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The Generation of a Membrane Potential by a Fermentative Bacterium

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In bacteria which operate a membrane-associated electron-transport system it is generally believed that the generation of a protonmotive force (Mitchell, 1961) results from protonmotive electron transport, whereas in those fermentative bacteria that lack this capacity the proton gradient is thought to arise from the protonmotive hydrolysis of ATP generated by substrate-level phosphorylation (Booth & Morris, 1975; Haddock & Hamilton, 1977; Harold & Papineau, 1972; Riebeling *et al.*, 1975). Surprisingly few studies have been undertaken of the magnitude of the protonmotive force so generated in fermentative bacteria, even though such organisms would seem to be particularly appropriate subjects for the investigation of the size and role of the membrane-potential component of the protonmotive force. It is the chief purpose of the present communication to report preliminary findings made with one obligately anaerobic fermentative bacterium; to assess the transmembrane potential generated by *Clostridium pasteurianum* we have used the lipophilic-cation method (Liberman & Skulachev, 1970; Rottenberg, 1975).

To determine the distribution of ions between medium and lumen of intact cell suspensions of *Cl. pasteurianum* we have chosen potentiometric methods (Kell *et al.*, 1978), which also allow on-line monitoring of glycolysis as acid production (Harold & Papineau, 1972). We have constructed an electrode selective for the lipophilic cation butyltriphenylphosphonium by a method analogous to that described by Muratsugu *et al.* (1977), whereas glass electrodes selective to K^+ and H^+ were obtained commercially.

In a typical experiment organisms (external pH 6.5–6.6) harvested from a mid-exponential-phase culture of low-K^+-grown cells took up butyltriphenylphosphonium in two kinetically distinct phases: an initial uncoupler-insensitive rapid uptake, followed by a slower uptake (equivalent to $a\Delta\psi$ of approx. 70 mV) that was reversed by the uncoupler tetrachlorosalicylanilide (2 μg/ml). Such organisms effluxed K^+ as endogenous glycolysis failed and the steady-state butyltriphenylphosphonium uptake slowly decayed. Addition of valinomycin or nigericin at this time (1 μg/ml of suspension; 1.2 nmol/mg of bacterial protein) caused negligible further K^+ efflux. Addition of glucose (120 mM) shortly after the cessation of endogenous glycolysis provoked re-uptake of the previously effluxed K^+ and a brief small enhancement in the uptake of butyltriphenylphosphonium that rapidly decayed to a significantly lower steady-state value (approx. 40 mV) as K^+ was taken up. Although at this stage valinomycin (1 μg/ml), nigericin (1 μg/ml) or sodium tetraphenylborate (10 μM) each provoked a rapid efflux of intracellular K^+, and thereby prevented glycolysis, only valinomycin and tetraphenylborate enhanced butyltriphenylphosphonium uptake, whereas nigericin, which exchanges H^+ for K^+ in an electroneutral manner (Ashton & Steinrauf, 1970), elicited H^+ uptake.

It has been shown by Harold & Papineau (1972) that the presence of extracellular K^+ dissipated the $\Delta\psi$ of artifically Na^+-loaded *Streptococcus faecalis* cells and that K^+-grown (KTY) cells demonstrated no $\Delta\psi$ as measured by the uptake of triphenylmethylphosphonium or of dibenzyldimethylammonium ions. In our experiments *Cl. pasteurianum* was grown in the medium described by Mackey & Morris (1972) lacking NH_4Cl but supplemented with casein hydrolysate (1%) and yeast extract (0.5%) and buffered with 50mM-sodium phosphate, pH7.0. Under these conditions, like the NaTY cells of *S. faecalis* (Harold & Papineau, 1972), the organism was able to accumulate K^+ from the medium. Indeed, *Cl. pasteurianum* shows an absolute requirement for K^+ and will not grow in the sodium phosphate-buffered minimal medium of Mackey & Morris (1972) unless this is supplemented with 1mM-K^+. We therefore cannot justify the use of monactin (Harold & Papineau, 1972) to deplete the cells of intracellular K^+ and load them with Na^+, for it would seem that under normal conditions of growth *Cl. pasteurianum* generates only a rather small $\Delta\psi$ compared with that observed in other micro-organisms (Haddock & Hamilton, 1977).

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Rotational Mobility of Membrane-Bound Cytochrome *o* of *Escherichia coli* and Cytochrome a_1 of *Thiobacillus ferro-oxidans*

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Photoselection methods can be used to measure the rotational relaxation times (τ) of those membrane-bound proteins with suitable natural chromophores that give a long-lasting ($>100\,\mu s$) and identifiable optical signal on flash illumination. For example, Cone (1972) reported that visual rhodopsin rotated rapidly, with $\tau = 20\,\mu s$. By contrast, bacteriorhodopsin in its native membrane was effectively immobile, with $\tau > 20$ ms (Razi Naqvi *et al.*, 1973). The photoselection method was extended to the CO complex of mitochondrial cytochrome a_3 by Junge (1972). Subsequently, after a detailed study of the wavelength-dependence of the Soret-band linear dichroism resulting from polarized laser-flash photolysis of the cytochrome a_3–CO complex, Kunze & Junge (1977) concluded that mitochondrial cytochrome a_3 did not rotate in the membrane over a time scale of 100ms or more. In all of these approaches it was assumed, on good grounds, that intrinsic membrane proteins would rotate, if they rotated at all, only about an axis normal to the plane of the membrane.

We have measured the Soret-band linear dichroism caused by laser-flash photolysis of the CO complexes of cytochrome *o* of *Escherichia coli* and cytochrome a_1 of *Thiobacillus ferro-oxidans*. We used the methods of Haddock *et al.* (1976) and Cobley & Haddock (1975) for the aerobic growth of *E. coli*, for the chemolithotrophic growth of *T. ferro-oxidans* and for the preparation of cytoplasmic membrane vesicles. Photo-