On the Current-Voltage Relationships of Energy-Transducing Membranes: Submitochondrial Particles

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A transmembrane electrochemical proton gradient is widely held to constitute the link between electron transport and ATP synthesis in biological membranes (Boyer *et al.*, 1977), but much debate surrounds the issue of the stoicheiometry of H^+ movement that either generates (by electron transport) or dissipates (by ATP synthesis) this proton gradient (proton-motive force, Δp) (Brand, 1977). Our estimates of both Δp and the phosphorylation potential generated by submitochondrial particles indicate that Δp is thermodynamically competent to serve as such a link provided that $3\,H^+$ ions are translocated through the mitochondrial adenosine triphosphatase for each molecule of ATP synthesized (Sorgato *et al.*, 1978).

Although the thermodynamic competence of Δp in submitochondrial particles has now been scrutinized in some detail (Azzone *et al.*, 1978; Sorgato *et al.*, 1978) rather less attention has been paid to two other-related matters: (1) the relationship between Δp and the rate of proton translocation; (2) the relationship between Δp and the rate of ATP synthesis.

It was found (Sorgato *et al.*, 1978) that the oxidation of either succinate or NADH by submitochondrial particles generated virtually the same Δp, despite the fact that, after correction for the slower rate of succinate oxidation, the rate of proton translocation was approximately 2-fold slower when succinate was the substrate. Decrease, by titration with malonate, of the rate of succinate oxidation to 18% of its maximal value is now found to cause only a small decline in Δp from approx. 140mV to approx. 135mV. [Δp was determined in a reaction mixture of 10mM-phosphate/Tris, 5mM-magnesium acetate, pH 7.3, by using a flow dialysis assay for SCN^- uptake to estimate $\Delta\psi$, the sole detectable component of Δp under these reaction conditions (Sorgato *et al.*, 1978).] These two sets of observations suggest either that the protic resistance of the inner mitochondrial membrane (at least in submitochondrial particles) is variable or that the membrane capacitance is strongly voltage-dependent. Thus as the rate of proton translocation is decreased there is a corresponding decrease in the rate at which protons are able to pass back across the membrane down their electrochemical gradient. This type of behaviour has also been suggested for rat liver mitochondria (Nicholls, 1974) and for chloroplast thylakoids (Schönfeld & Neumann, 1977), although it is noteworthy that with intact mitochondria a decline in Δp was observed when the rate of succinate oxidation was decreased by only 40% (Nicholls, 1974).

Titration of the rate of NADH oxidation by submitochondrial particles with rotenone indicated that, except at the highest respiratory rates, there was an almost ohmic relationship between Δp and the rate of proton translocation (Kell *et al.*, 1978). This result appears to contrast markedly with that found when succinate is the substrate. The reasons for this difference remain to be elucidated, but it may be relevant to note that Nicholls (1977) has suggested that the second and third segments of the respiratory chain have the ability, for a given rate of electron transfer, to maintain Δp at a higher value than that produced at the first segment between NADH and ubiquinone.

A knowledge of the resistive (and capacitative) characteristics of the membrane is essential both for the relationship between oxidation and phosphorylation to be treated in terms of irreversible thermodynamics, and for the identification of Δp as the deter-determinant (or otherwise) of respiratory control (Boyer *et al.*, 1977; Kupriyanov & Pobochin, 1978). The results in the present paper indicate that treatments (Hinkle *et al.*,

1975; van Dam & Westerhoff, 1977) that assume a constant proton conductance (leak) or a voltage-independent membrane capacitance are unlikely to be justified.

The data of Sorgato *et al.* (1978) show that although the rate of ATP synthesis is significantly decreased when NADH is replaced as substrate by succinate there is no significant decrease in Δp. This result is not obviously consistent with a minimal form of the chemiosmotic hypothesis, which we would interpret to predict a fixed rate of ATP synthesis at a given Δp (cf. Baccarini-Melandri *et al.*, 1977). A similar absence of a relationship between Δp and the rate of ATP synthesis has been observed in chromatophores from *Rhodopseudomonas capsulata* (Baccarini-Melandri *et al.*, 1977), whereas in the case of thylakoids Gräber & Witt (1976) suggested a linear relationship between the logarithm of the rate of ATP synthesis and Δp, provided that Δp exceeded a threshold value. This last type of behaviour resembles the Tafel relationship found for inorganic electrodes (Bockris & Reddy, 1970). Possible explanations of our data are: (1) there is a localized, membrane-associated, control on the rate of ATP synthesis; (2) the rate of ATP synthesis is responsive to very small changes in Δp, which present methods are insufficiently sensitive to detect; (3) a bulk phase Δp is not the sole and obligatory link between oxidation and phosphorylation.

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The Apparent Non-identity of Cytochrome *c* Reductase and Flavin-Dependent Azoreductase Activities

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Aromatic azo compounds are reduced, under anaerobic conditions, by hepatic microsomal NADPH-dependent enzymes. Hernandez *et al.* (1967*a*,*b*) characterized three pathways involved in this reduction: (*a*) a cytochrome *P*-450-dependent pathway; (*b*) NADPH–cytochrome *c* reductase (EC 1.6.2.4); (*c*) a pathway that is inducible by 3-methylcholanthrene and that differs from pathway (*a*) in being CO-insensitive and from pathway (*b*) in being more sensitive to solubilization.

Addition of flavin to the microsomal preparation enhances azo-reduction (Williams *et al.*, 1970; Mallett *et al.*, 1977) and diminishes the sensitivity to CO-inhibition (Fujita & Peisach, 1978; A. K. Mallett, R. Walker & L. J. King, unpublished work), which suggests an interaction between the soluble flavin and NADPH–cytochrome *c* reductase analogous to that proposed for a microbial azoreductase system (Gingell & Walker, 1971). However, flavin-stimulated azoreductase activity does not consistently correlate with NADPH–cytochrome *c* reductase activity (see below).