the phosphate and adenine nucleotide carriers, heart mitochondria have a feeble tricarboxylate transporter (England & Robinson, 1969) and kidney and ascites-tumour mitochondria have a slow glutamate-transport system (Kovacevik & Chappell, 1970).

The demonstration that certain thiol-blocking reagents at low concentration specifically inhibit phosphate transport without affecting a number of other mitochondrial functions (Fonyo & Bessman, 1966, 1968; Tyler, 1968, 1969), and that *n*-butylmalonate inhibits the dicarboxylate carrier (Robinson & Chappell, 1967; Chappell & Robinson, 1968), has enabled the interdependence of some of these carrier systems to be established. The phosphate transporter appears to carry only phosphate or arsenate. The dicarboxylate transporter catalyses either dicarboxylate-dicarboxylate exchange or dicarboxylate-phosphate exchange. Thus *N*-ethylmaleimide prevents the discharge of ^{32}P -labelled phosphate from mitochondria by added unlabelled phosphate (an expression of the activity of the phosphate carrier) but not that resulting from the addition of malate or succinate. This latter discharge is inhibited by *n*-butylmalonate, indicating that the dicarboxylate carrier catalyses a phosphate-dicarboxylate exchange (Johnson & Chappell, 1970). This finding had been foreshadowed by previous results with the ammonium swelling technique (Chappell & Haarhoff, 1967; Robinson & Chappell, 1967).

From evidence of this type it has been established that the oxoglutarate carrier catalyses an oxoglutarate-malate exchange and that the tricarboxylate transporter catalyses tricarboxylate-malate and tricarboxylate-tricarboxylate exchanges (see Chappell, 1969).

Other inhibitors that have provided evidence for the mechanism of action of the carriers are glutamate analogues, which inhibit glutamate transport and apparently activate the entry of aspartate (Azzi, Chappell & Robinson, 1967), and avenaciolide, an antibiotic produced by *Aspergillus avenaceus*, which specifically inhibits glutamate transport (McGivan & Chappell, 1970).

Aspartate is a potent competitive inhibitor of the oxoglutarate-transporting system (McGivan, Bradford & Chappell, 1969). In this case aspartate is competitive with oxoglutarate and not with malate. This may have important physiological implications, since under conditions appertaining *in vivo* the oxoglutarate transporter is probably essentially unidirectional, promoting only oxoglutarate efflux (and coupled malate entry).

In some respects *Pseudomonas fluorescens* appears to possess similar (inducible) carrier systems (Kogut & Podoski, 1953). However, the resemblance may be superficial, since, for example, *n*-butylmalonate does not inhibit the malate transporter, in contrast with its action on mitochondria.

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Anion Movement and Uncoupling in Mitochondria

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The movement of anions across the mitochondrial membrane is controlled by two factors. First, the presence of a number of relatively immobile positive charges (mainly K^+ ions) in the mitochondrial matrix gives the mitochondrion properties reminiscent of those of an anion-exchanger (Van Dam & Tsou, 1968). Anions are accumulated according to their charge and compete with each other for the available 'sites'. The accumulation pattern is mainly determined by the thermodynamic properties of the system. Secondly, there are specific mechanisms that allow some ions to pass the membrane while excluding others (cf., e.g., Chappell & Crofts, 1966; Pfaff, 1965). These translocating systems determine the kinetics of the movement of the different anions. A number of uncouplers of oxidative phosphorylation are weak acids and behave in many respects like the mitochondrial substrates: they are accumulated, and exhibit a certain specificity with regard to the way in which they are translocated. An energy-utilizing transport of uncoupler anions, combined with passive

diffusion of the uncharged uncoupler acid, has been proposed as the mechanism by which these compounds dissipate energy initially conserved during mitochondrial oxidations (Kraaijenhof & Van Dam, 1969).

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Aspects of the Chemiosmotic Hypothesis

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The chemiosmotic hypothesis has sometimes been received as though it were less in keeping with the fundamental tenets of biochemistry than the chemical or conformational coupling hypotheses. In this paper I have therefore sought to re-emphasize the chemically rational aspect of my hypothesis, both in its origins and in its subsequent development.

When an appropriate enzyme is orientated in a biochemical complex in such a way that the channel of entry of the group donor to the active centre is spatially separate from the channel of exit of the group acceptor, the conventionally scalar process of group transfer becomes the vectorial process of group translocation (Mitchell, 1954*a,b*, 1957; Mitchell & Moyle, 1958). The concept of enzyme-catalysed group translocation, generalizing cytochrome-catalysed electron translocation (Lundegardh, 1945; Ussing, 1949), led to the following corollary. Two enzyme-catalyzed reactions, involving a common donor/acceptor intermediate, could be coupled uniquely by the channelling of the intermediate between the two kinds of enzyme: either if the enzyme molecules were paired so that the intermediate passed from one active centre to the other through a microscopic internal domain, or if many of each type of enzyme molecule were appropriately orientated in a lipid membrane enclosing a macroscopic aqueous phase retaining the intermediate (Mitchell & Moyle, 1958).

Thus the question arose as to whether coupling between oxidoreduction and phosphorylation in oxidative and photosynthetic phosphorylation systems could be due to the channelling of translocation of protons, which could play the part of the donor/acceptor intermediate, between the complex oxidase and ATPase* systems; and a working hypothesis was proposed (Mitchell, 1961). This chemiosmotic hypothesis

led to certain experimentally testable questions. (i) Do the respiratory chain, photo-oxidoreduction chain and ATPase systems translocate protons across the membrane? (ii) What is the polarity and stoichiometry of the proton translocation, and is it compatible with the known phosphorylation quotients? (iii) Is the membrane sufficiently impermeable to protons and to other ions to account for the observed tightness of coupling and for reversibility between the oxidoreduction and ATPase systems? (iv) Will the rate of proton translocation observed during oxidoreduction account for the observed rate of ATP synthesis? (v) Do uncouplers like 2,4-dinitrophenol act by conducting protons chemically across the lipid membrane, thus short-circuiting the proton current? (vi) What is the magnitude of the total protonmotive force (Δp) across the membrane, and is it compatible with the driving force required to reverse ATP hydrolysis and electron transport under appropriate conditions? (vii) What proportions of Δp are accounted for by the electrical (membrane potential $\Delta\psi$) and chemical ($-Z\Delta pH$) components? (viii) Does the membrane contain proton-coupled porter systems required to regulate the internal pH, maintain osmotic stability and permit translocation of appropriate components across the membrane?

The results of exploratory experiments continued to be compatible with the basic chemiosmotic conception, and I continued to develop the chemiosmotic hypothesis in greater detail so as to provide the means of formulating more analytical questions (Mitchell, 1963, 1966, 1967, 1968).

In mitochondria from rat liver, the present published experimental knowledge, reviewed by Greville (1969), indicates that the above questions can be answered as follows. (i) Yes, either directly, or possibly via a 'proton pump' actuated by hydrolysis of an uncoupler-insensitive X~I intermediate. (ii) Outward translocation of 2 protons occurs per ATP molecule hydrolysed via the ATPase, or per 2 electrons traversing each 'coupling site' in the respiratory chain; and this is compatible with the known P/O quotients. In 'sonic-particles', but not in 'digitonin-particles', the polarity of proton translocation is reversed, corresponding to morphological inversion of the membrane, but membrane leakiness has prevented determination of translocation stoichiometries. (iii)-(v) Yes. (vi) About 230mV in State 4, and about 200mV in State 3; and this corresponds approximately to the requirement for ATP synthesis under normal conditions (but see Slater, 1967). (vii) Under State 4 and State 3 conditions Δp is mainly $\Delta\psi$, but in State 6 it is mainly $-59\Delta pH$. (viii) Yes.

The equivocal answer to question (i) is the least satisfactory. A related ambiguity arises in (ii) because the hydrolysis of externally added ATP occurs via the internal acceptor of the ATPase, and the

* Abbreviation: ATPase, adenosine triphosphatase.