JOURNAL OF BACTERIOLOGY, June 1976, p. 1224-1231
Vol. 126, No. 3

Printed in U.S.A.

Magnitude of the Protonmotive Force in Respiring *Staphylococcus aureus* and *Escherichia coli*

STEPHEN H. COLLINS[1] AND W. ALLAN HAMILTON*
Department of Microbiology, University of Aberdeen, Aberdeen, AB9 1AS, Scotland

Received for publication 26 January 1976

The membrane potential and pH gradient developed across the plasma membranes of whole cells of *Staphylococcus aureus* and spheroplasts of *Escherichia coli* were estimated. The distributions of potassium ions in the presence of valinomycin and the pH gradient across the membrane were determined from the changes in pK and pH observed in the external medium during transition from the energized respiring state to the de-energized resting condition. The protonmotive force in respiring cells was estimated at 211 mV for *S. aureus* and 230 mV for *E. coli* at external pH values of approximately 6.5. The adequacy of these protonmotive forces as a driving force for substrate accumulation or adenosine 5'-triphosphate synthesis is discussed.

The chemiosmotic hypothesis (15) states that during bacterial respiration, protons are pumped out of the cell to give an electrochemical gradient across the cell membrane. This protonmotive force has electrical and chemical components such that: $\Delta p = \Delta\psi - Z\Delta pH$, where Δp is the protonmotive force in millivolts, $\Delta\psi$ is the membrane potential (inside negative), also in millivolts, and $-\Delta pH$ is the pH gradient (inside alkaline). Z equals 59 at 25 C and is a constant converting the pH gradient to millivolts. The protonmotive force is envisaged as being capable of driving adenosine 5'-triphosphate (ATP) synthesis through the membrane-bound proton-translocating adenosine triphosphatase, and the transport of neutral and ionic nutrients through a series of proton symport and uniport carriers (5, 7, 16). Under anaerobic conditions, transport may be driven by the protonmotive force developed by the action of the proton-translocating ATPase on the glycolytically produced ATP of the cell.

In accordance with this chemiosmotic transport model, we have demonstrated (21, 22) that the membrane potential and pH gradient developed in resting cells is adequate to account for the accumulation of lysine and glutamate, respectively, that can be demonstrated under these conditions. In the next paper in this series (2), we discuss our own findings and those of other authors, with regard to the symport of protons by cells transporting a range of sugars, organic acids, and amino acids. The purpose of the experiments reported in this communication was to obtain a measure of the magnitude of the protonmotive force that is developed across the cytoplasmic membrane of respiring *Staphylococcus aureus* and *Escherichia coli*. In future studies, the concentration gradients achieved in nutrient accumulation assays will be compared with the values of the membrane potential and pH gradient recorded here. Against the background of the mechanisms discussed in the succeeding paper, the adequacy of the protonmotive force to drive transport in actively metabolizing cells also will be assessed.

Several groups of workers have demonstrated that respiring bacteria do in fact extrude protons (11, 14, 24). Griniuviene and his co-workers (3, 4), utilizing the distribution of cationic penetrants, have demonstrated a membrane potential of 140 mV developed by respiration in *E. coli*, but no estimate of the pH gradient was made in these experiments. In photosynthetic bacterial cells and chromatophores energized by illumination, estimates of the protonmotive force have been obtained which range from 200 mV to as high as 400 mV (1, 25). A protonmotive force is also developed from ATP hydrolysis by anaerobic streptococci. Harold et al. (8) obtained values from *Streptococcus faecalis* for a potential of 150 to 190 mV, and, in a separate experiment, for a pH gradient of 0.8 to 1.0 (9). Laris and Pershadsingh (13), using a fluorescent-probe technique, obtained a similar value of 140 mV for the membrane potential developed in *S. faecalis*. However, when Kashket and Wilson (12) applied this technique to *S. lactis*, they estimated the total protonmotive force to be as low as 80 mV, with the potential a

[1] Present address: Department of Botany and Microbiology, School of Biological Sciences, The University College of Wales, Penglais, Aberystwyth SY23 3DA, Wales.

mere 36 mV. It seems, though, that these differences are more likely a result of the different methods used for extrapolating the values for the membrane potential from the observed changes in fluorescence, rather than a consequence of any real difference in the potentials developed in these two closely related organisms.

It should be pointed out that it is not really possible to get a meaningful estimate of the protonmotive force simply by summing values obtained for the membrane potential and pH gradient in separate experiments, and possibly even by different laboratories. Mitchell and Moyle (17) have shown that although the total protonmotive force may be relatively constant, at least with mitochondrial systems, the relative contributions of the potential and pH gradient may vary markedly with experimental conditions. It was the specific ion electrode method developed by Mitchell and Moyle (17) that we used in the studies reported here on our simultaneous measurements of the membrane potentials and pH gradients developed by respiring *S. aureus* and *E. coli*. A preliminary report of some of this work has already appeared (10).

MATERIALS AND METHODS

Organisms and media. *S. aureus* was grown at 30 C with shaking in a medium containing 1% tryptone, 0.5% Lab-Lemco beef extract (Oxoid), 0.1% yeast extract, 0.5% Na_2HPO_4, and adjusted to pH 6.5 with HCl.

A deoxycholate-sensitive strain *E. coli* K-12 Doc S (*i⁻z⁺y⁺ pro⁻ trp⁻ his⁻ met⁻*) was the generous gift of B. A. Haddock, University of Dundee. This particular strain was used in these studies, as it is sensitive to lysozyme and valinomycin without the necessity of pretreatment with EDTA (ethylenediaminetetraacetic acid). The organism was grown at 37 C with shaking in a medium containing 0.4% peptone, 0.2% yeast extract, 1% K_2HPO_4, and adjusted to pH 6.8 with H_2SO_4.

Overnight cultures of both organisms were diluted 1 in 4 in fresh medium and re-incubated until the cell density had doubled. The cells were then harvested at 4 C, washed once in 150 mM KCl and twice in 250 mM sucrose-30 mM choline chloride before final resuspension in the sucrose-choline chloride solution to a density of approximately 10 mg of cells (dry weight) per ml and stored at 4 C up to a maximum period of 5 h.

Spheroplasts of E. coli. Spheroplasts rather than whole cells of *E. coli* were used, to allow rapid and complete lysis on the addition of detergent at the end of the experiment. Cells were diluted to approximately 5 mg/ml in sucrose-choline chloride and incubated at 37 C without shaking for 30 min in the presence of 1 mg of lysozyme per ml. Essentially, this procedure results in quantitative conversion of the cells to spheroplasts; no whole cells could be detected under the microscope after the addition of Triton X-100 (to 0.1%) to a sample of the suspension. A trace of deoxyribonuclease was added to the suspension, which was centrifuged, and the spheroplasts were then resuspended in sucrose-choline chloride at 4 C at a density equivalent to 10 mg of whole cells (dry weight) per ml.

Ion fluxes. The theoretical rationale for these experiments is described in detail by Mitchell and Moyle (17).

The fluxes of protons and potassium ions were measured using ion-specific electrodes. Suspensions were contained in a glass reaction vessel of approximately 50 ml capacity, surrounded by a temperature-controlled water jacket at 25 C; and sealed with a rubber bung through which were introduced the electrodes and sampling ports. Changes in pH were recorded with a Beckman glass electrode (part no. S 41263) and Research pH meter (model 101901). The reference calomel electrode was electrically coupled to the reaction medium through a remote liquid junction in a three-way ground-glass tap. Changes in pK were recorded with a Beckman cationic electrode (part no. 39137) connected to a second pH meter and using the same calomel electrode as a reference. The outputs from both meters were recorded simultaneously on a Rikadenki multichannel pen recorder (model B341). Suspensions (25-ml total volume) of *S. aureus* cells or *E. coli* spheroplasts at 0.5 to 1.0 mg/ml in the sucrose-choline chloride solution were used, with the addition of choline base-neutralized iodoacetic acid (1 mM) to inhibit glycolysis, and carbonic anhydrase (20 μg/ml) to catalyze carbonic acid/CO_2 equilibration (23).

Buffering power. For the determination of buffering power, KCl was added to these suspensions to 150 mM. For assay of B_T, the total extracellular and intracellular buffering, valinomycin and the proton-translocating uncoupler tetrachlorosalicylanilide (TCS) were added to 2 μg/ml. The suspensions were bubbled with water-saturated, oxygen-free nitrogen and stirred magnetically, and buffering powers were estimated from changes in pH observed on the addition of aliquots of 5 mM HCl that had also been rendered anaerobic by bubbling with oxygen-free nitrogen. Since buffering varies with each batch of cells, it must be determined each time.

Respiratory ion movements. For the determination of ion movements associated with respiration, suspensions contained 0.1 mM KCl and 2 μg of valinomycin per ml, and were buffered with either 0.2 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane-sulfonic acid (TES) (for *S. aureus*) or 0.2 mM glycylglycine (for *E. coli*). Then, 2 μl of C100 catalase suspension was added, and the suspension was bubbled with oxygen-free nitrogen until the pH was steady at a final value of 6.8 to 7.2, which was attained by adding HCl or choline hydroxide during this preliminary incubation. The bubbler was then raised so that a stream of nitrogen passed across the surface of the suspension, thereby preserving anaerobic conditions, and the buffering power of the external-phase B_o and the calibration of the recording system for potassium were determined by the addition of small aliquots of anaerobic 5 mM HCl or 10

mM KCl.

Oxygen was added as hydrogen peroxide. One hundred microliters of 2-volume solution was sufficient to maintain a finite oxygen concentration for 5 to 10 min under the conditions of these experiments; further additions of hydrogen peroxide were made as necessary during the course of experiments of longer duration. At the end of each experiment, after the suspension had become anaerobic and returned to a steady pH value, the cells were lysed by the addition of *n*-butanol (*S. aureus*) to a final concentration of 5%, or Triton X-100 (*E. coli*) to 0.1%.

Full details of the theory and method of the calculations are to be found in the analysis of Mitchell and Moyle (17). The symbols used have the following meanings. Subscripts O, I, and T refer to the extracellular, intracellular, and total extracellular plus intracellular phases, respectively. Superscripts o and ω refer to de-energized and energized (respiring) cells, respectively. The symbol Δ is used to denote a difference across the cell membrane, whereas δ denotes a difference with altered conditions in a single phase. The symbol d has the usual mathematical meaning of an infinitesimally small change.

The change in pH resulting from lysis was used to estimate the value of ΔpH^o, the transmembrane pH gradient under de-energized conditions. ΔpH^ω, the pH gradient under energized conditions, was then calculated from ΔpH^o using δpH_O, the change in external pH on exhaustion of oxygen to determine the movement of protons and the experimental curve for B_I, the internal buffering capacity, to estimate the change in internal pH of the cells.

$\Delta\psi^o$, the membrane potential under de-energized conditions, was calculated from the assumption that under de-energized conditions at equilibrium $\Delta p^o = 0$, and therefore $\Delta\psi^o = Z\Delta pH^o$. From the knowledge of the external concentration of potassium, application of the Nernst equation gave the internal potassium concentration under de-energized conditions. $\delta[K^+]_I$, the change in internal potassium concentration, was calculated from the movement of potassium ions and the estimated total internal water space of the cells. $\Delta\psi^\omega$ was then obtained from the ratio of internal to external potassium concentrations under energized conditions.

Estimation of intracellular water space. The internal volume of *S. aureus* was taken as 1.55 μl per mg of cells (dry weight) (19). The internal volume of *E. coli* spheroplasts was determined in each experiment by adding [^{3}H]water at 50 nCi/ml and [^{14}C]hydroxymethyl inulin at 25 nCi/ml to a sample of the stock spheroplast suspension. After 10 min of incubation, three 200-μl samples were taken and filtered through a cellulose nitrate membrane filter, which was sucked dry, but not washed, and added to a vial with 1 ml of water and 10 ml of a scintillation fluid containing: toluene, 667 ml; Triton X-100, 333 ml; 2,5-diphenyloxazole, 4 g; *p*-bis-2-(5-phenyloxazolyl)-benzene, 100 mg. Also at 10 min, three 50-μl samples of the suspension were taken directly to vials containing 10 ml of the toluene-Triton scintillation fluid, 1 ml of water, and a cellulose nitrate filter. The vials were stored at ambient temperature overnight, which allowed the establishment of a stable gel with the radioactivity evenly distributed. Radioactivity was counted on an ICN Coru/matic 200 liquid scintillation spectrometer. Since hydroxymethyl inulin does not enter the cells, the ^{14}C activity represents the extracellular water trapped on the filter, and the ^{3}H activity represents the intracellular plus extracellular water. Then V_i, the intracellular volume on the filter is given by $V_i = 50[(^3H_f/^3H_s) - (^{14}C_f/^{14}C_s)]$ where ^3H_f and $^{14}C_f$ represent the ^{3}H and ^{14}C counts on the filter, and ^3H_s and $^{14}C_s$ represent the counts in the 50-μl sample. The cell volume of an untreated *E. coli* whole-cell suspension estimated by this method was approximately 2.4 μl of cells (dry weight) per mg, which is in reasonably good agreement with the value of 2.7 μl/mg estimated by Winkler and Wilson (27), which we have used elsewhere (2).

Chemicals and stock solutions. Analytical-grade chemicals were used whenever possible. TES was obtained from British Drug Houses (Poole, England). Valinomycin, carbonic anhydrase, lysozyme, and catalase (C100 suspension) were obtained from Sigma Chemical Co. (London, England). Radiochemicals were obtained from the Radiochemical Centre (Amersham, England). 2,5-Diphenyloxazole and *p*-bis-2-(5-phenyloxazolyl)-benzene were purchased from Nuclear Enterprises Ltd. (Edinburgh, Scotland). Cellulose nitrate membrane filters (Sartorius 11306, 0.45 μm) were obtained from V. A. Howe (London, England). Bacteriological media were obtained from Oxoid Ltd. (London, England). Stock solutions of valinomycin and TCS were made up to 2 mg/ml in ethanol and stored at −20 C.

RESULTS

Buffering power. Figure 1 shows typical experimental curves of the buffering power (dH^+/dpH) of a suspension of cells of *S. aureus* expressed as nanogram-ions of H^+ per pH unit per milligram of cells (dry weight). B_O, the buffering power of the outer phase, was estimated from the instantaneous change in pH on addition of 50 μl of anaerobic HCl to the unbuffered suspension of cells. B_T is the total buffering power of the inner and outer phases estimated by addition of 100-μl aliquots of HCl to the cells rendered proton-permeable by the addition of valinomycin and TCS. B_I, the buffering power of the intracellular phase, is the difference between B_T and B_O. In fact, this value of B_I is an approximation, the true value being given by (17): $B_T - B_O = B_I [1 - (d\Delta pH^o/dpH_O)]$. However, determination of $d\Delta pH^o/dpH_O$ by lysis of cells (or spheroplasts), under the conditions used for the determination of B_T, showed that this function was always in the range 0 to −0.1, making a difference of not more than 10% in the estimated value of B_I. This in turn makes a difference of not more than 0.1 pH unit in the estimate of ΔpH^ω, or 6 mV in the final estimate of Δp^ω.

Figure 2 shows similar curves for the deter-

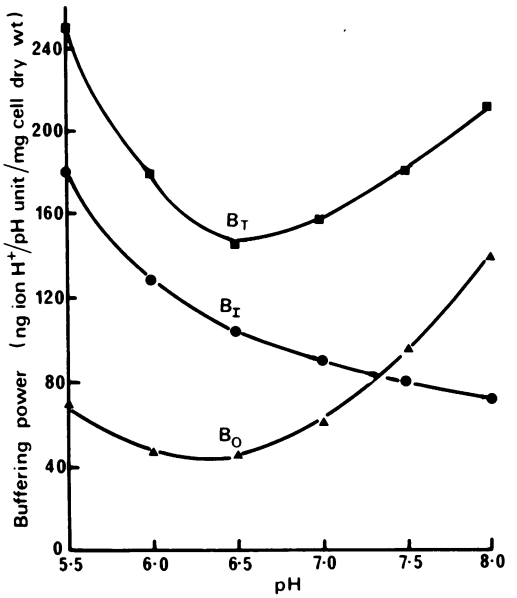

FIG. 1. *Buffering powers of extracellular (B_0; ▲), intracellular (B_I, ●), and total space (B_T, ■), of a suspension of S. aureus. Cells were harvested in exponential phase and allowed to equilibrate at a cell density of 0.83 mg/ml in sucrose-choline chloride-potassium chloride buffer as described in Materials and Methods. B_0 was determined from the instantaneous change in pH on addition of anaerobic HCl. B_T was determined from the change in pH on addition of HCl to cells treated with valinomycin and TCS (2 μg/ml each). B_I is the difference between B_0 and B_T.*

mination of B_0, B_T, and B_I for suspensions of spheroplasts of *E. coli*. Since dry weights of the spheroplast suspension could not be simply determined, cell volume was estimated in each experiment as a fraction of the total suspension volume, and the results in this case were expressed as nanogram-ions of H^+ per pH unit per milliliter of spheroplast suspension. The intracellular spheroplast volume in this particular case was 50 μl in the total volume of 25 ml.

Respiratory ion movements. The effect of adding oxygen to cells of *S. aureus* is shown in Fig. 3. The external pH fell, initially quite sharply. There was a corresponding rise in the external pK as potassium entered the cells in response to the increasing membrane potential. The actual values of the changes in pH and pK represent approximately equivalent amounts of protons and potassium ions, respectively, leaving and entering the cells. The rate of change in the external pH declined as the protonmotive force approached its steady value, but in the case of *S. aureus*, the pH never reached a constant minimum value during the course of the oxygen pulse, presumably because the products of oxidative metabolism are more acidic than the endogenous reserves from which they were generated. When oxygen was exhausted just after 21 min, the protonmotive force collapsed as protons diffused back into the cells. There was a corresponding decrease in the extracellular pK as potassium left the cells in response to the declining membrane potential. A new equilibrium was finally reached at a pH rather more acid than at the beginning of the experiment. The attainment of the final equilibration could be catalyzed by the addition of TCS shortly after the oxygen was exhausted. From the values of δpH_0 and δpK_0 on de-energization, i.e., from the differences between the values immediately preceding oxygen exhaustion and at final equilibrium, the total quantities of protons and potassium ions entering and leaving the cells were estimated at 1,800 ng-ions of H^+ and 1,670 ng-ions of K^+. Correction of the estimated potassium movement was made to allow for the slight response of the cationic electrode to protons. The value of ΔpH^0, estimated from the subsequent lysis of the cells with *n*-butanol, was +0.3. Now since at equilib-

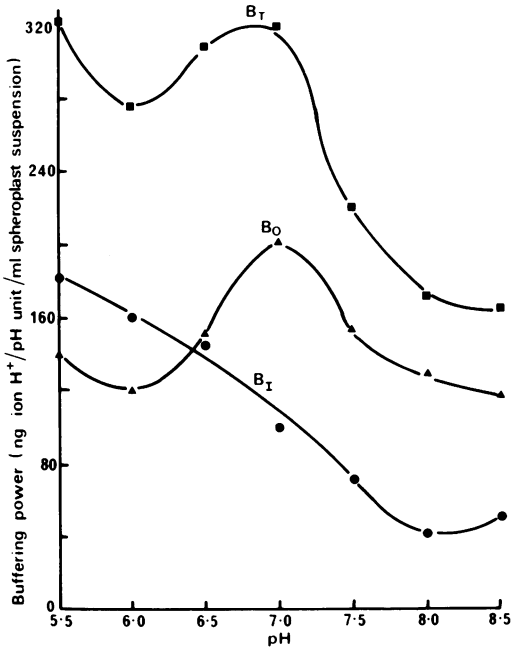

FIG. 2. *Buffering powers of extracellular (B_0; ▲), intracellular (B_I; ●), and total space (B_T; ■) of a suspension of spheroplasts of E. coli. Spheroplasts were prepared as described in Materials and Methods and allowed to equilibrate in 25 ml of sucrose-choline chloride-potassium chloride. Total spheroplast intracellular volume was 50 μl. B_0, B_T, and B_I determined as in Fig. 1.*

rium $\Delta p^{\omega} = 0$, then $\Delta\psi^{\omega} = Z\Delta pH^{\omega}$, or $\Delta pK^{\omega} = \Delta pH^{\omega}$. The extracellular potassium concentration of $[K^+]_0^{\omega}$ was 0.44 mM, and the intracellular concentration $[K^+]_i^{\omega}$ was therefore approximately 0.9 mM. The total cell volume was 31 μl, and thus the change in intracellular potassium concentration on de-energization was 1.67/31 = 54 mM. The value of $[K^+]_i^{\omega}$ was therefore 55 mM. The extracellular concentration of potassium under energized conditions $[K^+]_0^{\omega}$ was 0.37 mM. Therefore, $\Delta pK^{\omega} = \log_{10}([K^+]_i^{\omega})/([K^-]_0^{\omega}) = \log_{10} 149 = 2.17$, and $\Delta\psi^{\omega}$, the membrane potential under energized conditions was given by $Z\Delta pK^{\omega} = 2.17 \times 59 = 128$ mV.

The external pH under de-energized conditions, pH_0^{ω}, was 6.95. Thus, since ΔpH^{ω} was 0.3, pH_i^{ω} was 6.65. Now the movement of protons was 1,800 ng-ions, which, using the data in Fig. 1, allows us to calculate a value for pH_i^{ω} of 7.80 pH_0^{ω} was 6.65, and hence $-\Delta pH^{\omega} = pH_i^{\omega}$

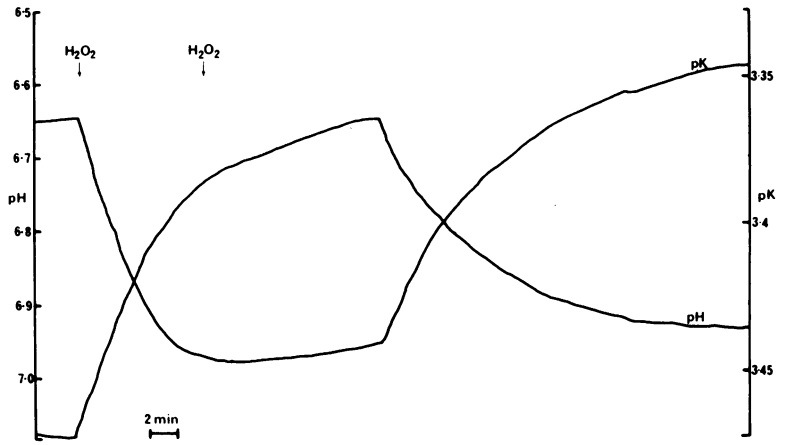

FIG. 3. *Changes in pH and pK observed on addition of hydrogen peroxide to resting potassium-permeable cells of S. aureus in the presence of catalase. Cells (0.83 mg/ml) were allowed to equilibrate in sucrose-choline chloride as described in Materials and Methods. Hydrogen peroxide (100 µl of 2 volume) was added at points indicated by arrows.*

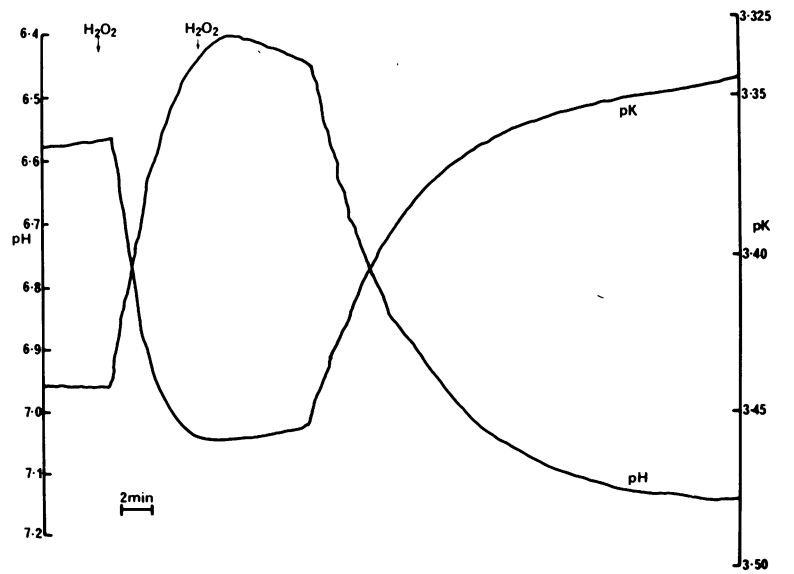

FIG. 4. *Changes in pH and pK observed on addition of hydrogen peroxide to potassium-permeable spheroplasts of E. coli in the presence of catalase. Spheroplasts (50 µl total intracellular volume) were allowed to equilibrate in sucrose-choline chloride solution as described in Materials and Methods. Hydrogen peroxide (100 µl of 2 volume) was added at points indicated by arrows.*

$- pH_0^{\omega} = 1.15$, or 68 mV. Thus, the total protonmotive force was 196 mV.

The traces of pH and pK obtained from a similar experiment, this time carried out on *E. coli* spheroplasts, are shown in Fig. 4. The general form of the traces is similar to that obtained with *S. aureus*, again showing the opposite and approximately equal movement of protons and potassium ions. In this case, however, the pH reached a minimum and began to rise again before the oxygen was exhausted. When the oxygen did run out after 13 min and the system had re-equilibrated, the final steady pH was higher than the original value before addition of hydrogen peroxide. Presumably, the products of endogenous respiration in *E. coli* are less acidic than the substrate. As in the case of *S. aureus*, we have assumed that no change in the buffering of the system occurs after respiration ceases and have estimated the magnitude of the ion movements across the cell membranes from the values of δpH_0 and δpK_0 occurring on exhaustion of oxygen.

Table 1 shows the data from the two experiments shown in Fig. 3 and 4, giving the actual values of pH_0^{ω} and $[K^+]_0^{\omega}$ under energized conditions, together with values of ΔpH^{o} estimated by lysis at the end of the experiments, and the various other parameters necessary for the calculation of ΔpH^{ω} and $\Delta\psi^{\omega}$ as described above for *S. aureus*. The value of $\Delta\psi^{\omega}$ for *E. coli*, estimated from the example shown in Fig. 2 and 4, was 137 mV, and $-\Delta pH^{\omega}$ was 1.7, or 100 mV, giving a total protonmotive force of 237 mV.

Table 2 shows averaged values of ΔpH^{ω}, $\Delta\psi^{\omega}$, and Δp^{ω} for six determinations with each organism. In each case, the initial external pH was adjusted so that the pH_0^{ω}, the external pH under energized conditions, was near to 6.5 (approximately the pH of the medium from which the cells were harvested). The values of Δp were 211 ± 15 mV for *S. aureus* and 230 ± 15 mV for *E. coli*.

DISCUSSION

In a recent paper Tedeschi (26) has questioned the existence of a metabolically induced membrane potential in mitochondria and, by implication, bacterial systems. Unfortunately, he failed to appreciate the significance of the finding that the influx of potassium ions against the chemical concentration gradient occurs only after the energization of the system, with the consequent efflux of protons and the creation of the potential that is inside negative. In resting cells, as Tedeschi himself commented, the addition of valinomycin causes the efflux of potassium and the generation of a diffusion potential, again inside negative. As we ourselves have shown (20), this potential is also capable of driving energy-requiring reactions such as amino acid transport. The crucial error made by Tedeschi (26) was his failure to understand that, for a potassium concentration gradient to exist across a potassium-permeable membrane without net flux of potassium, it must be opposed by an equivalent membrane potential.

The values of the protonmotive force that we have obtained in these experiments compare reasonably well with estimates of Δp for state 4 respiration in rat liver mitochondria (17, 18). This is to be expected, since according to the chemiosmotic hypothesis, the magnitude of Δp should depend mainly upon the relative concentrations of adenosine diphosphate (ADP) and

TABLE 1. *pH gradient (ΔpH^{ω}), membrane potential ($\Delta\psi^{\omega}$), and protonmotive force (Δp^{ω}) under energized conditions*[a]

Organism	Intracellular volume (μl)	pH_0^{o}	pH_0^{ω}	$[K^+]_0^{\omega}$ (mM)	H^+ movement (μg-ion)	K^+ movement (μg-ion)	ΔpH^{o}	ΔpH^{ω}	$\Delta\psi^{\omega}$	Δp^{ω}
S. aureus	31	6.95	6.65	0.363	1.80	1.67	0.30	−1.15	128	196
E. coli	50	7.15	6.45	0.350	3.90	3.70	0.65	−1.7	137	237

[a] Data from the experiments shown in Fig. 1-4 were used for the calculation of ΔpH^{ω}, $\Delta\psi^{\omega}$, and Δp^{ω} under energized conditions

TABLE 2. *pH gradient (ΔpH^{ω}), membrane potential ($\Delta\psi^{\omega}$), and protonmotive force (Δp^{ω}) developed by respiring S. aureus cells and E. coli spheroplasts*[a]

Organism	pH_0^{ω} (range)[b]	ΔpH^{ω}	$\Delta\psi^{\omega}$	Δp^{ω}
S. aureus	6.25-6.65	−1.3 ± 0.2	134 ± 10	211 ± 15
E. coli	6.45-6.75	−1.65 ± 0.2	132 ± 10	230 ± 15

[a] Averaged value with estimated random errors; mean of six determinations is given in each case.

[b] External pH (pH_0^{ω}) indicated near to 6.5.

ATP, provided that the proton conductivity of the plasma membrane (M phase) is adequately low. Mitchell and Moyle (17) have argued that the value of Δp required for ATP synthesis with an ATP to ADP ratio of 1 is approximately 210 mV at 25 C, assuming an intracellular concentration of phosphate at 10 mM. They argued further that an ATP to ADP ratio of 100, which is believed to occur in mitochondria during state 4 respiration, would require a Δp of 270 mV to drive ATP synthesis. Our estimates of Δp in bacteria fall between these values and therefore appear to be in reasonable agreement with the fundamental prediction of the chemiosmotic hypothesis.

There are two assumptions in the present work that might result in a systematic error in our estimate of Δp. First, we have assumed that the activity coefficients for potassium ions are equal inside and outside the cell so that we can equate our estimate of ΔpK with the true transmembrane concentration gradient of potassium. If this assumption were untrue, it would be most likely that the activity coefficient inside the cell, where the potassium concentration is higher, should be lower. This would result in our figures giving a slight overestimate of $\Delta\psi$. The second assumption is that the presence of valinomycin in the plasma membrane during assay does not affect the protonmotive force that the cell develops. However, Casadio et al. (1), using the shift in carotenoid absorption to measure $\Delta\psi$ in chromatophores of photosynthetic bacteria, found that the presence of valinomycin reduced $\Delta\psi$, Δp, and the phosphate potential by as much as 40%. Nicholls (18), on the other hand, measured phosphate potentials of the order of 270 mV in mitochondria treated with valinomycin and under conditions essentially similar to those in which he measured their protonmotive force.

In other work reported from this laboratory (6, 20-22), we have concluded that in *S. aureus* the cationic amino acid lysine is taken up by a uniporter in response to the membrane potential. The anionic amino acid glutamate and the two neutral amino acids glycine and isoleucine were found to respond to artifically imposed gradients of pH and electrical potential in a manner which indicated that these amino acids were translocated into the cell by proton symports, the driving forces being the pH gradient and the total protonmotive force for the anionic and neutral amino acids, respectively. Using the values obtained in the present work and assuming a fixed 1:1 stoichiometry of protons to amino acid and the absence of other control or energy input mechanisms, these driving forces would be adequate for accumulation ratios of up to approximately 200-fold for lysine, 20-fold for glutamate, and 4,000-fold for the neutral amino acids. We are at present attempting to measure the equilibrium levels of accumulation of these amino acids in metabolizing cells to determine whether in fact these driving forces are adequate to account for the levels of accumulation that can actually be observed.

ACKNOWLEDGMENTS

We are indebted to Ray Lindsay for skilled technical assistance. This work was financed by a Science Research Council grant B/RG/25183.

LITERATURE CITED

1. **Casadio, R., A. B. Melandri, C. Zannoni, and B. A. Melandri.** 1974. Electrochemical proton gradient and phosphate potential in bacterial chromatophores. FEBS Lett. 49:203-208.
2. **Collins, S. H., A. W. Jarvis, R. J. Lindsay, and W. A. Hamilton.** 1976. Proton movements coupled to lactate and alanine transport in *Escherichia coli:* isolation of mutants with altered stoichiometry in alanine transport. J. Bacteriol. 126:1232-1244.
3. **Griniuviene, B., V. Chmieliauskaite, and L. Grinius.** 1974. Energy linked transport of permeant ions in *Escherichia coli* cells. Evidence for membrane potential generation by proton pump. Biochem. Biophys. Res. Commun. 56:206-213.
4. **Griniuviene, B., V. Chmieliauskaite, V. Melvgdas, P. Dzneja, and L. Grinius.** 1975. Conversion of *Escherichia coli* cell produced energy to an electric form. J. Bioenerg. 7:17-38.
5. **Hamilton, W. A.** 1975. Energy coupling in microbial transport, p. 1-53. *In* A. H. Rose and D. W. Tempest (ed.), Advances in microbial physiology, vol. 12. Academic Press Inc., London.
6. **Hamilton, W. A., and D. F. Niven.** 1974. Transport driven by transmembrane gradients of chemical and electrical potential. Biochem. Soc. Trans. 2:797-800.
7. **Harold, F. M.** 1972. Conservation and transformation of energy by bacterial membranes. Bacteriol. Rev. 36:172-230.
8. **Harold, F. M., and J. M. Papineau.** 1972. Cation transport and electrogenesis by *Streptococcus faecalis*. I. The membrane potential. J. Membrane Biol. 8:27-44.
9. **Harold, F. M., E. Pavlasova, and J. R. Baarda.** 1970. The transmembrane pH gradient in *Streptococcus faecalis*. Origin and dissipation by proton conductors and N, N'-dicyclohexylcarbodiimide. Biochim. Biophys. Acta 196:235-244.
10. **Jeacocke, R. E., D. F. Niven, and W. A. Hamilton.** 1972. The protonmotive force in *Staphylococcus aureus*. Biochem. J. 127:p57.
11. **Jones, C. W., J. M. Brice, A. J. Downs, and J. W. Drozd.** 1975. Bacterial respiration linked proton translocation and its relationship to respiratory chain composition. Eur. J. Biochem. 52:265-272.
12. **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.
13. **Laris, P. C., and H. A. Pershadsingh.** 1974. Estimations of membrane potentials in *Streptococcus faecalis* by means of a fluorescent probe. Biochem. Biophys. Res. Commun. 57:620-626.
14. **Lawford, H. G., and B. A. Haddock.** 1973. Respiration driven proton translocation in *Escherichia coli*. Biochem. J. 136:217-220.

15. **Mitchell, P.** 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. Cambridge Philos. Soc. 41:445–502.
16. **Mitchell, P.** 1970. Membranes of cells and organelles: morphology, transport and metabolism, p. 121–166. *In* H. P. Charles and B. C. J. G. Knight (ed.), Organization and control in prokaryotic and eukaryotic cells. Society for General Microbiology 20th Symposium. Cambridge University Press, London.
17. **Mitchell, P., and J. Moyle.** 1969. Estimation of membrane potential and pH difference across the cristae membrane of rat liver mitochondria. Eur. J. Biochem. 7:471–484.
18. **Nicholls, D. G.** 1974. The influence of respiration and ATP hydrolysis on the proton-electrochemical gradient across the inner membrane of rat-liver mitochondria as determined by ion distribution. Eur. J. Biochem. 50:305–315.
19. **Niven, D. F., and W. A. Hamilton.** 1972. The mechanism of energy coupling in the active transport of amino acids by *Staphylococcus aureus*. Biochem. J. 127:p58.
20. **Niven, D. F., and W. A. Hamilton.** 1973. Valinomycin-induced amino acid uptake by *Staphylococcus aureus*. FEBS Lett. 37:244–248.
21. **Niven, D. F., and W. A. Hamilton.** 1974. Mechanisms of energy coupling to the transport of amino acids by *Staphylococcus aureus*. Eur. J. Biochem. 44:517–522.
22. **Niven, D. F., R. E. Jeacocke, and W. A. Hamilton.** 1973. The membrane potential as the driving force for the accumulation of lysine by *Staphylococcus aureus*. FEBS Lett. 29:248–252.
23. **Scholes, P., and P. Mitchell.** 1970. Acid-base titration across the plasma membrane of *Micrococcus denitrificans*. Factors affecting the effective proton conductance and respiratory rate. J. Bioenerg. 1:61–72.
24. **Scholes, P., and P. Mitchell.** 1970. Respiration driven proton translocation in *Micrococcus denitrificans*. J. Bioenerg. 1:309–323.
25. **Schuldiner, S., E. Padan, H. Rottenberg, Z. Gromet-Elhanan, and M. Avron.** 1974. pH and membrane potential in bacterial chromatophores. FEBS Lett. 49:174–177.
26. **Tedeschi, H.** 1975. Absence of a metabolically induced electrical potential across the mitochondrial semipermeable membrane. FEBS Lett. 59:1–2.
27. **Winkler, H. H. and T. H. Wilson.** 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli*. J. Biol. Chem. 241:2200–2211.