Commentary

A COMMENTARY ON ALTERNATIVE HYPOTHESES OF PROTONIC COUPLING IN THE MEMBRANE SYSTEMS CATALYSING OXIDATIVE AND PHOTOSYNTHETIC PHOSPHORYLATION

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1. Introduction

The elucidation of the molecular mechanism of ADP phosphorylation by the reversible ATPase complexes during oxidative phosphorylation in bacteria, mitochondria and chloroplasts is one of the most interesting, and perhaps most difficult, problems of biochemistry. It therefore seems all the more important that the discussion of alternative feasible types of mechanism, which may stimulate and guide appropriate experimental research, should not be confused either by misunderstandings about the relevant conceptual models that have been proposed, or about the implications of such models when they are developed in sufficient detail to make them practically realistic and experimentally testable.

Leaving aside purely mechanical or conformationally coupled models, two main alternative types of biochemical model have been described to account for the reversal of the ATPase reaction in the ATPase complexes. They are, on the one hand, the protonmotive chemiosmotic type of mechanism [1–3], which has been evolved in considerable detail from a membrane-dependent theory of transport [4–9]; and on the other hand, the protonic anhydride mechanism of Williams [10–13], which has been evolved in outline from a membrane-independent theory of 'dislocated phases' between neighbouring redox and reversible ATPase components in 'chains of catalysts' ([10] and see [8,9]). These alternative concepts involve a different approach to the chemical mechanism of ADP phosphorylation. They also involve a radically different view of the topological relationship between the reversible ATPase complexes and the redox complexes that act as the source of power for ADP phosphorylation.

Recent arguments about the relative merits of the protonmotive chemiosmotic mechanism and Williams' protonic anhydride mechanism have seemed to me to confuse as much as to resolve the major scientific issues with which we are concerned [14–22]. I have therefore thought it appropriate in this paper to review the origins of the alternative concepts and the relationships between them, and attempt to examine briefly their relative compatibility with existing experimental knowledge.

Abbreviations: P_i or POH, partially protonated inorganic orthophosphate; PO^-, deprotonated POH; ADPOH, ADP having an OH group on the β-phosphorus; $ADPO^-$, deprotonated ADPOH; ADPOP, ATP with the ADP and P parts of the molecule in the same state of protonation and salt formation as ADPOH or $ADPO^-$ and POH or PO^- respectively; DNP, 2,4-dinitrophenate; PCP, pentachlorophenate; $\Delta\bar{\mu}H^+$ or Δp, protonic electrochemical potential difference.

2. Williams' protonic anhydride concept

According to Williams [10], phosphorylation can be driven by oxidation of hydrogen atoms in hypothetical dislocated phases between neighbouring redox and reversible ATPase catalysts, because the phosphorylation of ADP 'is thermodynamically favourable at

low pH'. As the standard free energy of hydrolysis of ATP is not appreciably pH-dependent below pH 6 in dilute aqueous media [23], Williams [11] explained that 'it is not sufficient just to increase the hydrogen ion activity to unity in order to polymerise phosphates', but the proposed dislocated phases would have to contain 'a low activity of the solvent-water', as in 'strongly acidic non-aqueous media or aqueous syrups' known to favour phosphate polymerization in inorganic chemistry. The required low water activity was assumed to be caused by the liberation, into the dislocated phases, of anhydrous or partially hydrated protons produced by the oxidation of the hydrogen atoms of hydrogenated carriers or substrates. Williams' view of the coupling mechanism in oxidative phosphorylation was originally presented as being essentially membrane-independent [10]; but he later assumed that, to prevent hydration and irreversible de-energisation of the protonic anhydride in transit through the dislocated phases in the redox–ATPase complexes, the dislocated phases would have to be protected from the entry of the water by being suitably buried in the lipid of the membrane [11]. This view of membrane function seems somewhat surprising, since it is well known that lipid membranes have a comparatively high permeability to water. One might have expected that precisely engineered polypeptide complexes would have been better adapted than the lipid phase of the membrane for the specific exclusion of H_2O, as assumed implicitly in classical enzymology. At all events, according to Williams' protonic anhydride concept, the energy is transferred from the redox complexes to neighbouring ATPase complexes by anhydrous or only partially hydrated protons, which thus act as unstable energy-rich anhydride intermediaries [10–19,24]. In keeping with this view, Williams [17] recently introduced a standard free-energy term to define this energy-rich intermediary function that he assumed for the proton. To distinguish unambiguously between Williams' hypothetical protonic anhydride 'aqueous syrup' [11] and the hydrated proton in equilibrium with the dilute aqueous media, we shall sometimes find it convenient to represent them as H^+-anh, and as H^+-hyd, respectively.

Figure 1 shows a diagram of the protonic anhydride mechanism, corresponding to that recently given by Williams [17]. It is noteworthy that the ATPase reaction is depicted as being reversed by the withdrawal

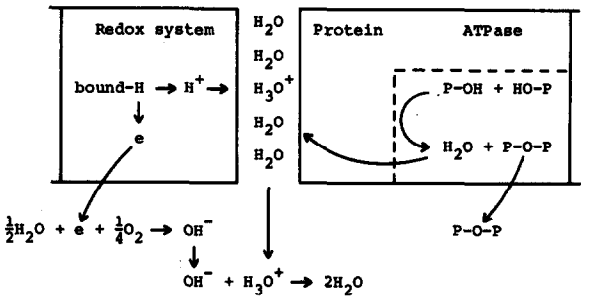

Fig.1. Diagram of protonic anhydride coupling mechanism according to Williams [17], showing 'the series of changes in the membrane which could be used to link proton production from redox centres with ATP formation'. This is a composite representation of fig.6 in Williams' paper [17], but with an adjustment of the quantity of oxygen and H_2O shown entering the redox reaction so as to balance the equation for the overall process. According to Williams [17] 'there could be conformational changes and minor leakage to outside solutions', but the diagram seems to be rather uninformative about the topological details, especially with respect to trans-membrane proton translocation. Readers are requested to consult Williams' paper [17].

of H_2O from the ATPase by the conversion of H^+-anh to H^+-hyd in the dislocated phase in the redox–ATPase complex. Thus, the ATPase of Williams' protonic anhydride coupling mechanism is not a protonmotive ATPase. As H^+-hyd in fig.1 is finally represented as H_3O^+, containing one H_2O more than H^+-anh, which is initially represented as H^+, it appears as though the stoicheiometry of coupling corresponds to one phosphorylation per electron accepted from 'bound-H' in each redox–ATPase coupling site. Williams does not explain how the stoicheiometry of this system may be reconciled with the experimentally observed P/O quotients, corresponding to one phosphorylation per pair of electrons traversing each coupling site in mitochondria. Nor does he explain why H_2O does not enter the dislocated phase by the pathway through which H_3O^+ is supposed to exit.

The protonic anhydride coupling mechanism would obviously not be feasible if the dislocated phase were equilibrated with an extensive dilute aqueous phase, such as the outer aqueous medium of a bacterial or mitochondrial suspension. The anhydride property of the protons would be lost and the system would be energetically uncoupled if the H^+-anh were extruded into the outer aqueous medium rather than into the

hypothetical protected dislocated phase. However, it has been proposed by Robertson and Boardman [14] that the anhydride of Williams' coupling mechanism may be anhydrous HCl, or some similar chemical intermediary, which somehow remains anhydrous while being freely diffusible in the lipid phase of the membrane – thus extending the confines of Williams' dislocated phase to the whole lipid phase of the membrane, and reverting to a practically classical type of chemical coupling hypothesis.

Williams' description of the protonic anhydride coupling mechanism is clearly not sufficiently complete to resolve questions of transport, topology, energetics, stoicheiometry and reversibility or tightness of coupling that are both theoretically and experimentally important. But before proceeding to examine these questions more closely, it will be helpful to compare the chemically conventional basis of Williams' mechanism – according to which coupling depends on the ATPase-catalysed transfer of H_2O from ADP + POH to the relatively anhydrous 'aqueous syrup' H^+-anh in a lipid phase at low H_2O potential – with the chemically vectorial chemiosmotic mechanism (fig.2), according to which coupling depends on the specific diffusion of H^+ and of OH^- or O^{2-} groups in opposite directions from ADP + POH (or to ATP) in the anisotropic active centre region of the ATPase system, plugged through the membrane between two phases that are at the same H_2O potential but at low and high protonic potentials respectively.

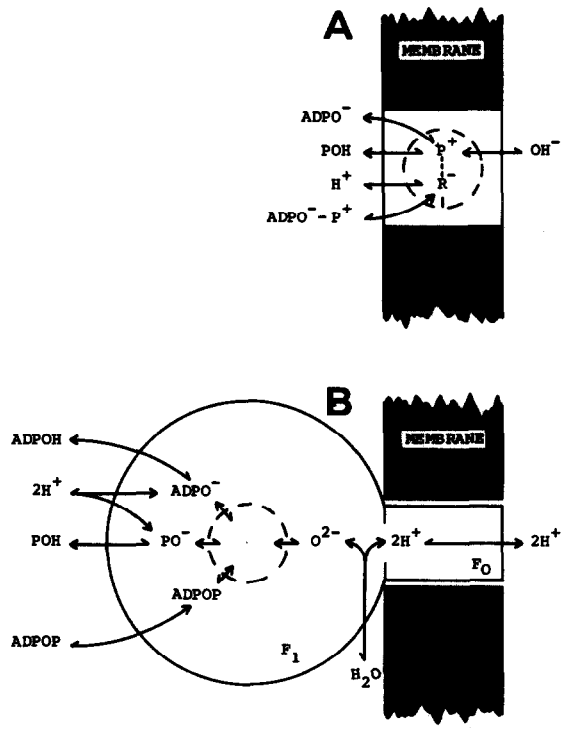

Fig.2. Protonmotive ATPase mechanisms: (A) As surmised in 1961 [1]. (B) Taking account of experimental observations described in the text. In (A) R^- represents a negatively charged group in the active centre and P^+ represents phosphorylium as described by Lipmann [23]. Further explanations in the text.

3. Chemiosmotic enzyme and carrier systems

Figure 2 illustrates the general principle of the protonmotive ATPase system: (A), as originally conceived [1], when the protonmotive property of mitochondrial, bacterial and chloroplast ATPases was entirely hypothetical; (B), taking account of some subsequent experimental observations that have led to the following main conclusions. A lipophilic F_0 component of the ATPase [25] functions as a specific proton conductor or proton well through the lipid (dielectric) phase of the membrane, the bulk of which has a very low proton conductance [26–31]. The complete F_0F_1 ATPase systems of mitochondria from rat liver and ox heart are protonmotive and translocate not one, but two protons across the coupling membrane per ATP hydrolysed [32–34].

The reaction catalysed by this ATPase system is described as chemiosmotic because the chemical processes of H^+ and of OH^- or O^{2-} transfer are conceived as being channelled vectorially so that they are integral with (or are tightly coupled to) the osmotic process of the net translocation of protons (or H^+-hyd) from the phase on one side of the ATPase system to that on the other.

The proton well through F_0 would be expected to convert the electric potential component ($\Delta\psi$) of the total protonic potential difference (Δp) across the membrane into an equivalent pH difference [26] in accordance with the equation

$$\Delta p = \Delta\psi - Z\Delta\mathrm{pH} \qquad (1)$$

where Z is the conventional factor 2.303 RT/F, which has a value of about 60 at 25°C when the potentials are given in millivolts. Thus, the high and low protonic potentials of the regions on either side of the active centre of the ATPase F_1 component should correspond largely to a difference of pH, as usually defined in dilute aqueous media. This pH difference Δ'pH would be given quantitatively by the simple relationship

$$\Delta'\text{pH} = \Delta p/Z \qquad (2)$$

Equation (2) shows that a total protonic potential difference Δp of, say, 250 mV corresponds to a pH difference Δ'pH across the ATPase F_1 component of about 4.2 units, and using a proton translocation stoicheiometry (→ H^+/P ratio) of 2, the equilibrium ATP phosphorylation potential would be 2 × 250 = 500 e-mV/molecule or 11.5 kcal/mol.

According to the chemiosmotic hypothesis of oxidative and photosynthetic phosphorylation, the coupling membrane of low proton conductance, which has the reversible protonmotive ATPase plugged through it, also has redox systems that are protonmotive plugged through it, so that the generation of a proton current by the redox systems at a trans-membrane protonic potential difference of the above order of magnitude can drive ADP phosphorylation via the proton current carried by the proton-conducting phases on either side of the membrane [1–3,30].

Williams [12,13,15–19,24] has long been a powerful critic and opponent of the chemiosmotic hypothesis, both specifically and in general principle; and I shall therefore consider his criticisms at some length here. According to Williams, the concept of chemiosmotic coupling is specifically deficient because, for example, 'the theoretical membrane of chemiosmosis, which is nothing but an insulating dielectric is really chemically non-existent, as in the physiologists' theories of nerve ' [19]. Or again: 'As Morowitz (1976) points out [see 21], chemiosmosis has no coupling device, but is a statement of energy equivalences. Now, the coupling device has to be in the membrane, and since I reject any suggestion that through-space fields will drive phosphorylation or transport, coupling is an in-membrane reaction of atoms obeying conventional chemistry'. This view is supported by Robertson and Boardman [14], because of what they describe as 'The need to understand what happens, not only across the membrane but also within it'.

When Williams says that the membrane of the chemiosmotic rationale is really chemically non-existent, or that chemiosmosis has no coupling device, he presumably does not intend to deny the fact that the chemiosmotic hypothesis has been concerned with the formulation and with the experimental investigation of the molecular mechanisms and topological arrangements by which in-membrane components of the protonmotive ATPase and of the protonmotive redox systems translocate protons or their equivalent from the phase on one side, into and through the membrane to the phase on the other side (see, for example [2,3,25–28,30,35–38]). Williams' specific objections [12,13,15–19,24], like those of Robertson and Boardman [14], apparently arise from the fact that the protonic anhydride concept treats part or all of the lipid of the membrane as a conventional chemical reaction phase so that the free-energy of what has been loosely called the energised membrane may be thought of as being stored in, and transmitted through, the membrane itself, in the form of the protonic anhydride, H^+-anh. By contrast, the chemiosmotic rationale treats the bulk of the lipid of the membrane as an insulating osmotic-barrier phase, having the protonmotive redox complexes and the reversible protonmotive ATPase complexes plugged through it. Thus, protons and their associated potential energy can be transferred between the in-membrane redox and ATPase complexes, not via the bulk of the membrane itself, but via the proton-conducting phases at different protonic energy potentials on either side of it. These considerations may explain some of Williams' specific objections to features of the chemiosmotic coupling concept. But his view, that the membrane of chemiosmosis is really chemically non-existent and contains no coupling device [19,21], is too far-reaching to depend only on specific objections. Presumably, this view depends on a more general or fundamental type of objection, in which Williams [13,15,19] has received the support, for example, of Wang [59] and of Weber [60,61].

Williams [15] has criticised protonmotive chemiosmotic mechanisms generally in the following terms: 'Clearly in a membrane of a biological system the reaction could go only if the redox energy which drives proton formation is not dissipated by allowing free

diffusion of the proton into water or elsewhere .... (in Mitchell's chemi-osmotic sense the reaction domain must be the whole cell or organelle and its surrounding media. The dissipation of energy in such an equilibration is quite foreign to my way of thinking and I have not seen an explanation of these energy losses in the chemi-osmotic hypothesis which from its creation has required such an equilibration)'. In an additional note in the same paper [15], Williams described his general objection more graphically by saying that 'if charge is thrown out into the medium, as in osmotic theories, then we face the problem of equilibration of the energy of a single cell on its outer side with the whole of the volume in which it is suspended, say the Pacific Ocean'.

As discussed above in section 2, it is, of course, true that, according to the protonic anhydride mechanism proposed by Williams, the anhydride property of the protons would be irreversibly lost and the system would be energetically uncoupled if the anhydrous protons were extruded into the outer aqueous medium. However, Williams does not seem to have realised that the chemiosmotic coupling mechanism behaves differently from the mechanism that he himself proposed, because it depends on the vectorial chemical process of specific trans-location of chemical groups through an anisotropic catalytic domain between one phase and another, rather than on the conventional chemical process of trans-formation in a phase.

In the chemiosmotic type of system, the hydrated H^+ ions do not appear in the outer aqueous medium without a corresponding disappearance of hydrated H^+ ions (or their equivalent) from the inner aqueous medium. As I have shown in a quantitative treatment of this matter [26,62], the work done in translocating H^+ ions across a membrane depends on the total protonmotive potential difference Δp between the dilute aqueous phases on either side, which includes electric ($\Delta\psi$) and chemical activity (given by ΔpH) components according to eq. (1). This total energy difference Δp of the hydrated hydrogen ions on either side of the membrane increases very rapidly as H^+ ions or their equivalent are pumped across, because the electrical capacitance of the lipid membrane is only about 1 $\mu F/cm^2$ membrane, practically independently of the volume of the outer phase [26,62]. Thus, the $\Delta\psi$ component of Δp increases fast 'as charge is thrown out into the medium' even if the outer medium is the Pacific Ocean; and the work done in charging the membrane system is conserved in the total protonic potential difference Δp. In mitochondria or bacteria, for example, it has been calculated that the transfer of only some 1 μg ion H^+ across the membrane/g protein is sufficient to raise the protonic potential from zero to around 250 mV – a process expected to take only a fraction of a second [26,62].

Even if the membrane were freely permeable to certain ion species so that the pumping of H^+ ions outwards were compensated electrically by other ion movements, and resulted in no significant change of $\Delta\psi$, but only in a significant change of ΔpH, the energy of the pH difference would still be conserved. In this case, although the outer buffering power might be practically infinite (as in the Pacific Ocean), the inner buffering power would be finite, so that ΔpH would be a function of the number of H^+ ions transferred across the membrane. This can be appreciated quantitatively [26,62] from the fact that the differential buffering power B across a membrane, defined as the number of equivalents of H^+ transferred across the membrane/unit change in ΔpH, is given by adding the reciprocals of the buffering powers of the inner and outer phases (denoted by suffixes I and O), as follows:

$$\frac{1}{B} = \frac{1}{B_I} + \frac{1}{B_O} \qquad (3)$$

Therefore, if B_O is infinite, $1/B_O$ is zero, and

$$\frac{1}{B} = \frac{1}{B_I} \text{ or } B = B_I \qquad (4)$$

In mitochondria and bacteria, B_I is of the order of 10 μg ion H^+/pH unit change; and so the translocation of only some 40 μg ion H^+ is sufficient to generate a ΔpH value of some 4 units or a Δp equivalent to around 250 mV.

Thus, it is not true that, in the protonmotive chemiosmotic type of mechanism, the energy of the H^+ ions is lost in an extensive outer aqueous phase, as would be the case for Williams' protonic anhydride mechanism. This is obviously particularly important in bacteria, where the chemiosmotic systems are used, not only for oxidative phosphorylation, but also for the uptake of nutrients through the plasma membrane of the free-living cells in extensive aqueous environments [57,58,63].

Ort et al. [64,65] recently observed the effect of changing the quantity of permeating ion species or of changing the differential pH buffering power on the time taken by chloroplasts to start synthesising a significant quantity of ATP, when illuminated by a series of light flashes of short duration. Their observations led them to conclude that, although a delay was caused by increasing the effective electric or pH buffering power capacitance, this delay was less than they expected it to be; and they therefore suggested that the protons were not equilibrating with the phases on either side of the membrane. However, they did not take account of the fact than an increase of capacitance should not only cause an extension of the rise-time of the protonic potential difference Δp, driving ATP synthesis via the reversible protonmotive ATPase, but it should also cause an increase in the energy-storage capacity of the system – enabling ATP synthesis to continue longer into the dark periods between the flashes of illumination. This illustrates nicely the energy conserving property, associated with pH buffering and ion diffusion in chemiosmotic systems, that may well seem foreign and surprising to those unaccustomed to thinking in the chemiosmotic idiom.

The objections, mentioned above, by Wang [59] and by Weber [60,61] to the chemiosmotic coupling rationale rest broadly on the idea that osmotic reactions, being diffusion processes, are necessarily irreversible, and cannot be tightly coupled to each other or to chemical reactions. This also represents an important and fundamental aspect of the objections by Williams [13,15,19]. Space will not allow a more detailed discussion and rebuttal of this misconception here (but see [39,63]). However, to dispel the idea that tightly coupled chemicomotive and chemiosmotic reactions may contravene fundamental physical or chemical principles, as argued most authoritatively by Weber [60,61], the following section shows how old-fashioned and well-founded are the physical and chemical origins of the concepts of reversible chemicomotive and chemiosmotic reactions, and how these concepts are related to conventional enzymology by means of the notion of group translocation, catalysed in and through coupling membranes via appropriately orientated enzymes and catalytic carriers.

4. Foundations of the chemicomotive concept of enzyme catalysed group translocation

In 1933, Guggenheim generalised the idea of electrochemical cells and circuits to include the chemically motivated transport of any two species of chemical particle around a suitably conducting circuit [66]. The implication of his rather abstract treatment of such systems, which he called chemicomotive cells, was not, perhaps, entirely self-evident; but it amounted to the principle that chemicomotive systems depend on the driving chemical process being split spatially into two half-reactions, connected internally by a specific conductor of one chemical species, and connected externally by a specific conductor of another chemical species needed to complete the overall chemical reaction. Thus, provided that the chemical conductors were sufficiently specific, the reaction would proceed, and chemical work would be done, only when the external circuit was closed, so that the circulating chemicals flowed down the through-space chemicomotive fields, corresponding to their chemical potential gradients, in their respective conductors ([66] and see [26,62]). The tight coupling and thermodynamic reversibility of such diffusion-regulated systems described by Guggenheim [66] is, of course, very relevant to the contrary views expressed by Wang [59], by Weber [60,61] and by Williams [13,15,19], discussed above.

As I have shown elsewhere [7,27,30], the general concept of chemical group translocation, catalysed by enzymes and catalytic carriers, is closely related to Guggenheim's idea of chemicomotive cells and circuits, and owes much to the work of Curie [67] Lipmann [68], Lundegardh [69], Davies and Ogston [70] and many others. Perhaps one should even trace back the notion of chemicomotive systems to the remarkable invention of the hydrogen/oxygen fuel cell by Grove [71] in 1839.

Mechanistically, the chemical group translocation concept is an extension of the idea put forward by Pauling [72], that enzymic catalysis depends on tight binding of the transition-state complex rather than of reactants and resultants. Pauling's idea required only a small adjustment to adapt it to the notion that the active centre regions of certain enzymes and of certain catalytic carriers (such as cytochromes) may be conceived, not simply as specific group-binding centres, but rather as specific group-translocating or group-conducting devices that facilitate the passage of chemical groups through a region of the catalytic complex between specific group-donating and group-accepting domains [4,5,8,9].

Figure 3 shows a diagrammatic representation of a hypothetical example of the concept of enzyme-

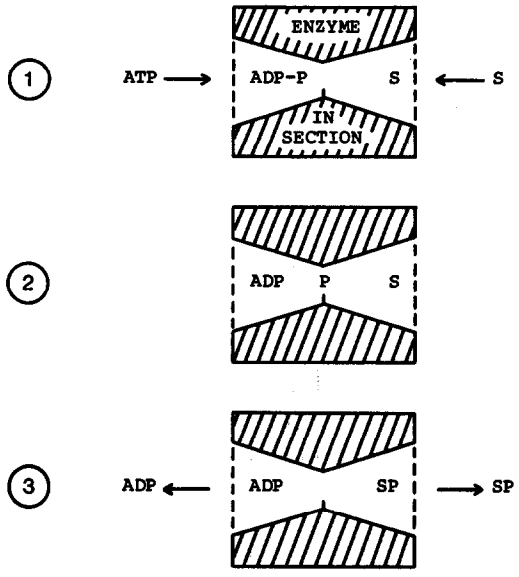

Fig.3. Enzyme-catalysed group translocation illustrated by a hypothetical example of phosphoryl transfer from ATP to a substrate S (from Mitchell and Moyle [8,9]).

catalysed group translocation. When this concept was explicitly formulated [8,9] in 1958, it was realised that the chemicomotive effect of group translocation would not be manifested unless the enzyme or catalytic-carrier molecules were inhomogeneously organised in space according to either of two main topological principles. According to one topological principle, the organisation could be at the macroscopic level in a membrane, thus giving rise to macroscopic chemiosmotic processes, of which some permutations and combinations were listed for a phosphokinase system by way of example [9], as reproduced in table 1. The table illustrates that the overall chemiosmotic process depends as much on the osmotic translocational specificities as on the chemical specificities of the catalytic system. It is in this respect that chemiosmotic systems differ fundamentally from conventional chemical ones. Figure 4A represents the macroscopic chemiosmotic group translocation principle applied to the phosphorylative translocation of the substrate S (which could, for instance, be a sugar), as in the second example shown in table 1.

According to the other topological principle, the organisation could be at the microscopic level, by pairing and enclosure of a 'microscopic internal phase' between neighbouring catalytic units, thus giving rise to a chemical coupling effect [8,9]. The NADP-linked isocitrate dehydrogenase or the malic enzyme, which catalyse consecutive oxidation and decarboxylation reactions (as illustrated in fig.4B), were cited as examples. It was further suggested that such pairing of catalytic units could be developed in three dimensions for branching or cycling reaction sequences in enzyme complexes [8,9]. Figure 5A illustrates, as a typical example of the application of this microscopic chemiosmotic catalytic pairing principle, consecutive hydrogen transfer and electron transfer reactions – as, perhaps, in NADH dehydrogenase. The microscopic and the macroscopic chemiosmotic principles of organisation can, of course, be employed together, as in the application of the chemiosmotic concepts of the redox loop and the proton well [2,3,26,62], as illustrated in figs.5B and 5C. For comparison, fig.6 illustrates the redox loop mechanisms of figs.5B and 5C,

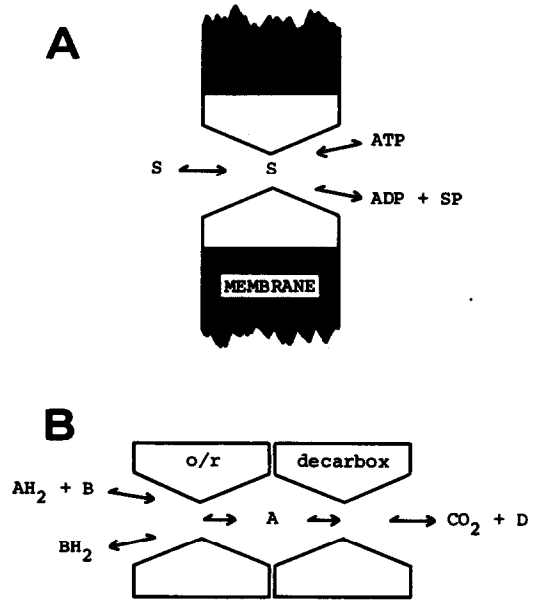

Fig.4. Chemicomotive effects of group translocation (after Mitchell and Moyle [8,9]): (A) By macroscopic chemiosmotic organisation of an S-translocating enzyme in a membrane. (B) By microscopic pairing and enclosure of a 'microscopic internal phase' between neighbouring catalytic units, as in some enzymes catalysing oxidative decarboxylation. Further details in the text.

Table 1
Illustration of different chemiosmotic processes catalysed by a hypothetical membrane-located phosphokinase, according to the translocational specificities (from Mitchell and Moyle [8,9])

EXAMPLE	CHEMI-OSMOTIC PROCESS			GROUPS TRANS-PORTED
	LEFT PHASE	PHOSPHOKINASE IN MEMBRANE	RIGHT PHASE	
1	ATP ADP	⇄	S SP	P-
2	ATP ADP + SP	⇄	S	S-
3	ATP + S ADP	⇄	SP	S- P-
4	ATP	⇄	S ADP + SP	ADP- P-
5	ADP	⇄	S + ATP SP	ADP-
6	ATP SP	⇄	S ADP	ADP- S-
7	ATP + S	⇄	ADP + SP	ADP- P- S-

The two aqueous phases are shown to left and right of the central line denoting the membrane, and, in each example, the chemical (group transfer) reaction is represented as progressing downwards, while the osmotic (group translocation) reaction is represented as progressing across the membrane. The table emphasises that the overall chemiosmotic process depends on the translocational specificities as much as on the chemical specificities of the catalytic system. Only the possibilities for homolytic phosphoryl transfer are shown here. Additional possibilities arise if the reaction is heterolytic [7].

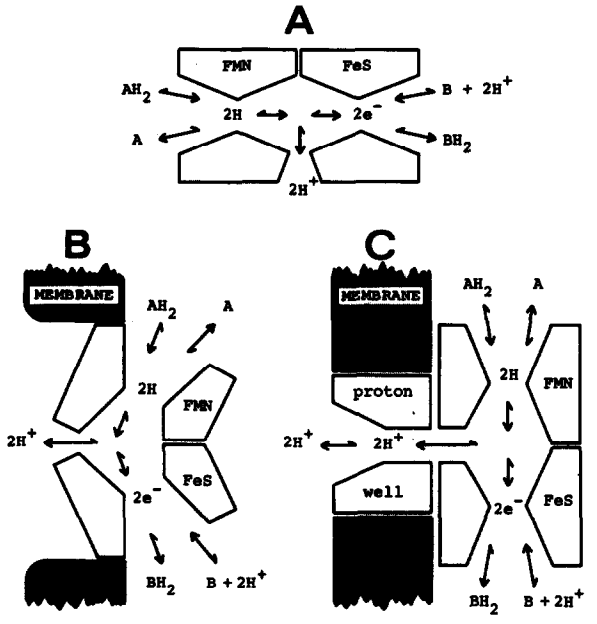

Fig.5. Development of microscopic and macroscopic topological principles together in chemiosmotic systems: (A) Microscopic pairing between hydrogen and electron transfer proteins, denoted FMN and FeS respectively, as, perhaps, in NADH dehydrogenase. (B) and (C) Macroscopic organisation of this paired system in a membrane to give chemiosmotic protonmotive redox loop systems. Further explanations in the text.

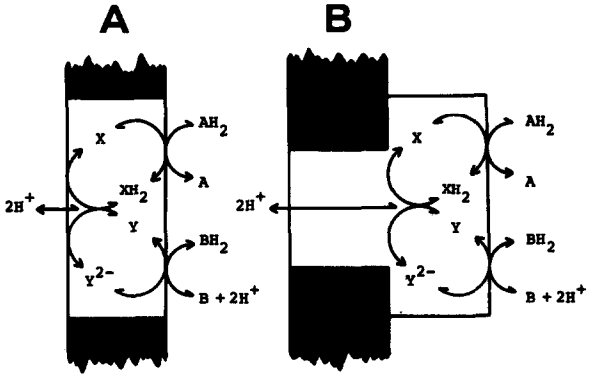

Fig.6. Redox loop mechanisms: (A) Looped across the membrane. (B) Involving a proton-conducting component or proton well: (A) and (B) correspond to fig.5B and C respectively, and X and Y represent hydrogen and electron carriers respectively. Further explanations in the text.

using a more formal and somewhat more explicit idiom that has become customary, where X and Y represent hydrogen and electron carriers, respectively. The protonmotive ATPase mechanism, illustrated by fig.2B, like the redox loop, is based on both the microscopic and the macroscopic chemiosmotic principles of organisation.

5. Further consideration of the protonic anhydride coupling concept

The microscopic chemiosmotic principle of pairing of specific group-translocating enzymes or carriers [8,9] enables us to obtain further insights concerning the protonic anhydride type of coupling mechanism proposed by Williams [10–13,17], because the chemiosmotic principle focuses attention on the catalysts of translocation of the groups entering and leaving the microscopic internal phase, the specificities of which determine the tightness of coupling – in accordance with the principles of reversible chemicomotive cells, described by Guggenheim [66].

In order to make the protonic anhydride coupling mechanism tightly coupled, we would have to develop it in some such way as illustrated in fig.7A. In this mechanism, it is assumed that the protonic exit pathway is specific for the translocation of H_3O^+, so that H_2O would be excluded from the microscopic internal phase in the hypothetical water-tight complex between the protonmotive redox system and the hydromotive ATPase system. Thus, we assume here that the exit pathway works as a tightly coupled proton–water symporter. Further, to explain the experimental fact that only one ADP is phosphorylated per pair of electrons traversing each coupling site in oxidative phosphorylation systems, we should either have to assume that only one of the two electrons at each site went by a pathway of the type represented by fig.7A, or we should have to make other assumptions about the properties of the putative redox–ATPase complexes: for example, we might assume that the exit pathway acted as a $2H^+$–H_2O symporter or as a H^+–H_3O^+ symporter, as indicated in fig.7B.

The more explicit developments of Williams' protonic anhydride coupling hypothesis raise interesting bioenergetic and kinetic questions because the free energy of hydration of the proton in dilute aqueous media is of the order of 100 kcal/mol [73] – some

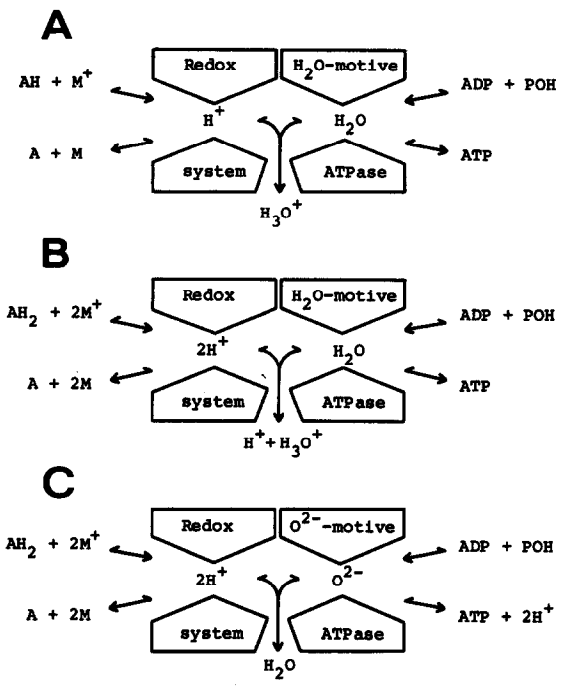

Fig.7. Microscopic chemiosmotic coupling mechanisms: (A) Protonic anhydride mechanism with protonic exit pathway specific for H_3O^+ uniport or H^+–H_2O symport. (B) as (A) but with protonic exit pathway specific for $2H^+$–H_2O symport. (C) Miniaturisation of macroscopic chemiosmotic mechanism.

ten times the standard free energy of hydrolysis of ATP. But, to be kinetically competent, energy-rich intermediaries must generally have a standard free-energy change corresponding to the systems between which they mediate. The hydrogen ions of aqueous solutions probably exist mainly in the form of the trihydrate of H_3O^+ [73]. Accordingly, the dehydrating 'aqueous syrup' in the dislocated phase assumed by Williams [11] must be represented as containing a considerable number of H_2O molecules (as in fig.1) to bring the free-energy difference between H^+-anh of the 'aqueous syrup' in the dislocated phase and H^+-hyd in the surrounding dilute aqueous media down to a value that is of the same order of magnitude as the free energy of hydrolysis of ATP. However, the same requirement – that the free energy of the protons in the dislocated phase should be brought down to a level that is only around 10–15 kcal higher than that of H^+-hyd – could alternatively be met by the protonation of a suitable group X^- in the hypothetical redox–ATPase complex, to give the chemically unstable energy-rich protonic intermediary $X \sim H$. My developments of Williams' concept shown in fig.7 might either include the 'aqueous syrup' of Williams [11], or an equivalent unstable intermediary $X \sim H$, in the microscopic internal phase. We are reminded, incidentally, of the rather close relationship between the protonic anhydride type of coupling hypothesis and the classical energy-rich chemical intermediate hypotheses of oxidative and photosynthetic phosphorylation that are now generally regarded as untenable [74].

The question may well be asked: why does coupling in the protonic anhydride mechanism depend on the specificity of translocations of chemical groups into and out of a microscopic internal phase (like a microscopic chemiosmotic reaction), whereas conventional chemical intermediary mechanisms do not? The answer is that conventional chemical intermediaries have some intrinsic chemical bonding stability, so that their reaction pathways may be determined by enzymic catalytic specificities, whereas the anhydrous or partially hydrated proton, as discussed by Williams [11], generally has virtually no intrinsic stability in a system where there is abundant water. Therefore, structural confinement and translocational specificities are required to determine and couple the pathways of the reactions of the protonic anhydride intermediary, where covalent bonding stability and enzymic specificities would determine and couple the pathways of the reactions of a conventional energy-conserving chemical intermediary [39].

In fig.7, the function of the translocationally specific exit pathway for H^+ (acting as a H_3O^+ uniporter or H^+–H_2O symporter in A, and as a $2H^+$–H_2O symporter in B) is to transform the protonic potential difference $\Delta\bar{\mu}H^+$, between the microscopic internal phase and the external aqueous media, into an equal and opposite potential difference of water ($\Delta\bar{\mu}H_2O$). Thus, for fig.7A,

$$\Delta\bar{\mu}H_2O = -\Delta\bar{\mu}H^+ \quad (5)$$

and for fig.7B

$$\Delta\bar{\mu}H_2O = -2\Delta\bar{\mu}H^+ \quad (6)$$

In the absence of the translocational specificities that determine such relationships as those of eq. (5) or eq. (6), the mechanism will have an unknown degree of coupling (if any), it will have no known stoicheiometry, and it will not be reversible.

It therefore transpires that, as the protonic anhydride coupling hypothesis formulated by Williams [10,11] does not include any protonic translocational specificity in the pathway between the internal 'aqueous syrup' and the external dilute aqueous media (fig.1), the mechanism has no known stoicheiometry, it is irreversible, and it is not a microscopic chemiosmotic mechanism. I emphasise this conclusion, because, in a special appendix to one of his papers, Williams [24] pointed out that I was previously mistaken [27] in describing his formulations as representing a microscopic chemiosmotic type of mechanism. It is true that I have been inclined to overlook the irreversibility of the protonic anhydride coupling mechanisms as formulated so far by Williams [10,11,17,19]. Perhaps this was because he has seemed to recognise the requirement for tight coupling, and thus of reversibility, in his criticisms of the chemiosmotic hypothesis. At all events, I now accept that it is only my reversible developments of Williams' idea, which he himself disowns [24], that can properly be described as microscopic chemiosmotic coupling mechanisms.

One is forced to the conclusion that Williams' formulations of the protonic anhydride coupling hypothesis do not, as they stand, define a practical reversible coupling mechanism. But, his protonic anhydride type of mechanism could theoretically acquire the required property of tight coupling and reversibility, either by the inclusion of protonic translocational specificity, so that it would become a microscopic chemiosmotic mechanism, as I have suggested, or by the inclusion of a stable protonic energy-rich intermediary $X \sim I$ that would react specifically with H_2O at the 'ATPase' active centre but not otherwise. In the latter case, the protonic anhydride coupling hypothesis would become a classical chemical type of coupling hypothesis, rather like that of Robertson and Boardman [14].

In order to continue this commentary on the merits of protonic anhydride hypotheses, in spite of the incompleteness and irreversibility of Williams' formulations, I propose to describe reversible developments of Williams' idea by phrases such as 'the Williams type of hypothesis' or as 'the protonic anhydride type of hypothesis'. Thus, we need not argue whether such developments are microchemiosmotic or not.

We are now in a position to note that the Williams type of protonic anhydride coupling concept, represented by fig.1 and by figs.7A and 7B, is different from the miniaturisation of the macroscopic protonmotive chemiosmotic coupling concept represented by fig.7C. The miniaturised macroscopic chemiosmotic mechanism of fig.7C involves a protonmotive and not a hydromotive ATPase, and the H_2O activity in the microscopic internal phase of this mechanism is assumed to be virtually the same as the H_2O activity of the dilute external aqueous media – as in the classical macroscopic protonmotive chemiosmotic system. Thus, it is incorrect to assume, as has sometimes been done, that a miniaturised version of the macroscopic chemiosmotic mechanism, in which the proton circulation is comparatively localised, corresponds to the Williams type of coupling mechanism.

Our commentary shows, as I have remarked elsewhere [40], that the Williams type of coupling hypothesis is open to much the same general criticisms as the more orthodox chemical coupling [75] or conformational coupling [76] hypotheses, because this type of hypothesis either requires tight (e.g. watertight) complexation between redox and ATPase catalytic units, or it depends on the transmission of energy between the redox systems and the ATP synthase by a chemically stable (e.g., protonic $X \sim H$) intermediary. Such general criticisms have been discussed previously at some length [30,40–42,74, 77–79]. The following are some of the more salient experimental facts which indicate that the protonic anhydride type of hypothesis of coupling in oxidative and photosynthetic phosphorylation is, like the chemical hypothesis [74], no longer tenable.

The redox systems in the membranes of bacteria, mitochondria and chloroplasts have been found to translocate protons during oxidoreduction, not into closed dislocated phases in the membrane, but from the proton-conducting phase on one side to that on the other [30,37,40–42,49,52, 53,80,81]. Topological and functional evidence is steadily accumulating in favour of plugged-through configurations of cytochromes, photosynthetic pigments and other carriers and enzymic components in the membranes of the systems that catalyse oxidative and photosynthetic phosphorylation [41–47,51,52,80–85]. Some experiments have drawn attention to the possibility that there may be regions of high proton

conductance close to the surfaces of the lipid membrane, and regions of lower proton conductance further away from these surfaces [50,64,65]; but there is no evidence that the suggested localisation of the proton current at the surfaces on either side of the membrane is associated with a difference of H_2O potential between these surfaces, as would be required by protonic anhydride coupling hypotheses.

The observations in favour of plugged-through configurations of the redox systems, independently of the presence of ATPase complexes, are more significant in the present context because they are not in accord with the protonic anhydride type of hypothesis than because they are in accord with the predictions of the macroscopic chemiosmotic hypothesis. However, the fact that the energy-rich property of the protonic anhydride of the Williams type of hypothesis would be lost if the protons were thrown out into an extensive aqueous medium – the 'Pacific Ocean effect' of Williams [15,19] – implies that positive evidence for the osmotic utilisation of the potential difference of H^+-hyd across the membrane can be counted as evidence against the protonic anhydride type of hypothesis. The fact, now well established, that the protonic energy-transfer mechanisms involved in oxidative and photosynthetic phosphorylation are used in bacteria and mitochondria for the import of essential metabolites from the external aqueous media via proton–solute symporters, plugged through the membrane [58,86,87], leaves little doubt that the protonic energy is represented by the protonic potential difference Δp between the outer and inner aqueous media and not by a significant potential difference of H_2O between these media or between either of them and some hypothetical in-membrane dislocated phase.

The reversible ATPases of oxidative and photosynthetic phosphorylation systems have been shown to function as protonmotive systems independently of the presence or activity of redox components. As in the case of the protonmotive redox systems, the protons are translocated stoicheiometrically from the phase on one side of the membrane to that on the other [30,31,40–42,49]. Moreover, there is evidence [31,40–42] for a plugged-through configuration of the F_0F_1 ATPase complex in the membrane, as illustrated in fig.2B. These experimental observations on the protonmotive ATPase system are not compatible with the protonic anhydride type of hypothesis, according to which the reversible ATPase itself is not a protonmotive system. Further, there is no satisfactory evidence for the various redox–ATPase complexes – corresponding to the coupling sites – that would be required. Finally, despite some claims to the contrary [88], ADP phosphorylation has never been demonstrated in systems definitely lacking a trans-membrane, or equivalent trans-interface protonic potential difference, Δp.

Boguslavsky et al. [89] found that, when the F_1 component of the protonmotive ATPase was added to the aqueous phase in a system containing a water/octane interface, the presence of ATP caused the appearance of an electric potential difference across the water/octane interface, positive in the octane phase, provided that the octane phase was made conducting to protons by a suitable lipid-soluble proton acceptor, such as DNP or PCP. They inferred that the F_1 ATPase was adsorbed at the water/octane interface, and that during ATP hydrolysis the F_1 molecules injected protons into the octane phase, where they were accepted by the DNP or PCP. Thus, F_1 appeared to function as a protonmotive ATPase, in accordance with the chemiosmotic hypothesis. Yaguzhinsky et al. [22] subsequently observed that the oxidation of NADH by a NADH dehydrogenase preparation would also produce an interfacial potential in this water/octane system, positive in the octane phase, provided that the octane phase contained DNP or PCP. They found that when F_1 was added to this system, and ADP and inorganic phosphate were also present at high concentrations, the interfacial electric potential due to NADH oxidation was not only lowered, but it was reversed in sign; and some evidence was obtained for ADP phosphorylation. These and other related findings were interpreted as showing that ADP phosphorylation can be induced in F_1 by a protonic activity difference across the water/octane interface, acidic in the octane, and that an electric potential across the interface is not a necessary condition for ATP synthesis by the ATPase. Yaguzhinsky et al. [22] concluded that their observations supported Williams' hypothesis, but not the chemiosmotic hypothesis – apparently on the misconceived basis that the chemiosmotic mechanism of the ATPase reaction must depend on an electric potential difference across the ATPase system.

According to the chemiosmotic hypothesis, the driving force for ADP phosphorylation is represented, not by the electric potential difference $\Delta\psi$, but by the protonic potential difference Δp, given by eq. (1). Moreover, as discussed earlier in connection with the protonmotive ATPase mechanism illustrated in fig.2B, the proton-conducting channel through the F_o component of the ATPase is expected to transform the electric potential difference across the membrane into a corresponding pH difference [26,30,31,39,40,62] so that, under normal conditions of ADP phosphorylation, F_1 would not be subjected to an electric potential difference, but only to a pH difference Δ'pH equivalent to $\Delta p/Z$, eq. (2).

It may be unwise to attempt to place firm interpretations on the experiments of Boguslavsky, Yaguzhinsky et al. [22,89] until further confirmatory work has been done. But, at all events, these interesting experiments are not inconsistent with the predictions of the chemiosmotic hypothesis, and they do not, so far, provide support for the protonic anhydride type of coupling hypothesis.

6. Molecular mechanics of chemiosmotic reactions

As discussed earlier in this commentary, the main stimulus for the development of the chemiosmotic hypothesis of coupling in oxidative and photosynthetic phosphorylation came via the concept of enzyme-catalysed and carrier-catalysed chemical-group translocation which evolved from studies of chemicomoti-

vated transport reactions. Williams [19] has correctly emphasised that the chemiosmotic rationale 'was always a transport theory (before 1960)'. Indeed, it continues to be a transport theory, and it is unfortunate that specialised preoccupations with the bioenergetic aspects of oxidative and photosynthetic phosphorylation systems have tended to isolate students of these important systems from those involved in the wider field of metabolically-coupled transport.

Generally speaking, the group translocation concept has encouraged the formulation of what I call direct chemiosmotic mechanisms [30,38–40]. In these direct mechanisms (illustrated by figs 2,5 and 6) the coupled solute translocation and chemical reaction are represented by an integral group translocation process in which the conformational movements of solute translocation overlap with those of chemical group transfer, and the stoicheiometry of coupling is determined by the enzyme and carrier specificities – as in conventional biochemistry.

A rather different and independent stimulus for the consideration of the molecular mechanics of chemiosmotic reactions came from the long-standing interest of cell physiologists, and especially of students of nerve, in the active transport of covalently unreactive ions, such as Na^+ and K^+ [90]. These solutes could not be involved in group transfer reactions in the conventional way, and they could not therefore be transported by the direct type of chemiosmotic mechanism – at least, not without modification [91,92]. A completely indirect (exclusively conformational) type of chemiosmotic mechanism was consequently defined [7] by analogy with the conformational changes of haemoglobin induced by oxygenation, and in keeping with the notion of conformational interaction in enzyme catalysis generally. This completely indirect type of mechanism was not originally intended to be applied to covalently reactive solute species, like the H^+ ion, that could readily participate directly in group transfer and translocation. But it has been so applied, for example, in schemes described by Skulachev [93], by Boyer [77,94] and by others [74,78,79]. The general principle of the completely indirect or exclusively conformational protonmotive mechanisms is illustrated in fig.8. In these systems, the proton translocations (by the translocators T) are supposed to occur through centres that are spatially separate from the chemical

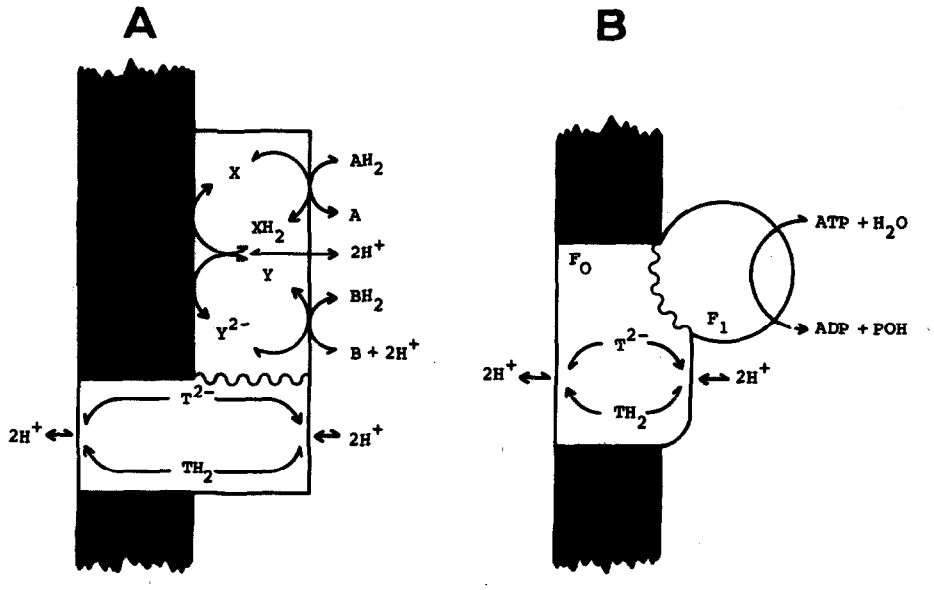

Fig.8. Completely indirect or exclusively conformational type of protonmotive chemiosmotic mechanism: (A) Protonmotive redox system corresponding to that of fig.6B. (B) Protonmotive ATPase system corresponding to that of fig.2B. The translocators T represent proton-pumping devices, energised by the conformational transitions transmitted from the separate chemical reaction systems, as indicated by the squiggly lines.

reaction centres, and coupling is supposed to be due exclusively to physical conformational movements communicated through the intervening polypeptide systems, as indicated by the squiggles in fig.8.

The completely indirect type of formulation, although theoretically feasible, is barren as to the biochemical details of the coupling mechanism. It has nothing to say about the stoicheiometry of coupling, and (as in fig.8) we may write in any numbers to suit the experiments of the day. Indeed, we may write in any mechanism for the chemical reaction and any mechanism for the translocation reaction, as long as we represent the coupling device by nothing more informative than the squiggly line between them (fig.8). Had Williams [19] applied his remark that 'chemiosmosis has no coupling device' only to this exclusively conformational type of mechanism, it would not, I think, have been entirely inappropriate.

With greatly increased knowledge of the polypeptide systems, the biochemically unsatisfactory 'black box' aspect of the completely indirect type of chemiosmotic formulation might ultimately change. But, meanwhile, the direct group translocation type of formulation is much more biochemically meaningful, and it is well known that it has, for some years, been acting as a valuable stimulus and guide for the experimental exploration of the protonmotive redox and ATPase systems.

A review on the NADH–ubiquinone reductase complex [54] shows that care should be taken not to confuse the direct type of mechanism shown in fig.6B with the indirect mechanism of fig.8A. In the indirect mechanism, there is supposed to be a separate proton pump T that does the work in pumping the protons. But in the direct mechanism of fig.6B, there is supposed to be a purely passive proton-conducting component or proton well connecting the proton pumping redox system through the membrane. Conversely, it is important to recognise that both the formulations of fig.6 have essentially the same overall protonmotive redox loop function. The protonmotive property of what I call the redox loop depends on the relative looped topological relationship between the redox chain and the membrane. Thus, as illustrated in fig.9, there is an infinite range of redox loop topologies between extremes in which either: (A), the redox chain may be looped across a flat osmotic barrier region of the membrane; or (B), the osmotic

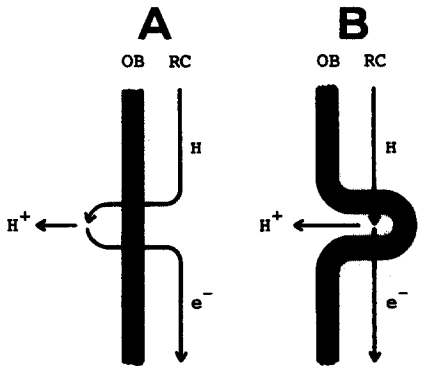

Fig.9. Possible types of redox loop topology: (A) Flat osmotic barrier (OB) with looped respiratory chain (RC). (B) Looped or invaginated osmotic barrier with straight respiratory chain. Further explanations in the text.

barrier component of the membrane may be looped across a straight redox chain region. This topological subtlety of the functional redox loop must, of course, be taken into account if one is to avoid being misled in practical prospecting for redox loops in redox chain systems. For example, the NADH–ubiquinone reductase system of mitochondria does not donate electrons directly to ferricyanide ions present in the aqueous phase on the C side of the cristae membrane. From that fact, De Pierre and Ernster [95] concluded, in a review on enzyme topology, that 'Complex I does not seem to form a redox loop across the membrane'. But, the concept of the proton well [26,62]. or of the equivalent infolding of the osmotic barrier region of the membrane, illustrated by figs 2B, 5C, 6B and 9B, shows that the lack of reactivity to ferricyanide could be due to the failure of ferricyanide to penetrate to the point of the redox loop in the well or crevice. The lack of ferricyanide reactivity should not therefore be regarded as diagnostic of the absence of a functional redox loop in Complex I.

Space will not allow further discussion of the mechanisms of protonmotive redox systems, including protonmotive Q cycle systems [96], in this commentary. But the reader may find relevant information in the following selection of references [38–40,53,55, 56,97–105].

7. The mechanism of protonmotivated ADP phosphorylation

It seems appropriate to conclude this paper with some comments on the direct type of chemiosmotic mechanism in protonmotive ATPases [1–3], because my efforts to develop more explicit descriptions of the molecular mechanism of O^{2-}-group translocation in protonmotive ATPase systems [27–29] have been greeted with such uncompromising condemnation by Boyer [94,106] and by Williams [15,16] that one might think these efforts altogether misguided, and that O^{2-}-group translocation is impossible or extremely improbable in general chemical principle.

Figure 10 summarises the essentially osmotic aspect of my proposals [28]. The double barbs on the arrows represent the purely formal forward direction of the reversible process of ADP phosphorylation. This diagram is slightly different from that given previously [28,30] in that H_2O is shown going off to the left instead of to the right. Thus, the net translocation is represented as the translocation of two protons rather than of one O^{2-} group. This alteration is only a formality because the membrane has a high permeability to water, and the water activity is virtually the same in the dilute aqueous phases on either side of it, so that O^{2-}-group translocation one way is strictly equivalent to $2H^+$ translocation the other way.

The biochemical implication of fig.10 is that F_0 and F_1 act as specific group conductors. The F_0 component of the ATPase acts as a specific proton conductor that enables the protons in aqueous phase R at a protonic potential equivalent to around pH 3 to equilibrate with the F_0 side of the active centre region of F_1. The F_1 component acts as a conductor of the substrates ADP, P_i and ATP into and out of the active centre region in specific protonation states, represented as $ADPO^-$, PO^- and ADPOP respectively. There are two less protons and one more O^{2-} group in $ADPO^- + PO^-$ than in ADPOP. The reaction represented as the dissociation of $2H^+$ from ADPOH +

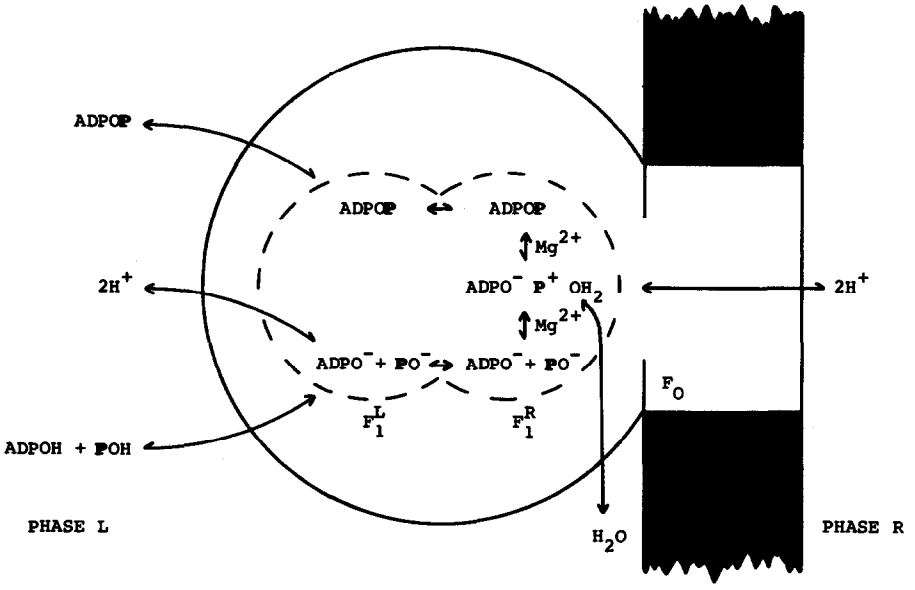

Fig.10. Proposed reversible O^{2-}-group translocating protonmotive ATPase mechanism (after Mitchell [28,30]). This diagram represents the overall flow of the reactants in the reversible F_0F_1 ATPase during protonmotivated ADP phosphorylation. The symbols $ADPO^-$ and PO^- represent ADP and phosphate each carrying one less proton than in the corresponding part of ADPOP, which represents ATP. The regions outlined by the broken line, labelled F_1^L and F_1^R, represent configurations and/or states of the F_1-substrates complexes in the active site region. As explained in the text, the diagram represents both translocational (conformational) and chemical changes in space and time, and it is not intended to indicate the actual scale or complexity of the movements in F_1.

POH on the left of the diagram, corresponding to the substrate side F_1^L of the active centre region of F_1, is attributed to the specific binding of the species $ADPO^-$ and PO^- by components of F_1 at a protonic potential equivalent to around pH 7 or 8. Of course, the species shown formally as ADPOH, POH and ADPOP in aqueous phase L will ionise to an extent that depends on the pH and Mg^{2+} concentration in this phase. For that reason, the stoicheiometric dissociation of $2H^+$ from ADPOH + POH, shown on the left of the diagram, which corresponds to $(ADPO^- + PO^-)$/ADPOP antiport across F_1, will not generally be the same as the net protonic dissociation change in phase L.

On the right side F_1^R of the active centre region of the F_1 complex, PO^- must come into a specific protonic equilibrium with phase R so that O^{2-} is detached from it as H_2O, whereas $ADPO^-$ must be protected from protonation. In that way, the P^+ produced by the removal of O^{2-} from PO^- may be attacked by $ADPO^-$ to form ADPOP. According to this scheme, PO^- represents the phosphorus centre of inorganic phosphate, complexed with F_1 in a specific (but, as yet, unknown*) state of protonation and salt formation, and P^+ represents this phosphorus centre after O^- and an additional electron have left it. Presumably the anionic charges of the species specifically complexed in F_1 would generally be neutralised by enzymic functional groups, including Mg^{2+}. Thus, the chemical transfer of O^{2-} from PO^- to $2H^+$ on the F_0 side of the F_1 complex can be conceived as occurring by a chemically orthodox phosphoryl transfer mechanism [107]. It would be immaterial whether this O^{2-} and phosphoryl transfer involved an associative (S_N2), a dissociative (S_N1), or a hybrid mechanism, or whether H_2O were produced directly by protonation of one O atom of the phosphate (possibly complexed with Mg) or, as suggested by Boyer [106], indirectly by elimination from two OH groups after protonation of two O atoms of the phosphate. It might even involve a pseudorotation [108]. The essential point is that the PO^- group would have to be complexed in the active centre region F_1^R of F_1 so that it could undergo a heterolytic phosphorylium-oxyanion dissociation promoted by two (and only two)** attacking H^+ ions conducted through the proton well in F_0, whereas $ADPO^-$ would have to be bound so that it was not susceptible to protonation from the F_0 side, but was so positioned that it could attack the positive phosphorus centre of the transitional phosphate–phosphorylium complex from the back. Functional groups of F_1, including Mg^{2+}, would presumably participate in the oxygen, phosphorus and proton transfers, and the O^{2-} (and phosphoryl) bond transfers would have to be facilitated by the tight complexation (and consequent increase of concentration) of the transitional complex, as in enzymic catalysis generally [109].

If we follow Lipmann [23], and describe P^+ as phosphorylium, and regard ADPOP as phosphorylium ADPate, the mechanism by which the translocation of the O^{2-} group through the F_1 enzyme complex may be coupled to the phosphorylation of ADP becomes relatively easy to appreciate. According to fig.10, the O^{2-} group leaves $2H^+$ on the left or substrate side F_1^L of the active site and joins $2H^+$ on the F_0 side F_1^R of the active site. The O^{2-} group potential difference $\Delta\bar{\mu}O^{2-}$ is therefore equal to twice the protonic potential difference $2\Delta p$ across the F_1 system at equilibrium. That accounts for the pulling effect of the protonic potential difference (or equivalent pH difference) on the O^{2-} group, which is therefore forced towards F_1^R from F_1^L. But the O^{2-} group travels across F_1 in combination with the phosphorylium group P^+ in the form of the F_1 complexed species of inorganic phosphate PO^-. Thus, the protonic force on the O^{2-} group would be transferred to the phosphorylium group P^+, so that the phosphorylium

* The specific state of protonation of the phosphate represented by PO^- in the F_1 complex could conceivably correspond to $H_2PO_4^-$, HPO_4^{2-} or PO_4^{3-}, when that of P^+ would formally correspond to $H_2P^+O_3$, $HP^+O_3^-$ or $P^+O_3^{2-}$ respectively, and that of the γ phosphoryl group of ADPOP would correspond to $-H_2PO_3$, $-HPO_3^-$ or $-PO_3^{2-}$ respectively. I have outlined a mechanism based on the third of these possibilities [30] for the sake of explicitness and in the light of certain experimental data [31]. But the more general treatment adopted here may be less open to misunderstanding.

** The stoicheiometry of the ATPase reaction described by fig.10 corresponds strictly to a $\rightarrow H^+/P$ quotient of 2, but higher stoicheiometries could be achieved, without fundamental change of mechanism, if the translocational specificities for ATP and for ADP + P_i in F_1 involved relatively higher deprotonation states in the latter [28].

group potential would be raised at F_1^R by an amount $\Delta\bar{\mu}P^+$ equal to $2\Delta p$ (which normally has a value of some 500–600 mV, corresponding to a concentration factor of around 10^9), and the phosphorylation of $ADPO^-$ would be promoted by an equivalent amount.

Perhaps the most important biochemical question concerning the general principle of the putative O^{2-}-group translocation mechanism of protonmotivated ADP phosphorylation by the F_0F_1 enzyme complex is: how may the polypeptide subunits of the F_1 protein participate in the translocational and chemical phenomena outlined here? Much experimental research will, of course, be required to give a definitive answer to that question; but it may be helpful to bear in mind, for the planning and interpretation of the required research, that the polypeptide subunits of F_1 would be expected to perform the following three interrelated functions, which are summarised by fig.10.

1. The F_1 polypeptide system, in a state or states that we shall call F_1^L, must catalyse deprotonation of ADPOH + POH relative to ADPOP by specific complexation of $ADPO^- + PO^-$ or of ADPOP at the substrate side of the active site region. But this protonic equilibration with aqueous phase L (around pH 7–8) on the substrate side of F_1 must not be accompanied by phosphorylium–oxyanion bond labilisation or dissociation.

2. The F_1 polypeptide system, in a state or states that we shall call F_1^R, must catalyse phosphorylium–oxyanion bond labilisation and dissociation at the F_0 side of the active site region, so that the transfer of O^{2-} from PO^- to $2H^+$ in equilibrium with phase R (around pH 3) raises the concentration of P^+ and promotes its transfer to $ADPO^-$, which is protected from protonation, and attacks P^+ from the back.

3. The F_1 polypeptide system must catalyse $(ADPO^- + PO^-)$/ADPOP antiport between the substrate side and the F_0-side of the active site region in a process corresponding to the transitions between the F_1^L and F_1^R states.

The specific translocational functions must depend on the migration of the substrates into and out of the F_1 complex. However, the translocational functions would presumably also require some mobility of the polypeptide subunits relative to one another, or conformational mobility of the polypeptide systems of the individual subunits, or both. Moreover, such movements of the polypeptides would have to be articulated in such a way as to prevent the leakage of H^+ ions through the polypeptide system of F_1 from the F_0 side to the substrate side. Incidentally, the wide separation between F_1^L and F_1^R, shown diagrammatically in fig.10, was dictated by the typographical requirements for constructing a clear diagram, and should not be taken to indicate the spatial extent or motion of components of the active site. I have previously suggested that there may be a kind of push–pull relationship between the entry of $ADPO^- + PO^-$ and the exit of ADPOP during ATP synthesis, much as in a proton-coupled solute porter system [110]. As there are pairs of α and β subunits in the F_1 polypeptide complex [111,112], the translocational transitions between the F_1^L- and F_1^R-states could involve a pair of translocational sites moving co-operatively in opposite directions between F_1^L and F_1^R, so that F_1 would oscillate between two equivalent F_1^{LR} states. Thus, the remarkable active/inactive state transitions of mitochondrial F_1, observed in my laboratory [113], and observations on molecular polymorphism and nucleotide binding in a bacterial ATPase [114,115], which have led Adolfsen and Moudrianakis to propose a 'flip–flop' type of alternation between allosterically interacting sites, may conceivably be manifestations of the translocational functions of the hypothetical O^{2-} group-translocating ATPase considered here.

I commend these hypothetical considerations to the reader as a basis for experimental exploration, because they do not involve any major biochemical innovation. The F_1 ATPase would not do any net work in this type of ADP phosphorylation mechanism, since the direct type of chemiosmotic reaction proceeds through local electrochemical equilibria – like classical group-transfer reactions. This is in contrast to the completely indirect or exclusively conformational type of chemiosmotic mechanism (fig.8B), advocated by Boyer [106], where the F_0F_1 enzyme polypeptide system is supposed to drive the chemical reaction conformationally (in F_1?) by means of the energy derived from the

spatially separate reversible proton-pumping system (in F_0?).

I think it can hardly be overemphasised, in the context of the mechanism of protonmotivated ADP phosphorylation, that the chemiosmotic rationale amounts to little more than the addition of an explicit spatial dimension to Lipmann's marvellously germane concept of group potential in enzyme-catalysed reactions [23,68], so that group potential gradients can be equated to the real through-space forces that cause, or are caused by, vectorial, chemical and osmotic processes [39].

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