The role of potassium transport in the generation of a pH gradient in *Escherichia coli*

R. G. KROLL and I. R. BOOTH*

Department of Microbiology, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, Scotland, U.K.

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The role of K⁺ transport in the generation of a pH gradient in *Escherichia coli* has been investigated. In K⁺-depleted cells, net K⁺ uptake dissipated $\Delta\psi$ (membrane potential) and led to an increase in ΔpH (pH gradient). The magnitude of the ΔpH formed bore a simple relationship to the net K⁺ uptake and was substantially independent of the respiratory rate. In K⁺-replete cells, generation of a pH gradient was again K⁺-dependent, although no net uptake of this cation occurred. The results are discussed in terms of K⁺ cycling, and it is suggested that ΔpH is in part a function of the rate of cycling and independent of the respiratory rate.

Since the advent of Mitchell's (1966, 1973) Chemiosmotic Hypothesis, the central role of the proton in microbial energetics has been recognized. In prokaryotes, solute transport, adenosine triphosphate synthesis and cell motility can be driven by a transmembrane electrochemical potential of protons, the protonmotive force. Generation of the protonmotive force may be achieved in a number of ways, all of which involve vectorially organized proton pumping (Garland, 1977). However, in all organisms performing oxidative phosphorylation, a proton cycle must be established between the proton-efflux mechanism and an uptake system, which is predominantly the adenosine triphosphatase (Mitchell, 1966; Kell, 1979). Consequently, generation of the protonmotive force must strike a balance between the requirement for synthesis of ATP and the requirement for an appropriate internal pH.

The protonmotive force (Δp) is composed of two components, the membrane potential ($\Delta\psi$) and the pH gradient (ΔpH) as follows:

$$\Delta p = \Delta\psi - Z\Delta\text{pH}$$

where Z equals $2.303RT/F$ and converts the transmembrane pH difference into mV. Over the past 5 years it has become apparent that the internal pH is relatively constant and that, as the external pH is raised from acidic to alkaline values, ΔpH and $\Delta\psi$ are exchanged as the principal components of Δp (Padan *et al.*, 1976; Booth *et al.*, 1978, 1979;

* To whom queries and reprint requests should be addressed.

Abbreviations used: Δp , protonmotive force; $\Delta\psi$, membrane potential; ΔpH , pH gradient; pH_i , internal pH; pH_o , external pH; TPP⁺, tetraphenylphosphonium ion.

Zilberstein *et al.*, 1979). Thus at low external pH the pH gradient generally exceeds $\Delta\psi$, whereas above pH 7.8 the membrane potential is the major component of the protonmotive force.

Mitchell (1966) has proposed that cation/proton antiports could play a major role in bringing about the exchange of $\Delta\psi$ for ΔpH . In prokaryotic cells there are antiports for several cations (Silver, 1978), but those for Na⁺ and K⁺ have received most attention as possible systems for the regulation of internal pH (pH_i), and hence the exchange of $\Delta\psi$ for ΔpH . Padan *et al.* (1976) and Schuldiner & Fishkes (1978) have both suggested that the Na⁺/H⁺ antiport may play a role in pH_i regulation in *Escherichia coli*. A mutant strain of *E. coli* that lacks the Na⁺/H⁺ antiport has been isolated and found not to grow at external pH 8 (Zilberstein *et al.*, 1980). However, the physiological basis of the inability to grow at alkaline pH was not elucidated, and measurements of pH_i were not made. Similarly, loss of the Na⁺/H⁺ antiport of the alkalophile *Bacillus alkalophilus* has been reported to shift the optimum pH for growth to a more acid external pH (Krulwich *et al.*, 1979). Thus Na⁺ may play a role in the regulation of pH_i in alkaline environments.

The possession by *E. coli* of a K⁺/H⁺ antiport (Brey *et al.*, 1980) seems anomalous in view of the central role of K⁺ in the metabolism of this organism (Epstein & Laimins, 1980). The intracellular K⁺ content appears to be largely a function of the osmotic pressure of the growth medium (Epstein & Schultz, 1965). Active uptake of the cation is mediated by both an inducible high-affinity K⁺-dependent ATPase (*Kdp*) and a constitutive lower-affinity system (*TrkA*), which may function as a

K⁺/H⁺ symport (Epstein & Laimins, 1980; Bakker, 1980). The presence of an extrusion mechanism for K⁺ suggests that the antiport has a regulatory role, either in the maintenance of intracellular K⁺ content or in pH_i regulation. Circumstantial evidence points to a role in pH_i regulation. Thus, in *E. coli*, the antiport has an alkaline pH optimum (Brey *et al.*, 1980), and loss of the antiport activity leads to inability to grow at alkaline pH (Plack & Rosen, 1980). Recent studies have shown that, in resting cells, rapid alkalization of the medium or stimulation of respiration at alkaline pH leads to K⁺ excretion from *E. coli* cells (Yamasaki *et al.*, 1980). Thus the evidence suggesting a role for K⁺ in pH_i regulation is quite strong.

The work described in the present paper was undertaken to increase our understanding of the role of cation transport in the regulation of internal pH. These studies confirm the importance of K⁺ as a regulatory cation and suggest that cycling of this cation is an intrinsic part of the pH_i control mechanism.

Materials and methods

Materials

All chemicals were purchased from BDH (AnalaR), except thiamin, Mes (4-morpholine-ethene-sulphonic acid), Tris, chloramphenicol and catalase, which were obtained from Sigma.

All radiochemicals were purchased from The Radiochemical Centre, Amersham, Bucks., U.K., except [7-¹⁴C]benzoic acid, which was purchased from New England Nuclear Corp. and was generously donated by Unilever Research, Colworth House, Sharnbrook, Bedford, U.K.

Bacterial strain

All experiments were performed with *E. coli* K12 *Frag 1* (*Kdp*⁺ *Trk* *A*⁺ *thi-1*, *rha-4*, *lac Z-82*, *gal-33*) supplied by Dr. W. Epstein, University of Chicago, Chicago, IL, U.S.A. The strain was maintained both as freeze-dried cell suspensions and on slopes of Yudkin's complex medium at 4°C as described previously (Booth *et al.*, 1979).

Growth conditions

Portions (100 ml) of growth medium (see below) in a 250 ml Erlenmeyer flask were loop-inoculated from a slope culture of *E. coli* *Frag 1*. Growth occurred overnight at 30°C on a New Brunswick shaking incubator. After overnight growth the stationary-phase cultures were diluted with fresh growth medium to an *A*₆₅₀ value of 0.5 and incubated until it increased to 1. An *A*₆₅₀ of 2 was taken to be equivalent to 1 mg dry weight of cells/ml (D. Strickland & I. R. Booth, unpublished work).

Two growth regimes were employed. (a) K⁺-replete cells (K20). These cells were grown in glucose minimal medium, which was 20 mM with respect to K⁺ (Epstein & Kim, 1971). In such cells the activity of the *Kdp* transport system was not expressed. (b) K⁺-depleted cells (K0). These cells were grown overnight in glucose minimal medium, which was 100 μM with respect to K⁺. Subsequently they were diluted with fresh K⁺-free glucose minimal medium (approx. 20 μM-K⁺; Epstein & Kim, 1971). This treatment decreased the K⁺ content from 282 ng-ions of K⁺/mg dry weight of K20 cells to 133 ng-ions of K⁺/mg dry weight of cells, and also de-repressed the *Kdp* transport system (Rhoads *et al.*, 1976).

Preparation of cell suspensions

Cell suspensions for transport experiments were harvested by centrifugation at 16 000 *g* at 4°C for 5 min and washed in a cold alkaline-metal-cation-free-buffer (contaminating K⁺ and Na⁺ not detectable by flame photometry) consisting of 3.07 mM-Mes, 6.93 mM-Tris and 150 mM-choline chloride, pH 6.3, containing 50 μg of chloramphenicol/ml to inhibit protein synthesis. The cells were washed three times in this manner and the final suspension of cells stored on ice for up to 4 h until required.

Measurement of internal pH

The magnitude of the pH gradient was estimated from the distribution of a weak acid, and pH_i was calculated from a knowledge of pH_o (Booth *et al.*, 1979). Cell suspensions (approx. 2 mg dry wt./ml) were incubated at 25°C in a stirred glass vessel with a large surface-area-to-volume ratio to ensure continuous aeration. The weak acids, [2-¹⁴C]dimethylloxidinedione (10 μM final concn.; sp. radioactivity 14.3 Ci/mol) and [7-¹⁴C]benzoic acid (2 μM final concn.; sp. radioactivity 24.4 Ci/mol), were added to the cell suspension together with [³H]inulin (0.5 μM final concn.; sp. radioactivity 2.2 Ci/nmol) as an extracellular water marker; catalase (250 μg · ml⁻¹ final concn.) was also present. After 5 min incubation, 500 μl samples were removed and transferred to Eppendorf centrifuge tubes (1.6 ml capacity) containing 10 μl of 2-volume (0.6%, w/v) H₂O₂. The action of catalase on H₂O₂ released O₂, which maintained aerobic conditions in the assay during the centrifugation step. In the absence of these precautions, ΔpH measurements showed considerably greater variability, and Δψ measurements were significantly decreased (S. Ahmed, & I. R. Booth, unpublished work). The cells were separated from the supernatant by rapid centrifugation at 12 000 *g* for 20 s in a Mechanika Preczyjna microcentrifuge type 320A modified for short time intervals by the supplier (Burkard Scientific Sales Ltd., Uxbridge, Middx., U.K.). A

portion (100 μ l) of the supernatant was removed and transferred to a similar centrifuge tube containing a similarly treated cell pellet that had not been incubated with radiochemicals. This enables calculation of the contribution of trapped and intracellular ¹⁴C label to the radioactivity in the experimental pellet (Booth *et al.*, 1979). The remaining supernatant was aspirated off with a vacuum line. The cell pellets were resuspended in 300 μ l of the Tris/Mes/choline chloride buffer and a portion (200 μ l) added to 2 ml of Triton X-100/toluene/PPO (2,5-diphenyloxazole)/POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene] scintillant (333 ml of Triton X-100, 667 ml of toluene, 4 g of PPO and 100 mg of POPOP). The samples of supernatant and pellet were counted for radioactivity on the preset ³H/¹⁴C program of a Packard 3255 liquid-scintillation counter. The pH gradient was calculated as described previously (Booth *et al.*, 1979).

Measurement of the membrane potential

The membrane potential was measured by the same experimental protocol as was used above for Δ pH. A lipophilic cation, tetraphenylphosphonium (TPP⁺) (Zilberstein *et al.*, 1979), was used as the indicator of the membrane potential. [U-¹⁴C]TPP⁺ at a concentration of 4 μ M (sp. radioactivity 8.7 Ci/mol) was incubated with cells and the transmembrane distribution of the ion estimated as described above. A correction based on the uncoupler-insensitive binding to cells was applied to the observed accumulation (Booth *et al.*, 1979). The membrane potential was calculated by substitution of the accumulation data into the Nernst equation.

K⁺-transport assays

The rate of uptake of K⁺ was determined by observing the disappearance of the ion from the medium in which the cells were suspended. Cells at a density of 2 mg dry weight/ml were incubated as described above, and at timed intervals 1 ml of cells were filtered through a Millipore filter (2.5 cm, 0.45 μ m pore size) and the filtrate collected in test tubes within the filtration assembly. The K⁺ content of the filtrate was assayed by flame photometry as described previously (Booth *et al.*, 1979).

The incubation buffer at pH 6.3 was as described above. Studies at pH 8.4 were performed in a similar Mes/Tris/choline chloride system in which the Mes was present at 2.0 mM and Tris at 8.0 mM.

Respiration studies

The rate of respiration was measured in a oxygen electrode as described previously (Booth *et al.*, 1979).

Measurement of internal volume

The cytoplasmic volume of K0 and K20 cells was

Table 1. *Cytoplasmic volume of E. coli Frag 1*
For details, see the Materials and methods section and Booth *et al.* (1979).

Additions	Volume (μ l/mg dry weight of cells)	
	K ⁺ -replete K20	K ⁺ -depleted K0 cells
None	1.25 ($\pm$ 0.07)	0.88 ($\pm$ 0.1)
2 mM-Glucose	1.34 ($\pm$ 0.05)	0.95 ($\pm$ 0.15)
2 mM-KCl	1.27 ($\pm$ 0.05)	1.07 ($\pm$ 0.14)
2 mM-Glucose + 2 mM-KCl	1.44 ($\pm$ 0.1)	1.39 ($\pm$ 0.14)

estimated by the exclusion of a low-molecular-weight marker, [U-¹⁴C]taurine (sp. radioactivity 114 Ci/mol; 0.9 μ M), in the presence of ³H₂O (Stock *et al.*, 1977). The true cytoplasmic volume was found to be a significant experimental variable (Table 1). Thus K⁺-depleted cells (K0) were smaller than those replete with K⁺ (K20). However, after incubation with glucose and K⁺, both sets of cells had similar volumes (Table 1). In terms of the parameters Δ pH and $\Delta\psi$, the volume changes in K0 cells have significant effects on the measured values. By assuming the largest volume under all conditions, one would underestimate $\Delta\psi$ and Δ pH by a maximum of 14 mV or 0.26 unit respectively.

When hydroxymethylinulin and inulin were used to measure the total cell volume (periplasm plus cytoplasm) in K20 cells, they registered 1.6 μ l/mg dry weight and 1.3 μ l/mg dry weight, respectively. Thus inulin and taurine gave similar values in K20 cells, and in subsequent experiments to measure pH_i and $\Delta\psi$, [³H]inulin was chosen as the marker of external water.

Results

As the transport of K⁺ has been implicated in the control of internal pH (Plack & Rosen, 1980), we examined the uptake of the cation into cells grown on K0 and K20 media. Although K0 cells had a low K⁺ content (133 ng-ions of K⁺/mg dry weight of cells), the actual internal concentration was estimated to be approx. 151 mM, owing to the cell shrinkage (Table 1). This is 60–70% of the K⁺ concentration of K20 cells (227 mM). Nonetheless, the K⁺-depleted K0 cells rapidly removed K⁺ from the incubation medium until a steady state was reached after 5–10 min (Fig. 1). Glucose (1 mM final concn.) stimulated both the rate and extent of K⁺ uptake, and at an external concentration of 400 μ M-KCl, the maximum uptake of 180 ng-ions of K⁺/mg of cells was observed. The internal K⁺ concentration rose in these cells to approx. 226 mM, since the uptake of 180 ng-ions of K⁺/mg dry weight

Fig. 1. Uptake of K^+ by K^+ -depleted $K0$ ($\square$, $\blacksquare$) and K^+ -replete $K20$ ($\circ$, $\bullet$) cells of *E. coli* strain Frag 1 in the presence of 1 mM-glucose ($\blacksquare$, $\bullet$) or in the absence of glucose ($\square$, $\circ$)

The cell suspension contained 2 mg dry weight of cells/ml and an initial K^+ concentration of 400 μM . Uptake was measured as K^+ disappearance from the medium. In subsequent experiments, measurement of $\Delta\psi$ and ΔpH was made 5 min after addition of K^+ and glucose.

of cells was accompanied by an expansion of the cell volume from 0.88 μl /mg dry weight of cells to 1.39 μl /mg dry weight of cells (Table 1). This value is almost identical with that found in K^+ -replete cells and gives some indication of the remarkable capacity of *E. coli* to regulate its internal concentration of K^+ .

Endogenously respiring $K20$ cells, on the other hand, showed a slow but significant leakage of K^+ (Fig. 1), the rate of which was substantially independent of the external K^+ concentration, at around 5 ng-ions of K^+ /min per mg dry weight of cells (results not shown). This leakage was prevented by glucose, but no net uptake of K^+ was observed at any K^+ concentration tested.

Harold (1977) has suggested that net K^+ uptake is essential for the generation of an alkaline internal pH and acts to compensate for the net extrusion of H^+ . It seemed reasonable to expect that this might be the case in $K0$ cells of *E. coli* Frag 1, but not in $K20$ cells, which did not take up K^+ . Thus we investigated the effect of external K^+ concentration on internal pH, membrane potential and oxygen consumption in the presence and absence of glucose at pH 6.3.

Potassium-depleted $K0$ cells

The pH gradient, and hence internal pH, and membrane potential were measured 5 min after the addition of K^+ , at which time net K^+ uptake had virtually ceased (Fig. 1). As the external K^+ concentration was raised from 0 to 250 μM the membrane potential fell by approx. 30 mV and ΔpH increased (Fig. 2). In the presence of glucose, a similar decline in $\Delta\psi$ was observed, but the increase in ΔpH was somewhat greater than in endogenously respiring cells (Fig. 2). Respiration was stimulated by the addition of K^+ , or by the addition of glucose, or by simultaneous addition of K^+ and glucose (Table 2). However, when the data are considered together, the following points emerge.

(a) When net K^+ uptake has occurred (external K^+ greater than zero), there appears to be a relatively simple relationship between the extent of K^+ uptake and the increase in ΔpH , independent of the presence of glucose and so consequently of the respiratory rate (Fig. 3 and Table 2).

(b) In the absence of external K^+ , the enhancement of respiration provoked by glucose addition causes a small but significant increase in ΔpH (Fig. 2 and Table 2).

(c) Thirdly, it is clear that the extent of the depolarization of $\Delta\psi$ provoked by net K^+ uptake is

Fig. 2. Effect of K^+ on $\Delta\psi$ ($\square$, $\blacksquare$) and ΔpH ($\circ$, $\bullet$) in $K0$ cells of *E. coli* Frag 1 in the presence ($\blacksquare$, $\bullet$) and absence ($\square$, $\circ$) of 1 mM-glucose. Other conditions were as in Fig. 1.

Table 2. Effect of K⁺ on respiratory rate, Δψ and ΔpH in *E. coli* Frag 1

	Addition		Respiratory rate (ng of O ₂ ·min ⁻¹ ·mg ⁻¹)	Δψ (mV)	ΔpH (mV)	Δp (mV)
	Glucose (1 mM)	KCl (1 mM)				
K ⁺ -depleted (K0) cells	—	—	38.2 ± 6.7	109 ± 5	9 ± 7	118
	—	+	50.6 ± 7.0	80 ± 5	42 ± 5	122
	+	—	163.3 ± 17.1	121 ± 3	24 ± 6	145
	+	+	211.8 ± 18.1	75 ± 3	64 ± 5	139
K ⁺ -replete (K20) cells	—	—	49.0 ± 4.8	98 ± 3	41 ± 6	138
	—	+	50.4 ± 4.7	99 ± 4	38 ± 4	137
	+	—	173.1 ± 3.6	137 ± 3	41 ± 6	178
	+	+	226.3 ± 20.1	112 ± 3	63 ± 3	175

Fig. 3. Δψ (□, ■) and ΔpH (○, ●) as a function of the net K⁺ accumulation. Other details are as in Fig. 2.

dependent upon the respiratory rate. The ability of cells to maintain Δψ while accumulating K⁺ is considerably increased by the enhancement of the respiratory rate in the presence of glucose (Fig. 3).

(d) Finally, the protonmotive force remained constant during K⁺ uptake, but its distribution between Δψ and ΔpH was dependent on the extent of net K⁺ uptake (Table 2 and Fig. 3).

Clearly, as predicted (Harold, 1977), the pH gradient, and hence internal pH_i, is a function of the net K⁺ translocation. The relative independence of pH_i from the respiratory rate indicates that it is possible for the cell to separate the H⁺ cycle from the regulation of the internal pH.

K⁺-replete K20 cells

From the foregoing results it was expected that pH_i would be independent of the presence of K⁺ in the incubation medium, since net cation movement

Fig. 4. Variation of Δψ (□, ■) and ΔpH (○, ●) in K⁺-replete cells K20 of *E. coli* Frag 1 as a function of K⁺ concentration in the presence (■, ●) and absence (□, ○) of glucose. Other details are as in Fig. 1.

did not occur. Indeed in endogenously respiring cells this appeared to be the case, both Δψ and pH_i being substantially independent of the external K⁺ concentration (Table 2 and Fig. 4). However, when glucose was added, both the membrane potential and the internal pH became K⁺-sensitive. The pH gradient increased between 0 and 100 μM-K⁺ and continued to rise slowly up to 2.5 mM-K⁺. A 'mirror-image' effect was seen on the membrane potential (Table 2 and Fig. 4). In the absence of net K⁺ uptake on the same scale as that in K⁺-depleted

cells, the simplest explanation of these results is that a K^+ cycle was established. This cycle must be electrogenic and dependent on both a rapidly metabolized carbon source and external K^+ . Thus, in the absence of external K^+ , glucose stimulated respiration 3.5-fold (Table 2), causing an increase in $\Delta\psi$ of 39 mV, but no significant change in pH_i (Fig. 4). Addition of K^+ caused a concentration-dependent rise in pH_i and a decline in $\Delta\psi$, but no net change in the magnitude of the protonmotive force (Table 2). The K^+ -dependent depolarization suggests that K^+ uptake is occurring, but the constancy of the external concentration (Fig. 1) indicates that uptake is matched by an equivalent efflux. Hence we must invoke a K^+ cycle to explain the data.

Effect of alkaline pH on K^+ transport

It has fairly recently been reported that a K^+ -excretion mechanism, which was possibly a K^+/H^+ antiport, was activated at alkaline pH

(Yamasaki *et al.*, 1980). The activity was biphasic when lactate was used as a respiratory substrate. We decided to investigate this phenomenon further, since it might represent one half of the proposed K^+ cycle.

In K^+ -replete K20 cells, excretion of K^+ was stimulated by glucose (Fig. 5b) and occurred at a rate that was substantially independent of the external K^+ concentration (results not shown). The extent of efflux was considerable and eventually led to a dramatic release of $A_{260/280}$ -absorbing material and a decrease in A_{650} , possibly indicating cell lysis. When NH_4^+ or PO_4^{3-} (1 mM each final concn.) was added (Fig. 5b) to the incubation medium, K^+ excretion, the decrease in A_{650} and increase in $A_{260/280}$ -absorbing material were considerably decreased (results not shown). The significance of this observation is not known.

K^+ -depleted K0 cells rapidly accumulated K^+ at pH 8.4, as they did at pH 6.3. However, the uptake of K^+ was followed by efflux of K^+ , the rate of which

Fig. 5. K^+ uptake and excretion at pH 8.4 in *E. coli* Frag 1

(a) K0 cells incubated at various K^+ concentrations in the presence of 1 mM-glucose: $\bullet$, 400 μM ; $\blacktriangle$, 200 μM ; $\blacksquare$, 100 μM ; Δ , no added K^+ ; $\circ$, cells incubated at pH 8.4 in the absence of glucose but in the presence of 400 μM - K^+ . (b) Efflux of K^+ from K20 cells of *E. coli* Frag 1 at pH 8.4 (∇ , $\blacktriangledown$, $\square$, $\blacksquare$) in the presence of 1 mM-glucose and (Δ) in the absence of 1 mM-glucose. At the time indicated (arrow), additions were made to cells with glucose: $\square$, control, no addition; ∇ , 1 mM- Na_3PO_4 ; $\blacksquare$, 1 mM- NH_4Cl ; $\blacktriangledown$, 1 mM- Na_3PO_4 plus 1 mM- NH_4Cl .

was stimulated by glucose (Fig. 5a). This effect was entirely dependent on the uptake phase, since cells incubated in the absence of K^+ did not excrete the cation even in the presence of glucose (Fig. 5a). Indeed the onset and rate of excretion appeared to be dependent on reaching a critical internal K^+ concentration, since both parameters were affected by the initial net K^+ uptake. Thus it seems possible that the excretion mechanism responds both to pH (Yamasaki *et al.*, 1980) and to intracellular K^+ concentration; however, this point requires further investigation.

Discussion

The present data confirm that K^+ fluxes play a significant role in the regulation of internal pH in *E. coli*. Net K^+ uptake into K0 cells of *E. coli* caused a significant depolarization of the membrane potential and a concomitant stimulation of the rate of respiration, leading to an increase in pH_i . However, in cells respiring glucose, high rates of oxygen consumption were achieved in the absence of K^+ without the generation of a significant pH gradient (Figs. 2 and 4). Assuming that the oxidation of glucose was fully coupled to proton extrusion, it seems that a stimulation of respiration on its own is not sufficient to bring about the generation of a pH gradient. Rather one must predict that rapid H^+ cycling is achieved, most probably via the ATPase, in the presence of glucose. The role of K^+ uptake would then be to break the cycle by allowing a positive charge to re-enter that is not H^+ . The result would be a decline in $\Delta\psi$ and a resultant increase in ΔpH caused by the net exodus of H^+ . One prediction arising out of this view is that ΔpH should be dependent on the net K^+ uptake, and indeed this was found to be the case (Fig. 3). We do not at present know what the individual roles of the *Kdp* and *TrkA* transport systems are; however, whereas the former should be saturated over the concentration range studied, the *TrkA* system has a much lower affinity for K^+ (Rhoads & Epstein, 1978).

Two cautionary points should be made about this simple view of the effects of glucose and K^+ . Firstly, Neijssel & Tempest (1979) have pointed out that K^+ limitation of *Klebsiella aerogenes* leads to changes in the pathway of glucose metabolism. The significance of this observation is that the oxidation of glucose may not be coupled to H^+ extrusion in the identical manner in the presence and absence of K^+ . Secondly, glucose uptake itself is stimulated by K^+ (D. Strickland, W. A. Hamilton & I. R. Booth, unpublished work), possibly owing to the decrease in $\Delta\psi$ (Reider *et al.*, 1979). Thus although it is clear that net K^+ uptake does lead to an increased internal

pH, several factors may contribute to the final pH_i achieved.

In K^+ -replete K20 cells, net uptake of K^+ was not observed and thus we invoked a K^+ cycle to explain the effects of this cation on ΔpH and $\Delta\psi$. Previous workers on K^+ transport have noted that K^+ exchange via the *Trk* system (*Kdp* is not present in K20 cells) occurs only in the presence of an external energy supply and may require both Δp and ATP (Rhoads & Epstein, 1978; Kepes *et al.*, 1976). However, these studies did not discriminate between exchange mediated solely by the *Trk* system and a cycle involving separate entry and exit routes. K^+ exchange should be electrically silent, unless there is a stoichiometric demand for ATP, and thus $\Delta\psi$ should not be influenced. Cycling, on the other hand, would lead to a net influx of H^+ dependent on the H^+/K^+ stoichiometries of the uptake and efflux pathways (Fig. 6). One might speculate that the K^+ efflux observed in the absence of glucose, continues in its presence, but is compensated for by uptake of K^+ . It also seems possible that the efflux might be stimulated by glucose, as is observed at pH 8.4, but there is at present no evidence for this. The K^+ cycle decreases $\Delta\psi$ through the extra H^+ influx, respiration is stimulated and pH_i increases as a result of the net difference between respiration-linked H^+ extrusion and K^+ -cycle-coupled H^+ re-entry. However, this is not a wholly satisfactory explanation, since a much larger increase in respiratory rate did not affect the internal pH but rather led to an elevated $\Delta\psi$ (Fig. 4). We do not at present have a solution to this problem; however, two possibilities are, firstly, that the major H^+ cycle, operating in the absence of K^+ , does not occur through the bulk phase but rather through membrane-localized proton channels (Kell, 1979). Secondly it may prove to be the case that the role of a K^+ cycle is to lower $\Delta\psi$

Fig. 6. Predicted H^+ and K^+ cycles across the *E. coli* membrane

Entry is via the *Trk* system (a) with a H^+/K^+ stoichiometry equal to n . Exit is via the K^+/H^+ antiport (b) with a stoichiometry equal to x . For each K^+ cycled a net influx of $x + n$ H^+ occurs.

and that this effects subtle changes in the pattern of H^+ re-entry, leading to an increase in pH_i .

In conclusion, therefore, we have presented evidence in support of the idea that K^+ transport plays a role in the regulation of ΔpH . In K^+ -depleted cells, net uptake and cycling of K^+ may be important, whereas in K^+ -replete cells it is the latter that predominates.

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