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Effects of K^+ and Na^+ on the Proton Motive Force of Respiring *Escherichia coli* at Alkaline pH

EVA R. KASHKET

Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts 02118

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The role of K^+ and Na^+ in the maintenance of the proton motive force (Δp) was studied in *Escherichia coli* incubated in alkaline media. Cells respiring in Tris buffer (pH 7.8) that contained less than 100 μEq of K^+ and Na^+ per liter had a normal Δp of about −165 mV. At pH 8.2, however, the Δp was reduced significantly. The decrease in Δp at pH 8.2 was due to a marked decrease in the transmembrane potential ($\Delta\psi$), while the internal pH remained at 7.5 to 7.7. When KCl or NaCl, but not LiCl or choline chloride, was added to the cells, the $\Delta\psi$ rose to the values seen at an external pH of 7.8. In addition, choline chloride inhibited the enhancement of $\Delta\psi$ by K^+. None of the salts had a significant effect on the internal pH. The effects can be attributed to alterations of K^+ or Na^+ cycling in and out of the cells via the known K^+ and Na^+ transport systems.

The proton motive force (Δp) of respiring gram-negative bacteria such as *Escherichia coli* is maintained at −150 to −200 mV (17, 50; reviewed in E. R. Kashket, Annu. Rev. Microbiol., in press). As the external pH (pH_o) is varied from below pH 6 to over pH 8, the range that permits growth, the internal pH (pH_i) remains constant at 7.6 to 7.8 (1, 2, 4, 40; reviewed in 31), while the transmembrane electrical gradient ($\Delta\psi$) varies in magnitude (17, 30). The mechanisms by which the Δp and its components, the pH gradient (ΔpH) and the $\Delta\psi$, are regulated have not yet been elucidated.

The pH_i is relatively alkaline when the pH_o is 7.8 or lower, as a result of H^+ extrusion by the respiratory chain (15, 24, 25). It is currently envisioned that proton extrusion would, in the absence of electrogenic fluxes of other ions, result in the generation of a high $\Delta\psi$ because of a relatively low electrical capacity of the membrane (24, 25, 38). Indeed, the $\Delta\psi$ has been shown to be converted to ΔpH by the exchange of K^+ for H^+ in a number of bacteria, including *E. coli* (4, 6, 18–20, 44). The K^+ gradient across the cell membrane, therefore, functions as a buffer for $\Delta\psi$ (38).

When the pH_o exceeds 7.8, it is necessary to postulate that H^+ ions return into the cells so that the interior is acidified. Two ion-proton antiporters have been implicated in such pH_i homeostasis: an Na^+/H^+ antiporter (5, 9, 31, 46, 49; reviewed in reference 21) and a K^+/H^+ antiporter (6, 9, 10, 32, 41). In the former case, Na^+ ions are seen as effluxing from the cells in exchange for H^+. However, it is not clear how the Na^+ ions that exchange for the H^+ enter the cells. Also, an Na^+ requirement for the growth of *E. coli* has not been demonstrated, whereas K^+ ions are essential. It seems, therefore, that the physiological function of the Na^+/H^+ antiporter is to rid *E. coli* cells of Na^+ that has entered during Na^+-driven solute transport (45; reviewed in 21), rather than being essential for pH_i regulation during growth (20, 32, 36). Note that in alkalophiles and halophiles the membrane transport of Na^+ ions occurs by a number of mechanisms not found in *E. coli* (39).

It was expected that respiring *E. coli* cells incubated in K^+- and Na^+-poor buffer at high pH_o would have a pH_i of >7.8 and a high $\Delta\psi$ (38). However, as reported here, the Δp under those incubation conditions was decreased significantly, compared with incubation at pH_o 7.8. The decrease in Δp was due to a reduced $\Delta\psi$, while the pH_i was unaltered.

MATERIALS AND METHODS

Growth of cells. Most of the experiments were carried out with *E. coli* ML308-225 (*lacI lacZ lacY*$^+$ *lacA*$^+$). *E. coli* ML308-831 (*lacI lacZ*$^+$ *lacY*$^+$ *lacA*) and *E. coli* K-12 T184 (39) were used where indicated. Strains ML308-221 and T184 were grown aerobically at 37°C to mid-exponential phase in minimal salts medium CC supplemented with 0.5% succinate. Medium CC, a modification of the medium of Neidhardt et al. (29), consists of (final concentrations) 50 mM 3-(*N*-morpholino)propane sulfonic acid (MOPS) adjusted to pH 6.5 with Tris(hydroxymethyl)aminomethane (Tris) base, 28 mM $(NH_4)_2SO_4$, 0.9 mM $MgSO_4$, 11 mM phosphoric acid pH adjusted with Tris base, 3 mM KCl, 3.4 μM $FeSO_4 \cdot 7H_2O$, and the micronutrient mixture of trace salts (29). For strain T184, medium CC was supplemented with methionine (20 μg/ml), proline (30 μg/ml), threonine (100 μg/ml), and 0.5% Tryptone (Difco Laboratories). *E. coli* ML308-831 was grown in medium 63 (12) supplemented with 0.5% glycerol and 0.1% Tryptone.

Measurement of Δp, ΔpH, and $\Delta\psi$. The cells were harvested, washed with either 50 mM *N*-Tris-(hydroxymethyl)methyl-3-aminopropane sulfonic acid (TAPS) adjusted to pH 8.5 with Tris base (TAPS-Tris buffer) or with 50 mM Tris hydrochloride adjusted to pH 8.3 with Tris base (Tris buffer), and suspended to a concentration of about 4 mg (dry weight) per ml. The uptake of radioactive Δp probes was carried out in assay mixtures consisting of either TAPS-Tris buffer (final pH 8.2) or Tris buffer (final pH 7.8), 0.5% succinate, 1 mM sodium phosphate, 10 mM EDTA for incubation at pH 7.8 to 7.9 or 5 mM EDTA for reactions carried out at pH 8.2, radioactive Δp probe, as described below, and added salt, as indicated. The Δp probes, [^{14}C]lactose for Δp_{lac} ($\Delta\mu_{lac}/F$); [^{14}C]benzoic acid, [^{14}C]benzylamine, or [^{14}C]methylamine for ΔpH; and [^{3}H]tetraphenyl phosphonium for $\Delta\psi$, were added to the final concentrations and specific activities described previously (17). The reactions were started by adding cells to concentrations of 0.3 to 0.4 mg (dry weight) per ml and were carried out for 15 min or as indicated, at 25°C in rapidly shaking

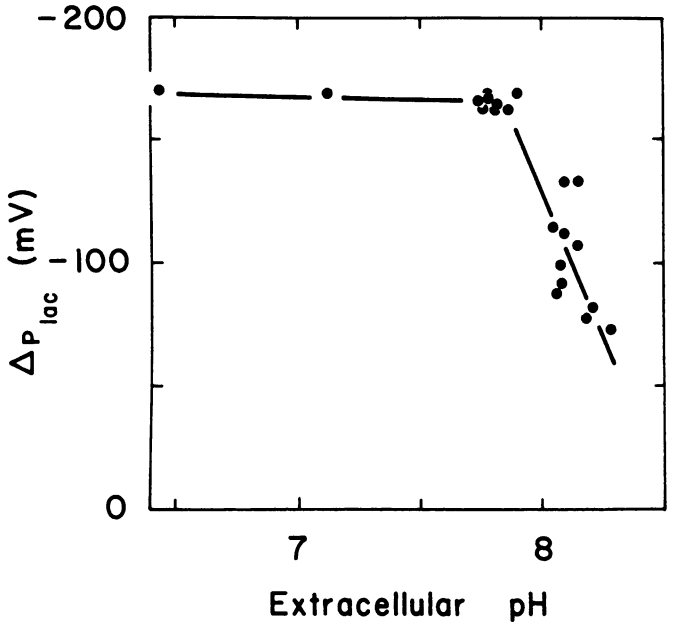

FIG. 1. Effect of pH_o on Δp_{lac} of respiring *E. coli*. Experiments were performed as described in the text. The external $[K^+]$ and $[Na^+]$ were <100 μEq/liter, as determined by flame photometry. Each Δp_{lac} value was the mean of triplicate determinations (standard deviation <2 mV) with separate cultures.

flasks. The cells were separated from the incubation mixtures either by membrane filtration (17) or by centrifugation for 2 min at 12,000 × *g* in a Microfuge (Fisher Scientific Co., Boston, Mass.). During centrifugation the cells were kept aerobic by the addition of catalase and H_2O_2 to the reaction mixture (1). In each experiment the volume of trapped medium was measured with the impermeant compound [1,2-^{3}H]polyethylene glycol (^{3}H-PEG), M_r 4,000. The accumulated radioactivity was counted, and the magnitudes of $-\Delta p_{lac}$ (−59 log[internal lactose]/[external lactose]), ΔpH (pH_i minus pH_o, inside alkaline), $-\Delta\psi$ (inside negative), and $-\Delta p$ (inside negative and alkaline) were calculated as described before (17, 22).

Other assays. Oxygen consumption was measured at 23°C with a Clark-type oxygen electrode (Rank Brothers, Bottisham, England) with strain ML308-225 cells grown and harvested as described above. The cells were suspended to a concentration of about 0.6 mg (dry weight) per ml in TAPS-Tris buffer (pH 8.1 to 8.2), MOPS-Tris buffer (pH 7.0), or 0.1 M sodium phosphate buffer (pH 7.0) and supplied with 0.5% succinate.

The in vivo production of *o*-nitrophenol from *o*-nitrophenyl-β-galactoside (ONPG) by intact *E. coli* ML308-831 cells (11) was measured at 420 nm, as described previously (47). Exponential-phase cells were washed and suspended in 50 mM potassium phosphate buffer (pH 7.5) to a concentration of about 7 mg (dry weight) per ml; these stock cells were kept on ice. The rate of ONPG hydrolysis by intact cells was determined over 10 min at 30°C by incubating the cells (about 25 μg (dry weight) per ml) in 50 mM potassium phosphate buffer (pH 7.5) with 0.5% glycerol and 2 mM ONPG. β-Galactosidase activity was measured in cells permeabilized with toluene and 2-deoxycholate for 15 min at 30°C.

The radioactive chemicals were obtained from New England Nuclear Corp., Boston, Mass., except for the [^{14}C]lactose, which was bought from Amersham Corp., Arlington Heights, Ill. The other reagents were of analytical grade and are available commercially.

RESULTS

Effect of salts on lactose accumulation. To estimate Δp, the accumulation of radiolabeled lactose was measured in *E. coli* ML308-225 (reviewed in Kashket, in press). Respiring cells incubated in Tris buffer had a Δp_{lac} of about −165 mV at pH_o 7.8 and below (Fig. 1). At pH_o of >7.8, the capacity of the cells to maintain this gradient decreased as the pH_o increased, reaching about −70 mV at pH 8.25 (Fig. 1).

The addition of salts to cells respiring at pH 8 or higher had a dramatic effect on Δp_{lac} (Fig. 2). KCl increased Δp_{lac} from −80 mV at 0 added KCl to −160 mV with 2 mM KCl present; more KCl had no further effect. NaCl also increased Δp_{lac}, with a maximum effect seen at 50 mM. The stimulatory effect was also seen with LiCl, and, like NaCl, LiCl had its optimum effect at 50 mM. However, at higher concentrations LiCl had a smaller effect, and at 200 mM LiCl, Δp_{lac} was reduced to the levels seen in the absence of the added salt. Choline chloride had no effect on Δp_{lac}.

In contrast, when the cells were incubated at pH 7.8, the Δp_{lac} was −160 to −170 mV in the absence of added salts (Fig. 1 and 3) and was not affected by the addition of KCl or choline chloride (Fig. 3). Addition of NaCl or LiCl decreased the Δp_{lac} somewhat, to −130 mV, when 200 mM salt was present.

Effect of choline on Δp_{lac}. Increasing the osmotic pressure of the medium can lead to plasmolysis of bacterial cells (26), which would result in apparent changes in the Δp_{lac}. Since statistically significant differences in the intraperiplasmic spaces could not be demonstrated with increasing external salt concentrations (data not shown), the effects of salt addition on Δp_{lac} were retested at pH 8.2 with choline chloride to bring the total added salt to a constant concentration of 200 mM (Fig. 4). The same pattern of salt effects was obtained as that obtained without choline chloride, but the Δp_{lac} values were lower, and more salt was needed to reach

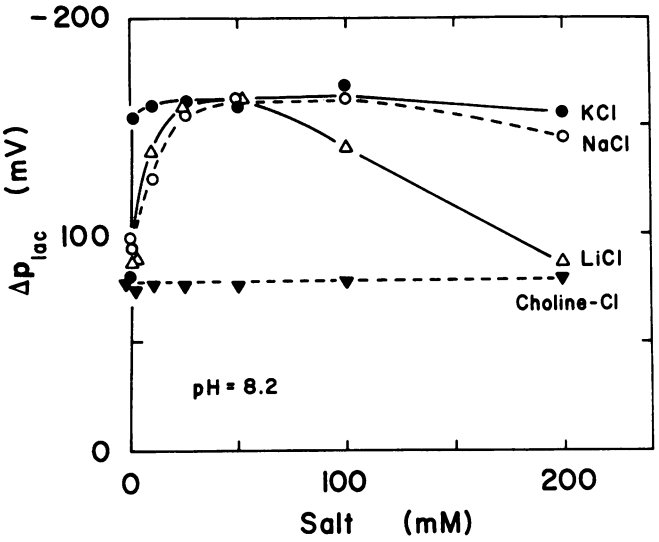

FIG. 2. Effect of various salts on Δp_{lac} at pH_o 8.2. Experiments were conducted as described in the text. The various salts were added to the indicated concentrations.

maximum values. It appeared, therefore, that choline inhibits the stimulatory effects of the other cations on Δp_{lac}.

The inhibition by choline was shown more directly in experiments in which cells were incubated at pH 8.15 with 2 mM KCl added (Table 1). Increasing the choline chloride concentration decreased the Δp_{lac} from the −158 mV seen with only 2 mM KCl added to −102 and −97 mV with 50 and 200 mM added choline chloride, respectively. Moreover, LiCl also inhibited the K^+-effected stimulation, although to a lesser extent than did choline chloride.

Effect of salts on $\Delta\psi$. Since addition of the K^+, Na^+, and Li^+ salts increased Δp_{lac}, it was of interest whether the $\Delta\psi$ or the ΔpH component of Δp was affected. Therefore, the effect of KCl addition was tested on the ability of the cells to accumulate the $\Delta\psi$ probe TPP^+ (Fig. 5). It can be seen that the $\Delta\psi$ decreased over the first 15 to 20 min of incubation of the cells in the absence of added K^+; the external K^+ was 50 to 60 μEq/liter, and the external Na^+ was about 40 μEq/liter. The addition of 9 mM KCl after 18 min caused a significant increase in the $\Delta\psi$ from about −105 mV to about −175 mV. The $\Delta\psi$ then decreased somewhat to −160 mV, where it remained over the next 40 min. There was an increase in the internal K^+ at the same time that the $\Delta\psi$ increased, from 85 mEq/liter to about 120 mEq/liter. The effect of NaCl addition (50 mM) on the $\Delta\psi$ had a time course similar to that seen with KCl (data not shown). Cells could be depleted of K^+ more extensively by incubating them at pH 8.2 for 40 min at 28°C and centrifuging them (data not shown). Such cells again showed a net influx of K^+ and an increase in the $\Delta\psi$ when 9 mM KCl was included in the medium. Finally, the effects of K^+ on $\Delta\psi$ were not peculiar to strain ML of *E. coli*, because similar patterns of depletion of cellular K^+ at pH 8.1, decrease in $\Delta\psi$, and the time course of $\Delta\psi$ increase by added K^+ were obtained in *E. coli* T184, which is a K-12 strain (data not shown).

At pH_o 8.15 the effect of KCl on $\Delta\psi$ paralleled its effect on Δp_{lac} (compare Fig. 6 with Fig. 2). The addition of 2 mM KCl increased the $\Delta\psi$ from −111 to −162 mV in cells incubated for 15 min at pH 8.15, and 10 mM salt increased the $\Delta\psi$ to its maximum value of −180 mV (Fig. 6). Further addition of KCl resulted in a decrease in the $\Delta\psi$. The effects of NaCl on

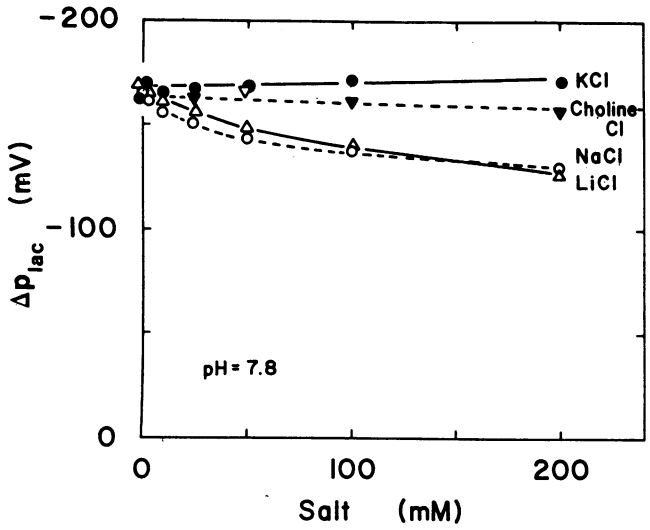

FIG. 3. Effect of salts of Δp_{lac} at pH_o 7.8. Experiments were carried out as described the legend to Fig. 2 and in the text.

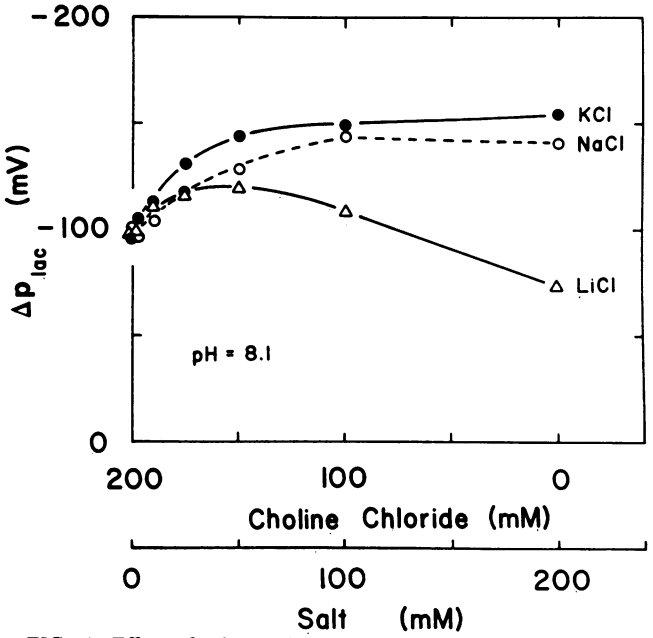

FIG. 4. Effect of salts on Δp_{lac} at pH_o 8.2 with choline chloride added to maintain a constant salt concentration. The experiment was carried out as described in the text. The salts were added to the indicated concentrations.

$\Delta\psi$ at pH 8.15 also were similar to those on Δp_{lac} (Table 2). The $\Delta\psi$ increased to −183 and −159 mV with 50 and 200 mM NaCl added, respectively.

At pH_o 7.9 the $\Delta\psi$ was higher than at pH_o 8.1 to 8.2 and was affected to a significantly lesser extent by salt addition (Table 2). The $\Delta\psi$ was increased by KCl, rising from −154 mV with no added salt to −172 and −176 mV with 2 or 10 mM KCl added, respectively. When 200 mM KCl was present, the $\Delta\psi$ decreased to −108 mV. Again, the stimulatory effect of NaCl at pH 7.9 was less marked than at pH 8.2, as the $\Delta\psi$ rose to −174 mV with 50 mM NaCl but decreased to −119 mV with 200 mM NaCl.

Choline chloride had little effect on the $\Delta\psi$ at pH 8.1 to 8.2 and decreased it at pH 7.9 (Table 2). Moreover, this ion inhibited the stimulatory effect of K^+ on the $\Delta\psi$ at the higher medium pH (Table 2). In contrast, at pH_o 8.1 to 8.2, LiCl did not stimulate the $\Delta\psi$, although it increased the Δp_{lac} (compare Table 2 with Fig. 2). At pH_o 7.9, Li^+ decreased the $\Delta\psi$.

Again, these salts had a similar effect on *E. coli* K-12 cells. After 15 min of respiration at pH 8.1, the $\Delta\psi$ was −124 mV. The addition of KCl increased the $\Delta\psi$ to a maximal value of −160 mV with 10 mM KCl. NaCl maximally raised the $\Delta\psi$ to −178 mV at 100 mM salt, while choline chloride had no

TABLE 1. Effects of choline and Li^+ on the stimulation of Δp_{lac} by K^+ (pH_o 8.15)[a]

Salt added	Δp_{lac} (mV)
None	−78
2 mM KCl	−158
2 mM KCl + 10 mM choline Cl	−157
2 mM KCl + 50 mM choline Cl	−102
2 mM KCl + 200 mM choline Cl	−97
2 mM KCl + 50 mM LiCl	−159
2 mM KCl + 200 mM LiCl	−78

[a] Experiments were carried out as described in the text.

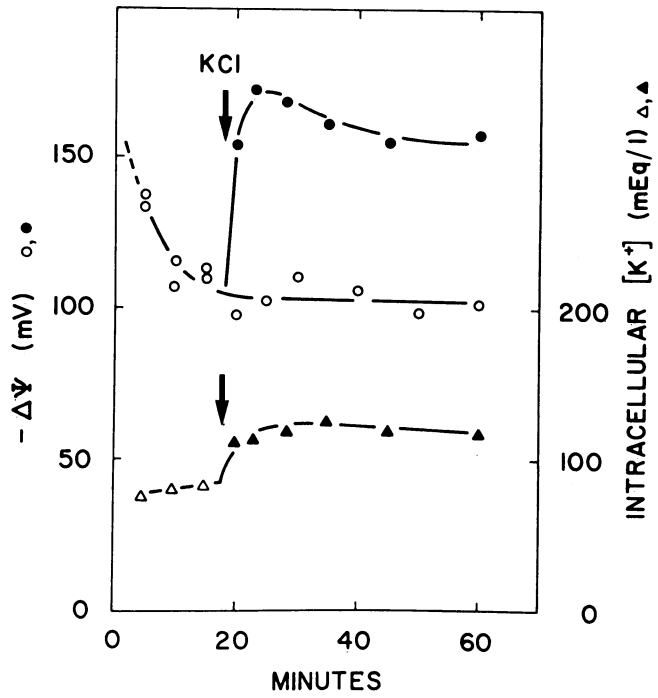

FIG. 5. Effect of KCl addition on the $\Delta\psi$ of *E. coli* at pH_o 8.1. The experiment was carried out as described in the text. KCl was added at 18 min as indicated by the arrows. The closed symbols indicate the mixtures with KCl added. The external $[K^+]$ was 50 to 60 μEq/liter before and 9.7 mM after KCl addition. Samples were removed for $\Delta\psi$ assay and flame photometry at the indicated times.

effect. However, LiCl decreased the $\Delta\psi$, which reached a value of −55 mV with 200 mM LiCl added.

Effect of salts on ΔpH. Cells incubated at a pH_o 8.1 to 8.2 in the absence of K^+ or Na^+ were expected to have a relatively alkaline internal pH, and the addition of K^+ or N^+ was expected to decrease the pH_i. However, the methods available for measuring ion gradients are inaccurate when the gradients are low (Kashket, in press). The addition of KCl apparently increased the pH_i of cells incubated at pH 8.15 (Fig. 6), but the magnitude of the effect was marginally significant (Table 2). The average pH_i of cells incubated at pH 8.15 was found to be 7.5 ± 0.2 (n = 11), and there was no significant effect of salt addition. Cells incubated at pH 7.9 had an average pH_i of 7.7 ± 0.2 (n = 10), and again there was no significant effect of salt addition.

Effect of K^+ on respiration. A possible reason for the stimulatory effect of salts on $\Delta\psi$ at high pH_o could be that K^+ or Na^+ stimulate respiration and hence Δp generation. However, that was not the case, since at pH 8.1 in TAPS-Tris buffer, with succinate as the electron donor, the cells consumed 162 ± 26 (n = 8) nmol of O_2 per min per mg (dry weight), and after the addition of 10 mM KCl or 50 mM NaCl, the cells consumed 188 ± 29 (n = 6) and 189 ± 34 (n = 3) nmol of O_2 per min per mg (dry weight) of cells, respectively. These values are not significantly different. At pH_o 7.8, however, K^+ did affect respiration, as has been reported (30). Cells incubated in the absence of K^+ respired at a rate of 37 nmol O_2 per min per mg (dry weight) in MOPS-Tris buffer, and the rate increased to 152 nmol of O_2 per min per mg (dry weight) when 10 mM KCl was added. When cells were incubated in 0.1 M sodium phosphate buffer (pH 7.0) instead of MOPS-Tris buffer, they respired at the same low rate, which was similarly stimulated by the addition of 10 mM KCl. This shows that Na^+ ions could not substitute for K^+.

Effect of respiration on ONPG hydrolysis by intact cells. In a recent report Ghazi et al. (14) concluded that rapid respiration inactivates the lactose carrier of *E. coli* cells. To distinguish between the effects of respiration on Δp generation and on carrier activity, I tested carrier activity under conditions in which it catalyzes facilitated diffusion, that is, Δp-independent downhill transport of β-galactoside (11). Respiring *E. coli* strain ML308-831 cells, incubated at 30°C in potassium phosphate buffer with glycerol as the energy substrate, hydrolyzed ONPG at a rate of 395 nmol/min per mg (dry weight). Membrane transport of the β-galactoside was rate limiting for ONPG hydrolysis, as shown by the fivefold increase in activity in toluene-permeabilized cells. To test whether rapid respiration affects the carrier, the cells were allowed to respire at 37°C for 30 or 40 min before the transport assay was begun. After this treatment the cells hydrolyzed ONPG at a rate of 420 nmol/min per mg (dry weight). ONPG hydrolysis by untreated cells was not increased by omitting glycerol or by adding 5 mM NaCN. Therefore, rapid respiration did not inactivate the *lac* carrier in these experiments.

DISCUSSION

The principal observation reported here is that respiring *E. coli* cells incubated in a low K^+ and Na^+ Tris-buffered medium maintained a normal pH_i of 7.6 to 7.8 and a Δp of −165 mV, as long as the pH_o was not more alkaline than the pH_i. However, at pH_o 8.1 to 8.2, there was net K^+ efflux, as has been reported previously (48), and a decrease in $\Delta\psi$, which also has been seen at high pH_o (1).

K^+ ions are accumulated in *E. coli* cells principally by a

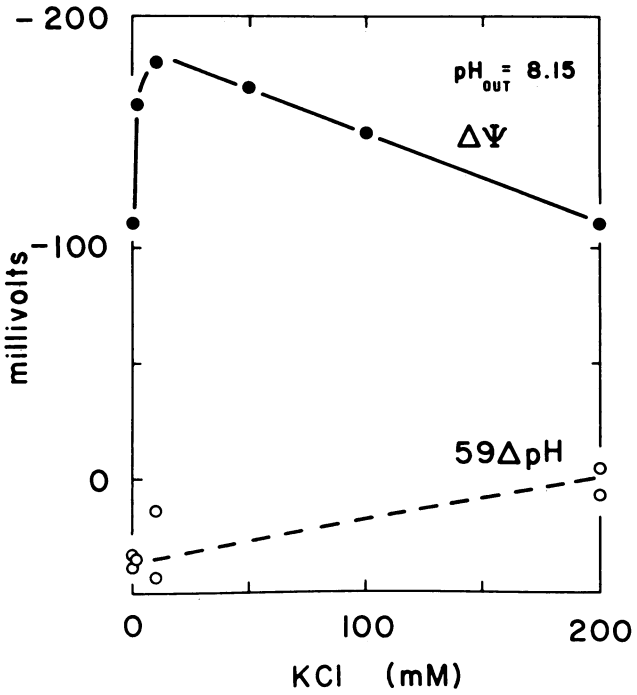

FIG. 6. Effect of KCl on the $\Delta\psi$ and ΔpH at pH_o 8.15. The experiments were carried out as described in the text.

TABLE 2. Effect of salts on the $\Delta\psi$ and pH_i of respiring *E. coli*[a]

Salt added (mM)	$\Delta\psi$ (mV) at pH_o:		pH_i[b] at pH_o:	
	8.15	7.9	8.15	7.9
None	−111	−154	7.5 ± 0.2 (11)	7.7 ± 0.2 (10)
KCl				
2	−162	−172	7.6 ± 0.2 (4)	7.6 ± 0.2 (3)
10	−180	−176	7.6 ± 0.2 (5)	7.9 ± 0.3 (4)
200	−111	−108	8.0 ± 0.5 (6)	8.1 ± 0.1 (3)
NaCl				
50	−183	−174	7.6 ± 0.2 (4)	7.9 ± 0.4 (3)
200	−159	−119	7.4 ± 0.2 (4)	7.6 ± 0.2 (3)
LiCl				
50	−93	−85	7.5 ± 0.2 (2)	7.9 ± 0.3 (3)
200	−76	−46	7.6 ± 0.1 (2)	7.9 ± 0.3 (3)
Choline Cl				
50	−105	−134	7.3 ± 0.1 (2)	8.0 ± 0.7 (3)
200	−95	−130	7.6 ± 0.3 (2)	8.3 ± 0.3 (3)
Choline Cl + KCl				
50 + 2	−142	−158	7.6 ± 0.3 (2)	7.9 ± 0.2 (3)
200 + 2	−122	−136	7.6 ± 0.1 (2)	8.1 ± 0.4 (3)

[a] Experiments were carried out as described in the text. The pH_i values at pH_o 8.15 were determined with [^{14}C]benzylamine and [^{14}C]methylamine, and those at pH_o 7.9 were determined with [^{14}C]benzylamine and [^{14}C]benzoate.

[b] Each pH_i value is the mean plus or minus the standard deviation of *n* experiments (in parentheses) with cells from separately grown cultures, each assayed in triplicate. The standard deviations of the $\Delta\psi$ values were <9 mV (*n* = 2 to 4).

low-affinity, high-rate K^+/H^+ symporter, the Trk system, and by a repressible, high affinity ATP-driven K^+ pump, the Kdp system (13, 16, 33, 34, 42). *E. coli* cells become depleted of potassium ions under a number of incubation conditions, such as incubation in alkaline media low in K^+ (30), treatment with metabolic inhibitors (35, 41), or incubation in the presence of permeant amines (27, 48). It is possible that in the present experiments Tris acted as a permeant amine, traversed the membrane in the uncharged form, and ionized within the cell where it substituted for K^+. However, an electroneutral exchange of Tris for K^+ would not explain the decrease in $\Delta\psi$. Moreover, permeant amines tend to raise pH_i (e.g., see reference 28). In the present experiments, loss of K^+ can be accounted for the following model. Efflux of K^+ may be due to a K^+-H^+ antiporter (9), which has an alkaline pH optimum (10, 32). The physiological function of the K^+/H^+ antiporter is to prevent excessive alkalinization of respiring cells and to acidify the interior of cells incubated at an alkaline pH (10). Thus, H^+ ions extruded during respiration (25) reenter the cells in exchange for K^+. This model accounts for the depletion of cellular K^+ and for the maintenance of the pH_i at 7.6 to 7.8. Replenishment of cellular K^+ would not take place because the external K^+ is too low to saturate the low-affinity Trk K^+ uptake system (K_m, 1.5 mM [35]). The high-affinity Kdp K^+ transport system is not functional, as it was repressed by the 3 mM KCl present during growth (35). In contrast, at pH_o 7.8, there was no decrease in $\Delta\psi$ in respiring cells in low K^+ medium. Here the K^+/H^+ antiporter would not function or would only function at a low rate. Therefore, there would be no net loss of cellular K^+, and uptake by the Trk system would be sufficient to maintain the high internal K^+. Indeed, it has been shown that K^+ uptake by *E. coli* cells occurs at a pH of <7.7 and that K^+ efflux occurs at a pH of >7.7 (48).

The decrease in $\Delta\psi$ seen at pH 8.1 to 8.2 cannot be attributed to the K^+/H^+ antiporter if this exchange is electroneutral (10). Movement of another ion must be invoked. Alternatively, Bourd and Martirosov (8) and Martirosov and Trchounian (23) have proposed that the dicyclohexycarbodiimide-sensitive exchange of two H^+ ions for one K^+ ion, seen in both respiring and glycolyzing bacteria, is catalyzed by a supercomplex consisting of the F_1F_0 of the H^+-translocating ATPase and the Trk system. Such an electrogenic H^+-K^+ pump operating in the direction of ATP synthesis, K^+ efflux, and H^+ influx would explain the decrease in $\Delta\psi$ at a pH_o of >7.8.

The addition of K^+ to the pH-8.2 system resulted in a marked increase in the $\Delta\psi$, as well as in net K^+ uptake. The net K^+ uptake can be explained as a result of the saturation of the Trk uptake system. If the *E. coli* Trk system is a K^+/H^+ symporter (42), H^+ reentry during K^+ influx would prevent excessive alkalinization of the interior, while H^+ extrusion by respiration would result in the increase in the $\Delta\psi$. A second mechanism could involve a decrease in K^+-H^+ exchange by the K^+/H^+ antiporter, due to the higher external K^+ or the lower ratio of internal K^+ to external K^+; this would result in less extensive K^+ efflux and hence net K^+ uptake by the cells. Again, the increase in $\Delta\psi$ would be due to the H^+ extrusion by respiration. Finally, a third mechanism would be net K^+ uptake and decreased dissipation of the $\Delta\psi$ resulting from a reversal in direction of the reactions catalyzed by the carrier complex described by Bourd and Martirosov (8) and Martirosov and Trchounian (23).

The effects of K^+ at pH_o 8.2 were shown not to be due to effects on respiratory activity, i.e., Δp generation. Interestingly, at pH_o 7.8 respiration was markedly reduced in the absence of K^+, compared with to respiration at pH_o 8.2. Oxygen consumption at pH_o 7.8 was specifically stimulated by K^+, in agreement with an earlier report (30). Presumably, at pH_o 7.8 respiratory H^+ efflux occurs against a proton gradient, and a cation is required for charge compensation. Potassium performs this function, as it is actively accumulated by the cells. In contrast, respiratory H^+ extrusion at pH_o 8.2 takes place down a proton concentration gradient and thus would be less limited by the availability of a compensating cation than activity at the higher pH_o.

The stimulatory effect of Na^+ on $\Delta\psi$ could be due to its effect of the Na^+/H^+ antiporter (21). The exchange is electrogenic at a high pH_o, with a stoichiometry ratio of $H^+/Na^+ > 1$, both in membrane vesicles (37) and in intact cells (7). Added Na^+ ions would have a tendency to enter the cells by means of this antiporter, down the large in-out gradient, leading to H^+ efflux and an increase in the $\Delta\psi$. In addition, Na^+ ions have been shown to have an affinity for the K^+/H^+ antiporter (10). Na^+ could inhibit K^+ efflux via the K^+/H^+ antiporter, leading to decreased K^+ efflux. However, an effect of Na^+ on the Na^+/H^+ antiporter seems more probable, since Li^+ ions are also substrates for the K^+/H^+ antiporter (10), yet did not increase the $\Delta\psi$.

In ML cells at pH 8.1 to 8.2, Li^+ was found to stimulate lactose uptake, yet it had no effect on the $\Delta\psi$; in K-12 cells Li^+ decreased the $\Delta\psi$. The simplest explanation, although speculative, is that Li^+ ions are able to drive the active transport of lactose, acting as analogs of H^+, but only when the [H^+] is low, i.e., at high pH_o. The driving force for this lactose accumulation would be the $\Delta\psi$ and the Li^+ in-out gradient.

The stimulatory effect of K^+ on $\Delta\psi$ was inhibited by choline chloride. Since K^+ depletion and pH_i maintenance were not affected by choline, this cation probably inhibits

the uptake of K^+. This effect is of considerable interest because choline is frequently used as an inert cation for maintaining a constant cation concentration (e.g., see references 1, 14, 48). As discussed in detail elsewhere (Kashket, in press), inhibition by choline of K^+ uptake may resolve the discrepancy in $\Delta\psi$ values measured by the distribution of two kinds of membrane-permeant cationic $\Delta\psi$ probes. In the presence of choline chloride, the $\Delta\psi$ values obtained by the two probes have been found to agree fairly well (e.g., see reference 1), while in its absence the values derived from the distribution of Rb^+ (with valinomycin) exceeded those determined with lipophilic cations, such as TPP^+, that passively traverse the cell membrane (e.g., see reference 3). Presumably, when active transport of Rb^+ via a K^+ carrier is inhibited by choline, the probe is passively transported in response to the $\Delta\psi$ only by means of ionophore; therefore, the accumulation of Rb^+ will match that of TPP^+.

Ghazi et al. (14) have concluded that rapid respiration irreversibly inactivates the lactose carrier of *E. coli*. This conclusion was based on the finding that lactose uptake by strain ML308-225 temporarily increased and then decreased within minutes when an energy substrate was added to cells incubated in a high-pH medium. The authors found that this effect was sensitive to external $[K^+]$, that the $\Delta\psi$ decreased, and that the cellular K^+ effluxed during the incubation. The conclusion that the lactose carrier is inactivated is not supported by the demonstration (see above) that the downhill Δp-independent transport of ONPG was unaffected by rapid respiration. Rather, as pointed by Booth (personal communication), the results of Ghazi et al. (14) can be explained in terms of a $\Delta\psi$ that is sensitive to pH_o and external $[K^+]$. Thus, the lactose accumulation seen by these investigators probably was a reflection of the Δp which, at alkaline pH_o, consists of only the $\Delta\psi$.

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