism, we examined the effects of NaN_3 and sucrose on L-leucine exit. One can see from Fig. 8 that the half-time of leucine exit in the absence of either NaN_3 or sucrose is comparable to that of glutamate (7.1 and 8.5 min, respectively). However, whereas azide accelerates the apparent rate of glutamate exit, it has the opposite effect on the exit of leucine, slowing it down by a factor of 3. On the other hand, addition of sucrose, which markedly reduces the rate of glutamate exit, increases the initial rate of leucine exit, both in the presence and in the absence of azide. It is noteworthy that leucine exit, which behaves as a first-order reaction in the absence of sucrose, shows a higher order dependence on internal leucine concentration when sucrose, 25%, is added. Reciprocal plots of leucine uptake under steady-state conditions, in the absence and in the presence of sucrose, are shown in Fig. 9. One can see that sucrose reduces the maximal steady-state concentration of intracellular radioactivity to about one-half, but does not seem to affect the affinity of the uptake system for external leucine.

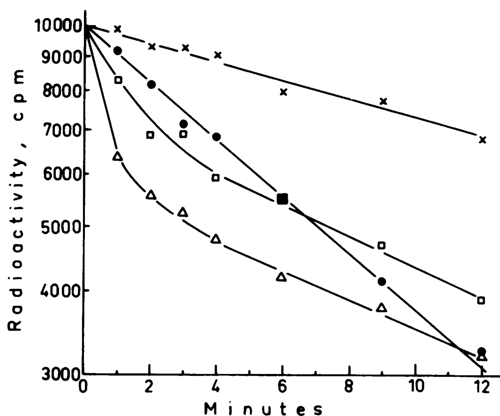

FIG. 8. *Loss of L-leucine by preloaded cells. Bacteria were grown in Davis-succinate (0.5%) medium at 37 C. Leucine uptake was carried out according to the protocol of glutamate uptake except that L-leucine, 344 μCi/μmole, 1.5×10^{-5} M, was used instead of glutamate. Incubation was at 30 C for 8 min. Where the effect of sucrose on exit was examined, sucrose, 25%, was added already to the uptake mixture. Exit was measured in the presence of the following additions: no additions, ●; NaN_3, 0.01 M, ×; sucrose, 25%, △; NaN_3, 0.01 M, plus sucrose, 25%, □.*

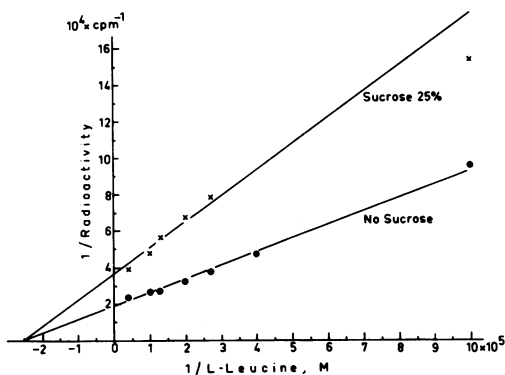

FIG. 9. *Reciprocal plots of* L*-leucine uptake in the presence and in the absence of sucrose. The bacteria were grown in Davis-glycerol (1%) medium at 37 C. Uptake was performed as with glutamate except that* ^{14}*C-*L*-leucine, 2 μCi/μmole, was used instead of glutamate. Bacterial density was 64 μg (dry weight)/ml in a total volume of 2 ml. The mixtures were incubated at 30 C for 6 min. Under these conditions, steady state of accumulation was reached both in the presence and in the absence of sucrose.*

DISCUSSION

Experiments presented in Fig. 2 and 4 demonstrate an almost threefold enhancement of the apparent rate of glutamate exit in the presence of 5×10^{-3} M nonradioactive glutamate in the exit medium. The occurrence of *trans* effects of substrate and related solutes on rates of efflux has been described in other systems (11, 18, 20). The generally accepted interpretation of these phenomena is based on the assumption that the influx and efflux reactions are mediated by the same carrier. It is assumed that the rates of movement of empty and loaded carrier molecules across the membrane are different. Therefore, binding of a solute molecule to the carrier on the *trans* side of the membrane may accelerate (or retard) the return of the carrier molecule to the *cis* side and thus affect the amount of carrier available for the exit reaction. The data presented in Fig. 3 argue against the validity of such an interpretation in the case of glutamate transport in *E. coli* K-12. If only an exchange flux were involved, one would expect the phenomenon to be symmetrical; that is, one would expect preloading of cells with cold glutamate to speed up subsequent uptake of radioactive glutamate by such cells. Figure 3 shows that this is not so; preloaded and control cells exhibited very similar rates of glutamate uptake. We therefore explain the apparent increase in the rate of exit in the presence of glutamate by the prevention of recapture of radioactive glutamate molecules at the outer membrane surface by the unrelated entry mechanism. This is supported by our earlier finding that glutamate-utilizing mutants, exhibiting several-fold higher rates of glutamate uptake than wild-type strains, showed rates of exit only 20% lower than that of the parent strain. Sodium azide, which inhibited glutamate uptake, increased the rate of exit in the mutant 20% more effectively than in the parent, which had a lesser capacity for uptake. In the presence of azide, the rates of glutamate exit were the same in mutant and wild-type strains (6). As shown in Fig. 4, there is no additive effect of NaN_3 and glutamate on exit, indicating that the two compounds affect the same reaction. All of these data are best interpreted on the assumption that both azide and glutamate increase the apparent rate of exit by inhibiting entry. The notion that exit itself is not directly affected is also in accord with the data in Fig. 2, which show that noncompetitive inhibitors of glutamate uptake were equally effective in accelerating glutamate exit as were glutamate itself and some of its structural analogues which inhibit glutamate uptake in a strictly competitive fashion (7). Furthermore, we have recently shown that competitive and noncompetitive inhibitors of glutamate uptake inhibit the binding of glutamate to a specific glutamate-binding protein releasable from the periplasmic space, in a competitive and noncompetitive fashion, respectively. It has been suggested by us that this glutamate-binding protein is a component of the glutamate uptake system (1).

An exit process independent of entry (at least to some extent) has been described previously for the transport of galactose in *E. coli* (10). The various models for galactoside transport proposed in the literature (12, 16) also include separate routes for exit, not dependent on the permease, as being responsible for some of the outward fluxes. In the early model, energy was considered to "push" the entry of galactosides by maintaining an excess of activated transporter at the outer boundary of the membrane. Later experiments showed that energy substrates and poisons, although greatly affecting efflux and accumulation, had little effect on *o*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolysis in vivo. In vivo ONPG hydrolysis does not require active transport, owing to an excess of β-galactosidase "pulling" the reaction. On the basis of these results, Koch placed the energy coupling inside the permeability membrane, "pulling" entry by facilitating dissociation of the sugar-

transporter complex inside the cell (16). However, recently Koch reexamined the problem using more sensitive means of energy depletion and found strong inhibition of ONPG hydrolysis after these procedures (17). He concluded that energy expenditure was obligatory even for downhill transport.

Studying the mechanism of the Na^+ and K^+ requirement of amino acid transport in a marine pseudomonad, Thompson and MacLeod have recently shown that K^+ is required for an energy-coupled step resulting in accumulation of the amino acid (α-amino-isobutyric acid, AIB) against a concentration gradient, whereas Na^+ facilitated the entry and equilibration of AIB into the cell by increasing the affinity of the carrier for it (21). Similar Na^+ and K^+ requirements for glutamate uptake by *E. coli* have recently been found in this laboratory (*unpublished data*). In this case too, *intracellular* potassium increases the cell's capacity for accumulating glutamate but does not affect the affinity of the carrier, whereas Na^+, to the contrary, reduces the K_m without affecting the maximal capacity for glutamate uptake. Recent experiments (*unpublished data*) showed that increasing the internal concentration of K^+ ions, although it enhanced the accumulation of glutamate, did not affect the rate constant of exit. These findings and the results presented in Fig. 5 to 7, showing that sucrose reduces the exit rate constant but has no effect on the affinity and rate of the entrance reaction, lend further support for our contention that the processes of entry and exit of glutamate in this organism are mediated by separate carriers.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Barash, H., and Y. S. Halpern. 1971. Glutamate-binding protein and its relation to glutamate transport in *Escherichia coli* K-12. Biochem. Biophys. Res. Commun. 45:681-688.
2. Christian, J. H. B., and J. M. Hall. 1972. Water relations of *Salmonella oranienburg*: accumulation of potassium and amino acids during respiration. J. Gen. Microbiol. **70**:497-506.
3. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17-28.
4. Epstein, W., and S. G. Schultz. 1965. Cation transport in *Escherichia coli* V. Regulation of cation content. J. Gen. Physiol. **49**:221-234.
5. Frank, L., and I. Hopkins. 1969. Sodium-stimulated transport of glutamate in *Escherichia coli*. J. Bacteriol. **100**:329-336.
6. Halpern, Y. S. 1967. Further studies of glutamate transport in *Escherichia coli*. Some features of the exit process. Biochim. Biophys. Acta **148**:718-724.
7. Halpern, Y. S., and A. Even-Shoshan. 1967. Properties of the glutamate transport system in *Escherichia coli*. J. Bacteriol. **93**:1009-1016.
8. Halpern, Y. S., and M. Lupo. 1965. Glutamate transport in wild-type and mutant strains of *Escherichia coli*. J. Bacteriol. **90**:1288-1295.
9. Hoffee, P., E. Englesberg, and F. Lamy. 1964. The glucose permease system in bacteria. Biochim. Biophys. Acta **79**:337-350.
10. Horecker, B. L., J. Thomas, and J. Monod. 1960. Galactose transport in *Escherichia coli*. II. Characteristics of the exit process. J. Biol. Chem. **235**:1586-1590.
11. Jacquez, J. A., and J. H. Sherman. 1965. The effect of metabolic inhibitors on transport and exchange of amino acids in Ehrlich ascites cells. Biochim. Biophys. Acta **109**:128-141.
12. Kepes, A. 1971. The β-galactoside permease of *Escherichia coli*. J. Membrane Biol. **4**:87-112.
13. Kepes, A., and G. N. Cohen. 1962. Permeation, p. 179-221. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 4, Academic Press Inc., New York.
14. Kessel, D., and M. Lubin. 1965. Stability of α-hydrogen of amino acids during active transport. Biochemistry **4**:561-565.
15. Knowles, C. J., and L. Smith. 1971. Effect of osmotic pressure of the medium on the volume of intact cells of *Azotobacter vinelandii* and the rate of respiration. Biochim. Biophys. Acta **234**:144-152.
16. Koch, A. L. 1964. The role of permease in transport. Biochim. Biophys. Acta **79**:177-200.
17. Koch, A. L. 1971. Energy expenditure is obligatory for the downhill transport of galactosides. J. Mol. Biol. **59**:447-459.
18. Levine, M., D. L. Oxender, and W. D. Stein. 1965. The substrate-facilitated transport of the glucose carrier across the human erythrocyte membrane. Biochim. Biophys. Acta **109**:151-163.
19. Marcus, M., and Y. S. Halpern. 1967. Genetic analysis of glutamate transport and glutamate decarboxylase in *Escherichia coli*. J. Bacteriol. **93**:1409-1415.
20. Robbie, J. P., and T. H. Wilson. 1969. Transmembrane effects of β-galactosides on thiomethyl-β-galactoside transport in *Escherichia coli*. Biochim. Biophys. Acta **173**:234-244.
21. Thompson, J., and R. A. MacLeod. 1971. Functions of Na^+ and K^+ in the active transport of α-aminoisobutyric acid in a marine pseudomonad. J. Biol. Chem. **246**:4066-4074.