Proton Motive Force During Growth of *Streptococcus lactis* Cells

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Experiments with the aerotolerant anaerobe *Streptococcus lactis* provide the opportunity for determining the proton motive force (Δp) in dividing cells. The two components of Δp, $\Delta \Psi$ (the transmembrane potential) and ΔpH (the chemical gradient of H^+), were determined by the accumulation of radiolabeled tetraphenylphosphonium (TPP^+) and benzoate ions. The $\Delta \Psi$ was calibrated with the K^+ diffusion potential in starved, valinomycin-treated cells. With resting, glycolyzing cells, the Δp was measured also by the accumulation of the non-metabolizable sugar thiomethyl-β-galactoside (TMG). In resting cells the Δp, calculated either by adding $\Delta \Psi$ and $Z\Delta pH$ or from the levels of TMG, was relatively constant between pH 5 to 7, decreasing from 160 to 150 mV and decreasing further to 100 mV at pH 8.0. With the TPP^+ probe for $\Delta \Psi$, we confirmed our previous finding that the K^+ ions dissipate $\Delta \Psi$ and increase ΔpH, whereas Na^+ ions have little effect on $\Delta \Psi$ and no effect on ΔpH. [^3H]TPP^+ and [^{14}C]benzoate were added during exponential phase to *S. lactis* cells growing at pH 5 to 7 at 28°C in a defined medium with glucose as energy source. As with resting cells, the ΔpH and $\Delta \Psi$ were dependent on the pH of the medium. At pH 5.1, the ΔpH was equivalent to 60 mV (alkaline inside) and decreased to 25 mV at pH 6.8. The $\Delta \Psi$ increased from 83 mV (negative inside) at pH 5.1 to 108 mV at pH 6.8. The Δp, therefore, was fairly constant between pH 5 and 7, decreasing from 143 to 133 mV. The values for Δp in growing cells, just as in resting cells, are consistent with a system in which the net efflux of H^+ ions is effected by a membrane-bound adenosine triphosphatase and glycolytically generated adenosine triphosphate. The data suggest that in both growing and resting cells the pH of the medium and its K^+ concentration are the two principal factors that determine the relative contribution of ΔpH and $\Delta \Psi$ to the proton motive force.

It is well established that bacteria conserve and transduce metabolic energy by means of an electrochemical gradient of hydrogen ions across the cytoplasmic membrane, in accordance with the chemiosmotic theory of Mitchell (reviewed in references 5, 6, 24, 25). This gradient, $\Delta \bar{\mu}_{H^+}$, gives rise to the proton motive force (PMF), Δp, and consists of a transmembrane potential ($\Delta \Psi$, negative inside) and a transmembrane pH gradient (ΔpH, alkaline inside). The relationship of these parameters is described by $\Delta p = \Delta \Psi - Z\Delta pH$, where ΔpH is pH_{out} (pH of the bulk medium) minus the pH_{in} (pH of the cytosol); the factor Z is 2.303 RT/F and equals 59 mV at 25°C (R, T, and F have the usual meanings). The PMF available under any condition is the net result of extrusion of hydrogen ions and their reentry into the cell. In anaerobes such as *Streptococcus lactis*, H^+ efflux is catalyzed by the membrane ATPase complex (EC 3.6.1.3; BF_0F_1), whereas H^+ influx is mediated by a number of proton-linked transport systems. Excretion of metabolic end products may, in theory, contrib-

ute to the PMF (23).

The PMF has been estimated in a number of fermenting and respiring bacteria (reviewed in reference 28; see also references 1, 3, 35), but not yet in growing cells. We wished to compare resting and growing cells in order to investigate the factors that determine the Δp and its components under the physiological condition of growth. In this report we show that in *S. lactis* cells growing in a defined medium, the Δp ranged from 135 to 145 mV, depending on the external pH. The relative contributions of the electrical and chemical components of the PMF varied depending on the pH of the medium, as in resting cells.

MATERIALS AND METHODS

Growth of cells. For the resting-cell experiments, *S. lactis* ATCC 7962 cells were grown to midexponential phase in medium D plus 0.5% galactose, as described previously (11). The cells were harvested and washed, and stock cell suspensions containing 2 to 3 mg (dry weight) of cells per ml were prepared either in 0.1 M Tris-citric acid buffer (TCB buffer) at pH 5.0

to 7.0 or in 0.1 M 3-(*N*-morpholino)propanesulfonic acid (MOPS)-Tris buffer (MTB buffer), pH 7.0 to 8.0. The intracellular aqueous volume was determined by incubating cells in MTB buffer, pH 7, with either ^3H_2O or [^{14}C]taurine and centrifuging through silicone oil (12). The ^3H_2O counts in the pellet reflected the total aqueous space (4.54 μl/mg of cells; standard deviation [SD] = 0.38, *n* = 3), whereas the [^{14}C]taurine was taken to occupy the aqueous region outside the cell membrane (2.42 μl/mg of cells, SD = 0.46, *n* = 3). This intracellular aqueous volume value of 2.11 μl per mg of cells is higher than the 1.54 μl/mg reported previously (12) for cells centrifuged without use of silicone oil.

For growing-cell experiments, *S. lactis* cells were shaken gently for at least three doublings at 28°C in medium P supplemented with 0.5% glucose. Medium P consists of (per liter): 50 mM MTB buffer, pH 7.25; 0.03 mM $FeSO_4$; 3 mM phosphoric acid adjusted to pH 7.25 with Tris base; 4.8 mM $MgSO_4$; 0.025 mM $MnCl_2$; 0.09 mM $CaCl_2$; 1.075 ml of glacial acetic acid; 0.5 g of ascorbic acid; 20 mg each of L-alanine, L-arginine-HCl; L-asparagine, L-aspartic acid, L-cysteine, L-cystine, L-glutamine, glycine, L-histidine-HCl, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine-HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine; 0.1 g of L-glutamic acid; 0.5 mg each of *p*-aminobenzoic acid, calcium *d*-pantothenate, pyridoxine-HCl, riboflavin, and thiamine-HCl; 0.1 mg of folic acid; 0.05 mg each of pyridoxal-HCl, pyridoxamine-dihydrochloride, DL-6,8-thioctic acid, spermine, and spermidine; 0.1 mg of *d*-biotin, nicotinamide, and putrescine; 3.8 mg each of adenine, cytosine, guanine, hypoxanthine, 5-methylcytosine-HCl, and xanthine; 38 mg of thymine; and 19 mg of uracil. The pH was adjusted to 7.25 with Tris base when necessary. The solution was sterilized by membrane filtration (0.45-μm pore size) after adding glucose (0.5%) and brewer's yeast extract, which contributed 6 meq of K^+. The aqueous yeast extract was prepared by E. E. Baker by dialyzing with water 50 g (dry weight) of brewer's yeast and concentrating the supernatant fluid to 50 ml by evaporation at reduced pressure and temperature; the concentrated extract was frozen for storage and used at a 1:10 dilution in medium P. Alternatively, medium P was supplemented with 3 mM KCl and 12 mM thioglycolic acid instead of the aqueous yeast extract, and briefly heated and cooled just before use. The two media gave the same results.

Measurement of ΔpH, sugar accumulation, and ΔΨ in resting cells. The ΔpH values were calculated from the uptake of [^{14}C]benzoate (20), and the accumulation of [^{14}C]methyl-β-thio-D-galactopyranoside ([^{14}C]TMG) was used as an index of Δ*p* (14, 15). For ΔΨ determinations, the uptake of [^3H]tetraphenylphosphonium bromide ([^3H]TPP$^+$) was used. The values for Δ*p* were calculated by adding ΔΨ and ZΔpH (20).

Cells were incubated at 23°C for 10 min in 1.5 ml of various mixtures, and 1.0-ml portions were centrifuged through silicone oil to separate the cells from the media as described previously (12). Each tube contained 80 mM MTB or TCB buffer at the indicated pH, 33 mM glucose, 0.5 to 0.7 mg of cells (dry weight),

and either 20 μM [^{14}C]benzoate at 25.6 Ci/mol, 1 μM [^3H]TPP$^+$ at 208 Ci/mol, or 2 μM [^{14}C]TMG at 43.9 Ci/mol. The radioactivity of the pellets and of portions of the supernatant fluids and the incubation mixtures was counted. Radioactivity attributable to extracellular fluid trapped in the pellets after centrifugation through silicone oil was determined in each experiment with cells treated with 7% *n*-butanol or with 1.7% toluene for 50 min at 34°C, and these counts were subtracted from the experimental values. Cells treated in this way could not accumulate [^{14}C]TMG when incubated with added glucose, showing disruption of the cell membrane. The contaminating radioactivity was insignificant for the benzoate anions and the uncharged sugar molecules. However, binding of cations occurred to a significant extent in these intact, gram-positive cells, probably because of the large number of negative charges on the surface, such as those of teichoic acids. Binding of monovalent cations to cells with disrupted membranes also has been observed with $^{204}Tl^+$ and $^{22}Na^+$ (E. R. Kashket, A. G. Blanchard, and W. C. Metzger, unpublished experiments). With [^3H]TPP$^+$, the binding to cells with disrupted membranes was equivalent to 4,500 cpm of [^3H]TPP$^+$ per 0.4 mg of cells (440 SD, *n* = 30). For comparison, when energized cells accumulated TPP$^+$ in response to ΔΨ of 120 mV, 15,000 cpm of [^3H]TPP$^+$ were associated with the cell pellet. The ΔΨ values obtained, however, were reproducible and significant (see figures). Even at low ΔΨ values, e.g., in the 50-mV range, the [^3H]TPP$^+$ counts of the intact cell pellets were significantly different from those of butanol-treated cells (*P* < 0.01). The binding of [^3H]TPP$^+$ to *n*-butanol-treated cells was not significantly different in media of pH 5.0 to 8.0, nor was there a significant effect of increasing the NaCl or KCl concentrations to the levels used in these experiments.

Measurement of ΔΨ and ΔpH in growing cells. Cells were shaken gently at 27 to 28°C in medium P plus 0.5% glucose, and the turbidity was followed for at least three generations until exponential phase (absorbancy at 625 nm < 1.0). If necessary, the pH of the culture was adjusted with Tris base or HCl at least one generation before assay. [^3H]TPP$^+$ or [^{14}C]benzoate was added to 7.5 ml of culture in duplicate flasks. The cultures were shaken for 15 min, and 1.0-ml portions were removed and centrifuged through silicone oil. Portions of radioactive cultures were treated with *n*-butanol (7%) for 50 min at 34°C, and then portions were centrifuged. The turbidity of the growing culture was monitored to establish that the addition of the ΔΨ and ΔpH probes had had no effect. In a third flask, the pH of the medium was measured and the cells were centrifuged through silicone oil; the K^+ and Na^+ concentrations of this supernatant fluid were determined by flame photometry.

Chemicals. [7-^{14}C]benzoic acid ([^{14}C]benzoic acid), [^{14}C]TMG, [1,2-^3H]polyethylene glycol, ^3H_2O, [^{14}C]taurine, and [^3H]TPP$^+$ were obtained from New England Nuclear Corp., Boston, Mass. Some of the [^3H]TPP$^+$ was kindly provided by H. R. Kaback of the Roche Institute of Molecular Biology, Nutley, N. J. Valinomycin was bought from Sigma Chemical Co., St. Louis, Mo.; all other reagents were of analytical grade and commercially available.

RESULTS

Accumulation of TPP⁺ in response to the potassium diffusion potential. To confirm that the TPP⁺ method (1, 17, 35) is valid for intact *S. lactis* cells, we first calibrated the accumulation of this lipophilic cation with the potassium diffusion potential (Fig. 1). In starved cells treated with valinomycin, which renders the membranes permeable to K^+ (B. C. Pressman, Fed. Proc. **27:** 1283-1288, 1968), a membrane potential (negative inside) is imposed by the efflux of K^+. The K^+ diffusion potential was varied by varying the medium KCl concentration. The $\Delta\Psi$ values were calculated from the distributions of the two cations, using the Nernst equation (reviewed in reference 20). A direct relationship was found between the $\Delta\Psi$ values, showing that TPP⁺ is a useful probe for measuring $\Delta\Psi$ in starved streptococcal cells.

Effect of extracellular pH on Δp in resting *S. lactis* cells. To determine whether TPP⁺ accumulation is a valid probe for $\Delta\Psi$ in the absence of valinomycin and in energized cells, *S. lactis* were tested while fermenting glucose in media of pH 5 to 8 (Fig. 2). As has been observed before in intact bacteria (3, 12, 13, 35), the relative contributions of $\Delta\Psi$ and ΔpH to Δp are dependent on the pH of the medium. In intact,
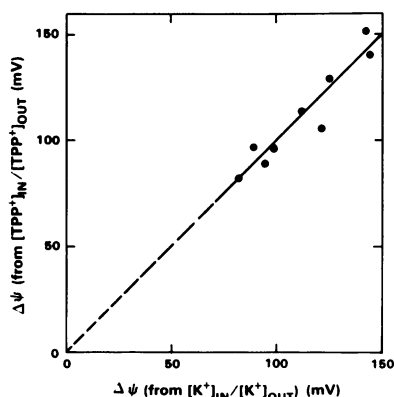

FIG. 1. *Accumulation of TPP⁺ in response to the K^+ diffusion potential. Starved S. lactis cells were incubated in 80 mM MTB buffer, pH 7.25, supplemented with 0 to 10 mM KCl, and treated with valinomycin as described in text. The uptake of [³H]-TPP⁺ and K^+ content were measured in parallel reaction vessels as described in the text. The trapped and nonspecifically bound counts of [³H]TPP⁺, determined from cells treated with n-butanol, were subtracted from the radioactivity in each pellet. The membrane potentials were calculated by the Nernst equation from the distributions of the two cations as described in the text. The regression line has a slope of 0.99 and intersects the y-axis at −0.2 mV.*
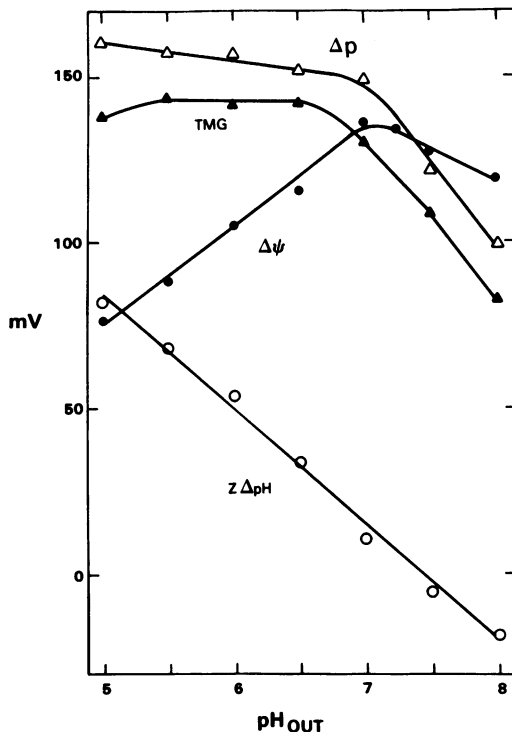

FIG. 2. *Effect of external pH on Δp of resting cells. The experiments were carried out as described in the text. Each value shown is the average from three to nine experiments. Symbols and standard errors of the means (SEM): ○, $Z\Delta pH$ (<3 mV SEM); ●, $\Delta\Psi$ (<3.3 mV SEM); ▲, TMG accumulation = Z log ([TMG]ᵢₙ/[TMG]ₒᵤₜ) (<4.7 mV SEM); △, Δp = sum of $Z\Delta pH$ and $\Delta\Psi$.*

fermenting *S. lactis* cells, the ΔpH decreased from a value equivalent to 85 mV (alkaline inside) at pH 5.0 to −21 mV (acid inside) at pH 8.0; this corresponds to an increase in pH_{in} from 6.5 to 7.6. The $\Delta\Psi$ increased from 75 mV (negative inside) at pH 5.0 to 135 mV at pH 7.0. At higher medium pH, the $\Delta\Psi$ decreased to 120 mV at pH 8.0. The Δp, calculated by adding these two parameters, was fairly constant up to pH 7.0, decreasing from 160 mV at pH 5 to 150 mV at pH 7.0. Beyond the maximum at pH 7, the Δp decreased to 100 mV at pH 8.0. Control experiments showed that Tris base in the concentrations used in these experiments did not dissipate $\Delta\Psi$ to a significant degree. Hence the decrease in Δp at pH greater than 7 is not due to such an artefact.

The intracellular levels reached by TMG, used as an independent measure of Δp (14, 15), paralleled the Δp values between pH 5 and 8, but were usually lower. A discrepancy between Δp and TMG accumulation of about 25 mV was

seen previously in K^+ diffusion potential-energized *S. lactis* cells (14). Wilson and co-workers have recently measured Δp and TMG accumulation in energized, resting *S. lactis* cells and also observed that the chemical gradient of TMG was 20 mV lower than Δp during high-Δp conditions (34). In those experiments, the $\Delta \Psi$ was determined from the ratio of K^+ concentrations in/out in valinomycin-treated cells. It is possible that TMG levels may underestimate Δp. Sugar accumulation may reach an upper limit because diffusion-like pathways may contribute significantly to sugar efflux at high intracellular sugar levels, in analogy to the *E. coli* system (3, 21). Nevertheless, the TMG accumulation values parallel the Δp, as also seen below and in Fig. 3 and 4, supporting the validity of TPP^+ as a $\Delta \Psi$ probe for intact cells.

Effects of KCl and NaCl on the Δp of resting *S. lactis* cells. We had shown previously that KCl addition to glycolyzing *S. lactis* cells resulted in a decrease in $\Delta \Psi$ and increase in ΔpH (12), whereas NaCl addition had a minor effect on $\Delta \Psi$ and no effect on ΔpH (2). In those experiments, the $\Delta \Psi$ was measured by using the membrane potential-sensitive dye 1,1'-dipropyl-2,2'-thiodicarbocyanine iodide (31). Because of the limitations imposed by the calibration of fluorescence quenching to potassium diffusion potential, it was only possible to test the effects of KCl and NaCl addition to cells incubated at pH 5.0. With the TPP^+ technique for $\Delta \Psi$, it is now possible to study the effects of these salts
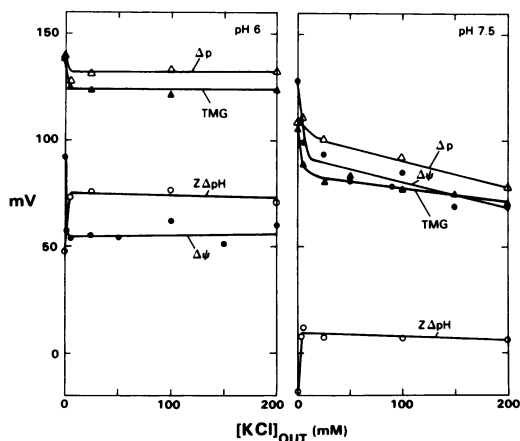

FIG. 3. *Effect of KCl on Δp of resting cells at pH 6.0 (left) and pH 7.5 (right). The experiments were carried out as described in the text, with KCl added as indicated. Each value is the average of three to six experiments. The symbols used are as in Fig. 2, and the values for standard error of the mean were <6 mV for each point.*
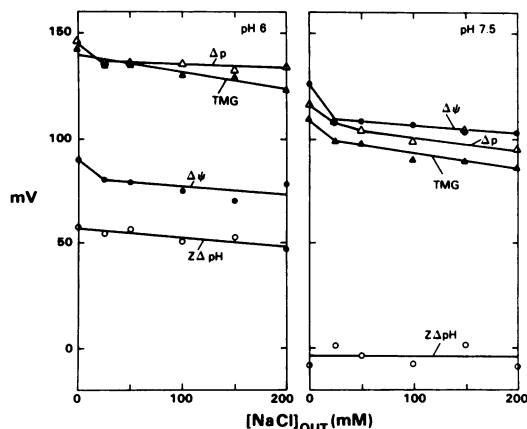

FIG. 4. *Effect of NaCl on Δp of resting cells at pH 6.0 (left) and pH 7.5 (right). The experiments were carried out as described in the text, with NaCl added as shown. Symbols are as in Fig. 2. The values for standard error of the mean at pH 6.0 were <9 mV for $\Delta \Psi$, <4.5 mV for $Z\Delta pH$, and <3 mV for TMG accumulation; at pH 7.5, the corresponding values were <4.5, <7.7, and <6.5 mV.*

at other medium pH values.

KCl added at concentrations of 2.5 mM or greater dissipated the $\Delta \Psi$ of cells incubated at pH 6 from 92 to 55 mV while increasing the $Z\Delta pH$ from 47 to 75 mV (Fig. 3, left). Thus, the Δp decreased slightly from 140 to 132 mV. At pH 7.5 a similar phenomenon was observed: $\Delta \Psi$ decreased from 128 to 90 mV or less, whereas ΔpH increased from -20 mV (acid inside) to about 8 mV (alkaline inside) (Fig. 3, right). The chemical gradients of TMG decreased in parallel with Δp in these experiments. The Δp was thus markedly affected by KCl addition at pH 7.5, probably because a rise in ΔpH could not compensate for the dissipation of $\Delta \Psi$. The limitation to the rise in ΔpH may be due to the buffering power of the cytosol and the limitations in H^+-extruding activity of the membrane ATPase complex.

In contrast, addition of NaCl at pH 6.0 had no effect on ΔpH (Fig. 4, left) but lowered the $\Delta \Psi$ from 90 to 80 mV and less, thus lowering the Δp slightly. TMG levels also decreased somewhat with increasing NaCl addition. At pH 7.5 (Fig. 4, right), NaCl addition again had no effect on ΔpH, but dissipated $\Delta \Psi$ by 15 to 20 mV.

In these experiments, the ratios in/out of the various radioactive probes were calculated by using the same intracellular aqueous spaces for the cells for all the salt concentrations. The lack of effect of increasing NaCl concentration on the ΔpH is consistent with a constant intracellular space. No significant shrinkage of the intracel-

lular space was measured in cells incubated with these salts at 200 mM (not shown), and therefore the decreases seen in TPP$^+$ and TMG levels are considered to reflect dissipation of $\Delta\Psi$.

$\Delta\Psi$ and ΔpH in growing *S. lactis* cells. After establishing TPP$^+$ as a valid probe for $\Delta\Psi$ in intact glycolyzing *S. lactis* cells, we turned to growing cells. It is important to note that TMG accumulation is not a feasible assay in growing cells because the membrane carrier must be induced by galactose, and galactose, which would have to be present during growth, competes strongly with TMG for entry (13).

The two components of Δp were measured in exponential phase in *S. lactis* cells growing at 28°C (Fig. 5). The doubling time was fairly constant between pH 5 and 7.

The ΔpH of cells growing in medium P was directly dependent on the external pH, as in resting cells. The $Z\Delta$pH in growing cells decreased from 60 mV at pH$_{out}$ = 5.1 to 25 mV at pH 6.8. The $\Delta\Psi$ in growing cells increased from 83 mV at pH 5.1 to 108 mV at pH 6.8. The Δp calculated from the $\Delta\Psi$ and $Z\Delta$pH values, therefore, was 143 mV at pH 5.1, decreasing to 133 mV at pH 6.8.

Comparing resting and growing cells at the same external pH and at similar K$^+$ and Na$^+$ concentrations (Table 1, rows A and C), it is evident that the Δp may be the same (136 and 133 mV at pH 6), and yet cells growing in this medium have a higher $\Delta\Psi$ (96 versus 62 mV) and a lower ΔpH (40 versus 71 mV) than the resting cells. In resting cells, increasing the Na$^+$ concentration (rows D and E) had no effect on the $\Delta\Psi$-dissipating effects of K$^+$ (rows C and E); therefore, the differences between the growing and resting cells were not due to Na$^+$. In other experiments, cells growing rapidly at pH 6.85 in medium D plus glucose had a Δp significantly lower than that of cells growing more slowly in medium P at the same pH. The lower Δp value (94 versus 132 mV for medium P) was the result of lower $\Delta\Psi$ (71 versus 109 mV), whereas the ΔpH was the same (23 mV) in the two media.

Medium D (11), a rich medium containing yeast autolysate and tryptic digest of casein, supports faster growth of these cells at 28°C (80-min. doubling time) than the defined medium P (140-min doubling time). The differences seen under the two growth conditions point out that the PMF of growing cells is not an invariant property of these streptococci.

DISCUSSION

A variety of techniques is available for measuring Δp in bacterial cells, including the distribution of permeant ions to determine $\Delta\Psi$ and of weak acids to measure ΔpH (reviewed in reference 20). Membrane potential-sensitive and pH gradient-sensitive fluorescent dyes have also been used. However, most of the techniques useful for resting cells are not suitable for growing cells. For example, the use of fluorescent cyanine dyes is feasible for only a narrow range
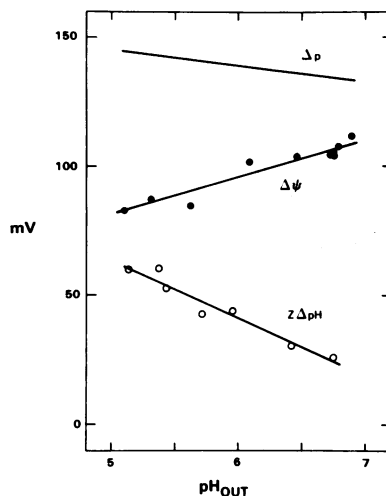

FIG. 5. Δp in growing cells. Cells were grown and tested as described in the text. Each value shown is the average of triplicate determinations in separate experiments. The Δp line was calculated by summing the regression lines for the $\Delta\Psi$ and the $Z\Delta$pH values.

TABLE 1. *Comparison of Δp at pH 6.0 in resting and growing S. lactis cells*[a]

Cells	Determination					
	[K$^+$]$_{out}$ (mM)	[Na$^+$]$_{out}$ (mM)	$\Delta\Psi$ (mV)	$Z\Delta$pH (mV)	Δp (mV)	Z log ([TMG$_{in}$]/[TMG$_{out}$])
Growing						
A	6.6	0.4	96	40	136	
Resting						
B	<0.05	<0.05	93	55	148	143
C	5	<0.05	62	71	133	131
D	<0.05	25	88	56	146	138
E	5	25	58	78	136	129

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

of dye and cell concentrations, and valinomycin cannot be used to render membranes permeable to K^+ in growing cells. However, the lipophilic cations first developed by Liberman and co-workers (16), which are available in radioactively labeled form, offer the opportunity for $\Delta\Psi$ measurements without affecting cell growth. We therefore turned to TPP^+, which, in a number of systems, has been shown to accumulate on the negatively charged side of membranes (1, 17, 35). In intact S. lactis cells, the related compound triphenylmethylphosphonium requires the presence of the lipophilic anion tetraphenylboron (TPB^-) (data not shown), which may raise difficulties because TPB^- may form complexes with K^+ (22). TPP^+ uptake does not require TPB^-. We have confirmed that [^3H]TPP^+ is useful as a $\Delta\Psi$ probe both for starved, valinomycin-treated cells and for intact, glycolyzing cells. Indeed, Porter and co-workers (J. S. Porter et al., Abstr. Annu. Meet. Fed. Am. Soc. Exp. Biol., p. 145, 1979) have shown that in Escherichia coli giant cells the $\Delta\Psi$ values measured with TPP^+ match those obtained with microelectrodes. It seemed reasonable, therefore, to apply the TPP^+ technique to growing cells. Also, at the low concentrations used, this cation had no effect on growth.

With resting cells, we have confirmed and expanded our previous findings on the effects of KCl and NaCl addition on Δp (2, 12). Transport of cations affected the PMF, presumably because BF_0F_1 activity could not compensate for the increase in H^+ flux. In streptococci, unlike E. coli (26), the K^+ transport carrier(s) has not been identified by genetic means. However, K^+ uptake occurs by at least one membrane carrier and is energized by a high-energy phosphate compound (8, 9, 28), which may be ATP or another compound derived from it (10). Bakker and Harold (1) have concluded that K^+ accumulation in S. faecalis involves both $\Delta\Psi$ and ATP (or a derivative) and is mediated either by an ATP-driven pump and regulated by Δp, or by a membrane carrier (symporter) that translocates K^+ inward with H^+ and is activated by ATP. Our data can be interpreted to support an electrogenic K^+ influx by one or more membrane carriers energized by ATP or a derivative. The alkalinization of the cytosol would then be explained by an increased activity of the BF_0F_1 due to relaxation of the PMF. A Na^+/H^+ antiporter system presumably operates in these cells, in analogy to the carrier described for E. coli cells (4, 29, 33). The relative lack of effect of NaCl on Δp in resting S. lactis may be due to a flux of H^+, in exchange for Na^+, that is low enough that the BF_0F_1 can compensate by increasing H^+ efflux.

In resting cells, the $\Delta\Psi$ and ΔpH were found to be functions of the extracellular pH, as has been observed for other bacterial cells (3, 35). The values for Δp in S. lactis cells are rather similar to those in other streptococci and generally lower than those of respiring cells or vesicles (27). However, the $\Delta\Psi$ maximum seen at pH 7 has not been reported from other systems. The decrease in $\Delta\Psi$ at high pH, and hence in Δp, was confirmed by the decrease seen above pH 7 in the chemical gradient of TMG. These data thus support a 1:1 stoichiometry for the β-galactoside/H^+ symporter (3, 32, 35) throughout the pH range tested.

It is not immediately obvious whether the PMF of resting and growing cells would be similar. One possibility is that growing cells may utilize ATP (e.g., for macromolecule synthesis) rapidly enough that the internal ATP is lowered, the BF_0F_1 cannot operate maximally, and the Δp is affected. It is possible that such an effect may only be seen in very rapidly growing cells. Another effect may be that components in growth media utilize energy-dissipating transport processes not involved in the simpler media that are used in experiments with resting cells. Our finding that the relative contributions of $\Delta\Psi$ and ΔpH are similarly affected by the pH of the medium in the two kinds of cell suspensions suggests that, as a whole, the same kinds of H^+ efflux and influx mechanisms are operative.

The Δp values measured in both growing and resting S. lactis cells are consonant with the PMF levels expected of cells deriving their metabolic energy from the ATP synthesized by substrate-level phosphorylation during glycolysis. In streptococci, Δp greater than 175 to 200 mV would be required for the H^+-translocating ATPase (BF_0F_1) to catalyze ATP synthesis (18, 19, 30). Therefore, any value for Δp less than about 175 mV would be compatible with the expected outward direction of H^+ movement through the BF_0F_1, energized by net ATP hydrolysis. The finding that the Δp was different in cells growing in the two media is acceptable, since both values were lower than about 175 mV. The reason for the differences seen in resting cells and cells growing in the defined medium is not clear. However, the two factors of primary importance in determining Δp in both growing and resting cells are the pH and the K^+ concentration of the medium. It remains to be seen whether other factors, such as the process of growth, also play a role.

LITERATURE CITED

1. **Bakker, E. P., and F. M. Harold.** 1980. Energy coupling to potassium transport in *Streptococcus faecalis*: interplay of ATP and the protonmotive force. J. Biol. Chem. **255**:433–440.

2. **Barker, S. L., and E. R. Kashket.** 1977. Effects of sodium ions on the electrical and pH gradients across the membrane of *Streptococcus lactis* cells. J. Supramol. Struct. **6**:383–388.

3. **Booth, I. R., W. J. Mitchell, and W. A. Hamilton.** 1979. Quantitative analysis of proton-linked transport systems: the lactose permease of *Escherichia coli*. Biochem. J. **182**:687–696.

4. **Brey, R. N., J. C. Beck, and B. P. Rosen.** 1978. Cation/proton antiport systems in *Escherichia coli*. Biochem. Biophys. Res. Commun. **83**:1588–1594.

5. **Harold, F. M.** 1972. Conservation and transformation of energy by bacterial membranes. Bacteriol. Rev. **36**:172–230.

6. **Harold, F. M.** 1977. Ion currents and physiological functions in microorganisms. Annu. Rev. Microbiol. **31**:181–203.

7. **Harold, F. M.** 1977. Membranes and energy transductions in bacteria. Curr. Top. Bioenerg. **6**:83–149.

8. **Harold, F. M., and K. Altendorf.** 1974. Cation transport in bacteria. Curr. Top. Membr. Transp. **5**:1–50.

9. **Harold, F. M., and D. Papineau.** 1972. Cation transport and electrogenesis by *Streptococcus faecalis*. J. Membr. Biol. **8**:45–62.

10. **Hong, J.-S., A. G. Hunt, P. S. Masters, and M. A. Lieberman.** 1979. Requirement of acetyl phosphate for the binding protein-dependent transport systems in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**;1213–1217.

11. **Kashket, E. R.** 1979. Active transport of thallous ions by *Streptococcus lactis*. J. Biol. Chem. **254**:8129–8131.

12. **Kashket, E. R., and S. L. Barker.** 1977. Effects of potassium ions on the electrical and pH gradients across the membrane of *Streptococcus lactis* cells. J. Bacteriol. **130**:1017–1023.

13. **Kashket, E. R., and T. H. Wilson.** 1972. Role of metabolic energy in the transport of β-galactosides by *Streptococcus lactis*. J. Bacteriol. **109**:784–789.

14. **Kashket, E. R., and T. H. Wilson.** 1973. Proton-coupled accumulation of galactoside in *Streptococcus lactis* 7962. Proc. Natl. Acad. Sci. U.S.A. **70**:2866–2869.

15. **Kashket, E. R., and T. H. Wilson.** 1974. Protonmotive force in fermenting *Streptococcus lactis* 7962 in relation to sugar accumulation. Biochem. Biophys. Res. Commun. **59**:879–886.

16. **Liberman, E. A., and V. P. Skulachev.** 1970. Conversion of biomembrane-produced energy into electrical form. IV. General discussion. Biochim. Biophys. Acta **216**:30–42.

17. **Lichtshtein, D., H. R. Kaback, and A. J. Blume.** 1979. Use of a lipophilic cation for determination of membrane potential in neuroblastoma X glioma hybrid cell suspensions. Proc. Natl. Acad. Sci. U.S.A. **76**:650–654.

18. **Maloney, P. C.** 1977. Obligatory coupling between proton entry and the synthesis of adenosine-5'-triphosphate in *Streptococcus lactis*. J. Bacteriol. **132**:564–575.

19. **Maloney, P. C., E. R. Kashket, and T.H. Wilson.** 1974. A protonmotive force drives ATP synthesis in bacteria. Proc. Natl. Acad. Sci. U.S.A. **71**:3896–3900.

20. **Maloney, P. C., E. R. Kashket, and T. H. Wilson.** 1975. Methods for studying transport in bacteria, p. 1–49. *In* E. Korn (ed.), Methods in membrane biology, vol. 5. Plenum Publishing Corp., New York.

21. **Maloney, P. C., and T. H. Wilson.** 1973. Quantitative aspects of active transport by the lactose transport system of *Escherichia coli*. Biochim. Biophys. Acta **330**:196–205.

22. **McClure, J. E., and G. A. Rechnitz.** 1966. Reaction rate measurements with cation-sensitive glass electrodes. A kinetic study of some tetraphenylborate precipitation reactions. Anal. Chem. **38**:136–139.

23. **Michels, P. A. M., J. P. J. Michels, J. Boonstra, and W. N. Konings.** 1979. Generation of an electrochemical gradient in bacteria by the excretion of metabolic end products. FEMS Microbiol. Lett. **5**:357–364.

24. **Mitchell, P.** 1963. Molecule, group and electron translocation through natural membranes. Biochem. Soc. Symp. **22**:142–168.

25. **Mitchell, P.** 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. **41**:445–502.

26. **Rhoads, D. B., and W. Epstein.** 1977. Energy coupling to net K⁺ transport in *Escherichia coli* K-12. J. Biol. Chem. **252**:1394–1401.

27. **Rosen, B. P., and E. R. Kashket.** 1978. Energetics of active transport, p. 559–620. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York

28. **Silver, S.** 1978. Transport of cations and anions, p. 221–234. *In* B. Rosen (ed.), Bacterial transport. Marcel Dekker, Inc., New York.

29. **Tsuchiya, T., and K. Takeda.** 1979. Extrusion of sodium ions energized by respiration and glycolysis in *Escherichia coli*. J. Biochem. (Tokyo) **86**:225–230.

30. **Van der Drift, C., D. B. Janssen, and P. M. G. F. van Wezenbeek.** 1978. Hydrolysis and synthesis of ATP by membrane-bound ATPase from a motile *Streptococcus*. Arch. Microbiol. **119**:31–36.

31. **Waggoner, A.** 1976. Optical probes of membrane potential. J. Membr. Biol. **27**:317–334.

32. **West, I. C., and P. Mitchell.** 1973. Stoichiometry of lactose-proton symport across the plasma membrane of *Escherichia coli*. Biochem. J. **132**:587–592.

33. **West, I. C., and P. Mitchell.** 1974. Proton/sodium ion antiport in *Escherichia coli*. Biochem. J. **144**:87–90.

34. **Wilson, T. H., J. L. Flagg, and D. M. Wilson.** 1978. H⁺-sugar cotransport in bacteria. Acta Physiol. Scand. Suppl., p. 389–397.

35. **Zilberstein, D., S. Schuldiner, and E. Padan.** 1979. Proton electrochemical gradient in *Escherichia coli* and its relation to active transport of lactose. Biochemistry **18**:669–673.