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 stoicheiometry 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?

* Abbreviation: ATPase, adenosine triphosphatase.

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 stoicheiometries. (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

porter-catalysed entry of ATP and exit of ADP and P_i in different states of deprotonation may contribute to the total proton translocation stoicheiometry (Mitchell & Moyle, 1968; Mitchell, 1969). These ambiguities can be resolved by a finer biochemical analysis based on questions connected with the proposed oxidoreduction-loop organization of the respiratory chain, the proposed duplex (X–I synthetase + X–I hydrolase) organization of the ATPase and the general concept of proton-coupled substrate-specific porter systems (Mitchell, 1963, 1966, 1967, 1968). For example: (ix) When electrons are donated to oxygen by cytochrome oxidase and the resulting base is protonated to give H_2O, are the protons taken up from the inner phase? (x) Do electrons travel across the cristae membrane via cytochrome *a* from cytochrome c_1 on the outside to cytochrome a_3 on the inside during oxygen reduction? (xi) Does cytochrome c_1 oxidize dihydroubiquinone (coenzyme QH_2), releasing protons in the outer phase? (xii) Is the initial inward migration of negative charge accompanying the entry of ATP into mitochondria (Mitchell & Moyle, 1968) due to the electrogenic property of the ATP/ADP antiport reaction rather than to activity in the ATPase system?

Recent experimental work, to be discussed, indicates that the answer to questions (ix)–(xii) is yes; and some interesting biochemical conclusions can be drawn.

In my opinion, the practical utility of a concept or of a hypothesis depends, not on the proof of its validity, but on the extent to which it opens up avenues for research and comprehension by providing a basis for the precise formulation of experimentally testable questions.

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Mitochondrial Coupling Factors

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A coupling factor may be defined as a compound of mitochondrial origin that on addition to a suitably depleted mitochondrial membrane preparation causes a stimulation of one or more of the reactions associated with the conservation of energy. There are several ways in which coupling factors may act, and these are not mutually exclusive. They can participate in the chemical reactions leading to ATP synthesis; they can cause conformational or structural changes in the mitochondrial membrane; they can remove inhibitors of the energy-conservation processes from the mitochondrial membrane preparations.

Many coupling-factor preparations have been described. These may be divided into those with ATPase* activity and those with no demonstrable enzyme activity. Of those with ATPase activity the most highly characterized are factor F_1 (Pullman, Penefsky & Racker, 1958) and factor A (Sanadi, Fluharty & Andreoli, 1962; Warshaw, Lam, Nagy & Sanadi, 1968). Factor F_1–X described by Vallejos, Van den Bergh & Slater (1968) appears to be a combination of factor F_1 and another coupling factor (Groot & Meyer, 1969). Other soluble mitochondrial ATPase preparations have been described, from liver (Lardy & Wellman, 1953) and from heart (Selwyn, 1967). Oligomycin-sensitive ATPase preparations have also been described (Kagawa & Racker, 1966; Kopaczyk, Asai, Allman, Oda & Green, 1968). Although oligomycin-sensitivity is a characteristic of the ATPase activity in intact mitochondria, no reports have appeared of the effects of these preparations on the reactions related to respiratory-chain phosphorylation.

The literature contains a large number of descriptions of preparations and properties of coupling factors that have no discernible enzymic activity: 'soluble factor' (Linnane, 1958; Linnane & Titchener, 1960); factor A (Sanadi *et al.* 1962; Warshaw, Lam, Nagy & Sanadi, 1968); 'soluble factor' (Hommes, 1963); factor F_4 (Conover, Prairie & Racker, 1963; Zalkin & Racker, 1965); factor F_3 (Racker, 1962; Fessenden & Racker, 1967); factors F_{3A} and F_{3B} (Prairie, Conover & Racker, 1966); 'soluble factor' (Ling & Wu, 1964; phosphoryltransferase (Beyer, 1964, 1968); 'soluble factor' (Sone & Hagihara, 1966); factor B (Lam, Warshaw & Sanadi, 1967); the OSCP (MacLennan & Tzagoloff, 1968); factor F_c (Bulos & Racker, 1968); factor F_5 (Fessenden-Raden & Racker, 1968). All these preparations have been shown to enhance one or more of the following

* Abbreviations: ATPase, adenosine triphosphatase; OSCP, oligomycin-sensitivity-conferring protein.