Linked Transport of Phosphate, Potassium Ions and Protons in *Escherichia coli*

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P_i entry into *Escherichia coli* cells through either of the two P_i -transport systems (Pit or Pst) prompts the influx of K^+ and H^+ in a ratio that depends on the external pH. The entry of P_i is absolutely dependent on the presence of K^+ , and the entry of K^+ is equally dependent on the presence of P_i . Experiments with a number of mutants carrying any one functional P_i -transport system and one or more of the individual K^+ -transport systems indicate a permissive type of linkage of the two transports, in that there is no obvious preference by any of the P_i -transport systems for a particular K^+ -transport system for the concomitant entry of the two ions.

Two major systems for the transport of P_i in *Escherichia coli* were first described by Willsky *et al.* (1973). These were designated Pit and Pst for phosphate (inorganic) transport and phosphate-specific transport respectively. Detailed studies of the properties of the two systems, and of the mode of energy coupling to each, have also been carried out (Rosenberg *et al.*, 1977, 1979).

Roberts *et al.* (1949) reported that in *E. coli* the uptake of P_i during glucose metabolism was greater in the presence of K^+ . They demonstrated that the transport of K^+ was an active process, and that the effect of K^+ concentration on P_i uptake showed saturation. Later work reinforced this association between K^+ and P_i transport, when the primary effect of K^+ limitation on cells was shown to be the inhibition of P_i uptake (Weiden *et al.*, 1967). If K^+ was omitted from the medium, *E. coli* lost the capacity to take up P_i . This K^+ requirement could be satisfied by Rb^+ (Medveczky & Rosenberg, 1971). An early report of an *E. coli* mutant impaired in P_i accumulation linked this with a defect in K^+ accumulation (Damadian, 1967). K^+ plays a major role in the osmoregulation of *E. coli* cells (Epstein & Schultz, 1965), but the critical role for its transport is believed to be in the control of the cytoplasmic pH (Harold, 1977a; Rhoads & Epstein, 1978).

The present work was undertaken in the course of our wider study of phosphate transport in *E. coli*. We aimed to determine the extent of mutual dependence of the processes of P_i and K^+ transport, and their possible quantitative relationship. New information about these systems is now available: P_i is transported by two independent systems with different modes of energy coupling (see above). K^+ is known to be transported by four independent systems, and the mode of energy coupling for each system has been elucidated (Rhoads & Epstein, 1977; Laimins *et al.*, 1978). Mutants for these individual transport systems have been isolated (Epstein & Kim, 1971; Rhoads & Epstein, 1977). We are now able to determine whether any of the K^+ -transport systems specifically interacts with one of the P_i -transport systems.

Finally, the movements of K^+ with P_i are of interest as part of the general question of cationic symport with the movement of P_i . The chemiosmotic theory predicts that the uptake of anions such as P_i involves the co-transport of H^+ (Mitchell, 1966). Such a H^+ symport has been demonstrated for P_i and arsenate in rat liver mitochondria (Chappell, 1968; Mitchell & Moyle, 1969). In the present work we have measured the movement of both K^+ and H^+ into *E. coli*, prompted by the addition of P_i , under a variety of conditions.

A preliminary account of this work has appeared (Russell & Rosenberg, 1978).

Materials and Methods

Chemicals

The chemicals used were of the highest purity available commercially. Triethanolamine (Purissimum grade) was from Roche-Sirius, Dee Why, N.S.W., Australia. Carrier-free [^{32}P]P_i was from the Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia.

Abbreviations used: Pit, P_i transport; Pst, phosphate-specific transport.

Table 1. *Strains of E. coli used*

Strain	Relevant genetic loci	Source and other information
AN710	<i>phoT101, arg, entA</i>	Rosenberg <i>et al.</i> (1977)
K10	<i>pit</i>	B. Bachmann, C.G.S.C. no. 5023, derivative of Hfr Cavalli (Echols <i>et al.</i> , 1961)
AN259	<i>argH, entA</i>	Gift from G. B. Cox (Butlin <i>et al.</i> , 1973)
AN1088	<i>pit, argH, entA</i>	Isolated after transduction with strain K10 as donor and strain AN259 as recipient, followed by selection for arsenate resistance
Frag 5	<i>kdpABC5</i>	B. Bachmann, C.G.S.C. no. 4832 (Epstein & Davies, 1970)
2k133	<i>thi-1, kdpABC5, trkA133</i>	B. Bachmann, C.G.S.C. no. 4836 (Epstein & Kim, 1971)
2k142	<i>thi-1, kdpABC5, trkE142</i>	B. Bachmann, C.G.S.C. no. 4837 (Epstein & Kim, 1971)
2k401	<i>thi-1, kdpABC5, trkD, trkA401</i>	B. Bachmann, C.G.S.C. no. 4838 (Epstein & Kim, 1971)
TAD110*	<i>thi-1, trkA, trkD</i>	Gift from Dr. K. H. Altendorf

* Strain TAD110 is probably identical with TK510 (Rhoads & Epstein, 1978).

Bacterial strains

The strains used in this work are listed in Table 1.

Media and buffers

Cells were grown in medium 56 (Monod *et al.*, 1951) supplemented with carbon source and thiamin (3 μ M). Arginine (1 mM) and 2,3-dihydroxybenzoate (10 μ M) were added where appropriate.

The phosphate-free medium, pH 6.5, contained triethanolamine/citrate (50 mM), KCl (15 mM), $(\text{NH}_4)_2\text{SO}_4$ (10 mM) and MgSO_4 (1 mM). (All pH values are given at 37°C.)

For H^+ -movement studies, cells were suspended in a lightly buffered medium containing KCl (100 mM), triethanolamine/citrate (3–10 mM) and MgSO_4 (1 mM); the pH was adjusted as required with KOH or HCl.

For the measurement of K^+ movements, the cells were suspended in a K^+ -free buffer containing LiCl (20 mM), triethanolamine/citrate (50 mM) and MgSO_4 (1 mM), and the pH was adjusted with citric acid or LiOH. Where stated in the text, some experiments were carried out with choline chloride substituted for LiCl in the same concentrations. For routine K^+ -uptake measurements, KCl was added to 0.5–1 mM.

Preparation of cells

Cell densities were measured as absorption at 660 nm in a Gilford 300 spectrophotometer, and expressed as mg dry wt./ml by using an experimentally derived factor ($A_{660}^{1\text{cm}}$ of 1.0 = 0.43 mg dry wt./ml).

Cells were grown to stationary phase overnight, with shaking at 37°C. Where needed, exponential-phase cells were obtained by growth on limiting-carbon source, followed by supplementation of the carbon source as described by Simoni & Shallenberger (1972). The harvested cells were washed twice in phosphate-free medium, then resuspended in this

medium at A_{660} 0.3, supplemented as for growth and shaken at 37°C for 2 h to deplete the cells of P_i .

The cells were then washed three times with the appropriate buffer, resuspended in that buffer at A_{660} 5.0 and stored at 3°C until required. Cells used for studies of the dependence of P_i transport on K^+ concentration were also depleted of K^+ by shaking in the K^+ -free buffer at A_{660} 0.3–0.4 for 1 h. They were then washed and resuspended in the same buffer at A_{660} about 5.0.

Measurement of ion movements

All assays were performed at 37°C. The measurement of H^+ movements was carried out by using a Radiometer electrode G202C. K^+ movements were monitored with a miniature K^+ -sensitive electrode designed and built in this laboratory. It is based on a liquid partition consisting of a solution of valinomycin in diphenyl ether. Complete details are given elsewhere (Rosenberg, 1979).

Both electrodes were incorporated into the same water-jacketed electrode vessel, the contents of which were aerated and stirred. A separate common reference electrode served both the H^+ - and K^+ -sensitive electrodes. It was connected to the main electrode vessel through a salt bridge of 1% agarose in the appropriate support medium. This prevented K^+ leakage into the main vessel.

Each electrode was connected to one input of a dual-pen Rikadenki recorder through a Radiometer PM63 meter and a voltage back-up device, set so that full-scale deflection equalled a change of 10 mV, representing 0.1 pH unit, or about $0.2 \log[\text{K}^+]$. The responses of the instruments (in mV) were converted to concentrations of ions transported by the use of appropriate standards.

The technique used for measuring the uptake of

P_i has been described elsewhere (Rosenberg *et al.*, 1975). The concomitant movements of H⁺, K⁺ and P_i were measured with the ion-sensitive electrodes and [³²P]P_i respectively. Details are given in the legend to Fig. 5.

Results

All experiments were carried out with glucose-grown cells. The exchange of intracellular and extracellular P_i, which is characteristic of the Pit system in cells grown on carbon sources other than glucose, is repressed when such cells are grown on glucose (Rosenberg *et al.*, 1977). Thus the use of glucose-grown cells avoids quantitative errors when cation fluxes are compared with the observed rates of [³²P]P_i uptake in cells carrying the Pit system.

K⁺-dependence of P_i uptake

In previous experiments with washed suspensions of *E. coli*, P_i uptake was shown to be enhanced by the addition of KCl, but a basic rate of uptake was still observed when no such addition was made (Medveczky & Rosenberg, 1971). We have now established (results not shown) that *E. coli*, even after being washed repeatedly in a K⁺-free buffer, leaked K⁺ into the medium at both 22 and 3°C, especially in the absence of an energy source. When introduced into the electrode cell at 37°C after three washes, such cell suspensions showed a K⁺ concentration in the medium ranging from 10 to 100 μM, and increasing steadily with time.

In order to deplete cells of K⁺ sufficiently to avoid this leakage, we aerated suspensions of washed *E. coli* at A₆₆₀ 0.3 in K⁺-free medium for 1 h in the absence of an energy source and in the presence of 1 mM-2,4-dinitrophenol. When these cells were washed and introduced under standard conditions into the electrode cell, the K⁺ concentration in the medium was below 0.3 μM and did not significantly increase over the next 10 min. Such cells showed total dependence on added K⁺ for P_i uptake. Neither strain AN710 (Pit system) nor strain AN1088 (Pst system) took up P_i at K⁺ concentrations below 5 μM. Maximal rates of P_i uptake were not observed before the external K⁺ concentration reached 1 mM (Fig. 1).

P_i-dependent K⁺ uptake

Transport of K⁺ into cells, after the addition of P_i, was studied in the isogenic pair, strains AN710 (*phoT101*) and AN1088 (*pit*) possessing only the Pit or Pst system respectively. In all cases the addition of P_i to the equilibrated cell suspension caused a rapid inflow of K⁺ (Fig. 2). The phenomenon depended absolutely on the presence of an energy source, in this case glucose (Fig. 2). DL-Lactate, glycerol and succinate were also effective energy

Fig. 1. K⁺-dependence of P_i uptake

Cells were depleted of K⁺ by treatment with dinitrophenol as described in the text, washed three times and suspended in the K⁺-free buffer containing 20 mM-glucose. KCl was added to the required concentration (monitored with the K⁺-selective electrode) and the initial rates of [³²P]P_i uptake were measured as given in the Materials and Methods section. ○, Strain AN1088 (Pst system); ●, strain AN710 (Pit system). Inset: cells of strain AN710 (Pit system) were treated with dinitrophenol and prepared as above. The uptake of [³²P]P_i was measured at 30 s intervals, with 20 mM-glucose as energy source. At 1.5 min (arrow), KCl was added to a final concentration of 1 mM.

sources in cells grown with these compounds as carbon sources. The P_i-dependent uptake of K⁺ was abolished by 5 min preincubation with 5 mM-*N*-ethylmaleimide, an inhibitor of P_i transport (Medveczky & Rosenberg, 1971). The ability of a variety of anions to replace P_i in prompting K⁺ influx was tested (Fig. 3). Acetate, propionate, nitrate and thiocyanate failed to replace P_i under these conditions (Fig. 3). Arsenate was tested as a potential inhibitor in this system by adding 0.2 mM-arsenate after the addition of P_i (Fig. 4). Arsenate did not affect K⁺ uptake into cells carrying the Pst system, but inhibited K⁺ transport in cells carrying the Pit system, which has been shown to be responsible for arsenate sensitivity (Rosenberg *et al.*, 1977). When arsenate was added before the P_i, it inhibited K⁺ uptake completely in both systems (results not shown).

In a study of the quantitative relationship between

P_i transport and K^+ entry (Fig. 5), both strains tested were found to take up K^+ with P_i in a ratio close to 2:1, within the pH range 6.8–7.2 (Table 2). This movement of ions into the cells occurred during a substantial efflux of H^+ , which followed the addition of 5 mM-glucose (results not shown).

The observed K^+/P_i ratio in the presence of excess KCl applied only to initial rates; the net uptake of K^+ declined after 1–2 min, while P_i entry continued, although at a lower rate.

Na^+ stimulation of glucose-supported K^+ uptake

Under standard conditions (with LiCl-based buffer), addition of NaCl to 5 mM, while K^+ was being taken up after the addition of P_i , caused a 2-fold increase in the net rate of K^+ uptake (Fig. 6). Addition of the NaCl before the P_i had the same effect. When cells were suspended in a choline chloride

Fig. 2. K^+ movements with P_i

Cells were grown overnight with 20 mM-glucose as carbon source and were starved as described in the Materials and Methods section. They were then washed three times in the K^+ -free buffer, pH 6.8, containing LiCl (20 mM), triethanolamine/citrate (50 mM) and $MgSO_4$ (1 mM). The cells were resuspended in the same buffer at $A_{660} = 5.0$ (2.2 mg dry wt./ml); 4 ml of this suspension was placed in the electrode cell and KCl was added to 0.5 mM and glucose to 5 mM (trace *a*). When cells reached ionic equilibrium a standard addition of 500 nmol of KCl was made to calibrate the apparatus (white arrow, K^+). Such an internal calibration was made with each run. Then 500 nmol of sodium phosphate was added (black arrow, P_i). Trace (b) shows the same experiment with glucose not added at the onset but at some time after the addition of phosphate (white arrow, Glc). All traces in this and following Figures are direct photographs.

Fig. 3. Counter ions for K^+ influx

Cell suspensions were prepared as described in Fig. 2. Sodium thiocyanate (500 nmol) (white arrow, C) was added as a counter ion, followed by P_i (500 nmol). Similar results were obtained when the sodium salts of acetate, propionate and nitrate (500 nmol) were also tried as counter ions. All other symbols are as in Fig. 2.

Fig. 4. Effect of arsenate on K^+ movements

K^+ -uptake experiments were conducted as for Fig. 2, but during the phase of K^+ uptake, 0.2 mM-sodium arsenate was added to the electrode vessel (black arrow, As). Other symbols are as in Fig. 2. Trace (a): strain AN1088; trace (b): strain AN710.

Fig. 5. Stoichiometry of K⁺ and P_i influx

Cell suspensions were prepared as described in Fig. 2, and the procedure for the measurement of K⁺ uptake was as previously described, except that 500 nmol of [³²P]P_i (10 μCi/μmol) was added to the equilibrated cell suspensions (black arrow, P_i). Samples of 100 μl were collected from the electrode vessel at 20 s intervals thereafter, diluted with 0.5 ml of suspending medium, filtered, washed and counted for radioactivity as described in the Materials and Methods section. Internal K⁺ standards (500 nmol; white arrow, K⁺) were added at the start of each experiment to calibrate the system. These data, and standard counts on the [³²P]P_i solution, appropriately corrected for background, were used to express the uptake of both ions in terms of mg/mg dry wt. The ordinate scale for K⁺ is double that of P_i. Trace (a): strain AN1088; trace (b): strain AN710.

Table 2. Stoichiometry of cation entry with P_i in strains of *E. coli* at pH 7.0

K⁺/P_i uptake ratios were determined as described in Fig. 5, with 5 mM-glucose as energy source. H⁺/P_i uptake ratios were determined as described in Fig. 7, after a pulse of 100 μM-glucose. Each ratio was derived from five determinations of initial rates and is shown as the mean ± S.E.M.

Strain used	AN710	AN1088
P-system operating	Pit	Pst
K ⁺ /P _i ratio	1.7 ± 0.13	1.8 ± 0.26
H ⁺ /P _i ratio	1.0 ± 0.22	0.79 ± 0.25

buffer, K⁺ uptake was not stimulated by LiCl at concentrations up to 20 mM (result not shown). Thus, in this system, unlike in the Na⁺/H⁺ exchange described by Schuldiner & Fishkes (1978), Li⁺ cannot replace Na⁺. This stimulation was found for both the Pit and Pst systems, and was observed to the same extent regardless of whether the cells had been grown and starved in the absence of Na⁺ or not. However, it was not seen in dinitrophenol-treated cells.

P_i uptake in K⁺-transport mutants

Genetic and kinetic studies have demonstrated the existence of at least four K⁺-transport systems in *E. coli*, with various affinities for K⁺ (Epstein & Kim, 1971; Rhoads & Epstein, 1977).

The system coded by the *kdp* genes is a repressible high-affinity system driven by phosphate-bond energy. Of the several constitutive systems coded by the *trk* loci, the TrkA system is responsible for the bulk of the K⁺ uptake by wild-type cells. This system, and the TrkD system, are singular in requiring both ATP and a protonmotive force for activity (Laimins *et al.*, 1978). The TrkF system is characterized by a low rate of uptake and is driven by the protonmotive force (Rhoads *et al.*, 1976).

Table 3 shows the rates of P_i uptake in several mutants defective in K⁺ uptake. Mutants carrying a *kdp* deletion took up P_i at normal rates. Introduction of a *trkA* mutation into such mutants decreased the rate of P_i uptake by about one-third, and the triple mutant, *kdp, trkA, trkD*, showed very low rates of P_i uptake. However, the presence of the system coded by the *kdp* gene cluster enabled a *trkA, trkD* mutant to take up P_i at a considerable rate.

Table 3. P_i uptake by K^+ -transport mutants

Glucose (20mM) was used as the carbon source in growth and as the energy source for P_i uptake. The uptake of P_i was measured as described in the Materials and Methods section.

Strain	P_i uptake (nmol/min per mg dry wt.)
Frag 5 (Kdp^-)	23.91
2k133 (Kdp^- , $TrkA^-$)	14.44
2k142 (Kdp^- , $TrkE^-$)*	13.28
2k401 (Kdp^- , $TrkA^-$, $TrkD^-$)	3.44
TAD110 (Kdp^+ , $TrkA^-$, $TrkD^-$)	20.18

* The product of the *trkE* gene is believed to be necessary for the highest rates of K^+ uptake in the *TrkA* and *TrkD* systems (Rhoads *et al.*, 1976).

Fig. 6. Effect of Na^+ on K^+ influx

The cells were washed and suspended in K^+ -free buffer as detailed in Fig. 2. Na^+ was excluded and all buffers were adjusted with LiOH. NaCl (5mM) was added where indicated (white arrow, Na^+).

Fig. 7. H^+ movements with P_i

Cells were grown and then depleted of P_i as described in Fig. 2. The cells were then harvested, washed three times in the lightly buffered medium, and resuspended in this buffer at A_{660} 5.0. Portions (2.5 ml) of the cell suspension were placed in the electrode vessel and aerated and stirred. When the cells reached ionic equilibrium they were adjusted to pH 6.9, and 40 μ M-glucose was added (white arrow, Glc). After the transient drop in pH (to about 6.8), [^{32}P] P_i (10 μ Ci/ μ mol) was added (black arrow, P_i) to 0.2mM [traces (a) and (b)]. Alternatively, the suspension was adjusted to pH 6.8 and [^{32}P] P_i was added as above, without the prior addition of glucose [traces (c) and (d)]. Samples of 0.1 ml were collected at 10s intervals, diluted with 0.5 ml of the suspending medium, filtered, washed and counted for radioactivity as described in the Materials and Methods section. The rate of P_i uptake was calculated as described in Fig. 5. The rates of H^+ movement were calculated from the traces, by using a standard deflection made on the recorder by the addition of 100nmol of HCl at the end of each run. The increment in the rate of H^+ movement caused by the addition of P_i was used in the calculation of H^+/P_i ratios. Traces (a) and (c); strain AN710. Traces (b) and (d): strain AN1088.

Fig. 8. K^+/P_i and H^+/P_i transport ratios as a function of pH

The ion ratios were determined for both the Pit (a) and Pst (b) systems as described in the text. All values are means $\pm$ S.E.M. for four determinations. $\bullet$, H^+/P_i ; $\blacksquare$, K^+/P_i ; $\square$, $(H^+ + K^+)/P_i$ Total negative charge on the P_i at the given pH, on the ordinate scale shown.

Fig. 9. Simultaneous measurement of the uptakes of K^+ , H^+ and P_i

Cells of strain AN710 were grown overnight as described (see the Materials and Methods section and Fig. 2), with 20mM-sodium succinate as carbon

source. These cells were harvested, washed twice with phosphate-free medium (pH 7.0), resuspended in this medium, supplemented as for growth, at A_{660} 0.3, and shaken at 37°C for 2h to deplete them of P_i . They were then harvested, washed three times in a modified K^+ -free buffer, pH 7.0 [LiCl (20mM)/triethanolamine/citrate (3mM)/MgSO₄ (1mM)] and resuspended in this buffer at A_{660} 5.0. Portions (4ml) of this cell suspension were placed in the electrode vessel, and 5mM-glucose was added. If necessary, the pH was adjusted to 6.9–7.0 with LiOH, and [^{32}P]P_i (10 μ Ci/ μ mol) (pH 7.0) was added. The K^+ and H^+ movements could be monitored simultaneously with the dual-pen Rikadenki recorder, and the uptake of [^{32}P]P_i was followed by withdrawing 100 μ l samples at 20s intervals. These were diluted with 0.5ml of suspending medium, filtered, washed and counted for radioactivity as described (see the Materials and Methods section). At the end of each run, 250nmol of K^+ and 50nmol of HCl were separately added to calibrate the system. Blanks and standards for [^{32}P]P_i were prepared as before (Fig. 5). The rates of simultaneous K^+ , H^+ and [^{32}P]P_i movements could thus be calculated. The traces (a, K^+ ; b, H^+) shown have been copied from the original recordings and arranged in time synchrony to compensate for the slight time-shift on the original recording caused by the dual-pen arrangement.

H⁺ movements

The addition of glucose to cells suspended in the lightly buffered medium resulted in the rapid net extrusion of H⁺. As the glucose was exhausted, net H⁺ removal from the medium was observed. In strains of both the Pit and Pst phenotype the rate of H⁺ 'fallback' was significantly increased on the addition of P_i (Figs. 7*a* and 7*b*), and the increment can be quantitatively compared with the rate of P_i uptake.

The addition of P_i without prior addition of glucose also resulted in a concomitant uptake of H⁺ and P_i, albeit at much reduced rates (Figs. 7*c* and 7*d*).

The H⁺/P_i uptake ratios were calculated from the results of experiments such as those shown in Fig. 7. For both strains AN710 and AN1088, over the neutral pH range 6.8–7.2, these ratios were close to 1 (see Table 2). Similar values for the H⁺/P_i uptake ratios were obtained in the presence and absence of glucose (results in the absence of glucose not shown).

Effects of pH on ion movements

The movements of both K⁺ and H⁺ with P_i were measured across the pH range 5.5–7.5 (Fig. 8). Outside the pH range 6.8–7.2, the K⁺/P_i uptake ratio decreased with a corresponding increase in H⁺ movement into the cell. Similar results were obtained for both P_i-transport systems. It should be noted that the respective movements of the two ions were measured under somewhat different conditions. The concentration of glucose required to support adequate K⁺ uptake (5 mM) was not suitable for the study of H⁺ symport, as it produced a continuing rapid efflux of H⁺ throughout the experimental period. The optimal glucose concentration for the measurement of H⁺ symport was 40 μM (Fig. 7).

Some experiments have been carried out with succinate-grown cells of strain AN710. With such cells suspended in a modified K⁺-free buffer [LiCl (20 mM)/triethanolamine/citrate (3 mM)/MgSO₄ (1 mM)] it has been possible to measure simultaneously H⁺ and K⁺ influxes (Fig. 9). In this case ratios of K⁺/P_i = 1.1 (±0.1) and H⁺/P_i = 1.0 (±0.2) (*n* = 5) (means ± s.e.m.) were obtained at pH 7.0. Such values are not significantly different from those obtained from the independent measurements of both ions in glucose-grown cells (Table 2).

Discussion

In this work, the relationship between K⁺ and P_i transport postulated by previous workers has been established as obligatory in both P_i-uptake systems.

In the pH range 6.8–7.2, the initial rates of entry of the two ions yield a K⁺/P_i ratio approaching 2. The rate of entry of K⁺ declines after 1–2 min, although

P_i entry continues. An explanation for this may be found in the observation (Medveczky & Rosenberg, 1971) that, in P_i-depleted cells, esterification of P_i is in progress after the first minute of uptake, when the internal pool of P_i appears to have reached capacity. The cessation of net K⁺ uptake at this time suggests a connection between the movements of this ion and the P_i pool in the cell.

The requirement of P_i for K⁺ transport is specific, as evidenced by the fact that none of a variety of anions tested could replace P_i, and that K⁺ uptake was inhibited by inhibitors of P_i transport, namely *N*-ethylmaleimide and arsenate.

The effect of arsenate deserves some comment. This ion competes effectively with P_i for entry in the Pit system, but not in the Pst system (Rosenberg *et al.*, 1977). Predictably, arsenate inhibited P_i-dependent K⁺ uptake only in the Pit system. The inhibition by arsenate of K⁺ transport in the Pit-carrying strain could be due to either inhibition of P_i transport, with concomitant inhibition of K⁺ uptake, or a direct effect on the K⁺-transport systems. The three Trk transport systems are known to be arsenate-sensitive (Rhoads & Epstein, 1977).

It follows from the results obtained with the K⁺-transport mutants that P_i is transported adequately by these strains, provided that at least one K⁺-transport system is functional. These experiments give no indication whether the K⁺-transport systems exhibit a similar lack of selectivity for the P_i-transport system, which provides the flux of the counter ion. The K⁺-transport mutants used here were derived from *E. coli* K12 (Epstein & Davies, 1970) and presumably have both P_i-transport systems present. Experiments with these K⁺-transport mutants, from which one or the other P_i-transport system has been eliminated, indicate that the individual K⁺-transport systems show no preference for a particular P_i-transport system, indicating that P_i and K⁺ do not use the same porter (L. M. Russell & H. Rosenberg, unpublished work).

The presented data show that H⁺ as well as K⁺ enters with P_i in the presence of excess K⁺. Recent work with the Pit system (H. Rosenberg, unpublished work) has shown HPO₄²⁻ to be the ionic species that interacts with the transport system. The negative charges on the P_i entering the cell can, in fact, be balanced, with a large margin, by the inward flow of H⁺ alone at low pH and by that of K⁺ alone at the higher pH values. If the independently measured ion movements are summed (Fig. 8), it appears that the phosphate-cation complex taken up bears an overall positive charge, especially at the more acidic pH values. It may be argued that such a summation is not valid, as the respective movements of K⁺ and H⁺ were studied under different conditions. However, optimal inward movements of the two ions during P_i transport require different glucose concentrations,

and this makes their simultaneous measurement impracticable.

The coupling of metabolite transport to H⁺ movements implies in most cases either symport or antiport of substrate with one or more H⁺ (Harold, 1977b). The question of H⁺ symport with P_i in *E. coli* must be approached with caution, especially when H⁺ appears to be symported with P_i in the Pst system, which involves a binding protein and requires phosphate-bond energy.

On theoretical grounds, Rottenberg (1976) predicts that the inwardly moving complex (substrate plus carrier) that crosses the membrane is always neutral. The net charge of this complex is determined by the charge on the substrate, the number of H⁺ co-transported and the charge on the carrier-binding sites. Ramos & Kaback (1977), however, argue that at external pH values higher than 6.0–6.5 the H⁺ gradient is thermodynamically insufficient to support protonmotive-force-driven transport, unless the stoichiometry between H⁺ and transported substrates increases. This does not appear to be the case for P_i transport, even in the Pit system, which requires a protonmotive force.

It is not clear how the addition of NaCl stimulates P_i-dependent K⁺ influx. A possible mechanism is for Na⁺ to enter the cell and be expelled by the Na⁺/H⁺ antiport (West & Mitchell, 1974), to which the additional influx of K⁺ can be coupled (Skulachev, 1978). The route by which the Na⁺ originally enters is not likely to be the H⁺/Na⁺ antiport. In that process Li⁺ is as effective as Na⁺ (Schuldiner & Fishkes, 1978), but we find that Li⁺ does not elicit the increase in K⁺ influx. It is more likely that the Na⁺ enters through the antiport, which Brey *et al.* (1978) designated 'system 1'. This system antiports H⁺/K⁺, H⁺/Na⁺ and H⁺/Rb⁺, but not H⁺/Li⁺.

The nature of the obligatory link between the transport of K⁺ and P_i can now be considered. The results presented above can be interpreted in two different ways. If P_i enters the cell with its charges balanced largely by H⁺, acidification of the cell cytoplasm would result. This could be relieved by the movement of H⁺ out of the cell, and the concomitant influx of K⁺ to compensate for the net electrogenic effect of the H⁺ flux.

Alternatively, it could be envisaged that a P_i carrier and a K⁺ carrier may be associated in the membrane in a manner such that their transport activities are stringently linked. The results with the mutant strains indicate that the association between the individual systems is permissive, in that the combination of any one of the two P_i-transport systems with any of the K⁺-transport systems is functional.

The systems depicted in either of the two schemes would show experimentally the same net result, namely the symport of K⁺ with P_i.

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