Commentary

THE HISTORY AND THE HYPOTHESES CONCERNING ATP-FORMATION BY ENERGISED PROTONS

R. J. P. WILLIAMS

Inorganic Chemistry Laboratory, South Parks Road, Oxford, OX1 3QR, England

Received 22 October 1977

1. Introduction

In a recent article Mitchell [1] has reviewed at length the origins of my views [2—4] as well as his own [5] on proton-driven ATP formation and has commented on 'the two main types of biochemical model' for ATP synthesis. I do not wish to attempt here so lengthy a review but since his historical perspective differs from mine, and I believe some of the scientific comments on the 'two biochemical models' are not correct, I wish to make some remarks. My historical references are confined to a short statement.

The origin of both our views was the desire to couple oxidative/reductive reactions to ADP + P_i condensation reactions. My idea of how this might be done using the intermediate energy of an energised proton was stated first in 1959 [2] and extended in 1961 [3,4]. In the opening paragraphs of the paper [3] I make it clear that there is a way of storing energy before using it by turning the initial energy into a proton concentration gradient. I illustrated this with a simple electrical cell connecting oxidation and proton production and I drew attention to the fact that this is a cell in which energy storage can be likened to a proton concentration gradient between two regions of space separated by a membrane, the membrane acting as a diffusion barrier. I quote from [3] 'This type of cell is particularly significant as one compartment contains the hydrogen ion at an entirely different activity from the other. In some biological systems this situation is realised.' On the same page I show the subsequent relationship of such charge separations to ATP production. I suggest that controlled diffusion of the proton makes ATP. This is also the essence of chemiosmosis. However I claim no priority over Mitchell for this hypothesis for Mitchell had an abstract in press of which I was unaware [6]. I trust that Mitchell does not wish to claim priority either. In our personal and friendly exchange of views in 1961 it happens that I initiated the discussion of these ideas. I leave the rest of history to matters which are now in print, see references in [1].

I now want to turn to the scientific differences between chemiosmosis and local proton theories which have become confused. I am not 'an opponent of the chemi-osmosis hypothesis' [1] as the above makes clear but I always wished and wish now to point out that it is only a special case of a more general idea. Moreover this special case does not have acceptable experimental support in my view and I have therefore stressed at all times the more general approach. I quote from the passage of my first paper [3] which follows directly the previous quotation 'The restriction of reaction by preventing mixing (i.e. by using a membrane or a salt bridge) is only one way of controlling reactions in space. For substances which need catalysts in order to react restriction of diffusion by a salt bridge or a membrane is unnecesary . . .' This clearly includes consideration of what is now called chemi-osmosis but makes it clear that chemi-osmosis is not a fundamental requirement. I then explain that the fundamental requirement for reaction systems of the Mitchell/Williams type is just diffusion control. In all my subsequent papers I have

been exploring the use of diffusion control either of the motions of electrons (electron transfer) or of protons and how these motions can be coupled to ATP formation, other reactions, and transport. Just as chemiosmosis is a special case of a more general theory so micro-chemiosmosis is another such special case but one which is a localised control of diffusion. It is a localised control of diffusion which is, I believe, the basis of the biological systems of interest. Micro-chemiosmosis is very close to my view, see [1]. The obvious advantages of the local control systems are:

(i) More efficient energy utilisation, see below;

(ii) Better control;

(iii) Discriminatory coupling of energy to different processes.

Before I present the general approach to these problems so as to show that chemiosmosis is only a special case I want to remove some other possible points of confusion. First the general ideas which are being discussed do not define the coupling device. That is a separate problem of enzyme chemistry. The present primary discussion is about the pathway of energy not about the molecular mechanism of how ATP is formed. Again the discussion should not be confused by the attribution of special features to one model rather than another, e.g., reversibility and vectorial processes. I believe that all the models are clearly chemically reversible, as they must be to obtain any measure of acceptance. However they are reversible only in the sense that they can be made to run backwards. All are irreversible in the thermodynamic sense. I make no further point about reversibility for both Mitchell and myself have described, reversible micro-schemes, local protons, and in effect we agree now on this point. We agreed in 1961 that chemiosmosis was a tenable hypothesis.

Another scientific point concerns vectors. If we are dealing with small isolated regions of space which are separated by a single phase boundary then direction is in fact defined [3]. It does not require a membrane to define a vector. This may seem peculiar but a vector is as real on the surface and within the earth in a planetary system as are the vectors between the planets. If the membrane is equated with space the analogy may be useful. When I use the idea of dislocated charges there is a local vector between the charges, see fig.1, but if the local region were to tumble rapidly this vector could average to zero and would not be found when an external device was used to search for it.

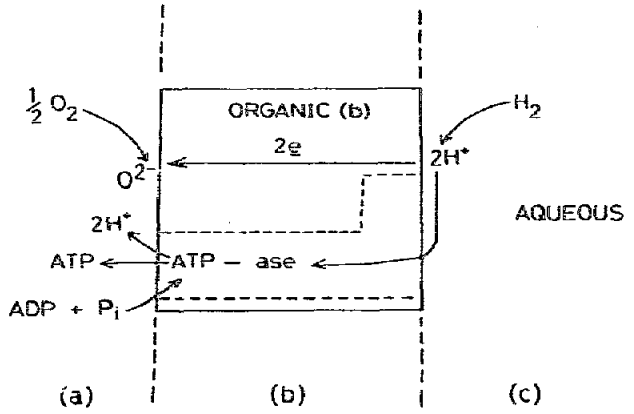

Fig.1. The localised model for ATP formation (even in a particle). The particle is illustrated by the box and the diffusion-controlled paths of $\underline{e}$ and H^+ are shown. The device can be converted to a local model in a membrane by putting the box into a membrane, see dashed vertical lines around (b), to give two aqueous phases (a) and (c) not in equilibrium with (b). This becomes the chemiosmosis model by releasing all H^+ to (c) and all O_2^{2-} to (a). These are three models (there are many more) for devices which can use an energised proton [2–4] to make ATP or to couple other energy-driven processes.

There are then no such differentiating general principles as reversibility or vectors which divide the approaches and they go step by step together (except at certain points of diffusion control). I have written the common and the differentiating points in table 1.

2. Pathways: diffusion control

In fig.1 I draw a box to represent the essential central scheme which I shall now relate to both local two-phase and chemiosmotic phenomena in a simple way. I start from the box defined by the full lines alone of fig.1. This will be the general case from which all others follow. The box is a particle (organic) in an aqueous phase. Reactants diffuse freely in the single

Table 1
Required features of energy conserving protons

	Model (a) localised or 'micro-chemiosmosis' [1,3]	Model (b) chemiosmosis [1,3]
Diffusion limitation	Two phases	Membrane (three phases)
Reversibility	Chemically reversible	Chemically reversible
Vectorial quality	Trans-interface	Trans-membrane
Osmotic term	Non-existent (or small)	Totally dominant
Aqueous capacity	Ill-defined	Well-defined
Bound charges	Totally dominant	Non-existent
Capacity	Bound charges (organic phase) and concentrations	Osmotic charges and concentration
Stoicheiometry nH^+/ATP	Not required (no postulate made)	Not required (extra postulate of two)
Electron transfer	Metal ions in non-aqueous phase	Metal ions in membrane
ATP formation	Proton-driven	Proton-driven
Coupling mechanism of ATP formation	Extra postulate (idea provided)[a]	Extra postulate (idea provided)[a]
Source of charge separation	$H_2 \rightarrow 2H^+ + 2e$	$H_2 \rightarrow 2H^+ + 2e$
	$h\nu \rightarrow (+) + e$	$h\nu \rightarrow (+) + e$
Aqueous pH in each water phase	Not at equilibrium with membrane	Two equilibria with different membrane faces
Potential across ATP site	Local across ATPase	General across membrane
Stoicheiometry ne/H^+	Not required (no postulate made)	Not required (extra postulate of one)
Chemical intermediates	Not required but not excluded	Not required but not excluded
Osmotic gradients of other ions and neutral sugars etc. across membranes	Indirect connection (see fig.1)	Direct obvious connection with exchange

[a] Several other authors have quite other ideas than those of Mitchell or Williams

surrounding aqueous phase but react only at certain catalyst centres. The initial products react through prescribed diffusion restricted paths.

Provided that the protons and oxide ions in fig.1 cannot diffuse together through water at a rate greater than the rate of diffusion of H^+ to O^{2-} through the prescribed ATPase path of fig.1, redox energy is not lost before ATP is formed. Local vectors exist in the required path for the proton, and the proton can not reach the left hand side of the above particle rapidly without moving along a path. Note the proton and oxide ions could remain absorbed at all times within the square box, which at this stage is not in a membrane and there is only one aqueous phase and one organic phase, the box. It is the separation in space of the catalysts which decides the diffusion control [3].

The figure shows the reactions on opposite sides

of the box but this is not essential. They can be placed on adjacent sides for example. The catalysts can even be placed side by side when H_2, O_2 and ADP + P_i all react on one surface of the box. If the box is now placed at the interface of two bulk phases, inorganic and organic then the interface could be as effective as any particle in ATP synthesis. Many biological reactions go at interfaces. This makes the point that biological systems could make ATP on the same side of a membrane (organic phase) as the one at which they pick up O_2 and H_2. The constraint remains that the energised ions, H^+ and OH^- from O_2 and H_2 (i.e., O^{2-} and $2H^+$) must not diffuse together faster through the aqueous phase than through the coupling device. There is a model device of this kind which makes ATP [7]. This device can be effective in vectorial transport [8].

Before generalising the device further other examples are worth noting. Nitrogenase is a particulate system of enzyme which uses the energy of hydrolysis of ATP. *P*-450 cytochrome systems work both in membranes and in particles. The energetics of these reactions are not clear but the diffusion paths of the electrons and protons (for nitrogenase) are well controlled. We do not yet know much about diffusion paths of protons since they do not travel through simply observable chromophores but it would be very rash to presume that biology had not learnt to control proton diffusion. The F_0 part of ATPase seems to be such a control, not just a channel.

It may be useful to elaborate the parallels a little further for they show how diffusion of the electron particularly is controlled. In the *P*-450 cytochromes the control of diffusion is governed by a sequence of reactions:

(i) Substrate uptake, diffusion of electrons does not occur without substrate;

(ii) Reduction (electron diffusion to the site), Fe(III) goes to Fe(II);

(iii) O_2-uptake which does not occur without reduction;

(iv) Proton reaction to give MO + H_2O (proton diffusion to the site but no further electron diffusion or proton diffusion is allowed;

(v) Oxidation of substrate;

(vi) Diffusion of product from the site.

In nitrogen fixation the reaction sequence is not yet known in equal details but again electrons enter first to react with N_2 and the diffusion of protons follows to give NH_3. The diffusion is controlled by the required binding and hydrolysis of ATP. In the reverse reaction of mitochondria in which succinate drives the reduction of NAD to NADH the hydrolysis of ATP is required in an analogous manner. Whether it is possible for biological systems to make ATP by reversing the nitrogenase reaction is not known and whether this method is how ATP is made in any biological system is for experimental decision. Theories describe the possible. Experimentalists find it very difficult to make lipid/water phase systems of the above kind but of course the same diffusion paths can be present in three phase systems too, i.e., in membrane-containing systems.

One step (there is a second) that is needed to go to a chemiosmosis model from fig.1, is to put the exact box of fig.1 into a membrane, see dashed lines of fig.1. This new model could be what Mitchell calls micro-chemiosmosis (see later) but for me it is a dislocated reaction system of [2]. It is clear that this is still a local proton model and that its description has no osmotic terms. All charges remain on localised paths in the membrane box.

The model still has the features that it can be coupled to transmembrane gradients of other chemicals but it does not have trans-membrane osmotic protons [8]. There could be local controls in the membrane so that energy is not indiscriminately spent on all carrier systems. The system works with a very small capacity. It is a very effective system and it is not easily distinguished from chemi-osmosis, see table 1.

Here I make an aside to a criticism put by Mitchell [1] who remarks that the proton energy in these models is 100 kcal. (This I take to be the ionisation energy of a hydrogen atom in a phase of dielectric = 3, a step which has no connection with any model previously described.) The actual energy required cannot exceed the redox potential difference between the couples which generate the proton, i.e., 10–20 kcal, and of course the proton is stabilised by inter-

action with charge centres of opposite charge within the membrane phase [4]. As as aside the production of O^{2-} in Mitchell's own reaction schemes [1] is not of lower energy cost than 100 kcal in a dielectric = 3.

A fundamental misconception in [1] is then that a proton in a membrane phase is a dehydrated proton. This is quite contrary to experimental studies of protons in organic solvents where any water is present and it has no connection with the local proton hypothesis. Nowhere in [2–4] is such a proton described. It is explicity said 'We would suggest that using oxidative phosphorylation pH equilibrium can not be established between these phases (lipid membrane and aqueous) and that hydrogen ions generated at a high equivalent aqueous activity inside the membrane phase diffuse slowly through the membrane phase to the outside' [4]. The next lines make it absolutely clear that these protons must pass through a series of hydrogen bonds, e.g., with oxygen, to reach the ATP-ase, giving energy captured as ATP. (In my own defence I add that paper [4] was submitted before any paper on chemiosmosis had been published. In [4] I thank Mitchell and state clearly that I had had an exchange of letters with him about proton-driven ATP-formation.) I trust that it is clear that the difference between the two models is not based on any of the distinctions, reversibility, vectors or hydrophobicity raised in [1] but solely on the restriction to the diffusion path of the protons.

It is convenient now to define the membrane phase rather than to draw it as a box in a membrane. The membrane phase is that volume associated with the membrane which is kinetically distinguishable by measurement of proton movement. The definition deliberately avoids the impossible task of defining a membrane by a line structure and leaves a clear cut experimental distinction between membrane and aqueous phases.

A wide variety of energy transducing devices based on diffusion control have now been elaborated and I can turn to a final one — chemiosmosis — which is only a special extension of diffusion control.

The second step which generates chemiosmosis from the above is the complete liberation of the protons and hydroxide ions from the box in the membranes to two different aqueous phases. If the ions are so liberated then these ions give osmotic terms. In the bound form they did not. Diffusion control is now due to the membrane, not the box. If the bound and unbound ions (protons) equilibrate then the equations of chemiosmosis will give a correct numerical value for the vector energy both trans-membrane (aqueous phase to aqueous phase) and in the membrane (across the box) since the two are in equilibrium. Thermodynamic analysis of this kind does not lead to a mechanism and cannot distinguish these cases. However only if the ions are totally unbound will chemiosmosis give the correct charging capacity. Again if there is no equilibrium between bound and unbound ions then the experimental terms ΔpH and ψ (chemiosmosis) will be incorrect and will not be adequate for ATP formation.

There are different degrees of localisation of proton gradients between the extremes of a strictly bound proton model and a strictly unbound proton model (chemiosmosis) but only in the cases of an equilibrium within each of the aqueous phases is an osmotic term meaningfully related to true measurement of ΔpH and 'osmotic' potentials. Once part of the system is not at equilibrium due to local (micro) events the word osmotic loses meaning. Thus micro-chemiosmosis is not an expression which has meaning different from a localised proton model and neither are related to osmotic terms as chemiosmosis is. This means that only chemiosmosis can be understood from simple ΔpH and trans-membrane potentials, ψ. This is one experimental point which decides if chemiosmosis is correct or if a localised model is correct.

It has always been clear therefore that chemiosmosis is more open to numerical analysis [1] but it has also been over-elaborated. Thus in any of the above systems which converts chemical energy to one form of electrical energy and then back to chemical energy in another form, there is no requirement for stoicheiometry. The chemiosmotic stoicheiometries proposed by Mitchell are interesting but quite unnecessary (and apparently incorrect). I have deliberately avoided the writing of such schemes. My work is less explicit [1], simply because there is no logical thinking that has lead me nor one that could have lead Mitchell to a defined stoicheiometry. We shall have to be guided by experiments as far as stoicheiometry is concerned. This is of the very essence of our disavowal of chemical coupling. All reference to stoicheiometry is from extra postulates, table 1.

While trying to incorporate the experiments of

Junge and Ausländer [10] and Ort, Dilley and Good [11] within the framework of chemiosmosis Mitchell [1] breaks the rules of chemiosmosis. It is not permissible within chemiosmosis to short circuit the bulk aqueous phase by proton paths closer to the membrane than the bulk phases. By so doing there is no equilibrium with bulk phases and the equation protomotive force = ψ + ΔpH is then incorrect. There is no longer an osmotic term at equilibrium. It is also quite untrue to say that the solvent activity near the membrane is not affected by these local charges [1]. Finally if the local circuit is in the kinetic confine of the membrane then this is part and parcel of a localised theory.

In what follows I direct attention to the differences between the extreme forms of the two hypotheses, table 1, and I turn to points which are not just related to the pathway of protons. Some points will be common ground within the framework of Mitchell/ Williams schemes.

3. Energetics

It will now be recognised that the two extreme forms of the ATP-synthetase systems have different energy states. In chemiosmosis the general energy across the whole membrane is given by

$$\Delta G = \psi + \Delta pH$$

(in appropriate units). ψ and ΔpH are measurable using electrodes in the two aqueous compartments. All chemical methods of making such measurements must be suspect as they can cause an equilibrium between any membrane bound charges and aqueous solutions which would be absent but for the presence of the added chemicals.

In the extreme local models the local energy across the ATP-forming site is

$$\Delta G = \Delta(\Delta G^\circ) + \psi'$$

where I have separated bound charges effects (ψ') i.e., electric potential from chemical potential $\Delta(\Delta G^\circ)$ and I have omitted concentration dependence within the particles. This model has no aqueous phase protons. Thus it should be observed that although the system occurs in a membrane no ΔpH is required to form ATP and that any potential will be thought to be too small when measured with electrodes. The examination of the biological systems with pH and potential determining electrodes leads me to suppose that this is so and that chemiosmosis is not operative [15]. (This does not mean that it is an invalid hypothesis.) Note that when the model box of fig.1, local system, is put in a membrane it will tend to leak toward chemiosmosis and it will discharge under the influence of chemicals towards the chemiosmotic energy. If excess energy is applied before ATP is allowed to be made it could yield osmotic gradients.

The capacity per ATPase site before ATP can be made is very small in the local model depending upon the sites which can absorb protons. As Mitchell [1] points out the capacity of the chemiosmosis system is relatively large. Its minimum capacity (no bound charges), if ΔpH alone is operative in making ATP, is given by buffering equations. Biological systems are well buffered and can absorb or release CO_2 on pH change. The formula given in [1] shows as has been stated earlier that the effect of the large external buffer volume of say a bacterial cell (the Pacific Ocean effect) is to impose a rigid external pH which means that half the proton/hydroxide energy gradient is lost since one side is an infinite buffer. The inside is also a good buffer and it now must go to pH $\gg$ 10 or pH $\ll$ 4 to give an adequate ΔpH ATP formation. The capacity of the inside of mitochondria or thylakoids for H^+ ions on going from pH 7–10 can be determined and is undoubtedly large. Again, is it really necessary to saturate the capacity of in vivo bacterial, mitochondrial, and thylakoid membranes which are very extended in a vast reticulum before any ATP is made?

The capacity for ATP synthesis dependent on ψ alone (chemiosmosis) is not small if the internal volume is large. There are large mitochondria. However it is experimentally observed that the protons are bound by the membrane of thylakoids and mitochondria (see more localised models) which makes the calculation of ψ just from known internal volumes of doubtful value in these systems. Do the surfaces of mitochondria need to be saturated before ATP is formed? The same question aaplies to bacteria. Some bacteria make ATP in a medium of pH > 10. Others do not make ATP in the absence of oxygen at pH 1.0. How?

The measured total capacity of the thylakoid membrane is equivalent to 0.5 H^+/chlorophyll molecule which would give a pH drop to about 1.0 from 7.0 [12]. Even allowing that dye measurements do not represent dye binding, the lowest pH found is around 3–4. Thus there is a massive proton binding on the thylakoid proteins. A local model is then extremely probably. Chemiosmosis would mean that ATP was not produced in any quantity until the membrane was saturated. This seems to be contrary to flash experiments [13], which generate ATP.

There is another objection to chemiosmosis in vivo as applied to the chloroplasts. The thickness of the inner thylakoid phase leads me to doubt whether there is an aqueous phase present at all. Certainly it is laced with proteins and possible these hold the inner surfaces together. The capacity of the inner surface of the thylakoid for protons greatly exceeds the predictions of chemiosmosis due to the surface binding of these charges. My overall view of this structure is that the inner aqueous phase is not required at all and that a completely functional system could be obtained without any osmotic protons in chloroplasts using the diffusion of charge as in fig.1. The negative charges in the membrane would retain the positive charges in their own vicinity making a system which is effectively a local particle. Note should be taken too of the high density of ATPases – roughly one per active site in both chloroplasts and mitochondria. While this is no proof of a localised model it makes a generalised osmotic system improbable.

4. The movement of the proton in the membrane

The rate of proton transfer along a net of hydrogen bonds which contain acid/base centres can be much faster than the rate of proton transfer in water at pH 7. This is well illustrated by the proton transfer on the surface of carbonic anhydrase [14]. Witt [15] is in error when he states that proton transfer in a membrane, which here is largely composed of proteins and some water, must be slower than through the bulk phase. It is likely that it is considerably fater. This leads me to suppose that Witt's experiments, which show there is no requirement for ΔpH in ATP formation, support the general notion that ATP is formed by protons which do not leave the membrane phase [15]. The potential Witt discusses is then trans-ATPase and not transmembrane in Mitchell's sense. Earlier indications of a kinetic barrier between the membrane and the bulk phase were given by Junge and Ausländer [10]. Further support comes from Ort, Dilley and Good [11] and from Van Dam [16]. As a large number of experiments show that ΔpH is not a requirement it is the nature of ψ which must be discovered.

If movement of a proton occurs in the membrane then a proton channel is required which cannot be just an alamethicin-type channel formed by n ($n \simeq 6$) helical linear polypeptides [17]. More likely it is a channel of fixed water molecules as shown in fig.2 and

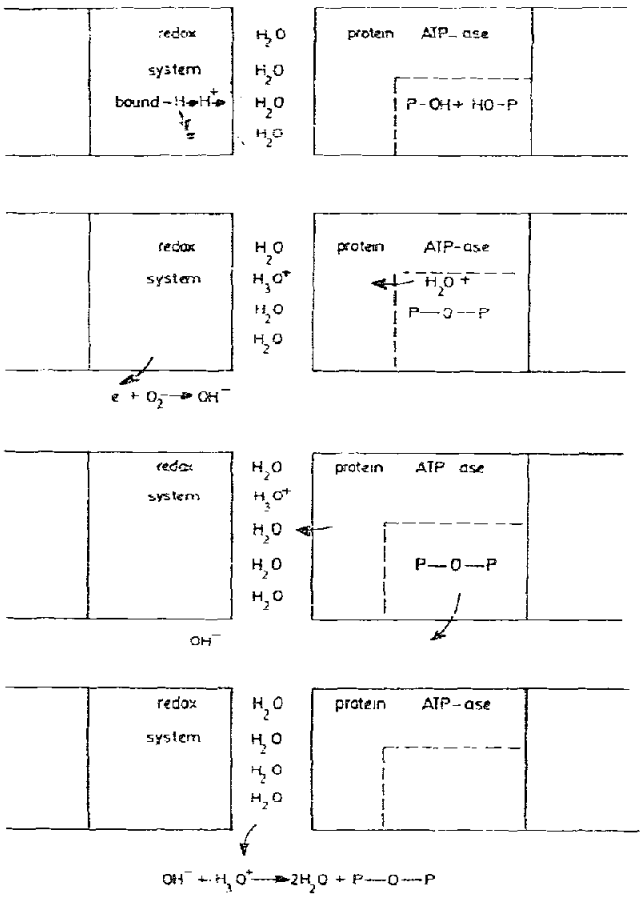

Fig.2. A coupling device [4]. This device can be related to any of the coupling models derived from fig.1. It is as suitable for chemiosmosis (with minor modification) as for a local model. It deliberately stresses the role of water in the reactions.

it is this channel of water in the membrane which must be connected to the ATPase. Thus the proton would have to be held in the channel and since there is the requirement for n protons not one, n protons must be accumulated before the protons drive the reaction $ADP + P_i \rightarrow ATP + H_2O$. The local proton hypothesis for coupling is that the protons are held in the membrane altering its energy and adjusting the activity of water in the ATPase region. The protons then move so as to drive the reaction forward by pulling additional water from the polyphosphate condensation site into this diffusion channel. The studies of Kagawa [18] are fully consistent with such a model for protons are bound at a site in a specific channel leading to the ATPase though as yet we do not know if there is a channel of fixed water molecules but its advantage is that it is specific for proton movement in and through a membrane and it is fast. Moreover it can be regulated by conformational changes in the proteins forming the walls of the channel. Note that the chemiosmotic model does not have these features but if the aqueous phases are treated as just a store of excess energy then it is possible to connect this local model for proton movement with the stores. As I explained in the article proposing the water channel, if it is open at both ends, as shown deliberately in fig.2 (see also fig.1 in [1]), then the connection to chemiosmotic store is readily made. The distinction between the models is one of diffusion barriers, gates.

In halobacteria the protons released upon illumination of the pigment are certainly retained by the membrane initially and I consider that they travel within the membrane phase to the ATPase. They would be retained in this phase by rapid diffusion and charge/charge interaction. The ability of halobacteria to make ATP with no or an inverted ΔpH is then explicable [19].

5. Control

One of the considerable advantages of the binding of protons to the membrane is control over the properties of the membrane by the state of energisation. The following possibilities exist:

(a) Allosteric control of the components of the energy capture devices;

(b) Release of bound metal ions, e.g., Mg^{2+} or other positive charges from the membrane and the subsequent use in the activation of metabolism;

(c) Release of bound proteins from the membrane into the medium so that new catalysts are generated.

No such controls exist in chemiosmosis for there are no bound protons. The control could be exercised locally or generally over the whole of the membrane. Let us suppose that the chemiosmotic store is put in equilibrium with the bound protons (which does not mean that the chemiosmosis pathway is correct but it is indistinguishable from such a situation). This implies that all parts of the membrane are activated and there is no local control, and (a), (b) and (c) above would all respond equally and simultaneously with the generation of ATP since we need an all-embracing steady state. Is this the case?

Alternatively under local diffusion control there are regions of the membrane which are activated and (a), (b) and (c) can be adjusted by their localisation and adjusted in degree so that as the level of ATP production rises so the membrane surface is gradually changed. Thus the switch-on of external metabolism is linked gradually to ATP levels and is not on a sudden-rise basis. It would appear that local control through proton binding has been observed at the level of the cytochrome oxidase by Wikstrom et al. [20]. It remains a moot point whether uncouplers act by generally adjusting the flow of protons across a membrane or if by local binding they affect the local proton states.

6. Electron transfer

The weight of concern with proton movement in some discussions of ATP formation [1] seems to me to be disproportionate. Of equal concern is the movement of electrons, fig.1. Here we know that the membrane contains a large number of metal binding centres and quinones which retain charge. (This is proven by many spectroscopic methods. The paths of transfer of electrons are much better known than those of protons since they can be followed so easily.) The electron cannot enter the aqueous phase. It is my contention that the charging of the membrane by electron flow is an essential feature of the ATP-

Table 2
Control features of electron transfer

Protein	Features allowing control	Ref.
Cytochrome *c*	Fe–methionine bond length changes adjust redox potentials	[23]
Cytochrome oxidase	Spin-state switches adjust redox potentials and rates of reaction	[20,22]
Fe/S proteins	Charge effects adjust redox potentials	[23]
Copper centres	Geometric switches allow coupling to external events	[22]
Quinones	Redox reactions have compulsory coupling with proton transfer	[4]

forming reactions and of their control. The electron and proton transfers are coupled for certain in hydroquinones, table 2. I do not want to review this topic here for it involves a major part of my research effort and is reviewed elsewhere in extenso [21,22], but there is a danger in chemiosmosis that the stress on the energised states of aqueous ions hides the joint role of protons and electrons and the membrane. Today some experimentalists do not even examine the membrane, treating it as an inert barrier. We know that the membrane is energised by both a change of electron charge distribution and a change in the number and distribution of bound protons.

Electron transfer in the membrane is localised and has some very special features of interest [21]. Table 2 gives some examples of the control of electron transfer which are to be seen in the mitochondrial electron-transfer chain. They are usually linked to conformation changes. Repeatedly we and others have stressed the in-membrane events, due to electron passage, which can be coupled to in-membrane electron proton, or ATP reactions but not to chemiosmosis, see [21–23].

7. Coupling models

Another area in which experiments do not lead to direct test of the different hypotheses is in the nature of coupling. The Mitchell/Williams models both have the pathway characteristic

Redox energy(light) → charge separation(protons) → ATP

Both models do not use chemical intermediates as written but neither excludes them of necessity. The point of departure between the old chemical theories and the newer views is the role of charge separation. Their difficulty is that it is harder to write a coupling between charge separation and ATP than between a chemical intermediate and ATP. There has been a proliferation of possible schemes all of which are now being tested. Any one of these models can be attached to the high energy proton and their validity does not affect the general truth of this energised proton approach. Some required features of this hypothesis which need stressing are:

1. The coupling has no compulsory stoicheiometry.

2. The coupling will require the binding of protons.

3. Chemical intermediates in the ATPase are not excluded.

4. Conformational changes must occur during the coupling.

In my view any attempt under the heading of chemiosmosis to ignore 1.–4. is unnecessary and confusing. Again the further points that:

5. The coupling must be vectorial and

6. The coupling must be reversible

go without saying (see above). In [1] Mitchell states that my coupling scheme fig.2, is irreversible. (Let me stress that even if this were so it does not affect the local proton versus chemiosmotic proton discussion.) It is clear however that the scheme is reversible for it has an obligatory return of H_3O^+ to the channel when ATP is bound for only ATP can remove the water from the interior of the channel. At the same time this removal of water yields a proton in the membrane which will drive the reversible reaction

$$H^+ + e \rightleftarrows H$$

toward reduction. All proposed coupling schemes are chemically reversible and vectorial, through the ATP-ase, but we do not know how the ATP synthetase works. This is to be decided by experiment but the appropriateness of the experiment as a test of the ideas can only be seen by examining all the ideas. For example, I think Mitchell's scheme for ATP formation is highly improbable. (This statement is consistent with the statement that chemiosmosis in totality is one possible valid hypothesis.)

8. Conclusion

It would appear to me that a range of experiments which have been partially explained by chemiosmosis are more convincingly covered by a more localised proton model. However, as both ideas belong in a general spectrum of possible models, a clear-cut decision between them is difficult.

It is worth stressing that the general ideas developed here do not just apply to proton and electron diffusion but can be generalised to any coupled energy-driven process. The sufficient and necessary condition are diffusion control. For groups other than H^+ or e special channels must be devised which can be likened to specific ring chelates for diffusion of ions or molecules or can be made of series of selected chemical bonds such as thiols for handling acetyl groups. In every case the generation of an energised agent at a point in space by a catalysed reaction plus a restriction on diffusion, of any of the above kinds, can be the basis of an energy capture device providing that the diffusion path leads the energised group to a (catalysed) coupling centre. Apart from ATP formation, and transport we should examine mechanical devices and the synthesis of polymeric materials in biology. The fundamental feature is not a membrane and there is no requirement for osmotic gradients but a single boundary akin to a phase boundary in a macroscopic system is a minimum requirement.

References

[1] Mitchell, P. (1977) FEBS Lett. 78, 1–20.
[2] Williams, R. J. P. (1959) in: The Enzymes, 2nd edn (Myrback, Boyer and Lardy eds) Academic Press, Vol. I, New York, p. 391.
[3] Williams, R. J P. (1961) J. Theoret. Biol. 1, 1–13.
[4] Williams, R. J. P. (1962) J. Theoret. Biol. 3, 209–229.
[5] Mitchell, P. (1961) Nature 191, 144–148.
[6] Mitchell, P. (1961) in: Biological Structure and Function (Goodwin, T. W. ed) Academic Press, New York.
[7] Yaguzhinsky, L. S., Boguskarvsky, L. I., Volker, A. G. and Rakhmaninova, A. B. (1976) Nature 259, 494–495.
[8] Williams, R. J. P. (1966) in: Protides of the Biological Fluids 14, 25–32.
[9] Williams, R. J. P. (1977) Trends Biochem. Sci. 1, N222–N224.
[10] Junge, W. and Ausländer, W. (1975) in: Electron Transfer Chains and Oxidative Phosphorylation (Quagliariello, E. et al. eds) pp. 265–270, North-Holland/American Elsevier, Amsterdam, New York.
[11] Ort, D. R. and Dilley, R. A. (1976) Biochim. Biophys. Acta 449, 95–107; Ort, D. R., Dilley, R. A. and Good, D. E. (1976) Biochim. Biophys. Acta 449, 108–124.
[12] Hind, G., Nakatani, H. Y. and Izawa, S. (1974) Proc. Natl. Acad. Sci. USA 71, 1484–1488.
[13] Witt, H. T. (1975) in: Bioenergetics of Photosynthesis (Govindjee ed) pp. 493–554, Academic Press, New York.
[14] Campbell, I. D., Lindskog, S. and White, A. I. (1975) J. Mol. Biol. 98, 597–614.
[15] Witt, H. T. (1977) in: Living Systems as Energy Converters (Buvet, R. et al. eds) Elsevier/North-Holland Biomedical Press, Amsterdam.
[16] Van Dam, K., Wiechmann, A. H. C. A., Hellingswerf, K. J., Avents, J. C. and Westerhoff, H. V. (1977) Abstracts 11th FEBS Mett., Copenhagen, Abstract No. A.4-13, L2, 9.
[17] Gordon, L. G. M. and Haydon, D. A. (1972) Biochim. Biophys. Acta 255, 1014–1018.
[18] Kagawa, Y., Wakabayashi, T., Yoshida, M., Hirata, H. and Sone, N. (1977) Abstracts 11th FEBS Meet., Copenhagen, Abstract No. A.4-13, 709.

[19] Bogomolni, R. A., Baker, R. A., Lozier, R. H. and Stoeckenius, W. (1976) Biochim. Biophys. Acta 440, 68–88 (see fig.12).
[20] Wikstrom, M. K. F. (1977) Abstracts 11th FEBS Meet., Copenhagen, Abstract No. A.4-12, L2, 5.
[21] Chance, B. (1977) Trends Biochem. Sci. 2, N112–N114.
[22] Williams, R. J. P. (1975) Trans. Biochem. Soc. 1, 1–29.
[23] Moore, G. R. and Williams, R. J. P. (1977) FEBS Lett. 79, 229–232.