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Proton Motive Force in Growing *Streptococcus lactis* and *Staphylococcus aureus* Cells Under Aerobic and Anaerobic Conditions

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Measurements of the electrochemical gradient of hydrogen ions, which gives rise to the proton motive force (PMF), were carried out with growing *Streptococcus lactis* and *Staphylococcus aureus* cells. The facultative anaerobe was chosen in order to compare the PMF of cells growning aerobically and anaerobically. It was expected that during aerobic growth the cells would have a higher PMF than during anaerobic growth, because the H^+-translocating ATPase (BF_0F_1) operates in the direction of H^+ influx and ATP synthesis during respiration, whereas under anaerobic conditions the BF_0F_1 hydrolyzes glycolytically generated ATP and establishes the proton gradient by extruding H^+. The electrical component of the PMF, $\Delta\psi$, and the chemical gradient of H^+, ΔpH, were measured with radiolabeled tetraphenylphosphonium and benzoate ions. In both *S. lactis* and *S. aureus* cells, the PMF was constant during the exponential phase of batch growth and decreased in the stationary phase. In both species of bacteria, the exponential-phase PMF was not affected by varying the growth rate by adding different sugars to the medium. The relative contributions of $\Delta\psi$ and ΔpH to the PMF, however, depended on the pH of the medium. The internal pH of *S. aureus* was constant at pH 7.4 to 7.6 under all conditions of growth tested. Under aerobic conditions, the $\Delta\psi$ of exponential phase *S. aureus* remained fairly constant at 160 to 170 mV. Thus, the PMF was 250 to 270 mV in cells growing aerobically in media at pH 6 and progressively lower in media of higher pH, reaching 195 to 205 mV at pH 7. Under anaerobic conditions, the $\Delta\psi$ ranged from 100 to 120 mV in cells at pH 6.3 to 7, resulting in a PMF of 150 to 140 mV. Thus, the mode of energy metabolism (i.e., respiration versus fermentation) and the pH of the medium are the two important factors influencing the PMF of these gram-positive cells during growth.

The electrochemical gradient of protons, $\Delta\tilde{\mu}_{H^+}$, which gives rise to the proton motive force (PMF or Δp), has been shown to be the mechanism by which bacteria couple metabolic energy to the transport of a number of nutrients and to ATP synthesis (for reviews, see references 7-9, 23, 24). According to the chemiosmotic theory of Mitchell (20, 21), this proton gradient consists of an electrical potential, $\Delta\psi$ (negative inside), and a pH gradient across the cytoplasmic membrane, ΔpH (alkaline inside); ΔpH is equal to the pH of the external medium minus the pH of the cytosol. The two components of the PMF can be added by using the relationship, $\Delta p = \Delta\psi - 59\Delta$pH, where 59 is a combination of constants for expressing ΔpH in millivolts at 25°C.

Bacteria establish the proton gradient by different mechanisms depending on whether they use respiratory or fermentative energy metabolism. Under anaerobic conditions, ATP is generated by fermentation, and the membrane ATPase (BF_0F_1) operates in the direction of ATP hydrolysis and H^+ extrusion. Under aerobic conditions, H^+ extrusion is effected by the respiratory chain, and the BF_0F_1 operates in the direction of H^+ influx and ATP synthesis. Thus, there should be a threshold value for the PMF. For example, in *Streptococcus lactis* cells, the imposition of a PMF of 175 to 200 mV resulted in net ATP synthesis by the BF_0F_1 in a process coupled to an obligatory H^+ influx (16, 18). When the PMF was below that value, the ATP was hydrolyzed by the same enzyme. The threshold PMF depends on a number of factors: the number of H^+ ions translocated per ATP molecule synthesized or hydrolyzed by the BF_0F_1 (the stoichiometry of the BF_0F_1), the relative concentrations of ATP to ADP and inorganic phosphate (from which the phosphorylation potential can be calculated), and the proton conduct-

ance of the membrane (17). In both growing and resting cells, therefore, one would expect the PMF to be below the threshold value during anaerobic energy metabolism, and at least equal to that value during respiration.

In a previous publication (14) my co-workers and I examined growing *S. lactis* cells and found that, like resting cells, they had a fairly constant PMF when incubated in media between pH 5.1 and 6.8. The contribution of $\Delta\psi$ and ΔpH to Δp varied depending on the pH of the medium, as it did in resting cells. The PMF was 133 to 143 mV in the defined medium tested, well below the putative threshold value required by the BF_0F_1 for energizing ATP synthesis in this organism.

I have now extended these studies to the facultative anaerobe *Staphylococcus aureus* so that I could compare the PMF in cells growing aerobically with that of cells growing anaerobically. In an accompanying paper (12), I compare the PMFs of gram-negative cells growing aerobically and anaerobically.

MATERIALS AND METHODS

Growth of cells. *S. lactis* cells (ATCC 7962) were agitated slowly at 28°C with 0.5% sugar (glucose, galactose, sucrose, or lactose) in either medium D (11) or medium X. Medium X is the same as the defined medium P (14), but with 5 mM KCl and 12 mM thioglycolate added instead of yeast extract. The cells were inoculated from overnight cultures of the same medium composition as that used in the experiment, and growth was followed by measuring the turbidity of the culture at intervals (14). The pH of the medium was adjusted by adding Tris base or HCl, and was kept at a constant value during growth by adding Tris base, as necessary.

S. aureus cells were grown at 28°C either aerobically by rapid agitation in a rotary water bath shaker (model G-76; New Brunswick Scientific Co., New Brunswick, N.J.) or anaerobically by continuous sparging with filtered, humidified gas consisting of 95% nitrogen or helium and 5% carbon dioxide, at a rate of 50 ml/min. The degree of anaerobiosis achieved by this technique is sufficient to allow cells of *Klebsiella pneumoniae* M5A1 to grow with N_2 as the sole nitrogen source (12), that is, the cells are able to fix nitrogen, which is an oxygen-sensitive process (10).

S. aureus W46 cells (ATCC 10832) were grown in medium 63S, which is medium 63 (4) supplemented with 0.5% Casamino Acids (Difco Laboratories, Detroit, Mich.), 0.5% glucose or other energy source as indicated, and the vitamin mixture used in the defined medium for *S. lactis* cells (14). For anaerobic growth, uracil (50 μg/ml) was also added.

S. aureus SG511A (wild type) and its heme-deficient mutant, H-14, a generous gift of J. Lascelles, were grown in medium SB (15) supplemented with 20 mM glucose or other energy source, as indicated. For anaerobic growth medium, SB was supplemented with 10 mM pyruvate, 0.2 mM uracil and 20 mM glucose; for H-14 cells, 2 μM hemin was added where indicated (15). The other medium used for the aerobic growth of SG511A cells was medium SG (2). The media were adjusted to the desired pH with Tris base or HCl and sterilized by membrane filtration. During aerobic growth, the desired pH was maintained by adding Tris base or KOH, as necessary.

Measurement of $\Delta\psi$ and ΔpH in growing cells. $\Delta\psi$ and ΔpH were calculated from the accumulation of [^{3}H]TPP^+ and [^{14}C]benzoate, as described previously (14). The radioactive compounds were added to separate cultures of cells at least 10 min before the first samples were taken. The cells were separated from the growth media by centrifugation through silicone oil in the case of anaerobic cultures, and by membrane filtration for aerobically growing cells, as described below.

For *S. aureus* cells growing anaerobically, samples were removed from the N_2-sparged cultures with syringes that were preflushed with N_2 or He. The samples were layered into 1.9-ml polyethylene Microfuge tubes under 0.4 ml of paraffin oil. A 0.5-ml amount of silicone oil (a mixture of 60% Fluid 550 and 40% Fluid 556 [vol/vol]; Dow-Corning Corp., Midland, Mich.) was layered on top, and the tubes were centrifuged for 2 min in an Eppendorf Microfuge at 12,8000 × *g*. Samples (50 μl) of the aqueous supernatant fluids, as well as of the cell cultures, were removed for the measurement of radioactivity. The aqueous layers and most of the silicone oil were removed with a Pasteur pipette, and the bottoms of the tubes containing the cell pellets were cut so that they fell directly into scintillation vials for counting. For aerobically growing *S. aureus* cultures, samples were filtered under suction with polycarbonate membrane filters, 0.4-μm pore size (Nuclepore Corp., Pleasanton, Calif.; or Unipore filters from Bio-Rad Laboratories, Richmond, Calif.) The samples filtered contained less than 0.045 mg (dry weight) of cells. I established that filtering more cells than that (i.e., either smaller samples of denser cultures or larger samples of less concentrated suspensions) resulted in lower [^{3}H]TPP^+ accumulation levels. Presumably, this effect is the result of an anaerobiosis that comes into effect during a longer filtration procedure. Indeed, centrifugation through oil of samples of aerobically growing *S. aureus* cells also resulted in lower [^{3}H]TPP^+ accumulation levels, presumably for the same reason. Using *S. lactis* cells, which are aerotolerant anaerobes, as controls, I established that membrane filtration and centrifugation gave the same $\Delta\psi$ values, confirming that the two methods for separating the cells from the medium were equivalent when the degree of aerobiosis was not a factor.

The intracellular volume of *S. aureus* cells was determined by using ^3H_2O for the total aqueous space and subtracting the space occupied by [^{3}H]polyethylene glycol (19). One milliliter of *S. aureus* cells with an absorbance at 625 nm of 1.0 was equivalent to 0.258 mg (dry weight) of cells (standard deviation [SD], 0.022; *n*, 4) and 0.40 μl of intracellular aqueous space. This equals 1.55 μl of space per mg (dry weight) of cells, which agrees with the value determined by Niven and Hamilton (22).

Before the ratios of the intracellular to extracellular concentrations of [^{14}C]benzoate were calculated, the

counts of radioactivity in the medium trapped in the cell pellets were subtracted from the total counts in the collected cell samples. The extracellular medium was measured by using the impermeant molecule [^{3}H]-polyethylene glycol. In *S. aureus* cells centrifuged through silicone oil, this volume was equal to 90% of the intracellular volume. For filtered samples, the extracellular fluid volume was greater and was measured in each experiment. For example, when 0.3-ml samples of cell cultures at absorbance values at 625 nm of 0.32 to 0.65 were filtered, the extracellular fluid volume was 0.82 μl (SD, 0.09; *n*, 7).

For the calculation of $\Delta\psi$, the radioactivity in the samples due to non-specifically bound [^{3}H]TPP$^+$ was subtracted from the total cell-associated counts. The bound radioactivity was measured by treating samples of each culture with *n*-butanol to disrupt the cell membranes and centrifuging the cells through silicone oil (14).

Chemicals. [7-^{14}C]benzoic acid, [^{3}H]polyethylene glycol, and [^{3}H]TPP$^+$ were obtained from New England Nuclear Corp., Boston, Mass. All other reagents were of analytical grade and are commercially available.

RESULTS

Effect of growth phase on the PMF in *S. lactis* cells. The PMF in the aerotolerant anaerobe *S. lactis* was measured during exponential and stationary phases of growth in batch culture (Fig. 1). Cells growing in the presence of air at pH 7 in the defined medium X with 0.5% glucose added as energy source were found to have a $\Delta\psi$ (negative inside) of 113.5 mV (SD, 3.0; *n*, 11) during exponential phase. The $\Delta\psi$ decreased to about 100 mV when the cells reached stationary phase. The ΔpH (alkaline inside) was equivalent to 20.8 mV (SD, 4.8; *n*, 13) during exponential phase and decreased to about 0 at stationary phase. Thus, the PMF was constant at about 135 mV during exponential phase, and then decreased in stationary phase to less than 100 mV.

When the cells were growing at pH 7 in the more complex medium D with glucose as energy source, the Δp was again constant during exponential phase of growth. In cultures with cells at densities ranging from absorbance values at 625 nm of 0.2 to 1.0, the $\Delta\psi$ was 89.4 mV (SD, 11.9; *n*, 49). The pH was equivalent to 14.1 mV (SD, 10.9; *n*, 19). Thus, the PMF was constant at 103.5 mV under these growth conditions.

The difference seen in the PMF in the two media cannot be attributed to differences in K^+ concentrations, since these were similar. At mid-exponential phase, medium D was found to contain 5.4 meq of K^+ per liter (SD, 0.54; *n*, 15), whereas medium X contained 4.4 meq/liter (SD, 0.17; *n*, 9). Such a small difference in K^+ concentration did not affect the Δp in non-growing *S. lactis* cells (13, 14). The difference in the Δp values in the two cultures cannot be due to the growth rate per se, since the cells grew at the same rate in two media (about a 60-min culture doubling time at 28°C with glucose, pH 7).

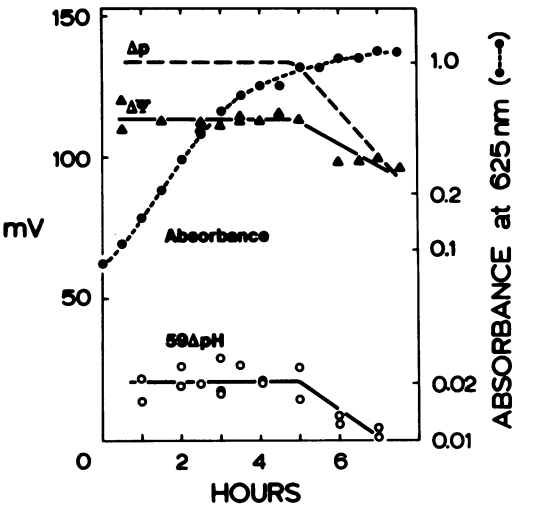

FIG. 1. *PMF of S. lactis cells during exponential and stationary phases of growth in batch culture. Cells were grown at pH 7.0 in medium X with glucose and tested for 59ΔpH* (○) *and* $\Delta\psi$ (▲), *as described in the text. The line for* Δp *was calculated by adding the lines for 59ΔpH and* $\Delta\psi$.

Effect of growth rate on the PMF in batch culture of *S. lactis* cells. The lack of effect of growth rate on the PMF was further demonstrated in other experiments where the growth rate was varied by adding galactose, lactose, or sucrose instead of glucose as the fermentable sugar. In medium X, the doubling time ranged from 120 to 610 min without an effect on the $\Delta\psi$ or the ΔpH (Fig. 2). In similar experiments with cells growing in medium D, the doubling time varied between 57 and 260 min, but the $\Delta\psi$ remained constant at about 90 mV, whereas the ΔpH stayed at about 15 mV. Thus, in batch cultures in both media, the faster growing cells had the same PMF as the cells that grew more slowly.

Effect of growth phase on the PMF in *S. aureus* cells growing aerobically. The PMF of *S. aureus* cells in batch culture was found to vary during the growth cycle, like that of *S. lactis* cells. When *S. aureus* W46 cells grew at pH 7 under aerobic conditions, the PMF was higher during early exponential phase than in later exponential and stationary phases (Fig. 3). The 59ΔpH was constant at 27 mV (alkaline inside), but the $\Delta\psi$ decreased from 167 mV (negative inside) in early exponential phase to about 123 mV in stationary phase. Thus, the PMF of

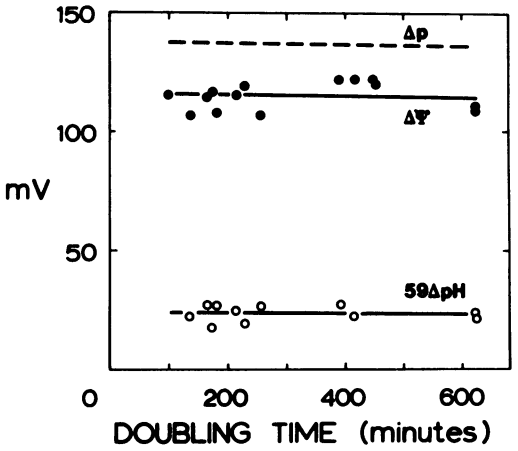

FIG. 2. *Effect of growth rate on the PMF of exponential-phase S. lactis cells. The experiment was carried out as described in the legend to Fig. 1. Samples from exponential-phase cultures for the 59ΔpH* (○) *and Δψ* (●) *measurments were taken at absorbance values at 625 nm of 0.3 to 0.5. The growth rate in medium X was varied by adding galactose, sucrose, or lactose as fermentable sugar. Each point is the average of triplicate determinations of individual cultures; the SDs were <7 mV for the Δψ values and <4 mV for the 59ΔpH values.*

these cells was 195 mV in early exponential phase and decreased to about 150 mV in stationary phase.

Effect of medium pH and energy source on PMF in *S. aureus* cells growing aerobically. Cells of *S. aureus* W46 growing at pH's of 6.15 to 7.25 were sampled during early exponential phase and assayed for the Δψ and the ΔpH (Fig. 4). The Δψ was found to be constant at 161 to 163 mV throughout this pH range, but the 59ΔpH values decreased as the pH of the medium increased. The ΔpH was equivalent to 90 mV at a medium pH of 6.0, and 32 mV at pH 7.0. Thus, the PMF was 252 mV at pH 6.0, and decreased to 194 mV when the cells grew at pH 7.0 in the same medium.

Cells of strain SG511A were grown aerobically with a variety of energy sources (glycerol, mannitol, pyruvate, or glutamate plus succinate added instead of glucose), and again, the Δψ was constant throughout the pH range tested while the ΔpH varied (Fig. 5). The Δψ was 170 to 174 mV in medium that was pH 6 to 7, whereas the 59ΔpH decreased from 102 mV at pH 6.0 to 33 mV at pH 7.0. Thus, the PMF was 272 mV at pH 6 and decreased to 207 mV at pH 7.0. The values obtained for strain SG511A under the these growth conditions were thus about 10 to 20 mV higher than those obtained for strain W46. As with *S. lactis* cells, there was no effect of the growth rate on the PMF or its components, with either strain of *S. aureus* cells. At pH 6.5, aerobically growing *S. aureus* W46 cells had a PMF of 220 mV, which was similar to that observed by Niven and Hamilton for respiring, valinomycin-treated, resting *S. aureus* cells (5).

The capacity of *S. aureus* cells to maintain a constant internal pH was evident in cells growing under high and low oxygen tension. The mean internal pH of aerobically growing cells during early exponential phase was 7.58 (SD, 0.14; *n*, 21) in experiments in which the medium pH ranged from 5.68 to 7.24. In cells growing anaerobically (see below) in media of pH 6.0 to 7.15, the mean internal pH was 7.35 (SD, 0.14; *n*, 18); this is not significantly different from the aerobic value. There was no difference between the two *S. aureus* strains.

PMF of *S. aureus* cells growing anaerobically. The PMF of *S. aureus* cells was measured at early exponential phase during growth conditions which are sufficiently anaerobic to abolish the Δψ of gram-negative *Escherichia coli* and *K. pneumoniae* (12). In *S. aureus* cells, however, the Δψ values ranged from 120.3 mV in cells growing at pH 6.0 to 148.5 mV in cells growing at pH 7.2 (Fig. 6). The ΔpH depended on the medium pH, as with aerobic cells. The resulting PMF, therefore, was 192 mV in cells at pH 6 and decreased to 163.5 mV in cells at pH 7.2. There was no difference between strains W46 and SG511A. Moreover, H-14 cells were indistinguishable from their parental cells when hemin was added to the medium to permit cytochrome synthesis (Fig. 6).

To insure that growth was dependent solely on glycolysis, H-14 cells were grown anaerobically without hemin. The experiment was carried out at various medium pH's, and I found that the ΔpH values were similar to those of cells in hemin-containing media (Fig. 7), but that the Δψ's were lower by about 20 mV (compare the Δψ lines in Fig. 6 and 7). The PMF value of H-14 cells was 153 mV at pH 6.3 and decreased to 141 mV at pH 7.0. It is probable that the higher Δψ values seen in Fig. 6 are due to trace amounts of oxygen available to the cells.

DISCUSSION

The PMF has been measured in a number of fermenting and respiring bacterial preparations, including intact cells, spheroplasts, and vesicles prepared from the cytoplasmic membrane (reviewed in reference 23). The resting cell experiments often have been carried out with membranes selectively permeabilized with ionophores so that the membrane potential could be calculated from the distribution of otherwise

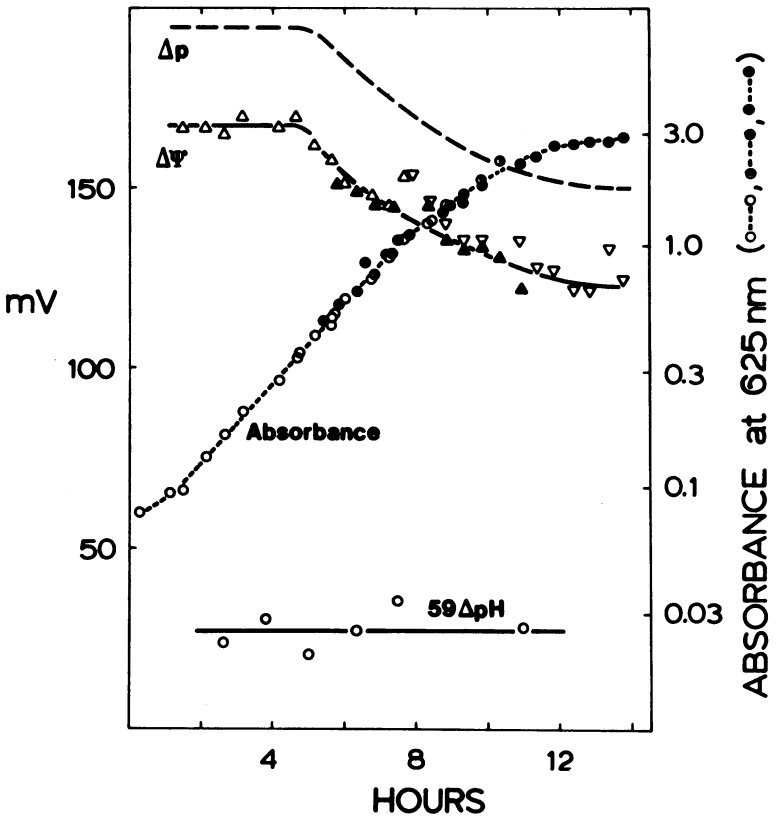

FIG. 3. *PMF during batch growth of S. aureus W46 cells under aerobic conditions at pH 7. Cells were grown in medium 63S with glucose, and the measurements were performed as described in the text. The points are derived from three typical experiments, as shown by the different symbols used for the absorbance and Δψ values. The 59ΔpH values shown are the means of 6 to 13 replicate determinations (SD, 2.2 to 8.8 mV). The Δψ values are derived from triplicate determinations by using three batches of cells, as denoted by different symbols (△, ▲, and ▽). The respective absorbance measurements of the three batches of cells are denoted by ○, ◑, and ●. The line designated as Δp was calculated by adding the Δψ line and the regression line through the 59ΔpH points. The doubling time of the culture during exponential phase was 108 min.*

impermeant ions. Also, vesicle preparations have been shown to be subject to eversions, partial eversions, or dislocation of enzymes in the membranes (reviewed in reference 6). I carried out PMF measurements in growing cells since it was important to examine the status of energy metabolism in the physiological condition of growth. Furthermore, in growing cells, there was no question of artifacts caused by leaks, and I did not use ionophore-permeabilized cells.

The expectation that aerobically growing cells would have a PMF greater than cells growing anaerobically was met in the present experiments. As shown in a later paper, the difference in PMF is much more evident for gram-negative cells than for gram-positive cells (12). The PMF of both strains of *S. aureus* growing aerobically was 180 mV (at pH 7.2) or greater, reaching 250 to 270 mV when the cells grew at pH 6. Anaerobically, the PMF of *S. aureus* was 160 mV (at pH 6.2) or less, and was at its lowest observed value (140 mV) when heme-deficient cells grew at pH 7. Thus, in these experiments the difference in PMF between respiring and fermenting cells was 40 to 70 mV or more, depending on the pH of the medium. In general, in both *S. lactis* and *S. aureus* cells growing anaerobically, the PMF values measured are compatible with H^+-extrusion effected by a BF_0F_1 system that is energized by glycolytically generated ATP. In aerobically growing *S. aureus* cells, the magnitude of the PMF is consonant with a putative threshold value required by the BF_0F_1 for catalyzing ATP synthesis which is energized by the proton gradient that is established by the respiratory chain.

The PMF of growing cells was maintained at constant levels during the exponential phase of

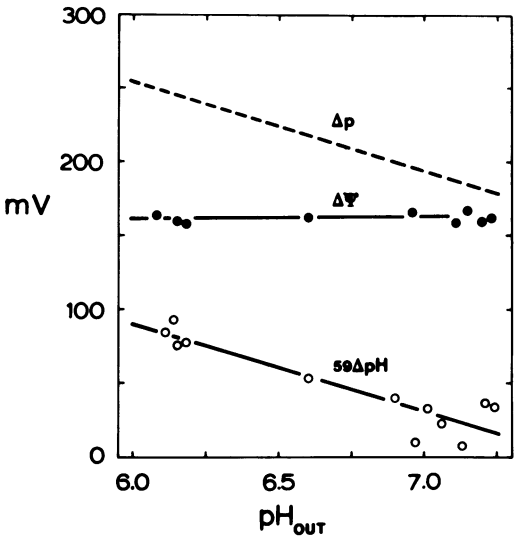

FIG. 4. *Effect of medium pH on the PMF of S. aureus W46 cells in early exponential phase during aerobic growth. Cells were sampled at absorbance values at 625 nm of 0.4 to 0.5 during growth on medium 63S with glucose, as described in the text. The pH of the medium was varied by adding HCl or Tris base. Each point is the mean of three to eight replicate determinations of the ΔpH (○) or the Δψ (●). The SDs were 1.1 to 14 mV for the 59ΔpH measurements, and 0.4 to 4.2 mV for the Δψ measurements. The Δp line is the sum of the regression lines for the 59ΔpH and Δψ values. The doubling times ranged from 80 to 210 min.*

batch growth. The constancy of the PMF suggests that the systems that establish and dissipate the proton gradient increase at the same rate during exponential phase, which is in line with Campbell's definition of balanced growth: growth during which every extensive property of the system increases by the same factor (3).

With both streptococci and staphylococci, in any one medium, the PMF was not affected by altering the growth rate, which suggests that this gradient is not a rate-limiting factor for growth in these cells. It can also be noted that the two *S. aureus* strains when tested anaerobically were indistinguishable, and that the PMFs of anaerobically growing *S. aureus* cells (Fig. 7) were quite similar to those of *S. lactis* cells (compare with Fig. 5 of reference 14).

In growing cells, the medium pH was the most influential factor in altering the PMF. In anaerobically growing *S. aureus* (Fig. 6, 7) and in *S. lactis* cells (14), as the medium pH increased, the contribution of the ΔpH to the PMF decreased, and the Δψ increased. Thus, the PMF was relatively constant in the pH ranges examined. In aerobic *S. aureus*, however, as the medium pH increased, the Δψ remained fairly constant. Since the internal pH remained constant, the PMF of the cells therefore was considerably higher (by about 60 mV) in cells growing at pH 6 as compared with those at pH 7. I found this surprising, since I assumed that the PMF-consuming systems, including nutrient carriers and the BF_0F_1, are in thermodynamic equilibrium with their driving force and since the growth rate per se did not seem to affect the PMF. However, as Booth et al. (1) have pointed out for the lactose transport system of *E. coli*, PMF-consuming systems may not have attained thermodynamic equilibrium, but rather a kinetic steady-state. Moreover, ion fluxes (e.g., cation-H^+ exchanges) could contribute to PMF dissipation, and these could be different at different external pH's. Such effects of medium pH on the PMF were also seen in gram-negative bacteria growing aerobically in minimal medium with oxidizable energy sources (12); thus, it is unlikely that the variation seen in staphylococci

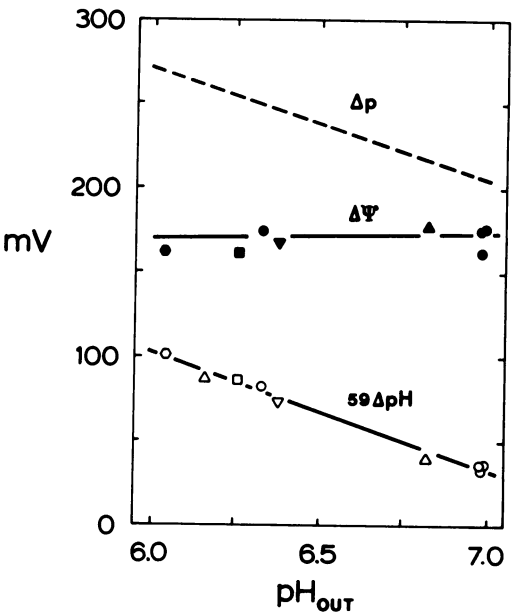

FIG. 5. *Effect of various energy substrates on the PMF of exponential phase S. aureus SG511A cells growing aerobically. The cells were grown either in medium SG (○), or in medium SB supplemented with one of the following: glycerol (△), glucose (○), mannitol (□), or pyruvate (▽). The Δψ (open symbols) and 59ΔpH (closed symbols) measurements were carried out as described in the text and in the legend to Fig. 4. The SDs of five replicate measurements were 3 to 16 mV for the 59ΔpH values, and 1.5 to 6.0 mV for the Δψ values. The doubling times ranged from 50 to 120 min.*

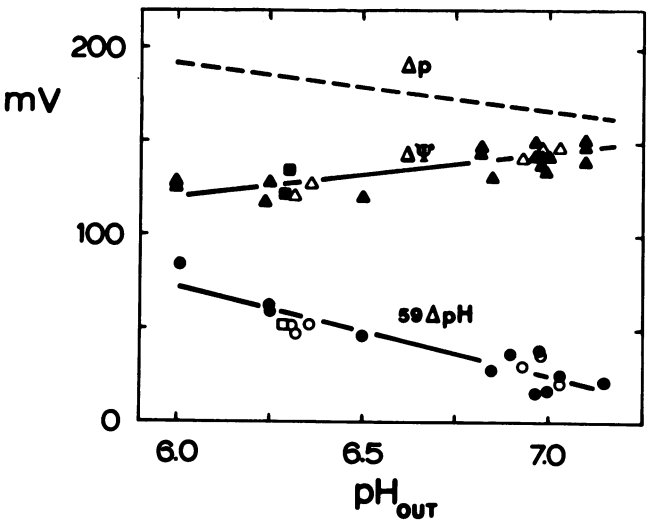

FIG. 6. *Effect of medium pH on the PMF of S. aureus growing anaerobically. Strain W46 cells (▲ and ●) in medium 63S and glucose and strain SG511A (△ and ○) in medium SB with glucose were tested for Δψ (△, ▲) and 59ΔpH (○, ●), as described in the text. The symbols ■ and □ denote the Δψ and the 59ΔpH of H-14 cells growing with 2 μM hemin added to the medium. Each value shown is the mean of three to five replicate determinations (SD < 6 mV). The regression lines for Δψ and 59ΔpH were added to give the Δp line.*

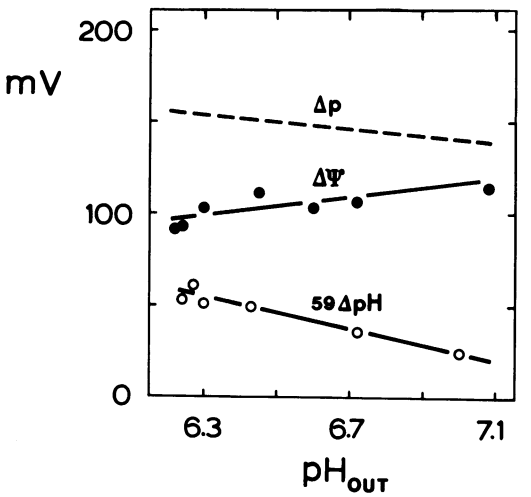

FIG. 7. *Effect of medium pH on the PMF of S. aureus H-14 cells during exponential phase under anaerobic conditions. The experiment was performed as described in the text and in the legend to Fig. 6. No hemin was added to the cultures, which were grown in medium SB with glucose. The cells were sampled at an absorbance value at 625 nm of 0.5. Each value for 59ΔpH (○) and Δψ (●) is from a separate culture and is the mean of five replicate determinations (SD < 8 mV). The Δp line is the sum of the regression lines through the 59ΔpH and Δψ points.*

is due to fermentation of components of the rich medium taking place at the higher pH and not the lower pH, even during aerobic conditions. It is premature to speculate whether the phosphorylation potential may be different in cells growing at different pH's.

ACKNOWLEDGMENTS

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