Energy Coupling to Potassium Transport in *Streptococcus faecalis*

INTERPLAY OF ATP AND THE PROTONMOTIVE FORCE

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We have studied the mechanism by which metabolic energy is coupled to potassium accumulation by the fermentative bacterium, *Streptococcus faecalis*. In starving cells, K⁺ movements into the cells or out are very slow; even 4K⁺/K⁺ exchange requires concurrent metabolism of glucose or arginine. Metabolizing cells accumulate K⁺, establishing a concentration gradient of some 50,000. Accumulation is prevented by reagents that block or short circuit the proton circulation, but 4K⁺/K⁺ exchange persists. In glycolyzing cells whose proton pump has been blocked with N,N-dicyclohexylcarbodiimide, net uptake of K⁺ can be induced by imposing an artificial membrane potential, interior negative. Net K⁺ efflux is also controlled by the interplay of ATP and the proton circulation. Addition of proton conductors to glycolyzing cells induces K⁺ efflux, but has no effect on starving cells; the rate of K⁺ efflux appears to be a function of the cells' ATP content. We conclude that K⁺ accumulation requires the cells to generate both a protonmotive force and ATP. K⁺ uptake is electricgenic and attains a concentration gradient far too steep to be in equilibrium with the membrane potential. We consider two alternative models for K⁺ transport: a primary ATP-driven pump regulated by the proton circulation or a secondary porter activated by ATP that mediates symport of K⁺ with H⁺.

Bacterial cells, like those of plants and animals, extrude Na⁺ from the cytoplasm but accumulate high concentrations of K⁺. The molecular mechanism is not understood, but it is clear that bacteria lack the (Na⁺, K⁺)-ATPase characteristic of animal cells. Instead, the movements of K⁺ inward and of Na⁺ and H⁺ outward are mediated by transport systems that are physiologically and genetically distinct. At least two of the cations, and possibly all three, are transported against the electrochemical potential gradient; passive leakage plays only a minor role (reviews: Harold and Altendorf, 1974; Silver, 1978).

Previous studies with *Streptococcus faecalis* (Harold and Papineau, 1972a and b) led to the proposal that the primary, energy-linked process is the electrogenic extrusion of protons by the membrane-bound ATPase. Potassium would then accumulate in response to the electrical potential, interior negative, while Na⁺ would be expelled by antiport for protons, in accordance with the chemiosmotic view of bacterial energetics (recent reviews include Mitchell, 1976; Harold, 1977; Rosen and Kashket, 1978; Eddy, 1978). However, as was pointed out at the time (Harold and Papineau, 1972b), two observations were not adequately accounted for by this elementary hypothesis. First, the K⁺ concentration gradient appeared to be too large to be in equilibrium with the membrane potential; second, movements of both K⁺ and Na⁺ across the membrane required concurrent metabolism, apparently the generation of ATP. More recently, Rhoads and Epstein (1977) provided strong evidence that K⁺ uptake via the TrKA system of *Escherichia coli* requires both ATP and the protonmotive force.

We report here that K⁺ accumulation by *S. faecalis* also requires the cells to generate both ATP and a proton circulation. Potassium uptake is electrogenic and attains a concentration gradient of the order of 50,000, too large to be in equilibrium with the membrane potential. The findings are considered in terms of two alternative models. The first invokes a primary potassium pump and a separate exit system regulated by the protonmotive force and by ATP, respectively. The second model proposes symport of K⁺ with protons, mediated by a secondary carrier that is energized by the protonmotive force but modulated by ATP.

**MATERIALS AND METHODS**

Organisms and Growth Media—*S. faecalis* (faecium) ATCC 9790 was grown overnight on the complex media NaTY (10 g of Difco tryptone, 5 g of Difco yeast extract, 8.5 g of Na₂HPO₄, and 10 g of glucose/liter) or KTY (tryptone, yeast extract, and glucose as above plus 10 g of K₂HPO₄). The cells were used in three modes. In some experiments, the cells were used directly ("NaTY cells"); in others, the overnight culture was neutralized with KOH and incubated with glucose for 20 min to allow the cells to take up potassium (K⁺-loaded cells). In either case, the cells were collected by centrifugation, washed, and suspended in buffer at pH 8; glucose was then added in order to activate glycolysis (Bakker, 1978). After 5 min, the cells were again centrifuged, washed three times, and resuspended in buffer as indicated for the particular experiments.

For most of the experiments, we used "sodium-loaded cells," whose potassium complement had been replaced by sodium. The procedure employed is based on that described by Rhoads et al. (1976). NaTY cells were suspended in 0.1 M Na⁺-Pipes' buffer, pH 7.1, at a density of guest on August 29, 2017

1. The abbreviations used are: Pipes, 1,4-piperazinediethanesulfonic acid; DiO-C₃(3), 3,3'-dihexyloxacarbocyanine; Heps, 4-(2-hydroxyethyl)-1-piperazinetanesulfonic acid; Mes, 2-(N-morpholino)ethanesulfonic acid; Hepes, 2,6-dihydroxy-1,1,1,7,7,7-hexafluoro-2,6-bis(trifluoromethyl)-heptanone-4; DCCD, N,N,N',N'-dicyclohexylcarbodiimide; TCS, tetrachlorosalicylanilide; FCCP, carbonyl cyanide p-trifluoromethoxyphenoxyhydrzone; TTPM⁺, triphenylmethyl phosphonium ion; TTP⁺, tetraphenylphosphonium ion; DDA⁺, dibenzyl-diethylammonium ion; Δψ, electrochemical potential of protons; ΔpH, membrane potential; ΔpH, pH gradient.

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of 5 mg/ml (dry weight) and incubated at 37°C; glucose was added (60 mM), followed after 5 min by 5 mM 2,4-dinitrophenol. After 90 min, the cells were harvested, activated, and washed three times with 2 mM MgSO4; this sufficed to remove both glucose and dinitrophenol. Sodium-loaded cells contain less than 5 nmol of K+/mg (dry weight) and 0.4 pmol of Na+.

**Transport Experiments**—Cells were suspended in buffer (usually Na+-Hepes) supplemented with glucose, arginine, and other reagents and incubated at 25°C. Cell samples were collected by filtration through Unipore polycarbonate filters (Bio-Rad Laboratories, Hercules, CA). The standard reagents were purchased from Sigma, TPMP+Br and various substances labeled with 14C or 3H were purchased from Du Pont de Nemours, Wilmington, Del. Other ionophores: TCS (Dr. A. Hamilton, Department of Biochemistry, University of Aberdeen, United Kingdom); monensin (Dr. R. J. Hasley, Eli Lilly and Co., Indianapolis); and FCCP and 1799 (Dr. P. G. Heylter, Du Pont de Nemours, Wilmington, Del.).

**RESULTS**

**Basic Features of K+ Transport**—When cells enriched in Na+ or H+ are transferred to fresh medium containing K+ and an energy source, they expel H+ and Na+ and accumulate K+ (Harold and Papineau, 1972a; Harold and Altendorf, 1974). Under the present conditions, cells replete with K+ contain as much as 600 to 700 nmol of K+/mg dry weight or about 0.4 n (Fig. 1; concurrent expulsion of the equivalent amounts of Na+ and H+ is not shown). Cells glycylizing in the presence of limited amounts of K+ (Fig. 1) absorbed K+ until the external concentration had fallen below 10 μM and established a concentration gradient as high as 50,000.

Net potassium uptake was characterized kinetically by a family of Michaelis-Menten curves with the following parameters: at pH 6.0, K+ 0.55 nmol, Vmax 55 nmol Mg2+/min; at pH 7.3, K+ 0.20 nmol, Vmax 68 nmol mg-1 min-1; at pH 8.0, K+ 0.12 nmol, Vmax 35 nmol mg-1 min-1. There was no evidence of sigmoidicity in the curves. The kinetic parameters were not altered by the presence of as much as 0.05 mM Na+ (data not shown).

Potassium accumulated by the cells continued to exchange with that in the medium (Figs. 2 and 3). However, the size of the K+ pool is not simply the resultant of a pump and a constant leak. Fig. 2 shows that, as the cells filled up with K+, the initial rate of 42K+ influx declined from 60 nmol min-1 mg-1 to 3 nmol min-1 mg-1; by contrast, the unidirectional rate of K+ efflux remained approximately constant at 7 nmol min-1 mg-1 (Fig. 3). Evidently, the K+ transport system is subject to some kind of feedback regulation. We shall see that 42K+/K+ exchange differs from net uptake with respect to energy requirements.

**Fig. 1. Accumulation of 42K+ by sodium-loaded cells.** Cells were suspended in 2 mM MgSO4 at a density of 1.0 mg/ml; the pH was maintained at pH 7.5 by continuous titration with LiOH. Glucose (20 mM) was added at -12 min, 42K+ at 0 min. O—O, Plus glucose and 2 mM 42KCl; •—•, plus glucose and 0.2 mM 42KCl; △—△, no glucose, 2 mM 42KCl.

**Fig. 2. Unidirectional influx of 42K+ into glycolyzing cells.** Sodium-loaded cells were suspended in buffer (100 mM Na+-Hepes, 0.2 mM MgSO4, pH 7.5) at a density of 1.0 mg of cells/ml. Glucose (20 mM) was added and the suspension was divided into four aliquots. Twelve minutes later, at 0 min, each aliquot received KCl to 1 mM. 42KCl (20 nCi/ml, 5 μM) was added as follows: •—•, 42K+ at 0 min; this curve represents the overall uptake of K+ by the cells; ○—○, 42K+ at 16 min; △—△, 42K+ at 32 min; [ ]—[ ], 42K+ at 48 min. The numbers indicate the unidirectional rate of 42K+ influx (nanomoles min-1 mg-1 cells) for each aliquot.
Accumulation of $K^+$ by exchange for $Na^+$ or $H^+$ requires an exogenous energy source; even autologous exchange of $K^+$ for $K^+$ in either direction is energy linked, as it occurs only in metabolizing cells (data not shown; see Harold and Altendorf, 1974). We now turn to experiments that address the question, how is the energy derived from metabolism coupled to $K^+$ transport?

Short Circuited Cells Cannot Accumulate $K^+$—Net uptake of $K^+$ is unavoidably linked to the efflux of $H^+$, $Na^+$, or both and cannot be studied in isolation. One can, however, ask whether $K^+$ can be accumulated by cells that have been rendered permeable to $H^+$ and $Na^+$ and are, therefore, unable to generate either $\Delta V$ or $\Delta pH$ across the plasma membrane (Harold and Van Brunt, 1977). This condition was approached by use of a combination of the proton conductor TCS and the sodium/proton ionophore monensin (For a recent review of ionophores see Bakker, 1979).

Results of one such experiment are shown in Fig. 4. Sodium-loaded cells were allowed to glycolyze in 0.2 M $Na^+$-Hepes buffer, pH 8, such that the external sodium concentration was close to the internal one. Addition of $42K^+$ to such cells resulted in rapid and extensive net uptake; this was almost unaffected by monensin, severely inhibited by TCS, and essentially abolished by TCS plus monensin. The ionophores did not inhibit glycolytic generation of ATP. Evidently, net uptake of $K^+$ by exchange for $Na^+$ requires an effective proton circulation. Analogous experiments (Harold and Papineau, 1972b, and new data not illustrated) indicated that accumulation of $K^+$ by exchange for protons likewise depends on the capacity of the cells to generate a proton motive force.

In contrast to net uptake, $K^+$/K$^+$ exchange was not inhibited by ionophores or by inhibitors of the proton-translocating ATPase (Harold and Altendorf, 1974). In one experiment, performed in conjunction with that of Fig. 3, addition of either TCS or DCCD increased the unidirectional efflux rate to as much as 20 nmol min$^{-1}$ mg$^{-1}$ (data not shown). Apparently, metabolic generation of ATP is sufficient to support $K^+$/K$^+$ exchange, but not net $K^+$ uptake.

Accumulation of $K^+$ in Response to an Artificial Membrane Potential—It will be recalled that, in S. faecalis 9790, glycolysis is the sole source of metabolic energy. Respiration and oxidative phosphorylation are absent and the proton circulation is generated by a proton-translocating ATPase (Harold, 1977). Cells whose ATPase has been blocked with DCCD can still generate ATP but no proton motive force. A membrane potential was imposed artificially by raising the pH of the cell suspension in the presence of a proton conductor; diffusion of $H^+$ out of the cells then generates an electrical potential, interior negative (for previous applications of this principle see Niven et al., 1973; Pick and Avron, 1976; Bakker, 1978). Such manipulations made it possible to explore the interplay of ATP and the proton circulation in $K^+$ accumulation.

The basic experiment is illustrated in Fig. 5. Cells fully

![Figure 3](http://www.jbc.org/) Unidirectional efflux of $42K^+$ from glycolyzing cells. Sodium-loaded cells were suspended in buffer and allowed to glycolyze as described in the legend to Fig. 2. At 0 min, $42KCl$ (0.15 mm) was added, followed by excess KCl (10 mm) at 22 min. $\bullet$ $42K^+$ in cells by flame photometry; $\circ$ $K^+$ content of the cells by flame photometry; $\circ$ $42K^+$ in cells, as per cent of the maximal level. The numbers give the unidirectional rates of $42K^+$ efflux at 22, 30, and 55 min in nanomoles min$^{-1}$ mg$^{-1}$ cells.

![Figure 4](http://www.jbc.org/) Inhibition of $42K^+$ accumulation by ionophores. Sodium-loaded cells were suspended in buffer (200 mm $Na^+$-Hepes; 0.2 mm $MgSO_4$, pH 8.0) at 0.5 mg of cells/ml and supplemented with glucose (20 mm). Aliquots received ionophores at $-5$ min and $42KCl$ (1 mm) at 0 min. $\bullet$ No inhibitors; $\circ$ monensin (MOS); $\circ$ TCS (5 PM) was added at $-5$ min; $\circ$ monensin plus TCS; $\circ$ no glucose. The ATP content of the cells was about 0.3 nmol/mg in the absence of glucose, 9 nmol/mg in the glycolyzing control cells, and 6 nmol/mg in the presence of TCS plus monensin.

![Figure 5](http://www.jbc.org/) Accumulation of $42K^+$ in response to an artificial membrane potential. Sodium-loaded cells were suspended in 2 mm $MgSO_4$ at a density of 1.0 mg/ml; the pH was maintained by continuous titration with LiOH. Three successive runs were made. 1) $\circ$ Glucose (20 mm) was added at 0 min and cells were allowed to glycolyze at pH 8.0. At 12 min, 0.3 mm DCCD was added to block the ATPase, and at 27 min the pH was lowered to 6.0 by addition of HCl; TCS (5 PM) was added at 30 min. At this point, we have cells that are glycolyzing and contain ATP (20 nmol/mg) but cannot generate $\Delta V$ due to the presence of DCCD and TCS. $42KCl$ (1.0 mm) was added at 35 min, but uptake was negligible. At 40 min, the pH was raised to 8.0 by addition of LiOH. This should generate an electrical potential, interior negative, by virtue of proton efflux catalyzed by the TCS. The cells now accumulate $42K^+$, generating a concentration gradient of 10-fold. The ATP level rose slowly to 3.0 nmol/mg of cells. 2) $\bullet$ Like Run 1, but without the addition of TCS. There was little uptake of $42K^+$ in response to the pH shift at 40 min; we interpret this to mean that proton efflux is required to generate $\Delta V$ of sufficient magnitude. 3) $\Delta$ Like Run 1 but without glucose. Under these conditions there was no $42K^+$ uptake at all.
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were added as described in the legend to Fig. 5. At pH 6.0, in the presence of both DCCD and TCS, there was no uptake of ⁴²K⁺ (Run 1; control cells, not treated with inhibitors, would accumulate ⁴²K⁺ at a rate of 55 nmol min⁻¹ mg⁻¹). At 49 min (arrow), the pH was abruptly raised to 8.0; this elicited a brief but considerable burst of ⁴²K⁺ uptake. This effect is not due to ATP synthesis, since the ATP level remained approximately constant throughout the run (legend). ⁴²K⁺ uptake is also not due to the pH shift per se. Run 2 shows that when TCS was omitted, ⁴²K⁺ uptake in response to the pH shift was greatly reduced, suggesting that Δψ is necessary. Finally, cells were subjected to the same sequence in the absence of glucose (Run 3); under these conditions, uptake of ⁴²K⁺ was insignificant.

The extent of ⁴²K⁺ accumulation by DCCD-blocked cells was a function of the pH shift imposed. Fig. 6 shows the effect of raising the pH from 6.0 by 1, 2, or 3 units. It is likely that the critical parameter is the membrane potential generated by the pH shift; if so, the data imply that the extent of K⁺ uptake increases continuously with Δψ rather than exhibiting a threshold. The initial rate of ⁴²K⁺ uptake was as high as 30 nmol min⁻¹ mg⁻¹, comparable to that of normal cells, but we were unable to determine the Kₑ. We infer that accumulation of K⁺ by these inhibited cells reflects a dual requirement for both ATP and an electrical potential.

Potassium Efflux Also Requires ATP Generation—Remarkably, not only net uptake of K⁺, but also its efflux is controlled by the interaction of the protonotive force with ATP. Representative data are illustrated in Fig. 7. Sodium-loaded cells were allowed to glycolyze in the presence of a limiting amount of K⁺, thereby maximizing the K⁺ concentration gradient between cells and medium. Addition of the proton conductor TCS elicited net efflux of K⁺ while glycolysis continued unabated; by contrast, TCS did not induce K⁺ efflux from starving cells. Analogous results were obtained with KTY cells in the absence of sodium.

It is likely that this dependence of K⁺ efflux upon concurrent metabolism reflects a requirement for ATP; data supporting this contention are presented in Fig. 8. NaTY cells, containing some 200 nmol of K⁺/mg, were allowed to glycolyze in the absence of external K⁺; their ATP level was about 2.0 nmol/mg. Addition of 2,4-dinitrophenol induced massive K⁺ efflux with little effect on the ATP pool. When, however, iodoacetate was also added, glycolysis ceased, the ATP pool declined to the level of starving cells (0.2 nmol/mg), and concomitantly the efflux of K⁺ came to a halt. Dinitrophenol did not induce K⁺ efflux from starving cells (Fig. 8). The experiment was repeated with cells metabolizing arginine (data not shown). Here again, dinitrophenol induced K⁺ efflux only in the presence of the energy source, but iodoacetate had no effect on either the ATP level or on K⁺ efflux (the inhibitor blocks glycolysis at the level of glyceraldehyde phosphate dehydrogenase; there is no susceptible step in arginine catabolism). We infer that ATP, or a substance derived from ATP, is required for K⁺ efflux.

The Effects of TCS and Dinitrophenol Reflect Proton Conduction—Although it is well established that TCS and 2,4-dinitrophenol do conduct protons across the plasma membrane of S. faecalis (Harold and Baarda, 1968; Bakker, 1979), we felt it necessary to exclude the possibility that their effects upon net K⁺ movement result from interaction with the transport system itself. To this end, we compared a number of...
of "uncouplers" (TCS, FCCP, 1799, and 2,4-dinitrophenol) over a range of concentrations. An extensive series of experiments is summarized in Fig. 9; it shows that for each reagent, the concentration required to inhibit K+ accumulation equaled that required for efficient proton conduction. DCCD, an inhibitor of the proton-translocating ATPase, also induced K+ efflux from glycolyzing cells, but not from starving ones (data not shown). We conclude that all of these reagents exert their effects via the proton circulation rather than upon the K+ transport system.

Potassium Transport Is Electrogenic—In order to determine whether the K+ transport system carries electrical charge, we monitored the membrane potential during K+ accumulation. Fig. 10A illustrates one experiment in which NaTY cells were allowed to glycolyze in Na+-Hepes buffer; Δψ was monitored by the distribution of ["HITPMP". The cells accumulated the lipophilic cation to a concentration gradient of 100, corresponding to a membrane potential of −119 mV. Addition of 2 mM KCl induced massive efflux of TPMP+, indicating that the membrane was depolarized by some 50 mV. Concurrently, the pH gradient (cytoplasmic acid) was dissipated by substitution of K+ for H+ (data not shown).

Analogous results were obtained when the membrane potential was monitored with ["HITTP", ["HIDDA", or by fluorimetry. Fig. 10B shows that glycolyzing cells quench the fluorescence of Dio-Cs-3 to an extent indicating a membrane potential near −145 mV. Addition of 2 mM KCl induced a rapid increase in fluorescence, which we attribute to depolarization of the membrane by as much as 50 mV. We conclude that K+ transport occurs by an electrogenic mechanism, as originally proposed by Harold and Papineau (1972b; see also Kashket and Barker, 1977).

Potassium is Not in Equilibrium with the Membrane Potential—The simplest relationship between K+ transport and the proton circulation envisages that, in glycolyzing cells, K+ behaves as a permeant cation and accumulates electrophoretically in response to Δψ (Mitchell, 1970; Harold and Papineau, 1972b; Rottenberg, 1973, Garty and Caplan, 1977). The distribution of K+ between cells and medium would then be described by the Nernst equation; moreover, the K+ ionophore valinomycin should have no effect on the K+ distribution since the membrane would be K+ permeable to begin with.

Fig. 11 shows that K+ movements do not conform to the predictions of the model. Glycolyzing NaTY cells accumulated ["HITTP" to a gradient of about 190, indicating a membrane potential of −134 mV. Concurrently, the same cells established a K+ concentration gradient of some 50,000, which would require a potential of −275 mV. Addition of valinomycin induced partial efflux of K+, lowering the concentration gradient to a level closer to the apparent membrane potential.

We conclude that, in the absence of valinomycin, K+ does not behave as a simple permeant cation but is accumulated by a mechanism capable of attaining far greater concentration gradients.

One such mechanism would be a primary K+ pump, energized by ATP or some other energy-rich compound. Alternatively, the steep K+ gradient may be achieved by a secondary carrier that links the movements of K+ to those of another ion. We could find no evidence for obligate coupling between the movements of K+ and Na+. Potassium accumulation does not require Na+ in either cells or medium, nor is it inhibited by monensin (Fig. 4). By contrast, symport of K+ with a proton deserves serious consideration; it can be shown (Mitchell, 1970, Rottenberg, 1976) that the driving force for K+/H+ symport would be:

$$58 \log \frac{[K^+]_{\text{cyt}}}{[K^+]_{\text{me}}} = 58\Delta pH - 2\Delta \psi$$

(ΔpH designates the pH gradient, cytoplasm minus medium; note that −2Δψ is a positive number.) It need not follow that the actual electrochemical potential of K+, 58 log([K+]cyt/[K+]me), be equal to (58 ΔpH − 2Δψ) since the cellular K+ level may be modulated both by inhibition of the pump (Fig. 2) and by separate exit pathways. However, the K+ potential may never exceed (58 ΔpH − 2Δψ). The data shown in Fig. 11 are pertinent to this point. In this experiment, the K+ distribution ratio would be in equilibrium with a potential of −275 mV. With Δψ estimated at −135 mV and ΔpH 1.1 unit (interior acid, corresponding to −65 mV), the driving force available comes to +207 mV. These data are typical of those we have collected with NaTY and sodium-loaded cells under conditions that maximize the K+ gradient. We find Δψ (from the distribution of ["HITTP"] ranging from −130 to −160 mV, depending on the batch of cells; ΔpH (from benzole or methylamine distribution; the cytoplasm is acidic.

![Fig. 9](image-url) Evidence that K+ release by uncouplers results from proton conduction. A. Potassium movements in glycolyzing cells as a function of uncoupler concentration. NaTY cells were allowed to glycolyze in the presence of 150 μM KCl as described in the legend to Fig. 7. When the K+ level in the medium had fallen to 40 μM, uncoupler was added and subsequent K+ movements were monitored with the K+-electrode. The ordinate shows the initial rate of K+ influx or efflux; the abscissa shows the concentration of uncoupler added. B, comparing the efficacy of various uncouplers with respect to K+ release and proton conduction. For each uncoupler, the ordinate shows the "effective concentration" required to induce K+ efflux as determined in A. The abscissa shows the concentration required to translocate protons across the membrane at the arbitrary rate of 50 nmol min⁻¹ mg⁻¹. The proton conductance was determined in the presence of valinomycin, as described by Harold and Baarda (1968). The correlation coefficient of the two scales is 0.99. Symbols as in A.

![Fig. 10](image-url) Potassium depolarizes the plasma membrane. A. NaTY cells were incubated in 0.1 M Na+-Hepes, pH 7.5, plus 0.2 mM MgCl₂ at a density of 1.5 mg/ml. The suspension was supplemented with 10 μM ["HITPMP" followed by glucose (25 mM) and KCl (2 mM) as shown. Samples were filtered at intervals, washed once, and counted. B, NaTY cells were incubated in 0.1 M Na+-Hepes buffer, pH 7.5, at a density of 0.4 mg/ml with 1.25 mM DIO Cs⁻(3). Glucose was added at 0 min; after 4 min, KCl (2 mM) was added to one portion (— — —). The membrane potential was monitored by fluorimetry.
under these conditions) ranged from 0 to -60 mV; while the total driving force often adds up to somewhat less than the possible to elicit K+ accumulation in response to a pH gradient metabolism for concurrent catabolism of glucose or arginine. Attempts to verify these predictions had to be abandoned and the distribution of Glucose was added at 10 min and valinomycin suspended at a density of 2.0 mg/ml in buffer containing 0.1 mM MgSO4, 0.2 mM KCl, and 10 µM [3H]TPP. Glucose addition at 10 min and valinomycin suspension at a density of 2.0 mg/ml in buffer containing 0.1 mM MgSO4, 0.2 mM KCl, and 10 µM [3H]TPP.

**FIG. 11. The K+ concentration gradient exceeds the membrane potential.** Cells grown in NaTY labeled with "K" were suspended at a density of 2.0 mg/ml in buffer containing 0.1 mM Na+. Hepes, pH 7.5, 0.2 mM MgSO4, 0.2 mM KCl, and 10 µM [3H]TPP! Glucose was added at 10 min and valinomycin (2 µM) at 40 min. At intervals, samples were withdrawn, centrifuged through silicone oil, and the distribution of "K" and of TPP was determined. A second aliquot, run in parallel, received [14C]methylamine and [3H]sorbitol. 

The model makes two other predictions. First, it should be possible to elicit K+ accumulation in response to a pH gradient alone; second, one should observe influx of H+ together with K+. Attempts to verify these predictions had to be abandoned because of technical difficulties that arose from the requirement for concurrent catabolism of glucose or arginine.

**DISCUSSION**

The potassium transport system of *S. faecalis*, like the TrKA system of *E. coli* (Rhoads and Epstein, 1977), appears to be linked to the metabolic machinery via both ATP and the proton circulation. The molecular basis of this dual requirement remains to be established, but our experiments define a number of features that any model for potassium transport in *S. faecalis* must accommodate. (i) Movement of K+ across the membrane, even influx down a steep concentration gradient or ΔG'/K' exchange, depends on concurrent metabolism of an exogenous energy source. (Figs. 7 and 8). Arginine supports K+ movements as well as glucose does; this suggests that transport requires either ATP (the only product common to these two pathways) or else some substance derived from ATP. Pending its chemical identification, we shall designate it "ATP." (ii) Net uptake of K+ requires the cells to generate a substantial membrane potential in addition to "ATP" (Figs. 4 to 6). Exchange of "K" for K+ can proceed in the absence of a membrane potential. (iii) Potassium influx is subject to feedback regulation (Fig. 2). (iv) Potassium movements are reversible. Reagents that block or dissipate the proton circulation induce net efflux of K+ from glycolyzing cells (Fig. 7 and 9). (v) Potassium uptake is electrogenic (Fig. 10); we have the impression that this is true for efflux as well, but the data were inconclusive. (vi) Potassium can be accumulated against a concentration gradient of some 50,000; this gradient would be in equilibrium with a membrane potential of -275 mV, far in excess of the maximum potential (-160 mV) measured by the distribution of lipophilic cations or by the quenching of carbocyanine dye fluorescence (Figs. 10 and 11). Membrane potentials obtained by the use of 2μM TMA- (Baker, 1978) were somewhat greater, but this method has now been found to be invalid for both *E. coli* (Damper et al., 1979) and *Streptococcus lactis*. It is helpful to consider K+ transport in terms of the two alternative models shown diagrammatically in Fig. 12.

**A Primary K+ Pump—** This model proposes that "ATP" serves as energy donor for a primary electrogenic pump that carries K+ inward, against the electrochemical potential gradient. The requirement for a proton motive force would reflect regulation of the pump. Perhaps it becomes functional only above some threshold value of Δψ or ΔG'/K', as is the case for neuronal sodium channels. Besides, an electrical potential (interior negative) will contribute to the driving force upon K+, reducing the free energy contribution that must come from "ATP"; the effect could be substantial, particularly if K'/ATP ratio is greater than 1, and may give the appearance of a requirement for Δψ. A primary K+ pump would probably be kinetically unidirectional so that K+ efflux must be attributed to an independent pathway. This cannot be a simple leak. To account for the data, one must postulate that the exit system is modulated by "ATP" and perhaps responds to Δψ or ΔG'/K' as well.

There is ample precedent for bacterial transport systems energized by ATP and other phosphoryl donors. Quite recently, Epstein et al. (1978) showed that the Kdp system for K+ transport in *E. coli* is energized by ATP hydrolysis; the work of Hong et al. (1979) suggests that the energy donor for shock-sensitive transport systems for ions, sugars, and amino acids may be acetyl phosphate. *S. faecalis* apparently expels calcium by a primary, ATP-dependent pump (Kobayashi et al., 1978). Examples of transport systems whose activity is regulated by the proton motive force are also beginning to appear in the literature. The proton-translocating ATPase is known to be "gated," admitting protons and synthesizing ATP only above a threshold value of Δψ (Maloney, 1977; Schonfeld and Neumann, 1977). A very recent paper by Lanyi and Silverman (1979) proposes that the sodium/proton antiporter and several amino acid/sodium symporters in *Halobacterium halobium* likewise exhibit gating by Δψ.

**A Secondary K+ Pump—** This model proposes that "ATP" but energetically coupled to the proton circulation— In this model "ATP" would render the proton mobile by binding or phosphorylation but need not be hydrolyzed with each turn of the cycle. The pump would mediate both K+ influx and efflux in response to the electrochemical potential gradient of protons. Evidence that halobacteria accumulate K+ by means of a secondary porter that responds to Δψ has been reported by Garty and Caplan (1977) and by Wagner et al. (1978). Precedent for modulation by "ATP" may be found in the proposal of Blaustein (1976) that extrusion of calcium from squid axons is effected by a Na+/Ca2+ antiporter that is driven by Δψ, but regulated by ATP.

The simplest mode of coupling K⁺ transport to the proton circulation, K⁺ unipor, must be rejected because the K⁺ gradient is too steep to be supported by the membrane potential. To account for the findings, K⁺ translocation must involve at least two positive charges per cycle. Co-transport of 2K⁺ seemed, for a while, a possible solution, but we now recognize that this mechanism is untenable. It violates the fundamental principle that the equilibrium position of a reaction is independent of the reaction pathway. A more attractive possibility is symport of K⁺ with H⁺, as shown in Fig. 12b. On this hypothesis, the driving force becomes 58ΔpH−2Δψ, of the order of magnitude required to account for the observed K⁺ distribution. If the H⁺/K⁺ ratio varies with the external pH (Rottenberg, 1976; Ramos and Kaback, 1977), the driving force may be still greater. We must emphasize that we have no direct evidence for symport of K⁺ with protons; more exotic modes of coupling K⁺ fluxes to those of other ions are conceivable but will not be considered here.

It is not possible to discriminate rigorously between the alternative models of Fig. 12, particularly as both could be rendered more flexible by invoking multiple parallel transport systems. These are known to exist in E. coli (Epstein and Kim, 1971; Rhoads et al., 1976; Rhoads and Epstein, 1978), but the genetic analysis required to demonstrate them is not feasible in streptococci. At first sight it may appear that secondary K⁺/H⁺ symport is ruled out by the finding (Fig. 11) that, under certain conditions, the electrochemical potential of K⁺ exceeds the available driving force, 58ΔpH−2Δψ. We are inclined to distrust this numerical argument for several reasons. The activity of K⁺ in the cytoplasm is not known, the stoichiometry of the carrier need not be 1:1, and, in truth, the accuracy of the chemical methods employed to measure ΔpH and Δψ remains open to doubt. We give more weight to the qualitative observations that favor the notion of secondary K⁺ transport regulated by “ATP.” An ATP-driven primary pump does not readily account for the finding that proton conductors and DCCD inhibit net uptake of K⁺, yet allow “K⁺/K⁻” exchange to continue. The necessity to invoke an elaborate exit system for K⁺ efflux in response to proton conductors detracts further from the simplicity of a primary potassium pump. We find it especially persuasive that reagents which decrease AilK⁺ induce net K⁺ efflux, despite the continued production of ATP (Figs. 7 and 8). Moreover, in cells whose native proton pump has been blocked by DCCD, the extent of K⁺ accumulation is a function of the membrane potential imposed across the membrane (Fig. 6). Taken together, these data suggest to us that the protonmotive force, rather than ATP, determines the direction of net K⁺ movement and provides the driving force for K⁺ accumulation.

Potassium accumulation by secondary symport with protons suggests a plausible explanation for the existence of mutants specifically defective in K⁺ “retention.” In mutants of this phenotype, K⁺ enters with fairly normal kinetics, but efflux is greatly enhanced, resulting in leakage of K⁺ from the cells (Harold et al., 1967). Earlier studies with one such mutant led to the conclusion (Harold and Papineau, 1972b) that the primary defect lay not in K⁺ transport per se, but in proton extrusion. This assignment, inexplicable at the time, makes good sense in terms of our present model (Fig. 12b). Any lesion that impairs generation of Δψ⁺ should drastically reduce the attainable K⁺ gradient without necessarily affecting the kinetics of influx.

Finally, we would draw attention to the parallels between the K⁺ transport system of S. faecalis and the TrKA system of E. coli, as defined chiefly by Epstein and co-workers. Aside from comparable kinetic parameters (Rhoads et al., 1976), the TrKA system requires both “ATP” and Δψ⁺ (Rhoads and Epstein, 1977). “ATP” is sufficient for K⁺ exchange but not for net uptake (Rhoads and Epstein, 1978). Dinitrophenol induces K⁺ efflux and was first used by Rhoads et al. (1976) to deplete cells of K⁺. Feedback regulation of K⁺ uptake has also been noted (Kepes et al., 1978; Rhoads and Epstein, 1977). Mutants deficient in K⁺ retention have been known for 15 years; in fact, they were among the first bacterial transport mutants to be isolated (Lubin and Ennis, 1964; Epstein and Kim, 1971; Rhoads et al., 1976). Rhoads and Epstein (1978) tentatively view the TrKA system as a primary ATP-driven K⁺ pump, but the data on hand may be compatible with a secondary porter, activated by “ATP” but energized by the proton circulation.

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Energy Coupling to Bacterial K⁺ Transport

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