Accumulation of Thallous Ions (Tl^+) as a Measure of the Electrical Potential Difference across the Cytoplasmic Membrane of Bacteria†

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ABSTRACT: The accumulation of thallous ions ($^{204}Tl^+$) by intact bacteria was investigated. I conclude that Tl^+ is a permeant cation, and that it therefore accumulates in response to the electrical potential difference ($\Delta\psi$) across the cytoplasmic membrane (interior negative). A comparison with other methods shows that the distribution ratio of $^{204}Tl^+$ serves as a reasonably satisfactory method for measuring the membrane potential of *Streptococcus faecalis*. Glycolyzing cells of this organism develop membrane potentials of up to 180 mV. Preliminary experiments with *Escherichia coli*, especially those with a mutant defective in the proton-translocating ATPase, indicate that the Tl^+ distribution also serves as a measure of the membrane potential in this organism. The particular advantage of Tl^+ over other indicators of the membrane potential is that the cells need not be pretreated in any way. By use of the Tl^+ distribution, it was calculated that respiring cells of *E. coli* develop a membrane potential of 160 mV with D-lactate and 180 mV with glucose as a substrate, respectively.

Over a decade ago, Mitchell (1966) proposed that the electrochemical potential gradient of protons ($\Delta\tilde{\mu}_{H^+}$)[1] across the plasma membrane of bacteria and mitochondria is an obligatory intermediate both in oxidative phosphorylation and in the accumulation of ions and metabolites (reviews by Mitchell, 1966; Harold, 1972, 1977; Simoni & Postma, 1975; Skulachev, 1977). Because of this central role, much interest attaches to methods for estimating $\Delta\tilde{\mu}_{H^+}$ and its elements, ΔpH and $\Delta\psi$. Since bacteria are too small to tolerate microelectrodes, indirect methods have been developed for measurement of $\Delta\psi$; these fall into two groups. The electrical potential has been estimated from the equilibrium distribution of permeant cations, including potassium in the presence of valinomycin (Mitchell & Moyle, 1969), and the organic cations introduced by Liberman, Skulachev, and their associates (Grinius et al., 1970; Harold & Papineau, 1972; Schuldiner & Kaback, 1975). More recently, potentials have been estimated from changes in the absorbance or the fluorescence intensity of various dyes, especially cationic ones (Sims et al., 1974; Waggoner, 1976; Åkerman & Wikström, 1976). Both methods are reasonably successful with mitochondria, gram-positive bacteria, and some gram-negative bacteria and indicate the generation of large electrical potentials, interior negative (Mitchell & Moyle, 1969; Åkerman & Wikström, 1976; Harold & Papineau, 1972; Laris & Pershadsingh, 1974; Kashket & Barker, 1977; Michel & Oesterhelt, 1976; Bakker et al., 1976; Bhattacharyya et al., 1977). In order to apply these reagents to other gram-negative bacteria, however, it is necessary to disrupt the outer membrane with unavoidable damage (Padan et al., 1976). Methods that are more generally applicable are therefore desirable.

I report here that, in both *Streptococcus faecalis* and *Escherichia coli*, the thallous ion (monovalent, Tl^+) is passively distributed across the plasma membrane in response to the electrical potential; accumulation of Tl^+ appears to be quite satisfactory as a quantitative index of the membrane potential.

Experimental Procedure

Preparation of Cells. Streptococcus faecalis (faecium) ATCC 9790 was grown overnight on the complex media KTY or NaTY (Harold & Papineau, 1972). *KTY cells* were fully loaded with K^+ ions as described by Harold & Papineau (1972) and were then harvested by centrifugation and washed three times with 2 mM $MgCl_2$. *NaTY cells* were harvested and washed once with 2 mM $MgCl_2$. In order to activate glycolysis, the NaTY cells were resuspended in a medium containing 100 mM Hepes-NaOH and 100 mM glucose, pH 8.0, and were allowed to glycolyze for 10 min at 37 °C. The cells were then washed three times with 2 mM $MgCl_2$. Both washed KTY and NaTY cells were resuspended at 10–20 mg (dry weight)/mL in buffer containing 100 mM Hepes-NaOH and 0.1 mM $MgCl_2$, pH 7.5 ("resuspension buffer"). The KTY cells contained about 1.0 μmol of K^+/mg dry weight and the NaTY cells about 0.2–0.3 μmol of K^+/mg dry weight.

Escherichia coli. ML 3088 and mutant DL 54 of strain ML 308-225 were grown for 20 h on a glucose-limited (= 10 mM glucose) medium, as described by Simoni & Schallenberger (1972). Glucose (10 mM) was added; cells were allowed to grow for half an hour and were then harvested by centrifugation, washed three times with 50 mM NaCl, and resuspended at about 20 mg (dry weight)/mL in 100 mM Hepes-NaOH buffer, pH 7.5. Wild type cells contain some internal energy reserves, which were depleted by treatment with 5 mM dinitrophenol (10 h, 30 °C, as described by Berger, 1973). Pretreatment of mutant cells was unnecessary.

Liposomes. Multilayered liposomes consisting of 95% egg lecithin (Sigma, type VA) plus 5% dicetyl phosphate (K & K Laboratories) (w/w) were prepared in 25 mM Tris-HCl, pH 7.5 (Bakker et al., 1973).

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[1] Abbreviations used: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; diO-C_6-(3), 3,3'-dihexyloxacarbocyanine; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; TCS, tetrachlorosalicylanilide; $TPMP^+$, the cation of the salt triphenylmethylphosphonium bromide; ΔpH, the pH gradient across the cytoplasmic membrane (interior alkaline); $\Delta\tilde{\mu}_{H^+}$, the electrochemical gradient of protons across the cytoplasmic membrane (interior negative); $\Delta\psi$, the electrical potential difference across the cytoplasmic membrane (interior negative); Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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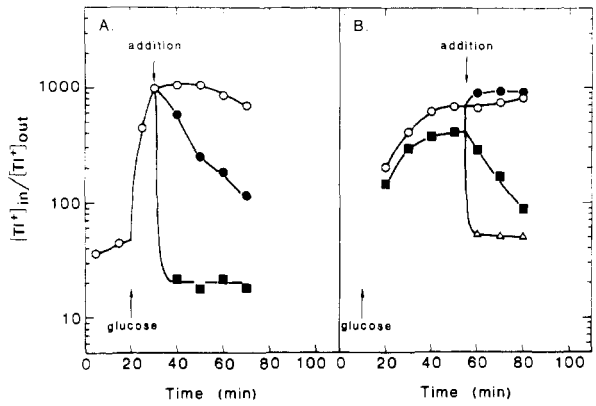

FIGURE 1: Energy-linked accumulation of $^{204}Tl^+$ by NaTY cells of *S. faecalis*. Starving cells were incubated in "resuspension buffer" at 22 °C; $^{204}Tl^+$ was added at zero time and glucose (20 mM) was added at the arrow. (A) Additions were made at 30 min as follows: (○—○) none; (●—●) 10^{-4} M DCCD; (■—■) 1 μg/mL gramicidin D. (B) Additions were made at 55 min as follows: (○—○) none; (●—●) 10^{-5} M valinomycin; (△—△) 10^{-6} M nigericin; (■—■) 10^{-5} M FCCP. In the last incubation the concentration of thallous acetate was 10^{-4} M (see text).

Intracellular Water Space. Intracellular water space was determined by using ^3H_2O and [^{14}C]sorbitol (Rottenberg et al., 1972; Bakker et al., 1976), except that the cells were centrifuged through a layer of silicon oil (Aldrich, *d* 1.05). For *S. faecalis* the total cell water was 2.60 ± 0.32 μL/mg dry weight (five preparations). The intracellular water space was found to be larger in the presence of glucose (about 1.75 μL/mg) than in its absence (about 1.45 μL/mg). These values are somewhat larger than those published for *S. lactis* (Kashket & Barker, 1977). For *E. coli* the total cell water was 2.60 ± 0.07 μL/mg dry weight (one preparation). The intracellular space was 1.10 μL/mg in the absence, and 1.40 μL/mg in the presence of glucose.

Uptake of $^{204}Tl^+$ and [^{3}H]TPMP$^+$. Cells of *S. faecalis* were diluted to 0.5 mg/mL in the "resuspension buffer" and incubated at 22 °C. In the standard protocol $^{204}Tl^+$ at 10–20 nCi/mL (about 10^{-8} M Tl_2SO_4) and 10^{-5} M unlabeled thallous acetate were added at zero time. At various times, 1.0-mL samples were filtered through Uni-pore polycarbonate filters (pore size 0.4 μm; Bio-Rad Laboratories). These filters do not bind any of the cations used and trap only about 3 μL of fluid. Filters were suspended in aquasol (New England Nuclear Co.) and radioactivity was counted. When TPMP$^+$ uptake was measured simultaneously with that of Tl^+, [^{3}H]-TPMP$^+$ Br^- (10 μM) was added at 200–400 nCi/mL at zero time as well. The spectra of ^3H and $^{204}Tl^+$ are sufficiently well separated for simultaneous counting of the two isotopes.

Uptake of $^{204}Tl^+$ by *E. coli* was determined in the same way except that the cell concentration was 0.2 mg/mL and that TlCl was used instead of thallous acetate. Assays were performed with shaking.

Data are given as the distribution ratio, $[\text{cation}]_{in}/[\text{cation}]_{out}$. The electrical potential across the cell membrane was calculated from the cation distribution in the steady state by use of the Nernst equation:

$$\Delta\psi = 58 \log([\text{cation}]_{in}/[\text{cation}]_{out})$$

Activity coefficients were neglected.

Fluorescence of diO-C_6-(3). Fluorescence of diO-C_6-(3) was measured at 90° angle in an Aminco-Bowman spectrofluorimeter. Excitation was centered at 470 and emission at 508 nm. For best results, cell suspensions of *S. faecalis*, diluted to 0.4 mg/mL in the resuspension buffer, were supplemented batchwise with 1.25 μM diO-C_6-(3) at least half an hour before the start of the experiment (Kashket & Barker, 1977). However, this procedure had the disadvantage that the dye induced a small, but measurable, loss of K^+ ions from the cells. When glucose-induced fluorescence quenching was to be compared with the distribution of $^{204}Tl^+$ and [^{3}H]TPMP$^+$, TPMP$^+$ Br^-, and thallous acetate were present at 10^{-5} M as well. The presence of these ions did not influence the fluorescence signal in any respect. For every batch of cells a calibration curve was determined to correlate fluorescence intensity with potassium diffusion potentials of known magnitude (Laris & Pershadsingh, 1974; Sims et al., 1974). For this purpose, valinomycin was added at 10^{-6} M to starving cells suspended in buffer containing various concentrations of potassium. At the moment that the change in fluorescence intensity had reached its maximum, a sample was taken for measurement of the potassium content of the cells (Kashket & Barker, 1977); the potassium concentration gradient at this time point could thus be calculated and converted to $\Delta\psi$ by the Nernst equation. The calibration curves obtained showed that fluorescence intensity was relatively sensitive to changes in $\Delta\psi$ between 50 and 120 mV, but that it became insensitive below 30 and above 130 to 150 mV. Fluorescence intensities observed for the cells in different metabolic states were converted to values of $\Delta\psi$ by the use of the calibration curve determined for that preparation.

Reagents and Radioisotopes. Valinomycin, Hepes, TlCl, and thallous acetate were purchased from Sigma Chemical Co.; TPMP$^+$ Br^- was from K & K Laboratories; gramicidin D was from Nutritional Biochemical Corp.; DCCD was from Calbiochem. I am grateful to the following investigators for gifts of the following reagents: to P. Heytler (E.I. Dupont de Nemours and Co., Wilmington, Del.) for FCCP; to A. Waggoner (Amherst College, Mass.) for diO-C_6-(3); to R. J. Hosley (Lilly Research Laboratories, Indianapolis) for nigericin; to A. W. Hamilton for TCS.

[^{14}C]Sorbitol (210 Ci/mol) and ^3H_2O (0.45 Ci/mol) were purchased from New England Nuclear Co; $^{204}Tl_2SO_4$ (500 Ci/mol) from Amersham/Searle. [^{3}H]TPMP$^+$ was a gift from H. R. Kaback, Roche Institute of Molecular Biology, Nutley, N.J.

Results

Uptake of Thallous Ions by S. faecalis. The basic findings on uptake of $^{204}Tl^+$ by NaTY cells of *S. faecalis* are shown in Figure 1. Starving cells took up a small amount of Tl^+; subsequent addition of glucose induced a 20- to 40-fold increase in the uptake ratio. A new steady-state level of Tl^+ uptake was usually reached within 10–25 min after glucose was added (Figure 1). In cells grown on arginine–glucose medium (Abrams et al., 1973), arginine and galactose, as well as glucose supported Tl^+ uptake (not shown).

Figure 1 also shows the effect of a number of ionophores and inhibitors on Tl^+ accumulation. Valinomycin, which specifically increases the potassium conductance of the membrane (Mueller & Rudin, 1967), hardly influenced the distribution ratio of Tl^+ (Figure 1B). DCCD, which inhibits ATP-driven electrogenic extrusion of protons by *S. faecalis* (Harold et al., 1970; Harold, 1972), slowly reversed Tl^+ uptake (Figure 1A). The same was true when either of the proton conductors FCCP (Figure 1B) or TCS (not shown) was added. Addition of gramicidin D (Figure 1A), or of nigericin (Figure 1B), quickly reversed Tl^+ accumulation. The effect of gramicidin is due to the fact that all monovalent cations present, K^+, Na^+, H^+, and indeed Tl^+ (Neher, 1975), move through the gramicidin

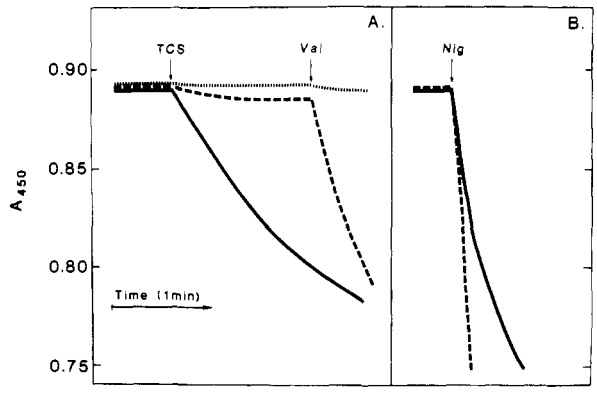

FIGURE 2: Swelling of liposomes caused by salt penetration. Liposomes were diluted 1:10 into 25 mM solutions of the acetate salts of various monovalent cations. Absorption at 450 nm (which is due to light scattering) was recorded as a function of time. In A, TCS (10^{-5} M) and valinomycin (10^{-6} M) were added at times indicated in the figure. In B, nigericin (10^{-6} M) was added. Traces: (—) liposomes suspended in thallous acetate; (- - -) liposomes suspended in potassium acetate; (· · ·) liposomes suspended in sodium acetate.

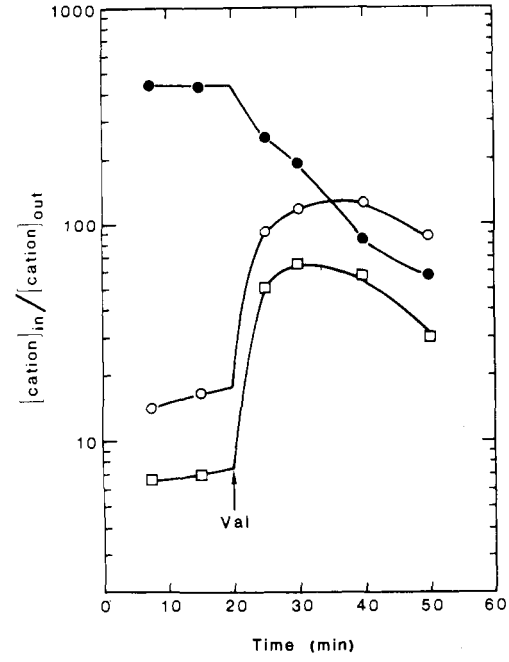

FIGURE 3: Accumulation of $^{204}Tl^+$ and [^{3}H]TPMP in response to a diffusion potential of K^+ ions. KTY cells of *S. faecalis* were incubated in the "resuspension buffer" in the presence of 1.0 mM KCl. At t = 20, valinomycin (10^{-6} M) was added. The distributions of $^{204}Tl^+$ (O—O), [^{3}H]TPMP (□—□), and K^+ (●—●) were determined as a function of time.

channel and collapse $\Delta\tilde{\mu}_{H^+}$. Nigericin probably acts by transporting Tl^+ in exchange for H^+ (see below).

The distribution ratio of Tl^+ was independent of the external concentration of Tl^+ up to 10^{-5} M, but began to decrease at 10^{-4} M (not shown). Therefore, all experiments reported here were carried out at 10^{-5} M Tl^+, except for that in which FCCP was added (Figure 1B). In that case the Tl^+ concentration was raised to 10^{-4} M, in order to circumvent possible binding of the ion pair Tl^+–$FCCP^-$; binding of the ion pair $TPMP^+$–$CCCP^-$ has been reported (Michel & Oesterhelt, 1976).

Uptake of Thallous Ions by Liposomes. The results shown in Figure 1 indicate that Tl^+ accumulation depends upon the generation of $\Delta\tilde{\mu}_H$, or one of its components. Several previous reports suggest that Tl^+ may be a permeant cation (Skulski et al., 1973; Melnick et al., 1976). In order to establish whether lipid bilayer membranes in general are permeable to thallous ions, I turned to multi-layered liposomes. Relative cation permeabilities in such a system can be determined by suspending the liposomes in isotonic acetate salts of different cations in the presence of a proton conductor. In this situation the rate of swelling is determined entirely by the cation permeability (Scarpa & de Gier 1971; Bakker et al., 1973). Figure 2A shows that liposomes suspended in thallous acetate did swell in the presence of the proton conductor TCS (10 μM). Liposomes suspended in potassium acetate required for swelling both TCS and valinomycin. Liposomes suspended in sodium acetate did not swell at all. Addition of valinomycin (1 μM), as well as TCS, to vesicles suspended in thallous acetate did not accelerate their swelling (not shown). These results indicate that Tl^+ is a permeant cation in a lipid-bilayer system, and that valinomycin-mediated Tl^+ movement is slow compared with the nonmediated movement. These and other observations lead me to believe that the contribution of valinomycin-mediated transport under the conditions of the experiments reported here can be safely neglected. Nigericin-mediated Tl^+ transport, however, cannot be neglected. This ionophore caused rapid swelling of liposomes suspended in either thallous acetate or potassium acetate and probably transports both cations in exchange for protons (Figure 2B, compare with Figure 1B).

The Distribution of Thallous Ions Is a Measure of $\Delta\psi$ in S. faecalis. The results presented so far indicate that Tl^+ is a permeant cation. Therefore, the results of Figure 1 can be explained by assuming that Tl^+ accumulation by *S. faecalis* is driven by the membrane potential (interior negative). In this section I shall consider whether the distribution ratio can serve as a quantitative measure of the electrical potential in *S. faecalis*. To this end, $\Delta\psi$ calculated from Tl^+ distribution was compared with that obtained by other methods. First, a diffusion potential was imposed in starving cells by addition of valinomycin. Figure 3 shows that both Tl^+ and $TPMP^+$ accumulated under these conditions. During the course of the experiment K^+ progressively leaked out of the cells, probably in exchange for protons (Harold & Baarda, 1967; Maloney, 1977). Note that after about 20 min the distribution ratio of the three ions approached similar, but not identical values. The experiment of Figure 3 was repeated at various concentrations of external K^+. Figure 4 shows the distribution ratios for Tl^+ and $TPMP^+$ as a function of the K^+ distribution at 20 and 30 min after addition of valinomycin. If all three ions were distributed in accordance with $\Delta\psi$, one would expect a line with a slope of 1.0 passing through the origin (Figure 4, dashed lines). In fact, the calculated lines for Tl^+ and $TPMP^+$ are approximately parallel with slopes of about 0.8, and intercepts on the positive ordinate (Figure 4, solid lines). I suggest that the deviations from the expected relationship indicate that Tl^+ and $TPMP^+$ do not equilibrate rapidly enough with the potential established by K^+.

The next set of experiments employed glycolyzing cells. The electrical potential was calculated by four different methods: from the distribution of Tl^+, $TPMP^+$, and K^+ (in the presence of saturating amounts of valinomycin), and from the quenching of diO-C_6-(3) fluorescence. The results (Table I) show several features of interest. First, glycolyzing KTY cells generate a much smaller potential than do NaTY cells (see Discussion). Second, in the KTY cells Tl^+, $TPMP^+$, and diO-C_6-(3) indicated approximately the same electrical potential (experiment 1). In the NaTY cells, however, $TPMP^+$ indicated a significantly lower potential than did the other cations (experiments 2 and 3). It appears that, at high values of $\Delta\psi$, $TPMP^+$ is not as suitable for measurement of $\Delta\psi$ as is Tl^+. Finally, it should

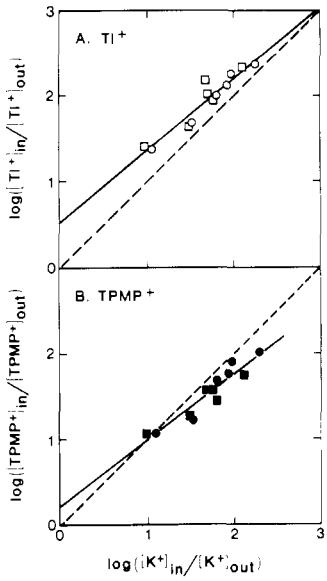

FIGURE 4: Comparison of $^{204}Tl^+$ (A) and [^{3}H]TPMP$^+$ (B) distribution with that of K^+ in the presence of valinomycin (10^{-6} M). Starving cells of *S. faecalis* were suspended as in Figure 3, except that the external KCl concentration varied between 100 μM and 30 mM. Symbols: (circles) points taken 20 min after addition of valinomycin (10^{-6} M); (squares) points taken 30 min after addition of valinomycin; (open symbols) $^{204}Tl^+$ distribution; (closed symbols) [^{3}H]TPMP$^+$ distribution. The solid lines were calculated for best fit by regression analysis. The dashed lines are the relations expected if the three cation distributions were entirely determined by $\Delta\psi$.

TABLE I: Comparison of Different Methods for Measurement of $\Delta\psi$ in Glycolyzing Cells of *S. faecalis*.[a]

experiment	cells	$\Delta\psi$ (mV) diO-C_6-(3)	Tl^+	TPMP$^+$	K^+
1	KTY	100	95	95	
2	NaTY	≥145[b]	165	115	
3	NaTY		180	110	190

[a] In experiments 1 and 2 cells were preincubated in the "resuspension buffer" in the presence of 1.25 μM diO-C_6-(3), 10^{-5} M Tl^+, and 10^{-5} M TPMP$^+$. Glucose (10 mM) was added, and either Tl^+ and TPMP$^+$ accumulation or diO-C_6-(3) fluorescence intensity were measured as a function of time. In experiment 3, cells were preincubated in a medium containing 2 mM $MgCl_2$, 180 μM KCl, 10^{-6} M valinomycin, 10^{-5} M Tl^+, and 10^{-5} M TPMP$^+$. Glucose (20 mM) was added. The pH was kept constant by automatic titration with 20 mM LiOH. Tl^+ and TPMP$^+$ accumulation were determined by sampling; K^+ accumulation was determined by continuous measurement of the external K^+ concentration with a cation-specific electrode (Beckmann 39137). The values of $\Delta\psi$ represent steady-state values (i.e., when net uptake ceased or when the fluorescence intensity became constant). In KTY cells such steady states were reached within 5 min. However, in NaTY cells fluorescence reached a constant level within a minute after addition of glucose, but cation uptake could take as long as 25 min to reach a steady-state value. [b] The fluorescence intensity was lower than that at which the calibration curve became independent of $\Delta\psi$ (145 mV, see Experimental Procedure).

be noted that in experiments 1 and 2 uptake of Tl^+ and TPMP$^+$ was measured in the simultaneous presence of diO-C_6-(3). This cationic dye appears to have a slightly toxic effect: In its absence the membrane potential calculated from cation distribution was higher by 15–20 mV (not shown, see also Pick & Avron, 1976). The reverse is not true: Tl^+ and TPMP$^+$ at the concentrations used (10^{-5} M) did not decrease $\Delta\psi$ as measured by diO-C_6-(3) fluoresence. Taken together, these results indicate that Tl distribution serves as a reasonably

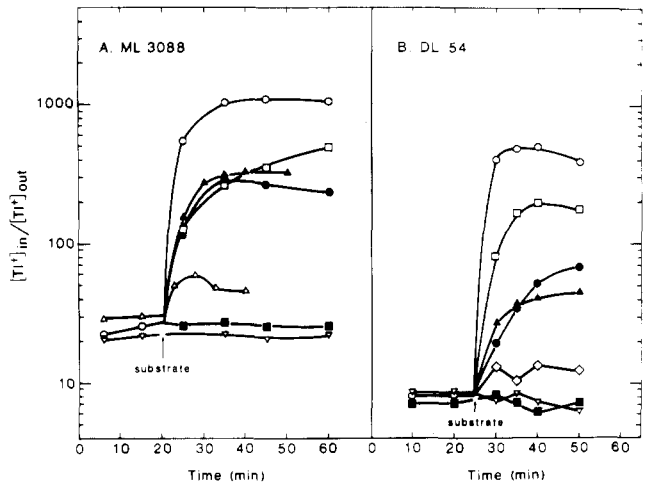

FIGURE 5: Energy-linked accumulation of $^{204}Tl^+$ by *Escherichia coli* ML 3088 (A) and mutant DL 54 (B). Cells were incubated as described under Experimental Procedure, in the absence or presence of metabolic inhibitors. Accumulation of $^{204}Tl^+$ by the cells was measured as a function of time. At t = 20 (A) or t = 25 (B) either glucose (10 mM) or D-lactate was added. Symbols: (○—○) glucose, no inhibitor; (●—●) glucose, 5 mM NaCN present; (▲—▲) glucose, anaerobic conditions; no inhibitor; (◇—◇) glucose, anaerobic conditions, 50 μM HQNO present; (△—△) glucose, 5 mM dinitrophenol present; (□—□) D-lactate, no inhibitor; (■—■) D-lactate, 5 mM CN^- present; (▽—▽) no substrate, no inhibitor.

satisfactory method for quantitative measurements of $\Delta\psi$ in *S. faecalis*.

Uptake of Thallous Ions by Escherichia coli. Measurement of the membrane potential in *E. coli* by use of permeant cations normally requires pretreatment of the cells with EDTA (Leive, 1968; Padan et al., 1976). This is not necessary with Tl^+. Figure 5A shows that depleted cells of strain ML 3088 accumulate a relatively small amount of Tl^+. Subsequent addition of glucose caused a 40-fold increase in the accumulation ratio. This uptake was almost completely prevented by the proton conductor dinitrophenol, but only partially so by anaerobiosis or by cyanide. Addition of D-lactate caused a 20-fold increase in the accumulation ratio of Tl^+; this process was completely sensitive to cyanide. These results indicate that Tl^+ accumulation by *E. coli* can be driven either by glycolysis or by respiration (or both).

Mutant DL 54 is defective in the proton-translocating ATPase (Simoni & Schallenberger, 1972). Thus, respiring cells of this mutant can generate $\Delta\tilde{\mu}_{H^+}$, but oxidative phosphorylation is blocked; conversely, glycolyzing cells generate ATP but, due to the defective ATPase, should be unable to generate $\Delta\tilde{\mu}_{H^+}$. If Tl^+ uptake were driven by $\Delta\psi$, one would expect the mutant to accumulate Tl^+ under aerobic conditions, but not under anaerobic ones. Figure 5 compares Tl^+ accumulation by the wild type (A) with that by the mutant (B). It will be noted that in the wild type oxidation of D-lactate and anaerobic glycolysis supported Tl^+ accumulation to about the same extent. By contrast, in the mutant D-lactate caused an accumulation of Tl^+ which was about fivefold greater than that supported by glucose under anaerobic conditions. These results suggest that accumulation of Tl^+ by *E. coli* depends upon the generation of $\Delta\tilde{\mu}_{H^+}$ and does not require ATP. It is not entirely clear why glucose, anaerobically, supported some Tl^+ accumulation by DL 54. However, the fact that this small uptake was completely abolished by HQNO (Figure 5B) points toward the generation of a small $\Delta\tilde{\mu}_{H^+}$ by anaerobic electron transport (Gutowski & Rosenberg, 1976). Note also that, with all substrates Tl^+ accumulation in the mutant was lower than

that in the wild type (Figure 5). This is probably due to the fact that a mutant with a defective proton-translocating ATPase cannot maintain as large a $\Delta\tilde{\mu}_{H^+}$ across its membrane as can the wild type (Rosen, 1973; Altendorf et al., 1974).

Since *E. coli* is insensitive to valinomycin, it is difficult to generate a K^+ diffusion potential. I therefore studied Tl^+ uptake in response to a diffusion potential of protons, generated with the help of proton conductors (Figure 6). Cells were preincubated at pH 6.2 with cyanide and the proton conductors dinitrophenol (5 mM) and TCS (10 μM). A sudden increase in the pH of the suspension to 8.5 caused a substantial, transient accumulation of Tl^+. This result shows that an artificially generated electrical potential (negative inside) also drives accumulation of Tl^+ by intact cells of *E. coli*.

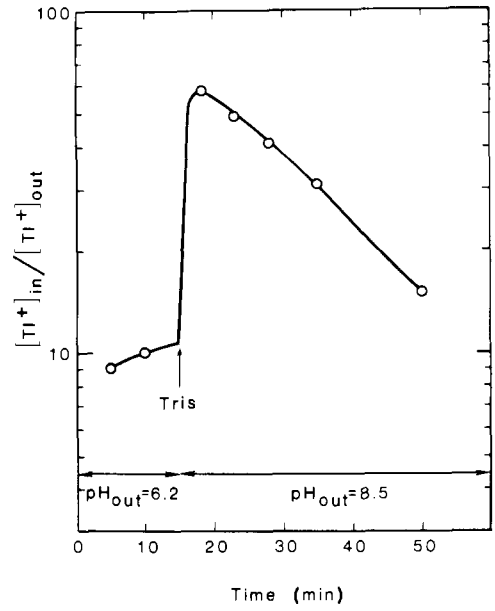

FIGURE 6: Accumulation of $^{204}Tl^+$ in response to a diffusion potential of protons. Cells of *E. coli* ML 3088 were preincubated in a medium containing 50 mM NaCl, 5 mM NaCN, 5 mM dinitrophenol, 10^{-5} M TCS, and 10 mM Hepes-NaOH, final pH 6.2. At t = 15 a little 2 M Tris was added causing the external pH to rise to 8.5. Accumulation of $^{204}Tl^+$ was measured as a function of time.

Discussion

The main findings reported in this communication are that Tl^+ behaves as a permeant cation and accumulates in bacterial cells in response to the electrical potential difference across the cytoplasmic membrane (interior negative); the distribution ratio of $^{204}Tl^+$ can be used to calculate the electrical potential difference. This conclusion rests on the following observations: First, artificially generated potentials (interior negative) caused substantial Tl^+ accumulation in both *S. faecalis* (Figure 3) and *E. coli* (Figure 6) as well as in liposomes (Figure 2). Second, in presence of valinomycin the Tl^+ distribution ratio of starving and metabolizing cells of *S. faecalis* was approximately the same as the distribution ratio of K^+ (Figure 4 and Table I, respectively). Third, in glycolyzing cells of *S. faecalis* the membrane potential calculated from the Tl^+ distribution is in good agreement with that calculated by other methods, especially diO-C_6-(3) fluorescence (Table I). For *S. faecalis*, at least, the distribution ratio of $^{204}Tl^+$ appears to serve as a quantitative measure of the electrical potential.

Two additional points should be made. First, even starving cells of *S. faecalis* accumulate a small, but significant amount of $^{204}Tl^+$. Part of this basal uptake may reflect binding of Tl^+ to the surface of the cells. However, the observation that, in NaTY cells which are known to have a low internal pH (Zarlengo & Abrams, 1963; Harold et al., 1970), the basal uptake increased with the external pH between 6.0 and 8.0 suggests that the basal uptake may also reflect a diffusion potential for protons. More importantly, the present data (Table I) reconfirm earlier observations that Na^+-loaded cells of *S. faecalis* develop larger potentials than do K^+-loaded cells (Harold & Papineau, 1972). My NaTY cells (which are comparable in properties to the Na^+-loaded cells used by Harold & Papineau, 1972) develop a membrane potential of about 180 mV; that of K^+-loaded cells is only about 120 mV. The basis for this difference is under investigation.

None of the methods currently available for measuring membrane potentials in bacteria is ideal. Fluorescent carbocyanine dyes (Sims et al., 1974; Laris & Pershadsingh, 1974; Waggoner, 1976) respond rapidly to changes in the membrane potential, but are not always metabolically innocuous (Pick & Avron, 1976; Manson et al., 1977; and Results). Tl^+ and $TPMP^+$ can be used at concentrations so low that they do not appear to interfere with the physiology of the cells, but equilibrate relatively slowly (Figures 1 and 4 and Komor & Tanner, 1976). In my experience Tl^+ indicated larger potentials than did $TPMP^+$ (Table I); the reason is not fully understood. It may be related to the hindered penetration of the bulky $TPMP^+$ cation that has been noted in several other organisms (Komor & Tanner, 1976; Miller & Budd, 1976). Finally, the disadvantages of Tl^+ should not be overlooked: Both nigericin (Figure 2B) and gramicidin (Neher, 1975) were found to translocate Tl^+. Valinomycin has been reported to form a complex with Tl^+ (Grell et al., 1972), but I found no evidence for significant valinomycin-mediated Tl^+ transport (Figure 2A and Results section). Another potential source of error is the oxidation of Tl^+ to Tl^{3+} and to insoluble Tl_2O_3 (standard redox potentials at pH 7.0, 1.25 and 0.65 V, respectively). The fact that $^{204}Tl^+$ accumulation in respiring *E. coli* was readily reversible by metabolic inhibitors (data not shown) suggests that this is not a serious problem under our conditions. However, Lindegren & Lindegren (1973) reported accumulation of Tl_2O_3 in mitochondria of starving yeast and I, too, observed slow oxidation of Tl^+ by starving cells of *E. coli* (about 5 natoms of O_2 per min per mg at 5×10^{-4}M TlCl). It should also be remembered that TlCl is insoluble above 10^{-2} M. The most serious objection to the use of Tl^+ as an indicator of $\Delta\psi$ is that Tl^+ is an analogue of K^+. Several authors have attributed Tl^+ accumulation to the activity of a potassium transport system (Landowne, 1975; Norris et al., 1976). The data reported here indicate clearly that in *S. faecalis* Tl^+ is not accumulated by active transport, since, unlike K^+ (results not shown), Tl^+ is in equilibrium with $\Delta\psi$ (Table I). Moreover, the K_m for Tl^+ uptake is at least 50 times higher than that for K^+ uptake (results not shown). Therefore, there is no evidence that the K^+ transport system of *S. faecalis* accepts Tl^+, but this point must be established anew for every organism. The data available suggest that Tl^+ behaves as a permeant cation in erythrocytes (Skulskii et al., 1973) and in mitochondria (Melnick et al., 1976), but in squid axons (Landowne, 1975) and in Ehrlich ascites cells (O. A. Bakker-Grunwald, unpublished) Tl^+ distribution is perturbed by the Na^+, K^+-ATPase.

The particular virtue of Tl^+, aside from its commercial availability, is that it can apparently be used as a measure of $\Delta\psi$ in *E. coli*, and presumably in other gram-negative bacteria. This assertion is based on the observation that metabolic substrates greatly enhanced Tl^+ accumulation by *E. coli*, under conditions known to generate $\Delta\tilde{\mu}_{H^+}$, but not ATP

(Figure 5B). Since an artificial membrane potential also supports Tl^+ accumulation (Figure 6), it seems reasonable to regard Tl^+ as an index of the electrical potential in metabolizing cells as well. I found no evidence for ATP-linked Tl^+ uptake (Figure 5).

By use of the Tl^+ distribution one calculates the electrical potential of respiring *E. coli* to be about 160 mV for D-lactate and 180 mV for glucose (Figure 5A). It will be noted that these values are considerably higher than the 120 mV reported for valinomycin-EDTA treated cells by Padan et al. (1976), let alone the 50–100 mV generated by membrane vesicles (Hirata et al., 1973; Ramos et al., 1976; Bhattacharyya et al., 1977). The higher values observed here are presumably due to the use of intact cells subject to minimal mistreatment.

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