JOURNAL OF BACTERIOLOGY, Apr. 1973, p. 53-58
Vol. 114, No. 1

Printed in U.S.A.

Sodium and Potassium Requirements for Active Transport of Glutamate by *Escherichia coli* K-12

Y. S. HALPERN, H. BARASH, S. DOVER, AND KARNINA DRUCK

Department of Molecular Biology, Institute of Microbiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Received for publication 27 September 1972

Active transport of glutamate by *Escherichia coli* K-12 requires both Na^+ and K^+ ions. Increasing the concentration of Na^+ in the medium results in a decrease in the K_m of the uptake system for glutamate; the capacity is not affected. Glutamate uptake by untreated cells is not stimulated by K^+. K^+-depleted cells show a greatly reduced capacity for glutamate uptake. Preincubation of such cells in the presence of K^+ fully restores their capacity for glutamate uptake when Na^+ ions are also present in the uptake medium. Addition of either K^+ or Na^+ alone restores glutamate uptake to only about 20% of its maximum capacity in the presence of both cations. Changes in K^+ concentration affect the capacity for glutamate uptake but have no effect on the K_m of the glutamate transport system. Ouabain does not inhibit the (Na^+-K^+)-stimulated glutamate uptake by intact cells or spheroplasts of *E. coli* K-12.

The involvement of sodium ions in the active transport of amino acids and sugars by animal cells has been established quite generally (17). Although active transport in bacteria earned considerable attention in the last two decades, the requirements for cations and their role in bacterial transport systems had been largely neglected until quite recently.

In earlier studies on glutamate transport in *Escherichia coli* K-12 (9) we observed a peculiar effect of the energy source used on the saturation kinetics of the system. Straight-line reciprocal plots of uptake versus glutamate concentration were obtained when sodium succinate served as the energy source. However, in the presence of glucose or glycerol, peculiar nonlinear kinetics was observed. That this effect might have been due to the concentration of sodium ions in the medium was very strongly suggested by a later study of Frank and Hopkins (6), who demonstrated a sodium requirement for glutamate uptake by *E. coli* B. We therefore investigated the cation requirements of the glutamate active transport system of *E. coli* K-12, the various other aspects of which are now under active study in our laboratory (1, 15).

MATERIALS AND METHODS

Bacterial strain. *E. coli* K-12 CS7, a methionine auxotroph capable of utilizing L-glutamate as the sole source of carbon (14), was used.

Growth media. The basal medium of Davis and Mingioli (3) from which citrate was omitted and the basal salts media of Frank and Hopkins (B7) (6) and of Tatum and Lederberg (20) were used as indicated. The media were supplemented with 50 μg of DL-methionine per ml, and 1% sodium succinate or 0.5% glycerol, as indicated, served as the carbon source.

Cultivation. The cultures were grown with aeration at 37 C. The bacteria were harvested from culture in the exponential phase (turbidity of 120 to 150 Klett units, filter no. 42).

Preparation of spheroplasts. Spheroplasts were prepared according to Repaske (16), as previously described (1).

^{14}C-L-glutamate uptake. Uptake experiments with intact cells were carried out according to Kessel and Lubin (13), as described by Halpern and Lupo (10), with some modifications in the composition of the uptake medium, where indicated. Glutamate uptake by spheroplasts was carried out according to Barash and Halpern (1).

RESULTS

As shown in Fig. 1, cells grown in a medium to which no sodium salts were added exhibited low capacity for glutamate uptake, when sodium ions were not added to the uptake mixture. Addition of NaCl up to a concentration of 500 mM greatly increased glutamate uptake. The fact that saturation was not achieved at the high salt concentrations used suggested an osmotic, rather than, or in addition to, a

specific sodium ion effect on glutamate transport. Figure 2 indeed shows that increasing the osmotic activity of the uptake medium by the addition of sucrose up to 20%, in the presence of a constant amount of Na^+ (37 mM), resulted in a threefold increase in glutamate uptake. The specific effect of Na^+ on glutamate uptake was therefore tested in a medium containing 25% sucrose in the presence of 104 mM of K^+. One can see in Fig. 3 that Na^+ specifically enhances (up to sixfold) glutamate uptake, with maximum stimulation at 15 mM Na^+.

Kinetic analysis of Na^+-stimulated glutamate uptake. The kinetics of the effect of Na^+ on glutamate uptake was examined in experiments in which glutamate concentration was varied in the presence and in the absence of 15 mM NaCl in mixtures containing 104 and 119 mM of K^+, respectively. A double reciprocal plot of the data (Fig. 4) shows that Na^+ increases the affinity of the transport system for glutamate 11-fold, but does not affect its capacity.

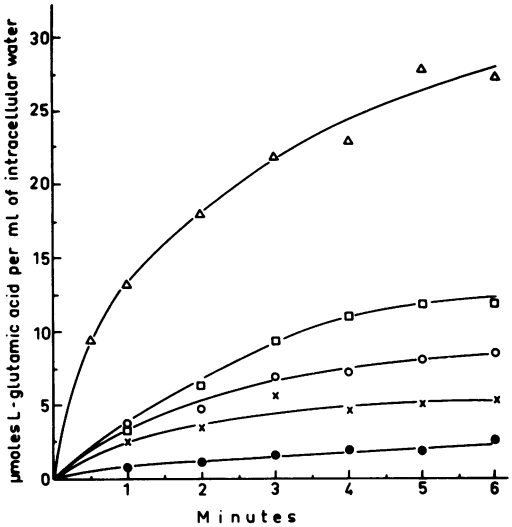

FIG. 1. *Effect of Na^+ on glutamate uptake by E. coli K-12 CS7. The bacteria were grown in B7 salts medium with 0.5% glycerol, as the carbon source. The uptake mixtures in fresh B7-glycerol medium with 200 µg of chloramphenicol per ml and bacteria at a turbidity of 60 Klett units were supplemented with NaCl as follows: no additions (●), 5 mM (×), 15 mM (○), 50 mM (□), 500 mM (△). The mixtures were incubated at 30 C for 10 min, ^{14}C-L-glutamate (1 µCi/µmol) was added to a concentration of 5 × 10^{-5} M, and incubation was continued. Samples of 0.5 ml were taken at indicated time intervals, washed with 5 ml of the respective uptake medium without glutamate, and further treated as previously described (10).*

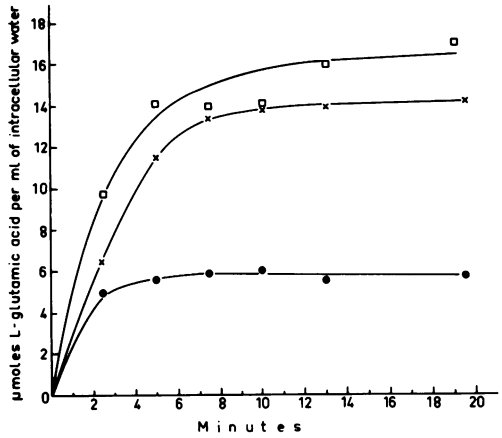

FIG. 2. *Effect of osmotic activity of the medium on glutamate uptake. The bacteria were grown in Davis medium (3) without citrate, with 0.5% sodium succinate, as the carbon source. The uptake mixtures in Davis-succinate medium with chloramphenicol and bacteria (73 Klett units) were supplemented as follows: no additions (●), 10% sucrose (×), 20% sucrose (□). Other details were as in Fig. 1 and reference 10, except that the samples were filtered on 2.5-cm Whatman GF/A glass-fiber paper filters.*

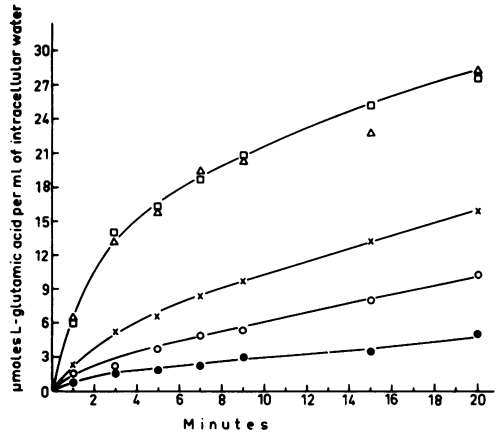

FIG. 3. *Effect of NaCl on glutamate uptake in media with high osmotic activity. The bacteria were grown in B7-glycerol medium. The uptake mixtures in B7-glycerol medium with chloramphenicol and bacteria (80 Klett units) were supplemented with 25% sucrose and NaCl as follows: without NaCl (●), 5 mM (×), 15 mM (□), 50 mM (△), 500 mM (○). For further details see Fig. 2.*

Effect of K^+ on glutamate uptake by E. coli K-12 CS7. As mentioned above, K^+ could not replace Na^+, nor did K^+ exert any inhibitory effect on Na^+-stimulated glutamate transport. However, since *E. coli* has a high capacity for accumulating potassium (18), the effect of K^+ had to be reexamined using K^+-depleted

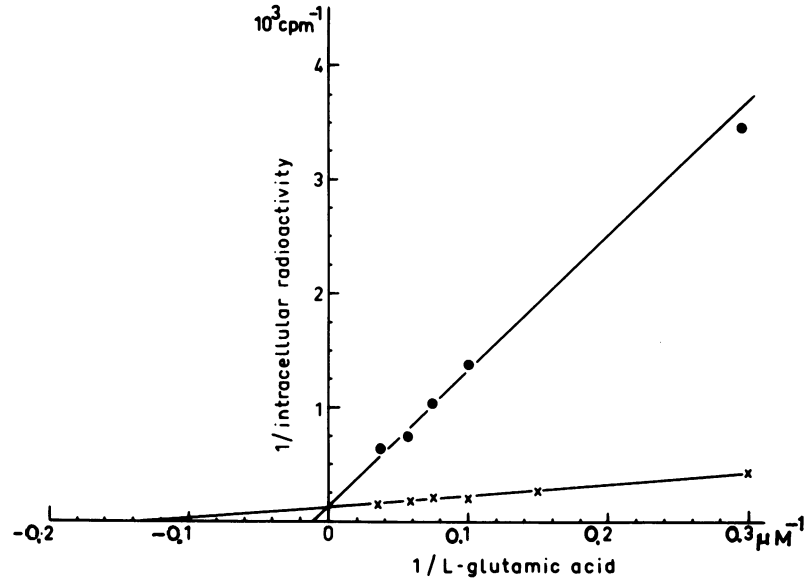

FIG. 4. *Effect of Na^+ on the substrate saturation curve of glutamate uptake-Lineweaver-Burk plot. The bacteria were grown in B7-potassium succinate medium; the uptake experiments were carried out in B7-glucose (0.5%) medium at a bacterial density of 37 Klett units, in a total volume of 2 ml, in the presence (×), or in the absence (●) of 15 mM NaCl. In the latter case the ionic strength was balanced by the addition of 15 mM KCl. The intracellular radioactivity was determined on the entire volume of the reaction mixture after incubation for 6 min at 30 C.*

cells. The K^+-depletion procedure of Thompson and MacLeod (21) consisting of three washings with cold 0.05 M $MgSO_4$ was employed. One can see from Fig. 5 that K^+-depleted cells showed very little glutamate uptake in a medium to which neither K^+ nor Na^+ ions were added. Addition of KCl and NaCl to a concentration of 10 mM each resulted in a 24-fold increase in the rate of uptake of the amino acid. In the presence of either K^+ or Na^+ alone, the rate of glutamate uptake was approximately 20% of that observed when both cations were present. The stimulation of glutamate uptake by K^+-depleted cells in the presence of K^+ and the failure of K^+ to stimulate glutamate uptake by nondepleted cells suggest that it is the intracellular potassium which is involved in the transport process. This is supported further by the data presented in Fig. 6. K^+-depleted cells were preincubated for various time periods (from 0 to 60 min) in the presence of 7×10^{-5} M KCl. Radioactive 5×10^{-5} M glutamate was then added, and uptake was followed for 16 min. One can see that the longer the cells were preincubated with potassium, the higher was the rate at which they took up glutamate from the medium.

Kinetics of K^+-stimulated glutamate uptake. The effect of K^+ was analyzed in experiments in which both K^+ and L-glutamate concentrations were varied simultaneously in reaction mixtures containing 15 mM Na^+ and 25% sucrose. The double reciprocal plot presented in Fig. 7 shows that increasing the concentration of K^+ causes an increase in capacity for glutamate but does not affect the K_m of the glutamate transport system.

Effect of ouabain on glutamate uptake by E. coli K-12. Hafkenscheid and Bonting (8) detected (Na^+-K^+)-dependent, ouabain-sensitive adenosine triphosphatase (ATPase) activity in *E. coli*, which accounted for about 10% of the total ATPase activity of the cells. In view of our finding that the simultaneous presence of Na^+ and K^+ was essential for maximum activity of the glutamate transport system in *E. coli* K-12, we tested the possibility that this transport is coupled to the activity of (Na^+-K^+)-dependent ATPase. As shown in Fig. 8, ouabain at a concentration as high as 10^{-3} M did not affect glutamate uptake by *E. coli* K-12 CS7 in the presence of Na^+ and K^+ ions. To minimize any possible interference of the cell wall with free access of ouabain to the ATPase site in the cytoplasmic membrane, experiments similar to that described in Fig. 8 were also performed with spheroplasts. Here too, ouabain had no effect on the (Na^+-K^+)-stimulated

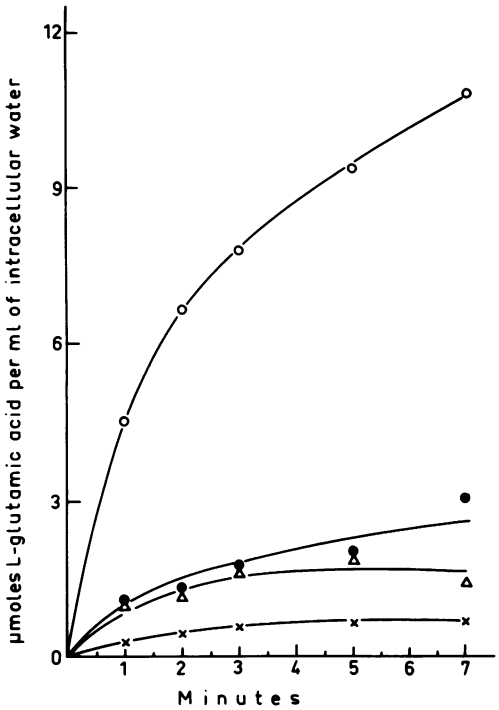

FIG. 5. *Effect of K^+ on glutamate uptake. Bacteria were grown in B7-glycerol medium and subjected to the K^+-depletion procedure of Thompson and MacLeod (21). The K^+-depleted cells were tested for glutamate uptake in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.2, containing 0.05 M $MgSO_4$, 0.5% glycerol, and 200 µg of chloramphenicol per ml and the following additions: no additions (×), 10 mM KCl (△), 10 mM NaCl (●), KCl and NaCl, 10 mM each (○). The reaction mixtures were preincubated for 10 min at 30 C before the addition of radioactive glutamate. The basic uptake solution (with no KCl or NaCl added) contained 15 µeq of Na^+ and 1.5 µeq of K^+ as measured by flame photometry, by using the Eppendorf 700 flamephotometer.*

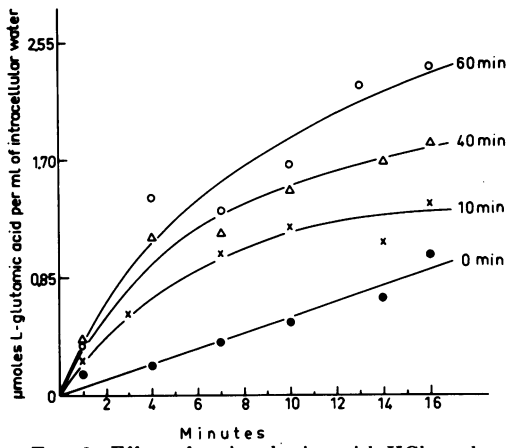

FIG. 6. *Effect of preincubation with KCl on glutamate uptake by K^+-depleted cells. The bacteria were grown in the medium of Tatum and Lederberg (20) with 0.5% glycerol, depleted of potassium as in Fig. 5, and suspended in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.2, containing 0.05 M $MgSO_4$, 0.015 M NaCl, 7 × 10^{-5} M KCl, 25% sucrose, 0.5% glycerol, and 200 µg of chloramphenicol per ml, to a density of 113 Klett units. The mixture was incubated for the indicated time periods, and glutamate uptake was measured as in Fig. 1.*

glutamate uptake (Table 1).

DISCUSSION

The experiments described in this paper demonstrate the dependence of the active transport of glutamate in *E. coli* K-12 on Na^+ and K^+ ions. The two cations are required simultaneously; neither replacement of one cation by the other nor competition between them was observed. Furthermore, the effects of the two cations on the kinetics of glutamate uptake are remarkably different. Na^+ greatly increases the affinity of the uptake system for glutamate, without affecting the capacity of the cells for accumulating glutamate. K^+, to the contrary, greatly increases the cell's capacity for accumulating glutamate, but does not affect its affinity for external glutamate. Moreover, although the effect of Na^+ is readily observed with untreated cells, K^+ requirement can only be demonstrated with cells depleted of internally accumulated K^+. The rate of uptake and the capacity for glutamate accumulation by K^+-depleted cells increase with time of preincubation in the presence of K^+ ions.

Interactions among K^+ or Na^+ and different bacterial transport systems have been observed in other laboratories. Thus, the uptake of glutamate and aspartate by *Staphylococcus aureus* is coupled to K^+ translocation, when there is a K^+ gradient from outside to inside the cell (2, 7). The inhibition of glutamate, aspartate, and alanine uptake in *Streptococcus faecalis* by valinomycin and its reversal by high concentrations of K^+ (7, 11, 12) indicate that the transport of these substances may depend on the dissipation of a K^+ concentration gradient from inside to outside the cell. A potassium-dependent citric acid transport system in *Aerobacter aerogenes* has been recently described by Eagon and Wilkerson (5). The authors believe that the transport of citric acid in this system depends on a K^+ concentration gradient from inside to outside the cell. In a

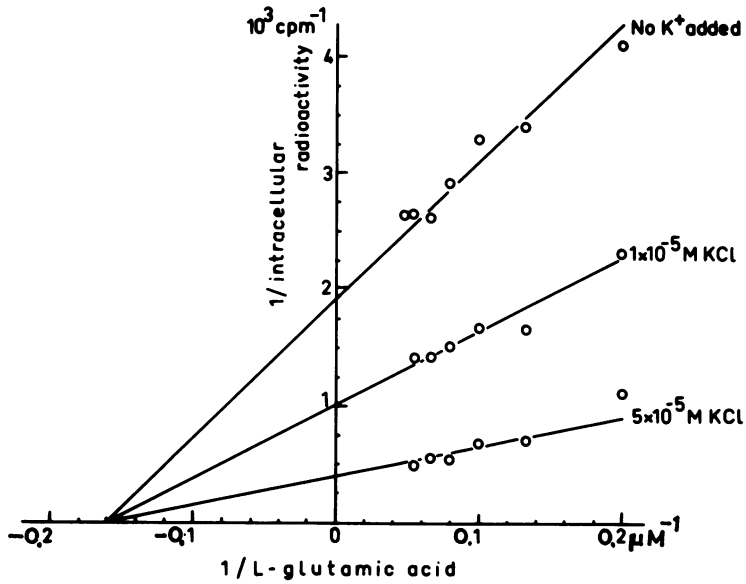

FIG. 7. *Effect of K^+ on the kinetic parameters of glutamate uptake-Lineweaver-Burk plots. The bacteria were grown and treated to deplete them of potassium as in Fig. 6. The K^+-depleted cells (0.1 mg dry weight) were suspended in 1 ml of uptake medium as in Fig. 6, in the presence of various concentrations of KCl as indicated, and incubated for 40 min at 30 C. ^{14}C-L-glutamate was then added, and uptake was measured after 15 min on the entire volume of the reaction mixture.*

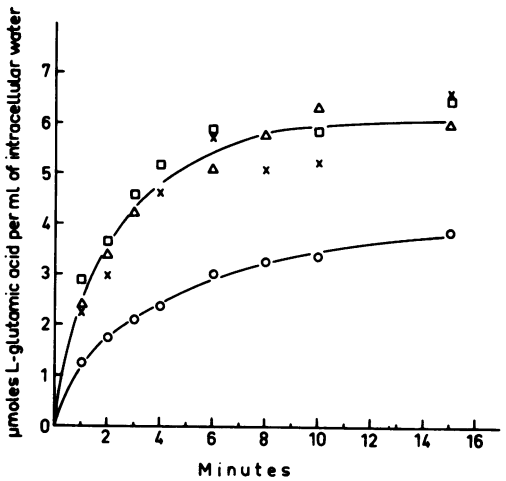

FIG. 8. *Effect of ouabain on L-glutamate uptake by intact cells of E. coli K-12 CS7. The bacteria were grown in B7-glycerol medium. The uptake mixtures in B7-glycerol medium with chloramphenicol were supplemented as follows: no additions (O), 15 mM NaCl (Δ), 15 mM NaCl plus 10^{-4} M ouabain (×), 15 mM NaCl plus 10^{-3} M ouabain (□). Further details were as in Fig. 1.*

TABLE 1. *Effect of ouabain on glutamate uptake by E. coli K-12 CS7 spheroplasts in the presence of Na^+ and K^+ ions*[a]

Expt no.	^{14}C-Glutamate uptake (counts/min)	
	No ouabain added (control)	In the presence of ouabain 10^{-3} M
1	900	1,031
	1,032	926
2	1,532	1,302
	1,289	1,551

[a] Cells were grown in Davis-glycerol medium. Spheroplasts were prepared as earlier described (1) and suspended to a density corresponding to 53 Klett units of whole-cell turbidity, in an uptake medium containing: 25% sucrose, 15 mM KCl, 2.5 mM KH_2PO_4, 15 mM NaCl, 20 mM $MgSO_4$, 0.5% glycerol, and 200 μg of chloramphenicol/ml. Ouabain, 1 mM, was added where indicated, and glutamate uptake was measured as in Fig. 1 after incubation at 25 C for 10 min.

recent report from Roseman's laboratory the melibiose permease (TMG permease II) of *Salmonella typhimurium* has been identified as a sodium-dependent co-transport system (19).

A double requirement for both Na^+ and K^+ in amino acid transport has been described for α-aminoisobutyric acid (AIB) uptake by a marine pseudomonad (4). In an elegant study

by Thompson and MacLeod (21) the authors demonstrated that Na^+ is required for entry and equilibration of AIB into the cells, whereas K^+, acting at the intracellular level, brings about the accumulation of AIB against a concentration gradient. Kinetic analysis indicated that an increase in Na^+ concentration was followed by a decrease in the K_m of the carrier system (22), whereas changes in the concentration of K^+ affected the capacity but had no effect on the K_m (21). The authors proposed a tentative model to account for their data. According to their scheme, Na^+ combines with the carrier, putting it into a conformation having affinity for the substrate. The ternary complex crosses the membrane. In the presence of energy and intracellular K^+, the affinity of the Na^+-carrier complex for the substrate is reduced somehow, and the substrate is released and accumulates within the cell. Our data on the (Na^+, K^+)-dependent glutamate transport system in *E. coli* presented in this report are in agreement with this model. An alternative explanation for the simultaneous requirement for Na^+ and K^+, based on the assumption that the energy required for the accumulation of glutamate is provided by the (Na^++K^+)-activated ATPase complex, is not supported by our findings that the *E. coli* glutamate transport system was not affected by ouabain.

Finally, recent experiments showed that the increased capacity of glutamate uptake in media of high osmotic activity (Fig. 1 and 2) was due to a three- to fourfold decrease in the rate of glutamate exit from the cells.

ACKNOWLEDGMENT

This work was supported by grants from the Reuben Kunin-Samuel Lunenfeld Medical Research Foundation and the Hebrew University-Hadassah Joint Research Fund.

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. Davies, R., J. P. Folkes, E. F. Gale, and L. C. Bigger. 1953. Changes in sodium and potassium accompanying the accumulation of glutamic acid or lysine by bacteria and yeast. Biochem. J. **54**:430–437.
3. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **62**:17–28.
4. Drapeau, G. R., T. I. Matula, and R. A. MacLeod. 1966. Nutrition and metabolism of marine bacteria. XV. Relation of Na^+-activated transport to the Na^+-requirement of a marine pseudomonad for growth. J. Bacteriol. **92**:63–71.
5. Eagon, R. G., and L. S. Wilkerson. 1972. A potassium-dependent citric acid transport system in *Aerobacter aerogenes*. Biochem. Biophys. Res. Commun. **46**:1944–1950.
6. Frank, L., and I. Hopkins. 1969. Sodium-stimulated transport of glutamate in *Escherichia coli*. J Bacteriol. **100**:329–336.
7. Gale, E. F. 1971. Don't talk to me about permeability. The Tenth Marjory Stephenson Memorial Lecture. J. Gen. Microbiol. **68**:1–14.
8. Hafkenscheid, J. C. M., and S. L. Bonting. 1968. Studies on (Na^+-K^+)-activated ATPase XIX. Occurrence and properties of a (Na^+-K^+)-activated ATPase in *Escherichia coli*. Biochim. Biophys. Acta **151**:204–211.
9. Halpern, Y. S., and A. Even-Shoshan. 1967. Properties of the glutamate transport system in *Escherichia coli*. J. Bacteriol. **93**:1009–1016.
10. Halpern, Y. S., and M. Lupo. 1965. Glutamate transport in wild-type and mutant strains of *Escherichia coli*. J. Bacteriol. **90**:1288–1295.
11. Harold, F. M., and J. R. Baarda. 1967. Gramicidin, valinomycin and cation permeability of *Streptococcus faecalis*. J. Bacteriol. **94**:53–60.
12. Harold, F. M., E. Pavlasová, and J. R. Baarda. 1970. A transmembrane pH gradient in *Streptococcus faecalis*: origin and dissipation by proton conductors and N,N'-dicyclohexylcarbodiimide. Biochim. Biophys. Acta **196**:235–244.
13. Kessel, D., and M. Lubin. 1965. Stability of α-hydrogen of amino acids during active transport. Biochemistry **4**:561–565.
14. Marcus, M., and Y. S. Halpern. 1967. Genetic analysis of glutamate transport and glutamate decarboxylase in *Escherichia coli*. J. Bacteriol. **93**:1409–1415.
15. Marcus, M., and Y. S. Halpern. 1969. Genetic analysis of the glutamate permease in *Escherichia coli* K-12. J. Bacteriol. **97**:1118–1128.
16. Repaske, R. 1956. Lysis of gram-negative bacteria by lysozyme. Biochim. Biophys. Acta **22**:189–191.
17. Schultz, S. G., and P. F. Curran. 1970. Coupled transport of sodium and organic solutes. Physiol. Rev. **50**:637–718.
18. Schultz, S. G., and A. K. Solomon. 1961. Cation transport in *Escherichia coli* I. Intracellular Na and K concentrations and net cation movement. J. Gen. Physiol. **45**:355–369.
19. Stock, J., and S. Roseman. 1971. A sodium-dependent sugar co-transport system in bacteria. Biochem. Biophys. Res. Commun. **44**:132–138.
20. Tatum, E. L., and J. Lederberg. 1947. Gene recombination in the bacterium *Escherichia coli*. J. Bacteriol. **53**:673–684.
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.
22. Wong, P. T. S., J. Thompson, and R. A. MacLeod. 1969. Nutrition and metabolism of marine bacteria. XVII. Ion-dependent retention of α-aminoisobutyric acid and its relation to Na^+-dependent transport in a marine pseudomonad. J. Biol. Chem. **244**:1016–1025.