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Sodium-Stimulated Transport of Glutamate in *Escherichia coli*

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Wild-type *Escherichia coli* B grew poorly on glutamate as the sole carbon source, except at very high concentrations of the amino acid. The addition of sodium ion markedly stimulated the growth. It had the same effect in a mutant of *E. coli* B selected for the ability to grow at low glutamate concentrations. Sodium ion also potentiated growth inhibition by analogues of glutamate. The uptake of glutamate by nongrowing cells of the mutant was markedly stimulated by sodium ion in the presence of an energy source, chloramphenicol, and arsenite, which retarded glutamate degradation.

Although the transport of amino acids and sugars in bacteria has been studied extensively, there has been little attention given to the possible involvement of inorganic cations in the process. In contrast, investigations of both amino acid and sugar transport in cells from higher organisms have indicated the frequent participation of sodium ion (2, 3, 19).

We became interested in this aspect of transport when we observed that the growth of *Escherichia coli* B on glutamate as the sole carbon source was markedly stimulated by sodium salts. In fact, growth on concentrations of less than 20 mM of glutamate displayed a requirement for sodium by the wild-type organism. This paper documents these observations and produces evidence that a locus of action of sodium in this system is, indeed, at the level of transport.

MATERIALS AND METHODS

Bacteria. *E. coli* strain B was obtained from the American Type Culture Collection (Catalog no. 11303). It was maintained on slants of nutrient agar. *E. coli* strain 29-78 was a derived mutant (*see* Results) selected for the ability to grow on low concentrations of glutamate. It was maintained on slants of the synthetic medium (solidified with 1.5% Oxoid Ion-Agar no. 2) containing potassium glutamate (10 mM) and sodium chloride (3 mM). Both strains were transferred bimonthly and stored at 4 C after development of the cultures at 37 C.

Growth studies. Growth media were prepared by appropriate additions to a salt solution ("B7 salts") whose final composition in the medium was as follows: K_2HPO_4, 40 mM; KH_2PO_4, 22 mM; K_2SO_4, 1 mM; $MgSO_4$, 0.4 mM; ferric citrate, 2 μM. When used, glycerol was present at a concentration of 20 mM and ammonium chloride at 15 mM. L-Glutamate was added as a filter-sterilized solution of the potassium salt, prepared by titration of L-glutamic acid to *p*H 7.2 with KOH. Cultures were grown in 10-ml quantities of medium contained in 25 by 150 mm glass tubes fitted with Morton closures (Bellco Glass, Inc., Vineland, N.J.). They were shaken at constant temperature in a Psychrotherm incubator-shaker operated at 350 rev/min (New Brunswick Scientific Co., New Brunswick, N.J.). Bacterial concentration was estimated from the absorbance of cultures at 600 nm in a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.) fitted with an adapter to accomodate the growth tubes. An absorbance of 0.1 represented 26 μg (dry wt) of bacteria and 3×10^7 viable cells per ml.

Transport studies. *E. coli* 29-78 was grown in B7 salts supplemented with glycerol and ammonium chloride. Stationary-phase cells (grown for 16 hr at 37 C) were harvested by centrifugation at room temperature, washed twice in B7 salts, and resuspended in B7 salts to the original culture density. After starving for 1 hr at 37 C on the shaker, the cells were centrifuged and resuspended in B7 salts for addition to the incubation mixtures. All incubations contained B7 salts at a final concentration identical to that in the growth medium; also, when applicable, the incubations contained glycerol (10 mM), ammonium chloride (20 mM), potassium arsenite (10 mM), chloramphenicol (200 μg/ml), and ^{14}C-L-glutamate. The cell concentration in the reaction mixture was 100 μg (dry wt) per ml for the experiments in which an energy source was not added, and 13 μg (dry wt) per ml for the experiments in which glycerol was used as an energy source. All components of the reaction mixture, except for ^{14}C-glutamate, were pre-incubated for 5 min before adding the isotope. Incubations were carried out in beakers on a water-bath shaker at the desired temperature.

Samples (0.5 ml) were removed at timed intervals with an Eppendorf pipette and the cells were collected on membrane filters (type B6, 0.45 μm pore size; Schleicher & Schuell Co., Keene, N.H.). Immediately after collection, the cells were washed in the filtration apparatus with 20 ml of 0.25 M sucrose at room temperature and the filter was transferred to a scintillation vial containing 10 ml of Bray's solution (1) for radioassay in a Unilux II instrument Nuclear-Chicago Corp., Des Plaines, Ill. Absolute activity levels were ^{14}C-L-glutamic acid (uniformly labeled) was obtained from New England Nuclear Corp., Boston, Mass.; it was diluted with potassium glutamate to the desired specific activity. All of the uptake data presented have been calculated to a standard specific activity of 10 μc/μmole and 1 mg (dry wt) of cells.

Chemicals. All reagents were of the best grade obtainable from the usual commercial sources. Sucrose was the "enzyme grade" preparation sold by Mann Fine Chemicals, Inc., New York, N.Y. Arsenite was used as a solution of primary standard arsenic trioxide. The compound was dissolved in 0.1 N KOH and this solution was mixed with one volume of double-strength B7 salts; the *p*H was adjusted to 7.1 with HCl and the resulting solution standardized by iodometric titration. The water used throughout this work was centrally-supplied, distilled water which was redistilled in a Corning glass still.

Metabolic studies. The radioactivity balance experiments reported in Results were conducted as follows. Cells were incubated with ^{14}C-glutamate and other additions, as indicated in the text, as for studies of transport. After 10 min, the reaction mixture was filtered through membrane filters and the filtrate saved for subsequent analysis. The cells were washed on the filters with 0.25 M sucrose; the filters, with their collected cells, were extracted with 80% ethyl alcohol in the cold for several hours to obtain the cellular pools of radioactivity. The original filtrate was acidified with trichloroacetic acid and shaken at 37 C to remove residual labeled carbon dioxide. After careful determination of the total radioactivity in each solution, the filtrate and the ethyl alcohol extract were separately chromatographed on Dowex 1 (12; Dow Chemical Co., Midland, Mich.) for the isolation of glutamic acid. In some cases, the glutamic acid obtained from the Dowex chromatography was contaminated with unidentified radioactive products. Hence, the pool fractions were subjected to high voltage paper electrophoresis at *p*H 5.3 on strips of Whatman 3-mm paper (H. Reeve Angel & Co., Clifton, N.J.). The strips were scanned in a radiochromatogram scanner (Packard Instrument Co., Inc., Downers Grove, Ill.); the relative quantity of glutamic acid on the strips was estimated from the areas of radioactivity depicted on the chart paper. The production of radioactive carbon dioxide during incubation was estimated from parallel incubations carried out in closed tubes. After 10 min of incubation with ^{14}C-glutamate, the mixture was acidified with trichloroacetic acid and radioactive CO_2 was flushed out with a stream of CO_2-free air. The $^{14}CO_2$ was trapped in a series of bubblers containing a solution composed of one part ethanolamine and two parts monomethyl ethylene glycol. Samples were radioassayed in Bray's solution.

RESULTS

Utilization of glutamate by E. coli B (wild type). When cells growing exponentially with glucose, succinate, or glycerol were washed and resuspended in the B7 salts medium containing moderate concentrations of glutamate (e.g., 25 mM) as the sole carbon and nitrogen source, vigorous growth resumed only when sodium ion was present. The relationship between growth rate and glutamate concentration, as affected by sodium addition, is shown in Fig. 1 for cells pregrown in glycerol-ammonium medium. These data illustrate the obligatory dependence on sodium at concentrations of glutamate lower than 20 mM; at very high glutamate concentration (>100 mM), rapid growth did not require sodium addition. Similar results were obtained whether or not ammonium chloride was added as an additional nitrogen source. With 12.5 mM glutamate, the growth rate passed through an optimum at 3 mM sodium chloride.

Repetition of this kind of growth experiment always yielded the same qualitative result, i.e., sodium ion stimulated the rate of growth with glutamate as carbon source. However, at the lower concentrations of glutamate, different stocks of *E. coli* B sometimes revealed long lag periods preceding exponential growth, even in the presence of sodium. This and other observations suggested that the growth response to glutamate was complicated by the selection of mutants able to utilize glutamate more efficiently than the bulk of the population. Accordingly, a substrain was isolated by sequential transfer and, finally, single colony isolation on media

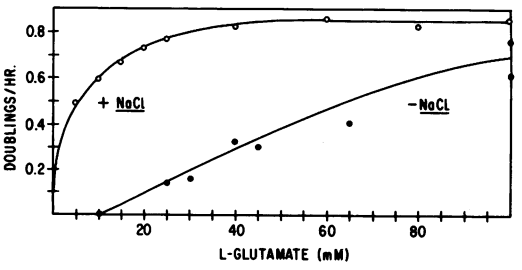

FIG. 1. *Effect of sodium ion on the growth rate of E. coli B as a function of glutamate concentration. Cells grown on glycerol and ammonium chloride were washed and incubated with B7 salts containing graded concentrations of potassium glutamate. Sodium chloride (3 mM) was added to one series of tubes and potassium chloride (3 mM) to the series lacking sodium ion. The growth temperature was 37 C.*

containing 10 mM glutamate and 3 mM sodium chloride. That this strain, designated *E. coli* 29-78, was truly a mutant is indicated by retention of the ability to grow efficiently at a low glutamate concentration without lag, even after 53 generations of growth in glycerol medium devoid of added glutamate.

Utilization of glutamate by E. coli 29-78 (mutant). As in the wild-type strain, sodium stimulated growth of the mutant on glutamate (Fig. 2). However, growth rate of the mutant was saturated at lower levels of glutamate (*compare* Fig. 1 and 2).

The growth rate reflected the concentration of both glutamate and sodium ion in the sense that higher levels of cation were needed to produce a given rate of growth at lower glutamate concentrations (Fig. 3). Unlike the parent strain, the mutant's growth rate did not pass through an optimum in the range of 0 to 10 mM sodium chloride, but displayed saturation with respect to sodium (Fig. 3).

The stimulation of growth was specifically a property of sodium ion; the chlorides of rubidium, lithium, cesium, choline, and ammonium had no such effect. Potassium was a standard component of the growth medium at 104 mM. Sodium sulfate was as effective as sodium chloride in a medium free of added halide ion. Growth rate was not affected by sodium ion when other carbon sources were used in place of glutamate; these included glucose, glycerol, pyruvate, succinate, and aspartate. The cells did not grow on α-ketoglutarate, γ-aminobutyrate, or glutamine in the presence or absence of sodium. It appears that there was a high degree of specificity for the sodium-glutamate interaction in this system.

Previous work (10, 11) with other strains of *E. coli* revealed a permeability barrier to glutamate. Our growth data were easily interpreted on this basis and suggested that a locale of action of sodium ion in this system was at the level of transport of glutamate into the cell. We pursued the investigation from this point of view and restricted our experiments to the mutant strain.

The pattern of growth inhibition by certain analogs of glutamate supported the idea that sodium accelerated glutamate transport. Halpern and Umbarger (11) showed that α-methylglutamate inhibited the growth only of *E. coli* mutants which were able to transport glutamate rapidly. Halpern and Even-Shoshan (9) later found that this analogue was a competitive inhibitor of glutamate transport in appropriate strains derived from *E. coli* K-12. We observed (Fig. 4) that α-methylglutamate and γ-hydroxyglutamate inhibited the growth of *E. coli* 29-78 in a glycerol-ammonium medium only when sodium ion was added as well.

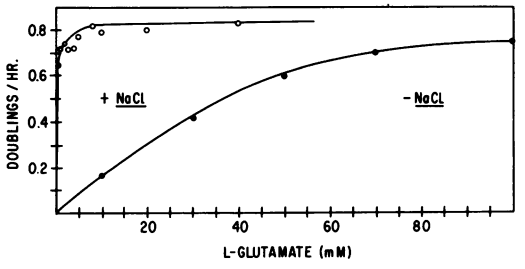

FIG. 2. *E. coli 29-78, growth rate as a function of glutamate concentration. Conditions were those described under Fig. 1, except for the use of sodium chloride (and the control, potassium chloride) at 10 mM.*

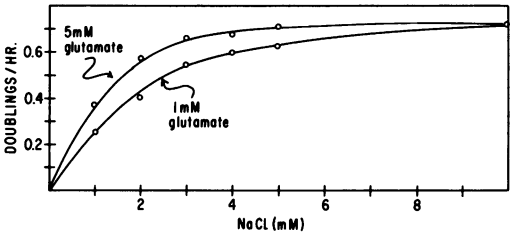

FIG. 3. *Growth rate of E. coli 29-78 as a function both of glutamate and sodium ion concentration. Conditions were those described under Fig. 1, with the concentrations of glutamate and sodium chloride indicated on the graph above.*

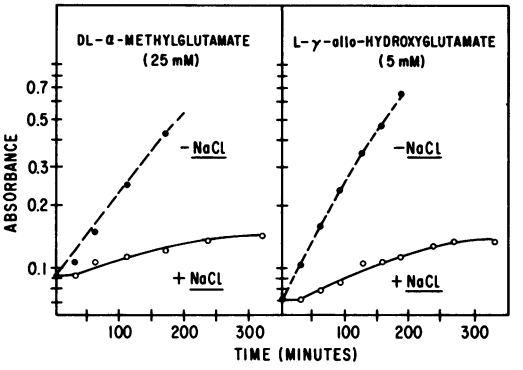

FIG. 4. *Sodium ion-dependent inhibition of growth of E. coli 29-78 by two analogues of glutamate. Cells grown in glycerol and ammonium chloride were diluted into fresh medium of the same composition but supplemented with either DL-α-methylglutamate or with L-γ-allohydroxyglutamate. Each inhibitor was tested in the absence and the presence of sodium chloride (10 mM). The dashed lines represent growth of the cells in control tubes lacking inhibitor, but the points associated with these lines are those for the cultures containing inhibitor and no sodium chloride. The growth temperature was 37 C.*

Sodium stimulation of glutamate transport in E. coli 29-78. Washed cells that had been grown in glycerol-ammonium medium readily accumulated radioactivity from ^{14}C-glutamate when incubated in B7 salts; chloramphenicol was added to prevent incorporation into protein. Sodium ion markedly stimulated uptake under these conditions (Fig. 5). These results are consistent with the explanation suggested for the growth data, i.e., that sodium ion stimulated the rate of transport of glutamate into the cells. However, in neither case do the data exclude a primary effect of the cation on the metabolism of glutamate, since glutamate was forced to serve as the energy source for its own accumulation in both systems. It was not surprising to find that these cells, capable of rapid growth on glutamate, effected extensive metabolism of the compound, even during the brief incubations in which uptake was measured. After 10 min of incubation with ^{14}C-glutamate, approximately half of the radioactivity in the cells was present as nonvolatile metabolic products of glutamate. Thus, we sought conditions in which the metabolism of glutamate could be curtailed to the point that ^{14}C accumulation would primarily reflect glutamate uptake alone.

Sodium stimulation of glutamate transport in an inhibited system. Arsenite was chosen as an inhibitor (indirect) of glutamate degradation because of its known effects on α-keto acid oxidations and because it permitted near-stoichiometric accumulation of glutamate from proline in earlier work (6) with *E. coli* B. In the presence of arsenite, the accumulation of radioactivity was greatly inhibited when starved cells were incubated with ^{14}C-glutamate. Addition of glycerol as a presumptive energy source restored uptake. However, in the presence of glycerol, accumulation was so rapid at 37 C that its rate could not be easily measured; hence, the incubation temperature was reduced to 25 C.

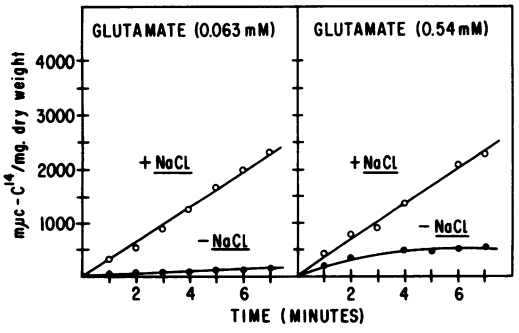

FIG. 5. *Effect of sodium ion on glutamate uptake by E. coli 29-78 at 37 C. Cells were incubated with the indicated concentrations of* ^{14}C-L-*glutamate in B7 salts containing chloramphenicol and no added energy source with and without 10 mM sodium chloride. Potassium chloride (10 mM) was present in those incubations not containing sodium. For other details see Materials and Methods.*

In incubations containing glycerol, chloramphenicol, and sodium ion, arsenite virtually abolished carbon dioxide production from ^{14}C-glutamate, but glutamate degradation was still extensive. [Halpern and Lupo (10) analyzed the radioactivity accumulated by a glutamate-utilizing mutant of *E. coli* K-12 after a 3-min incubation at 25 C with ^{14}C-glutamate in the presence of chloramphenicol and sodium succinate. They found that 70 to 75% of the activity was present as glutamate. However, they do not state whether radioactive CO_2 was produced.] The further addition of ammonium ion inhibited glutamate disappearance, so that after 10 min of incubation, at least 90% of the accumulated radioactivity was present as glutamate (Table 1).

Under these conditions of greatly limited metabolism of glutamate, sodium ion addition was still essential for vigorous accumulation of glutamate from low external concentration. The cation increased both the initial rate of uptake of glutamate and the ultimate pool (Fig. 6). With 10 mM sodium chloride and 2 μM ^{14}C-glutamate, the amino acid attained concentration in the cell water approximately 40,000-fold greater than that existing in the medium (assuming that pool glutamate was, in fact, present in free solution).

Kinetic analysis at several levels of sodium ion indicates that the cation increased the affinity of the system for glutamate (Fig. 7). When sodium ion concentration was varied from 3 to 10 mM, the K_m for glutamate decreased from 42 to 7 μM, although the maximal velocity (V_{max}) was not sensibly affected. These kinetic estimates agree closely with results obtained by Marcus and Halpern (14) in their studies of glutamate uptake by some mutants of *E. coli* K-12. In their work, sodium ion was a standard component of the reaction mixtures since sodium succinate was used as an energy source.

As in the growing system, the cation effect was specific for sodium; rubidium, lithium, cesium, and choline could not substitute for it, nor could they partially replace sodium when the latter was supplied at lower concentration. Potassium and ammonium ions were standard components of the incubation system.

In the arsenite-poisoned system, glutamate, once accumulated, tended not to exit from the cells when they were exposed to media lacking the amino acid. (Addition of unlabeled glutamate,

TABLE 1. *Degradation of glutamate by nongrowing cells of E. coli 29-78 as influenced by arsenite and ammonium ion*[a]

Radioactivity	Additions			
	With AsO_2^-		Without AsO_2^-	
	With NH_4Cl	Without NH_4Cl	With NH_4Cl	Without NH_4Cl
	%	%	%	%
In overall experiment				
Total amount added	100	100	100	100
Accumulated in cells	45	42	30	30
Evolved as CO_2	0.2	0.2	17	27
Remaining in medium	55	57	48	40
In glutamate				
Cellular pools	91	61	52	29
Reaction mixture minus cells	76	49	67	52
Total reaction mixture	83	54	48	30

[a] Washed and starved cells were incubated for 10 min at 25 C with 2 μM ^{14}C-L-glutamate, glycerol, and chloramphenicol with and without the indicated combinations of arsenite and ammonium chloride. For other details of the incubation and analysis, *see* Materials and Methods.

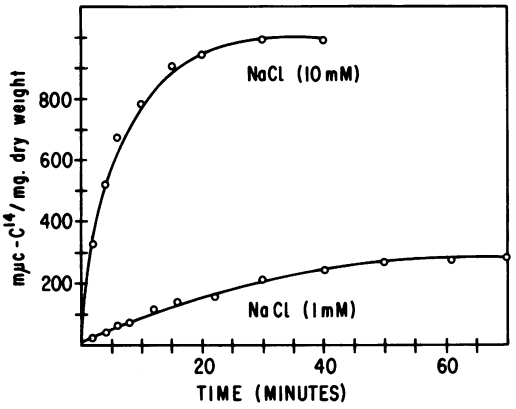

FIG. 6. *Effect of sodium ion on the time course of glutamate uptake by E. coli 29-78 at 25 C in the presence of glycerol and arsenite. Cells were incubated with 2 μM ^{14}C-L-glutamate and either 10 mM or 1 mM sodium chloride, as indicated. Glycerol, ammonium chloride, chloramphenicol, and arsenite were present as described.*

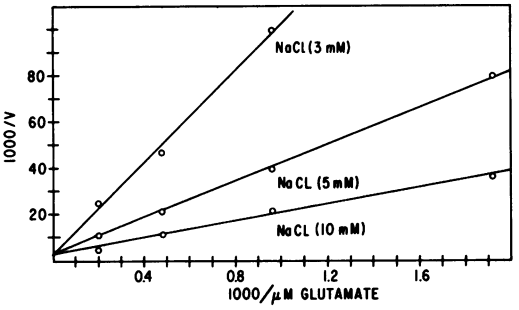

FIG. 7. *Kinetic analysis of the effect of sodium ion on glutamate uptake by E. coli 29-78 at 25 C in the presence of glycerol and arsenite. Conditions were those described under Fig. 6, except for the indicated concentrations of sodium chloride and glutamate. Initial rates were estimated from time courses obtained at the indicated concentrations of sodium and glutamate. The lines drawn through the points on the double-reciprocal plot were calculated by least squares. The K_m for glutamate, obtained from the least squares fit, were 6.9, 13.9, and 42.3 μM for sodium chloride concentrations of 10, 5, and 3 mM, respectively. The V_{max} estimates were 386, 354, and 424 nc of ^{14}C per min per mg for the three concentrations of sodium chloride. All results are calculated to a ^{14}C-glutamate specific activity of 10 μc/μmole.*

however, rapidly caused the release of radioactivity from cells preloaded with ^{14}C-glutamate.) The very slow exit of radioactivity (approximately 10% in 30 min) that was observed may simply be correlated with a measured 10% decrease in viability over the same interval. No difference in the exit was observed when cells were preloaded in the presence of sodium and then centrifuged, washed, and incubated under the same conditions with or without added sodium. That the cation was effectively removed from the cells by this treatment was indicated by the necessity for its addition to promote glutamate uptake in control experiments.

Relationship of the glutamate transport system to growth. In the presence of arsenite, chloramphenicol, sodium, and glycerol, the cells took up glutamate at a maximal rate of approximately 2.3 μmoles per hr per mg (dry wt) at 25 C (Fig. 7). We wondered whether this rate was sufficient

to account for growth behavior under nutritive conditions which simulate those of the transport system and at this temperature. Hence, we grew *E. coli* 29-78 at 25 C on glutamate as the sole nitrogen source, with glycerol as an additional energy-carbon source, and with 10 mM sodium chloride. The growth rate was 0.32 doublings per hr. The minimal rate of glutamate transport required to produce this growth rate can be estimated as follows. The rate of decrease of medium glutamate concentration (G) should be proportional to the rate of increase in cell concentration (X): $-(dG/dt) = p(dX/dt)$.

The proportionality factor (p) is, therefore, the quantity of glutamate required for the production of a unit quantity of cells. Assuming nitrogen content of 11% (dry wt) and stoichiometric conversion of glutamate-nitrogen to cellular-nitrogen, p = 8 μmoles of glutamate per mg (dry wt) of cells produced.

For cells growing exponentially at D doublings per hour, the rate of increase in cell concentration is: $dX/dt = 0.693(D)(X)$.

The specific rate of glutamate disappearance from the medium is then obtained by combining the two equations in the form: $-(dG/dt)/X = 0.693(D)(p)$. The experimental values for p and D were 8 and 0.32, respectively, yielding the estimate, 1.8 μmoles per hr per mg (dry wt) for the minimal rate of entry of glutamate as nitrogen source. The V_{max} of our transport system (about 2.3 μmoles $\times$ hr^{-1} $\times$ mg^{-1}) is, then, consistent with function of the system during growth.

DISCUSSION

The requirement for very high concentrations of glutamate by wild-type *E. coli* B for growth on this compound as carbon (or carbon and nitrogen) source in a medium free of added sodium ion suggests that glutamate entry is rate-limiting for growth. In this respect, strain B resembles other laboratory strains of *E. coli* (W, H, K-12) which have been shown to transport glutamate with difficulty (10, 11). The dramatic increase in growth rate at moderate concentrations of glutamate brought about by addition of sodium ion is, then, easily understood in terms of sodium-stimulated transport of glutamate. But additional metabolic effects of sodium cannot be ruled out a priori; it is conceivable that the cation also stimulates the degradation of glutamate, once accumulated. However, Kahana and Avi-Dor (13) observed no stimulation of glutamate oxidation by sodium in broken cell preparations of *E. coli* B, whereas the cation did stimulate glutamate oxidation by intact cells. These authors inferred a role for sodium ion in glutamate transport on this basis.

Because we have no assurance that poor growth on glutamate is due solely to its slow penetration, we cannot deduce with great confidence the nature of the mutational change in our mutant, *E. coli* 29-78. Most simply, the vigorous growth of this strain at low concentrations of glutamate is due to an enchanced ability to transport the amino acid. Effective growth of this strain on low concentrations of glutamate, however, is still dependent on the addition of sodium ion. Again, it seems reasonable to infer sodium-stimulated glutamate transport as a significant action of the cation during growth of the mutant strain. The sodium-promoted inhibition of growth by analogues of glutamate also suggests a transport-level effect (*see* Results). We do not know whether either of the analogues tested (α-methylglutamate or γ-hydroxyglutamate) is metabolized by the cells.

Our orientation in this work was to deduce whether sodium does stimulate transport of glutamate, rather than to attempt a complete description of the growth behavior. The study was complicated by the metabolic lability of glutamate; although sodium clearly stimulated the rate of accumulation of radioactivity from ^{14}C-glutamate, the conversion of the amino acid to metabolic products was rapid enough to prevent the secure conclusion that the cation effect was on glutamate transport, per se. However, by adding a supplementary energy source, and arsenite and ammonium ion, we obtained conditions in which sodium still stimulated the uptake of radioactivity with preservation of 90% of the activity as glutamate in the cellular pools. We conclude that sodium does markedly stimulate the transport of glutamate.

The very large glutamate concentration gradient established in the arsenite-poisoned system suggests that the rate of exit of glutamate is insignificant relative to its rate of entry. In experiments with preloaded cells, we could observe little or no loss of accumulated radioactivity whether or not sodium was present during the exit. This result differs materially from Halpern's (8) studies of glutamate exit in mutants of *E. coli* K-12 in which cells preloaded with ^{14}C-glutamate lost radioactivity to the medium with half-times of 20 min or less. Perhaps our result is due to arsenite inhibition of a normal exit mechanism. On the other hand, it is not clear to us whether the exit of radioactivity observed by Halpern actually reflects efflux of glutamate or possible metabolic products of the amino acid, since he assumed that glutamate is preserved as such in the cellular pools during the required incubations. Our system is too inflexible to permit a proper study of exit because we

require a metabolic poison and, even in its presence, glutamate degradation becomes a problem in long incubations or at high cell densities.

We have not yet measured sodium fluxes associated with the transport of glutamate and, therefore, cannot speculate on the mechanism of action of the cation. In growing *E. coli*, sodium tends to equilibrate between cells and medium (5), but cation distribution has not been studied from the point of view of simultaneous transport of a specific organic solute. MacLeod and associates (24) have studied a sodium-requiring marine pseudomonad in which the transport of α-aminoisobutyrate is specifically stimulated by this cation. In earlier work (22), they showed that sodium tends to equilibrate between cells and medium, but have not reported its distribution in temporal association with the accumulation of α-aminoisobutyrate. At lower levels of sodium ion, the cation caused a decrease in the K_m for the amino acid analogue, but did not affect the V_{max} of transport. Our kinetic results with sodium-stimulated glutamate transport were similar.

In animal cells, where sodium is often required for the concentrative uptake of amino acids, sugars, and other compounds, the uphill movement of organic solute is associated with the downhill movement of sodium into the cells (17, 19). The sodium gradient is maintained by the almost ubiquitous sodium exit "pump" found in animal cells—the (Na,K)-activated membrane adenosine triphosphatase. It is postulated (2, 3, 4, 17, 19) that the downhill (exergonic) gradient of sodium satisfies the thermodynamic requirement for the uphill (endergonic) transport of organic solute into the cell. A membrane carrier which interacts with both sodium and the organic solute could, therefore, be responsible for the vectorial movement of organic solute and the latter would not, itself, have to be coupled to "metabolic energy." The energetic coupling, in this mechanism, would be between metabolic reactions and the sodium pump. It is of interest that (Na,K)-adenosine triphosphatase activity has been detected in a number of bacteria including *E. coli* (7).

Reports of specific physiological effects of sodium in bacteria are rare. Among strains that show a general sodium requirement for growth, the most extensive studies of mechanism are those of MacLeod and associates (22, 24). Stevenson (21) found stimulation of glutamate uptake by a halophile dependent upon both sodium and chloride. Rhodes and Payne (16) interpreted some growth studies of another halophilic species in terms of a sodium-stimulated induction of a transport system. Sistrom (18) found a sodium requirement for growth of *Rhodopseudomonas* in a synthetic medium. *E. coli* does not require sodium for growth, except under the special conditions reported here. Similarly, *Aerobacter aerogenes*, although not normally requiring sodium, displays such a requirement for anaerobic growth on citrate (15). In this case, the sodium requirement could be correlated with a sodium-activated oxaloacetic decarboxylase (20) whose function is essential for anaerobic degradation of citrate. Another system (23) in which sodium seems to specifically affect a metabolic step is the fermentation of glutamic acid by *Peptococcus aerogenes*. The common practice of adding sodium salts to bacterial media (on a traditional, rather than a rational basis as far as we can tell) has probably obscured additional specific actions of the cation.

ACKNOWLEDGMENTS

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