DISTINCTION BETWEEN CHANGES IN MEMBRANE POTENTIAL AND SURFACE CHARGE UPON CHEMOTACTIC STIMULATION OF *ESCHERICHIA COLI*

MICHAEL EISENBACH, YAEL MARGOLIN, ADINA CIOBOTARIU, AND HAGAI ROTTENBERG
Department of Membrane Research, The Weizmann Institute of Science, 76100 Rehovot, Israel

ABSTRACT Galactose and other chemotactic attractants have been shown to trigger an apparent hyperpolarization in *Escherichia coli* (Eisenbach, M., 1982, *Biochemistry*, 21:6818–6825). The probe used to measure membrane potential in that study, tetraphenylphosphonium (TPP^+), may respond also to surface-charge changes in the membrane. The distinction between true changes in membrane potential and changes in the surface charge of the membrane is crucial for the study of this phenomenon in bacterial chemotaxis. To distinguish between these parameters, we compared the response to galactose with different techniques: K^+ distribution in the presence of valinomycin (measured with a K^+-selective electrode), TPP^+ distribution (measured with a TPP^+-selective electrode) at different ionic strengths, absorbance changes of bis(3-phenyl-5-oxoisoxazol-4-yl)pentamethineoxonol (oxonol V), and fluorescence changes of three probes with different mechanisms of response. All the techniques revealed stimulation by galactose of a transient hyperpolarization, of comparable magnitude. This indicates the involvement of ion currents rather than alterations of local surface properties.

INTRODUCTION

The galactose-binding protein of *Escherichia coli* participates in both transport of D-galactose and chemotaxis towards galactose (Hazelbauer and Adler, 1971). It has recently been shown that chemoattractants, including galactose, stimulate an apparent increase in membrane potential of *E. coli*, as monitored by a TPP^+[1]-electrode and detectable in the presence of a respiratory inhibitor (Eisenbach, 1982). For the case of galactose, it has been shown that this apparent hyperpolarization is correlated with chemotaxis (Eisenbach, 1983; Eisenbach et al., 1983).

TPP^+, a lipophilic cation, permeates the cytoplasmic membrane and equilibrates according to the membrane potential (Grinius et al, 1970; Rottenberg, 1979; Felle et al., 1980). Its lipophilic properties also cause it to bind in large quantities to the negatively charged cytoplasmic membrane (Zaritsky et al., 1981; Ahmed and Booth, 1981; Ten Brink et al., 1981; Lolkema et al., 1982; Bakker, 1982; Eisenbach, 1982; H. Rottenberg, unpublished). The observed changes in the concentration of TPP^+ in the medium, in response to stimulation by galactose, could thus be the consequence of changes in membrane potential and/or surface charge of the membrane. Distinguishing between these alternatives is very important. A real change in membrane potential stimulated by galactose would mean the involvement of ion current(s) in the chemotaxis process to galactose. A change in the surface charge of the membrane, caused by the same stimulus, would indicate either vertical displacement of one or more membrane proteins (Borochov and Shinitzky, 1976) or conformational changes of these proteins (Boos, 1974; Eisenbach and Caplan, 1979; Kell, 1979) ocurring, e.g., as a consequence of the binding of the attractant/binding-protein complex to the cytoplasmic membrane (Wang and Koshland, 1980).

We have therefore examined the response to galactose, using a variety of probes of membrane potential, some of which also respond to surface charge changes. Surface charge changes are reflected as surface potential changes at low ionic strength (Mclaughlin, 1977). To distinguish between changes in the external surface potential[2] and changes in the membrane potential, we studied the effect of the ionic strength of the medium on the apparent

Dr. Rottenberg's current address is the Department of Pathology, Hahnemann Medical University, Philadelphia, PA.

[1] *Abbreviations used in this paper:* ANS, 1-anilinonaphthalene-8-sulfonate; DCCD, *N*, *N'*-dicyclohexylcarbodiimide; diS-C_3-(5), 3,3'-dipropylthiodicarbocyanine iodide; oxonol V, bis (3-phenyl-5-oxoisoxazol-4-yl) pentamethineoxonol; RH160, anhydro-4-(4'-P-dibutyl aminophenyl-buta 1':3' dienyl) 1-δ-sulfobutyl pyridinium hydroxide; TPP^+, tetraphenylphosphonium; $\Delta\psi$, membrane potential (defined to be positive for an outwardly directed potential gradient [inside-positive]); in this study, hyperpolarization means an increase in the absolute value of $\Delta\psi$, or that $\Delta\psi$ becomes more negative; σ_s, surface charge.

[2] Surface potential is defined as the potential difference between the bulk solution and the surface of the membrane; membrane potential is defined as the potential difference between the bulk solutions across the membrane.

hyperpolarization detected by TPP^+. Change in ionic strength should affect the surface potential but not the membrane potential (Mclaughlin, 1977). Finally, presumed true changes in membrane potential were measured by K^+ distribution in the presence of valinomycin. The results with all these techniques indicate that galactose stimulates changes in membrane potential, rather than changes in surface charge.

EXPERIMENTAL PROCEDURES

Chemicals

Anhydro-4-(4'-P-dibutyl aminophenyl-buta 1':3' dienyl) 1-δ-sulfobutyl pyridinium hydroxide (RH160) and bis(3-phenyl-5-oxoisoxazol-4-yl) pentamethineoxonol (oxonol V) were gifts from Dr. A. Grinvald, and 3,3'-dipropylthiodicarbocyanine (diS-C_3-(5)) was a gift from Dr. P. Lelkes (both of the Weizmann Institute of Science, Rehovot, Israel). These dyes were dissolved in ethanol, and were added to the bacterial suspension in a volume not exceeding 0.1% ethanol. 1-anilinonaphthalene-8-sulfonate (ANS) was received from Molecular Probes (Junction City, OR) and dissolved in water. Tetraphenylphosphonium (TPP^+) was synthesized as previously described (Eisenbach, 1982). Essentially glucose-free D-galactose was from Sigma Chemical Co. (St. Louis, MO). D-fucose was purified before use by liquid chromatography as described previously (Eisenbach et al., 1983). All other chemicals were of the highest purity commercially available.

Bacteria and Preparation

The strains used in this study are *E. coli* K12 derivatives. AW546 is wild-type for chemotaxis; AW550 is an *mglB* mutant derivative of AW546, with a defective galactose-binding protein (Hazelbauer and Adler, 1971; in that paper AW546 is called B275*his*). They were obtained from Dr. J. Adler (University of Wisconsin at Madison). The bacteria were grown and treated as previously described (Eisenbach, 1982). All the probes used in this study, excluding diS-C_3-(5), required cel-wall permeabilization. This was performed by using the Tris-EDTA technique of Leive (1965), as modified by Szmelcman and Adler (1976). For consistency, permeabilized cells were used also with diS-C_3-(5) as a probe. As a control for probe adsorption, heat-treated cells were prepared from the same batch of permeabilized bacteria, as described (Muratsugu et al., 1979). All bacterial preparations were finally resuspended in the specified medium to a concentration of 0.5 mg of protein/ml (TPP^+ and K^+ electrodes) or 0.08–0.12 mg of protein/ml (optical probes).

TPP^+- and K^+-selective Electrodes

The TPP^+-selective electrode was constructed as previously described (Eisenbach, 1982). The K^+-selective electrode was from Orion Research Inc., Cambridge, MA (model 93-19). The electrodes were used with a calomel reference electrode, indirectly connected to the measuring vessel through an agar salt bridge. The measuring vessel was thermostated at 30°C. When the K^+ electrode was used, the suspending medium contained 10 mM NaPi instead of 10 mM KPi.

Fluorescence Measurements

The measurements were carried out in thermostated cuvettes with one of the following fluorometers: Spex fluorolog (magnetic stirring) (Spex Industries, Inc., Metuchen, NJ), Perkin Elmer MPF-44A (no stirring) (Perkin-Elmer Corp., Norwalk, CT), and Eppendorf (no stirring) (Eppendorf Gerätebau, Hamburg, FRG). The latter fluorometer, measuring front-face fluorescence, allowed the use of more concentrated cell suspensions. Under each set of conditions, controls in the absence of the dye were done to correct for any light-scattering effects. For each probe, the optimal ratio [dye]/[protein] and the optimal set of wavelengths were determined. The excitation-emission pairs of wavelengths were 480, 620 nm; 622, 670 nm; and 380, 490 nm for RH160, diS-C_3-(5), and ANS, respectively.

Absorbance Measurements

These were carried out with an Aminco (American Instrument Company, Silver Spring, MD) dual-wavelength spectrophotometer in a thermostated cuvette. Here, too, an optimal ratio of [dye]/[protein] and optimal set of wavelengths were predetermined.

Internal Water Space of the Bacteria

The centrifugation technique was applied for this purpose as described (Rottenberg, 1979), with ^3H_2O and [^{14}C] inulin as markers for the total and external water volume, respectively.

RESULTS

At high ionic strengths, such as in the presence of 50 mM KPi plus 5 mM $MgSO_4$, the external surface potential of the bacterial cell is practically abolished (Mclaughlin, 1977). Fig. 1 *A* shows that TPP^+ disappearance from the medium, stimulated by D-galactose, was detectable even under such conditions and even with a relatively low concentration of D-galactose (20 μM; cf. Fig. 5 in Eisenbach et al., 1983, for the dependence of the magnitude and duration of the TPP^+ response on the D-galactose concentration). The magnitude of the TPP^+ response under these conditions, -16 ± 6 mV (±SD), was very similar to the response with 10 mM KPi (Table I). Under both conditions the apparent level before D-galactose addition was -134 ± 12 mV. Further reduction of the KPi concentration to 2 mM and elimination of $MgSO_4$ from the suspending medium caused an apparent depolarization of the cells (cf. Skulachev, 1980), reaching a value undetectable by the TPP^+ electrode ($|60 \log [TPP^+]_{int}/[TPP^+]_{ext}| \leq 35$ mV). Addition of 20 μM D-galactose at this stage caused an apparent change in potential of -52 ± 22 mV, assuming a basal level of -35 mV.

The use of K^+ (or Rb^+) in the presence of valinomycin is accepted as a technique that measures membrane potential per se in *E. coli* (Padan et al., 1976; Ghazi et al., 1981; Ahmed and Booth, 1981; Bakker, 1982), and is considered to be relatively free of artifacts caused by surface charge. To circumvent the need to use short-lived radioisotopes ($^{42}K^+$ or $^{86}Rb^+$), we used a K^+-selective electrode. A typical experiment is shown in Fig. 1 *B*. Addition of D-galactose caused transient uptake of K^+ from the medium, indicative of hyperpolarization. The calculated magnitude of the hyperpolarization, measured with a K^+-selective electrode, was larger than the hyperpolarization measured with a TPP^+-selective electrode (cf. Bakker, 1982). No change in the K^+ level was detected in a control experiment with AW550, an *mglB* mutant derivative of AW546 (Ordal and Adler, 1974), that cannot bind, transport, or respond chemotactically to galactose (data not shown).

To eliminate possible effects of galactose metabolism,

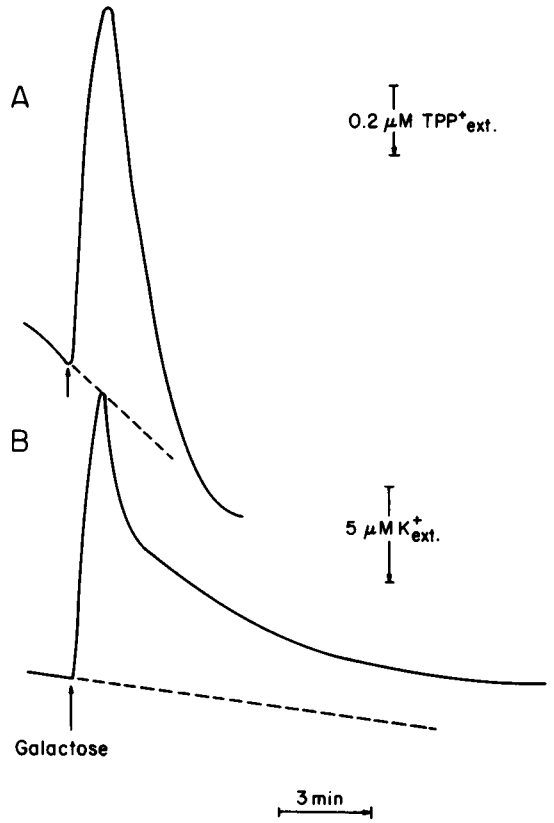

FIGURE 1 Galactose-stimulated TPP^+ and K^+ uptake. (*A*) TPP^+ uptake monitored via the external TPP^+ concentration ($TPP_{ext.}^+$) by a TPP^+ electrode. Prior to the addition of 5 μl D-galactose (20 μM final concentration) the suspension contained 1.5 ml of permeabilized bacteria (strain AW546) at a concentration of 0.5 mg of protein/ml, in 50 mM KPi, 5 mM $MgSO_4$, 2 mM amytal, 0.1 mM L-methionine, 0.1 mM EDTA, 20 μM DCCD, and 5 μM TPP^+ (final pH 7.0). (*B*) K^+ uptake monitored via the external K^+ concentration ($K_{ext.}^+$) by a K^+ electrode. The experimental conditions were as in *A*, except that NaPi (10 mM), valinomycin (10 μM), and KCl (27 μM) were present instead of KPi and TPP^+. At the end of each experiment TPP^+ or KCl was added for calibration. Temperature was 30°C. The dashed lines are extrapolations of the baselines.

we also measured K^+ distribution with D-fucose, a nonmetabolizable analogue of D-galactose (Adler, 1969). As shown in Table I, D-fucose caused hyperpolarization as did D-galactose. The apparently smaller hyperpolarization by D-fucose was possibly due to the nonsaturating concentration of this attractant (cf. Fig. 2 in Eisenbach et al., 1983).

To eliminate artifacts caused by possible changes in the internal water space of the bacteria, we measured this volume before and after stimulation by D-galactose or D-fucose. The internal water space of the cells was not affected by the attractants and remained constant, 4.1 ± 0.4 μl/mg protein in either case. Furthermore, attractant-stimulated changes in $\Delta\psi$, similar to those in Table I, were measured with the centrifugation technique, using either [^{14}C]TPP^+ or $^{86}Rb^+$ and valinomycin as probes. The values obtained with this technique were independent of changes

TABLE I
APPARENT VALUES OF HYPERPOLARIZATION STIMULATED BY GALACTOSE AND FUCOSE

Probe/technique*	Major possible interference	Attractant‡	Hyperpolarization§ ± SD
			(mV)
TPP^+ electrode	Internal σ_s	D-galactose	14 ± 3
		D-fucose	10 ± 3
K^+ electrode	None	D-galactose	26 ± 2
		D-fucose	11 ± 6
RH160	Not known	D-galactose	15 ± 2
DiS-C_3-(5)	Internal σ_s	D-galactose	16
ANS	External σ_s	D-galactose	17 ± 2

*All measurements were carried out in a medium containing 10 mM KPi (10 mM NaPi plus 20–40 μM KCl for K^+ electrode), 5 mM $MgSO_4$, 2 mM amytal, 0.1 mM L-methionine, 0.1 mM EDTA, and 20 μM DCCD. The magnitude of the hyperpolarization was independent of whether KPi or NaPi was present. Temperature = 30°C.

‡The final concentrations of galactose and fucose were 20 μM and 0.5 mM, respectively.

§The values shown were calculated as previously described (Eisenbach, 1982). They include corrections for dilution by the attractant and for nonspecific binding of the probe (using heat-treated bacteria). These are apparent values due to the uncertainty involved in quantitative corrections for adsorption (Ahmed and Booth, 1981; Ten Brink et al., 1981; Zaritsky et al., 1981; Bakker, 1982; Lolkema et al., 1982).

in the internal water space of the cells, due to the corrections made with ^3H_2O (Rottenberg, 1979).

DiS-C_3-(5) is a positively charged dye commonly used for measurements of membrane potential in bacteria and other organisms (Letellier and Schechter, 1979; Waggoner, 1979). The response of diS-C_3-(5) to galactose was a 12% transient decrease in the fluorescence, 9 min long (Fig. 2). (The relatively long duration of the response with diS-C_3-(5) and with the other dyes [see below] is because of the slower consumption of the attractant from the medium, due to the four- to sixfold lower bacterial concentrations that were used in these measurements.) No change

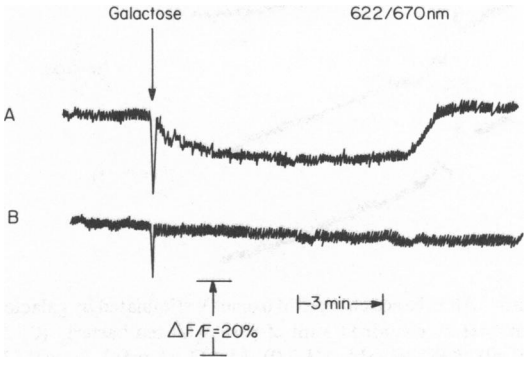

FIGURE 2 Fluorescence changes of diS-C_3-(5) stimulated by galactose. Fluorescence was measured at 30°C with a 3 ml suspension of permeabilized bacteria (0.08 mg of protein/ml), containing KPi (10 mM; pH 7.0), $MgSO_4$ (10 mM), amytal (3 mM), DCCD (13 μM), and diS-C_3-(5) (0.1 μM). D-galactose (20 μM final concentration) was added where indicated. *A*, strain AW546. *B*, strain AW550.

was observed in the mutant strain AW550 (Fig. 2B). Inclusion of 6 μM TPP^+ in the suspending medium did not affect the transient decrease in the fluorescence (data not shown; cf. results obtained in *Bacillus subtilis* [Zaritsky et al., 1981]).

ANS, a negatively-charged dye, has been used to measure both membrane potential and external surface potential (Haynes, 1974; Azzi, 1975). The same apparent hyperpolarization by D-galactose was detected with ANS (Table I).

RH160, a styryl dye, is a fluorescent voltage-sensitive probe, with a relatively high signal-to-noise ratio that has been used to measure rapid changes in the membrane potential of single neuroblastoma cells in culture (Grinvald et al., 1982). It is assumed to be an electrochromic dye (Loew and Simpson, 1981), though other mechanisms of its response cannot be excluded. Addition of galactose to the wild-type strain, AW546, stimulated a 15 mV transient hyperpolarization as measured by RH160 (Table I). No change in fluorescence was observed with the mutant AW550, or with heat-treated AW546 cells.

Oxonol V responds with absorbance changes to changes in membrane potential (Bashford and Smith, 1979). A transient (5 min) decrease in absorbance was observed upon addition of D-galactose to AW546 (Fig. 3). Maltose, a sugar to which AW546 does not respond chemotactically or metabolically (AW546 is a Mal^- strain), had no effect on oxonol absorbance.

DISCUSSION

This study shows that the chemotaxis-correlated apparent hyperpolarization (Eisenbach, 1982; Eisenbach et al., 1983) stimulated by D-galactose is indeed a change in membrane potential per se. Changes in surface charge as a parameter reflected by the measuring probes are excluded by the evidence, which can be summarized as follows: (*a*) K^+, in the presence of valinomycin, responds only to membrane potential. D-galactose stimulated uptake of K^+ under these conditions, indicative of a true hyperpolarization (Fig. 1 and Table I). (*b*) DiS-C_3-(5), ANS, RH160, and oxonol V differ in their charge and their mechanism of response to membrane potential, and yet all responded similarly to D-galactose (Table I and Figs. 2 and 3). (*c*) The magnitude and occurrence of the hyperpolarization was practically independent of the ionic strength of the medium (Fig. 1).

These results point to the conclusion that chemotaxis towards galactose (Eisenbach et al., 1983), and possibly other attractants (Eisenbach, 1982), is accompanied by ion currents, revealed under the conditions of these studies as changes in membrane potential. The identity of these ion currents as well as their significance in chemotaxis are under investigation.

We would like to thank Dr. E. Padan for suggesting the measurement of membrane potential by following K^+ distribution with a K^+-selective electrode in the presence of valinomycin, Dr. R. M. Macnab for constructive comments on this manuscript, and Dr. J. Friede for carrying out preliminary experiments.

This study was supported by research grants to Dr. Eisenbach from the U.S. National Institute of Allergy and Infectious Diseases and from the U.S.-Israel Binational Science Foundation, Jerusalem, Israel. Dr. Eisenbach holds the Barecha Foundation Career Development Chair.

Received for publication 9 June 1983 and in final form 12 August 1983.

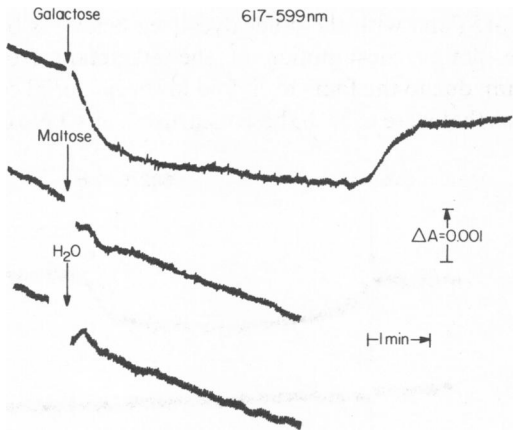

FIGURE 3 Absorbance changes of oxonol V stimulated by galactose. The reaction mixture contained 3 ml of permeabilized bacteria (0.12 mg of protein/ml), KPi (10 mM; pH 7.0), $MgSO_4$ (5 mM), amytal (3 mM), EDTA (0.1 mM), L-methionine (0.1 mM), oxonol V (33 μM), and *N*, *N'*-dicyclohexylcarbodiimide (DCCD) (13 μM). Following the equilibration of the dye, 10 μl of D-galactose were added, where indicated, to a final concentration of 20 μM (*upper* trace). For controls, 10 μl of either maltose (20 μM final concentration; *middle* trace) or H_2O (*lower* trace) were added where indicated.

REFERENCES

Adler, J. 1969. Chemoreceptors in bacteria. *Science (Wash. D.C.).* 166:1588–1597.

Ahmed, S., and I. R. Booth. 1981. Quantitative measurements of the proton-motive force and its relation to steady state lactose accumulation in *Escherichia coli*. *Biochem. J.* 100:573–581.

Azzi, A. 1975. The application of fluorescent probes in membrane studies. *Q. Rev. Biophys.* 8:237–316.

Bakker, E. P. 1982. Membrane potential in a potassium transport-negative mutant of *Escherichia coli* K-12. The distribution of rubidium in the presence of valinomycin indicates a higher potential than that of the tetraphenylphosphonium cation. *Biochim. Biophys. Acta.* 681:474–483.

Bashford, C. L., and J. C. Smith. 1979. The use of optical probes to monitor membrane potential. *Methods Enzymol.* 55:569–586.

Boos, W. 1974. The properties of the galactose-binding protein, the possible chemoreceptor for galactose chemotaxis in *Escherichia coli*. *Antibiot. Chemother. (Basel).* 19:21–54.

Borochov, H., and M. Shinitzky. 1976. Vertical displacement of membrane proteins mediated by changes in microviscosity. *Proc. Natl. Acad. Sci. USA.* 73:4526–4530.

Eisenbach, M. 1982. Changes in membrane potential of *Escherichia coli* in response to temporal gradients of chemicals. *Biochemistry.* 21:6818–6825.

Eisenbach, M. 1983. Changes in membrane potential of *Escherichia coli* stimulated by galactose. *In* Biological Structures and Coupled Flows.

A. Oplatka and M. Balaban, editors. Academic Press, Inc., New York. 349–352.

Eisenbach, M., and S. R. Caplan. 1979. The light-driven proton pump of *Halobacterium halobium:* mechanism and function. *Curr. Top. Membr. Transp.* 12:165–248.

Eisenbach, M., T. Raz, and A. Ciobotariu. 1983. A process related to membrane potential involved in bacterial chemotaxis to galactose. *Biochemistry.* 22:3293–3298.

Felle, H., J. S. Porter, C. L. Slayman, and H. R. Kaback. 1980. Quantitative measurements of membrane potential in *Escherichia coli. Biochemistry.* 19:3585–3590.

Gahzi, A., E. Schechter, L. Letellier, and B. Labedan. 1981. Probes of membrane potential in *Escherichia coli* cells. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 125:197–200.

Grinius, L. L., A. A. Jasaitis, Yu. P. Kadziauskas, E. A. Liberman, V. P. Skulachev, V. P. Topali, L. M. Tsofina, and M. A. Vladimirova. 1970. Conversion of biomembrane-produced energy into electric form. I. Submitochondrial particles. *Biochim. Biophys. Acta.* 216:1–12.

Grinvald, A., R. Hildesheim, I. C. Farber, and L. Anglister. 1982. Improved fluorescent probes for the measurement of rapid changes in membrane potential. *Biophys. J.* 39:301–308.

Haynes, D. H. 1974. 1-anilino-8-naphthalenesulfonate: a fluorescent indicator of ion binding and electrostatic potential on the membrane surface. *J. Membr. Biol.* 17:341–366.

Hazelbauer, G. L., and J. Adler. 1971. Role of the galactose binding protein in chemotaxis of *Escherichia coli* toward galactose. *Nat. New Biol.* 230:101–104.

Kell, D. B. 1979. On the functional proton current pathway of electron transport phosphorylation. An electrodic view. *Biochim. Biophys. Acta* 549:55–99.

Leive, L. 1965. Release of lipopolysaccharide by EDTA treatment of *E. coli. Biochem. Biophys. Res. Commun.* 21:290–296.

Letellier, L., and E. Schechter. 1979. Cyanine dye as monitor of membrane potentials in *Escherichia coli* cells and membrane vesicles. *Eur. J. Biochem.* 102:441–447.

Loew, L. M., and L. L. Simpson. 1981. Charge-shift probes of membrane potential. A probable electrochromic mechanism for *p*-aminostyrylpyridinium probes on a hemispherical lipid bilayer. *Biophys. J.* 34:353–365.

Lolkema, J. S., K. J. Hellingwerf, and W. N. Konings. 1982. The effect of "probe binding" on the quantitative determination of the proton-motive force in bacteria. *Biochim. Biophys. Acta.* 681:85–94.

Mclaughlin, S. 1977. Electrostatic potentials at membrane-solution interfaces. *Curr. Top. Membr. Transp.* 9:71–144.

Muratsugu, M., N. Kamo, Y. Kobatake, and K. Kimura. 1979. Determination of membrane potential of *Escherichia coli* with use of an electrode sensitive to tetraphenyl phosphonium. *Bioelectrochem. Bioenerg.* 6:477–491.

Ordal, G. W., and J. Adler. 1974. Properties of mutants in galactose taxis and transport. *J. Bacteriol.* 117:517–526.

Padan, E., D. Zilberstein, and H. Rottenberg., 1976. The proton electrochemical gradient in *Escherichia coli* cells. *Eur. J. Biochem.* 63:533–541.

Rottenberg, H. 1979. The measurement of membrane potential and ΔpH in cells, organelles, and vesicles. *Methods Enzymol.* 55:547–569.

Skulachev, V. P. 1980. Membrane electricity as a convertible energy currency for the cell. *Can. J. Biochem.* 58:161–175.

Szmelcman, S. and J. Adler, 1976. Change in membrane potential during bacterial chemotaxis. *Proc. Natl. Acad. Sci. USA.* 73:4387–4391.

Ten Brink, B., J. S. Lolkema, K. J. Hellingwerf, and W. N. Konings. 1981. Variable stoichiometry of proton: lactose symport in *Escherichia coli* cells. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 12:237–240.

Waggoner, A. S. 1979. The use of cyanine dyes for the determination of membrane potentials in cells, organelles, and vesicles. *Methods Enzymol.* 55:689–695.

Wang, E. A., and D. E. Koshland, Jr. 1980. Receptor structure in bacterial sensing system. *Proc. Natl. Acad. Sci. USA.* 77:7157–7161.

Zaritsky, A., M. Kihara, and R. M. Macnab. 1981. Measurement of membrane potential in *Bacillus subtilis:* a comparison of lipophilic cations, rubidium ion, and a cyanine dye as probes. *J. Membr. Biol.* 63:215–231.