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 stoichiometry 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. 140 mV to approx. 135 mV. [Δp was determined in a reaction mixture of 10 mM-phosphate/Tris, 5 mM-magnesium acetate, pH 7.3, by using a flow dialysis assay for SCN⁻ uptake to estimate Δψ, 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 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.,...
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. (1967a,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).