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Mechanism of Coupling of Oxidative Phosphorylation and the Membrane Potential of Mitochondria

THE existence of an electrical potential difference at the mitochondrial membrane is one of the postulates of Mitchell's chemiosmotic theory of oxidative phosphorylation[1,2]. A shunting of the mitochondrial membrane leads to the uncoupling of oxidative phosphorylation. It has been demonstrated that uncoupling agents increase the proton permeability of phospholipid micelle membranes[3] and artificial bimolecular membranes[4–7]. Mitchell and Moyle[8] showed that uncouplers can operate as proton carriers in mitochondrial membranes.

This communication is an attempt to prove directly the existence of a potential difference at the mitochondrial membrane. If such a potential exists, compounds inducing equal permeability for H^+ ions should produce equal uncoupling effects. The penetrating cations should be actively transported through the membrane into mitochondria while penetrating anions should go out of mitochondria but into sonic mitochondrial particles (SMP) which have the opposite membrane polarity. Cation transport in mitochondria should decrease the *p*H of extramitochondrial space while anion transport in SMP should increase the *p*H of extra-SMP space.

Our methods have been described in refs. 4, 9–12. Fig. 1*A* is a plot of uncoupling efficiency in mitochondria against that in bimolecular membranes prepared from mitochondrial phospholipids. The concentrations of uncouplers inducing two-fold stimulation of succinate oxidation in state 4 are plotted against the concentrations of the same agents that increase the membrane proton conductance by 5×10^{-9} mho/cm^2. There is a good correlation between these two parameters for most of the uncouplers.

A number of synthetic ions have been found which possess hydrophobic groups and penetrate the membrane without changing the membrane permeability for H^+, OH^- or other ions present in the incubation mixture (Fig. 1*B*).

Fig. 1*C* shows the *p*H responses on the addition of such ions to mitochondria and SMP in state 4. These results and other tests show that synthetic penetrating cations brought about the same alterations in mitochondrial functions as did Ca^{++} ions or K^+ ions in the presence of valinomycin. The active transport of K^+ into mitochondria in the presence of carriers has been described by Pressman and coworkers[13]. Our experiments suggest that such an effect takes place with all penetrating cations.