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ON THE FUNCTIONAL PROTON CURRENT PATHWAY OF ELECTRON TRANSPORT PHOSPHORYLATION

AN ELECTRODIC VIEW

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Abbreviations: ANS, 1-anilinonaphthalene-8-sulphonate; FCCP, carbonyl cyanide-*p*-trifluoromethoxy phenylhydrazone; PCB^-, phenyldicarbaundecaborane ion; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylene diamine.

"In many respects, then, the study of ion transport through membranes runs parallel to that of electrode kinetics, which is facing (and solving) strikingly similar problems."

De Levie [1]

"Electrodics is the study of processes which occur at the surface of an electronic conductor in contact with a liquid phase."

Bockris and Reddy [2]

I. Introduction

The thermodynamically most significant fact about living systems is that they are not at equilibrium with their surroundings. Realisation of this fact, and its apparent contradiction of the Second Law of classical thermodynamics [3], has led to the use of non-equilibrium or 'irreversible' thermodynamics (see, for example, Refs. 4–9) to describe the manner in which biological 'dissipative structures' can maintain a high degree of organisation at the expense of energy extracted from their environments. Now the phenomenological equations which describe the rate of extraction of energy from the environment required to maintain a system at a given degree of organisation (low negative Gibbs free energy) differ from those of classical equilibrium thermodynamics in the particularly important aspect that they contain kinetic, i.e. time-dependent, terms.

Although non-equilibrium thermodynamics has been applied by several authors to the process of electron transport phosphorylation (see for example, Refs. 10–14), a purely kinetic approach has not so far been presented to describe even semi-quantitatively the protonic coupling between electron transport and the phosphorylation of ADP catalysed by biological membranes. The prime purpose of this review, therefore, is to offer a preliminary analysis of certain thermodynamic and kinetic controls defining a possible path-

way for protons during electron transport phosphorylation. Related topics, such as proton-coupled solute porter systems (e.g. Ref. 15) will not be discussed, although similar principles may apply.

II. Protonic coupling in electron transport phosphorylation

IIA. Background: the chemiosmotic and local proton hypotheses

The evolution of ideas concerning the nature and pathways of protons as a coupling intermediate in electron transport phosphorylation has recently been reviewed by their chief exponents (Refs. 16–18 and references therein). Thus whilst Williams's views [17, 18] of a rather localised protonic coupling between electron transport and phosphorylation, subject to a kinetic or diffusion control, are of a more general nature, it is commonly believed (e.g. Ref. 19) that the chemiosmotic hypothesis of biological energy transduction (reviews: Refs. 16, 20, 21, and references therein), by its nature of being experimentally falsifiable (cf. Ref. 22), represents the more useful working hypothesis for experiments designed to gain an insight into the molecular mechanisms underlying the protonic coupling between electron transport and the phosphorylation of ADP catalysed by biological membranes.

It is therefore appropriate to begin with a brief review of some of the premises upon which the chemiosmotic hypothesis is based (cf. Refs. 23–26).

(a) Electron transport or ATP hydrolysis in an energy-transducing membrane results in the transmembrane electrogenic translocation of protons from one bulk aqueous phase to another, such that in the steady state an electrochemical potential difference of protons is set up across the proton-impermeable membrane phase, whilst within each aqueous phase there is no protonic potential gradient.

(b) The magnitude of the transmembrane electrochemical proton gradient ($\Delta\tilde{\mu}_{H^+}$) or protonmotive force (Δp) is given by the sum of an electrical ($\Delta\psi$) and a concentration (ΔpH) component, according to the equation

$$\Delta p = \Delta\tilde{\mu}_{H^+} = \Delta\psi - 2.303\frac{RT}{F}\Delta\text{pH}$$

where

Δp, $\Delta\tilde{\mu}_{H^+}$ and $\Delta\psi$

are in mV, ΔpH is in pH units and the symbol Δ signifies a difference between the two transmembrane bulk aqueous phases. R, T and F have their usual thermodynamic meaning, and the factor $2.303\,RT/F$ is approximately equal to 60 mV at 30°C.

(c) Since proton movements are primary, the electrical and concentration terms of the equation

$$\Delta p = \Delta\psi - 60\Delta\text{pH}$$

are energetically interchangeable under appropriate conditions of charge- or pH-neutralisation.

(d) The proton gradient generated by electron transport may be utilised to drive the endergonic synthesis of ATP from ADP and P_i such that, under 'static head' conditions (in which the rate of entropy production of the system is at a minimum), the free energy stored in ATP is poised at equilibrium against the protonmotive force according to the relationship

$$\Delta G_p = -zF\Delta p$$

where the phosphorylation potential

$$\Delta G_p = \Delta G^{\circ\prime} + RT \ln \frac{[ATP]}{[ADP][P_i]} ,$$

and z = the number of protons translocated across the membrane for each ATP molecule synthesised, the $\rightarrow H^+/ATP$ ratio. Thus ΔG_p should bear a constant relationship to Δp.

Additionally, z, the $\rightarrow H^+/ATP$ ratio is also related, under fully coupled conditions, to the stoichiometry of proton- and ATP-generation, according to the relationship

$$z = \rightarrow H^+/ATP = (\rightarrow H^+/e^-)/(ATP/e^-) .$$

where $\rightarrow H^+/e^-$ is the number of protons translocated across the membrane per electron transported down the electron transport chain and ATP/e^- is the number of ATP molecules synthesised per electron transferred. Although the actual stoichiometries themselves are not an integral part of the chemiosmotic hypothesis, the number obtained by different experimental methods should be constant under given reaction conditions.

(e) The membrane itself acts purely as a diffusion barrier between osmotic compartments, and is not itself the site of energy storage.

That the chemiosmotic hypothesis has currently gained such wide (if slow) acceptance as a heuristic tool in studies of membrane bioenergetics may be ascribed perhaps to four factors:

(1) Vectorial, transmembrane movements of protons and other ions do accompany both electron transport, active transport and ATP hydrolysis (e.g. Refs. 25–31);

(2) Electrochemical gradients of protons are formed across energy-transducing membranes (e.g. Ref. 32);

(3) The action of some uncouplers is indicated by their ability to dissipate transmembrane proton gradients, since almost all are lipophilic weak acids (e.g. Refs. 33–36, but see Refs. 37, 38);

(4) An artificially generated electrochemical proton gradient is kinetically competent in driving ATP synthesis [39,40].

Such considerations have led Slater [41] to the view that, "I hope most workers will now use this [chemiosmotic] framework as the starting point for the presentation of their work."

Clearly, one of the most appropriate methods for assessing the chemiosmotic hypothesis is to measure the magnitude and properties of the protonmotive force, and a wide variety of methods have been utilised in attempts to measure this parameter [32]. However, excluding microelectrode measurements, which are not applicable to membrane vesicle systems, no reliable absolute calibration is available for the majority of these methods. Only in the case of ion-distribution methods using hydrophilic ions which do not bind to biological membranes to any significant degree is it credible that the Nernst potential and bulk phase transmembrane pH gradients are being measured. In terms of a chemiosmotic hypothesis it is of course these bulk phase parameters which constitute the protonmotive force. I shall review later some of the data which have been obtained using other probes of the protonmotive force, but since the experimental observations which have led me to formulate the electrodic analogy which I shall be presenting in the following sections were concerned with measurements on the bulk phase protonmotive force using a hydrophilic ion-distribution technique, I do not think it inappropriate to review these studies here. Additionally, it seems likely that the experimental methods used are of general applicability.

IIB. Some recent experimental work concerned with the measurement of Δp

In recent experimental work we have used a flow dialysis assay of the distribution of hydrophilic solutes (SCN^- and methylamine) to determine the magnitude of the components of the protonmotive force generated by three different 'inverted' preparations of biological membrane vesicles under different conditions, and compared this with the free energy stored in ATP under 'static head' conditions ('d' above). An extensive validation of the methods used, and of the homogeneity of energy-coupling properties of the vesicle preparations, has been given elsewhere [42–46], and in the following discussion it will be taken that the parameters measured by the flow dialysis method are indeed the observable components of the bulk aqueous phases, corresponding to the transmembrane protonmotive force as defined by Mitchell (Refs. 23, 24, and see 32).

IIB-1. Results from chromatophores: chemiosmotic interpretation. In experiments with *Rhodospirillum rubrum* chromatophores [42] it was found that light-driven electron transport caused the formation of a transmembrane electrical potential of magnitude 100 mV in a P_i-Tris/magnesium acetate medium. Increasing concentrations of thiocyanate caused a conversion of the membrane potential component of the protonmotive force $\Delta\psi$ into a transmembrane pH gradient, ΔpH, such that their sum (in mV) remained essentially constant. At low concentrations of thiocyanate the pH gradient was energetically insignificant. Comparison of this value (100 mV) of the protonmotive force with the free energy stored in ATP under static head conditions (approximately 14 kcal $\cdot$ mol^{-1}) indicated that five or six protons must be translocated across the membrane for each molecule of ATP synthesised. Arguments were presented in an attempt to rule out the possibility of systematic error in the measurements. The only other quantitative measurement of the $\rightarrow H^+$/ATP ratio of chromatophores (of *Rhodopseudomonas sphaeroides,* [47]) for which no technical criticism could be offered gave a value of 2. Although an octamolecular reaction for the *R. rubrum* ATPase (6 H^+, 1 ADP, 1 P_i) seems very unlikely indeed mechanistically, no explanation, based on reasoning within the chemiosmotic idiom, could be offered for the discrepancy between the kinetic work of Jackson et al. [47] and the above (thermodynamic) measurements.

IIB-2. Results from submitochondrial particles: chemiosmotic interpretation. Experiments similar to those described for chromatophores were carried out using bovine heart submitochondrial particles (Ref. 45, cf. Ref. 48). Under a variety of conditions it was established that the bulk phase protonmotive force could serve as a thermodynamically competent intermediate between electron transport and ATP synthesis provided that an $\rightarrow H^+$/ATP ratio of 3 was accepted. The electrical and chemical (concentration) components of the protonmotive force were essentially interchangeable when increasing concentrations of the permeant nitrate ion were added to the usual reaction mixture. It was concluded, despite some controversy in the literature concerning this point, that the results were broadly consistent with a chemiosmotic interpretation in which the $\rightarrow H^+$/ATP ratio was equal to 3. However, two additional points of significance emerged from the results of these experiments [45]. First, the value of Δp generated by a given substrate varied markedly depending on the incubation medium used, without a corresponding variation in ΔG_p. This result is not expected if a bulk phase electrochemical proton gradient constitutes the sole and obligatory link between electron transport and ATP synthesis. Secondly, an independence between the measured bulk phase protonmotive force and the ΔG_p generated by submitochondrial particles was observed when the substrate was varied, in particular when ascorbate/*N,N,N',N'* tetramethyl-*p*-phenylene diamine (TMPD) was the substrate. Further, there did not seem to be any relationship between the value of the

steady-state protonmotive force and the rate of ATP synthesis [45,46].

Whereas the chemiosmotic hypothesis requires a constant ratio between Δp and ΔG_p, at least under conditions of constant pH, it was found that the value of the $\rightarrow H^+/ATP$ ratio calculated from a comparison of Δp with ΔG_p in fact varied significantly. Additionally, it was found that concentrations of the permeant nitrate ion sufficient to raise ΔpH at the expense of $\Delta\psi$ when NADH was the substrate were unable to convert the $\Delta\psi$ generated by TMPD-mediated ascorbate oxidation into a ΔpH. Again this behaviour would not be expected if a fully delocalised bulk phase transmembrane protonmotive force were the sole and obligatory functional intermediate between electron transport and phosphorylation.

IIIB-3. Results from Paracoccus denitrificans vesicles: chemiosmotic interpretation. In the case of phosphorylating *P. denitrificans* membrane vesicles, measurement of the protonmotive force generated by NADH oxidation in the usual P_i-Tris/magnesium acetate reaction medium suggested that the protonmotive force consisted of a transmembrane electrical potential of 145 mV, with no contribution from a transmembrane pH gradient [43]. Comparison of this value with the phosphorylation potential attained was consistent with the idea that at least four protons must be translocated across the membrane per ATP synthesised if a chemiosmotic type of mechanism is operative. However, the addition of 10 mM nitrate to this system caused a decrease in $\Delta\psi$ to an undetectable level with no increase in the ΔpH component of the protonmotive force. Under these latter conditions there was no decrease in the ΔG_p generated by the particles, and it was concluded that under such conditions a bulk phase transmembrane electrochemical proton gradient did not constitute a thermodynamically competent intermediate between electron transport and ATP synthesis.

In summary, therefore, it would seem from our own work that although a number of the experimental observations described above may be accommodated within the framework of the chemiosmotic hypothesis of biological energy transduction, there are a number of observations which cannot be so accommodated. Particularly in view of the large magnitude and the variability of the $\rightarrow H^+/ATP$ ratio calculated from these measurements, and from a large amount of data in the literature reviewed later, it seemed appropriate to seek an interpretation of these observations that is alternative to the equilibrium thermodynamic chemiosmotic viewpoint [49].

III. An electrodic model for electron transport phosphorylation

IIIA. Introduction

As noted above, biological, electrical and other processes in general are subject to a variety of kinetic or diffusion regulations. Examples include metabolic transformations [50], the transmission of nerve impulses [51,52], fluid movements [53–55] and heat flow [56]. Since Mitchell himself suggested a fuel cell analogy for protonmotive systems [57] it seemed to the present author that a closer examination of this analogy might allow a harmonisation of chemiosmotic principles, and their advantages of falsifiability, with the more general but difficult-to-falsify concepts of a kinetic or diffusion control of proton current flow championed by Williams [17,18]. Thus the fuel cell requires electrodes to perform chemical work on its (aqueous) surroundings, and the wide study of inorganic electrodes has led to a detailed understanding of molecular events at the solution/electrode interface. It is now known by electrochemists (e.g. Refs. 2 and 58–62) that working electrodes (like phosphorylating mitochondria) are not at true electrochemical

equilibrium with their surroundings, but in a steady state, and for this reason the description of electrode processes leans heavily on the realisation that significant kinetic (and thermodynamic) barriers to the field-induced flow of current, especially in the electrode/solution interphase, are operative. The major purpose of the following, then, is to ask whether the results of electrode kinetic studies can offer useful modifications to chemiosmotic theory, which I believe are required to explain a growing body of unexplained experimental data in the literature, so as to bring together the more widely used chemiosmotic principles and those based on a (more localised) proton diffusion control.

Accepting the widely held view that transmembrane phase proton gradient are intimately involved as an intermediate in electron transport phosphorylation, therefore, I present next a preliminary and qualitative statement of five postulates of a hypothesis of proton current flow during electron transport phosphorylation that I believe does incorporate the most attractive features of other current models of energy coupling in this process [19], and which may be of some explanatory and predictive value. This hypothesis is based upon (a) the application of current ideas concerning electrode processes to energy-transducing biological membranes, (b) the recognition that transmembrane protonic charge separation *is* a primary result of coupled electron transport, (c) the realisation that the membrane-solution interfaces represent a kinetically significant barrier to interfacial charge transfer, and (d) the belief that evolutionary factors will tend to promote a subtle and comprehensive structural and functional organisation within biological membranes. I shall refer to the present formulation, for the purposes of semantic distinction, as an electrodic view.

IIIA-1. The model.

Postulate 1: Transmembrane-phase electrochemical proton gradients of some kind do provide a functional link between electron transport and phosphorylation, but the steady-state electrochemical proton gradient across the membrane phase itself is greater than that measured across the bulk aqueous phases.

Postulate 2: Most of the functional proton current of electron transport phosphorylation does not normally enter the bulk aqueous phases, but is carried along relative localised channels at the surface of the membrane in specific interphase 'S phases'. Specific proton conduction, both across the membrane and within the interphases, is effected by charge-relay systems, Grotthus transfer along chains of adsorbed water molecules and by proton tunnelling. Therefore the electrochemical proton potential at the F_0 part of the ATPase [63], which is itself within the interphase 'S phase', is displaced from equilibrium with that in the bulk phase to which it is adjacent.

Postulate 3: Treatments which act to decrease the native proton current flow within the S phases affect electron transport phosphorylation in a manner which may be predicted from their known effects on surface potentials described by the Gouy-Chapman-Stern-Grahame theory of the double layer [2].

Postulate 4: The generation of surface charges is an integral part of electron transport phosphorylation. It is brought about by changes in ionisation, pK and/or conformation of electron transport carriers (and the ATPase enzyme) which are responsible for vectorial proton adsorption and release. Although the term surface is of somewhat arbitrary nature I shall retain it to describe the specific interphase S phases, within the Stern-Grahame layer, in which it is postulated that the majority of the functional proton current is carried.

Postulate 5: The membrane phase should not be considered only as an inert diffusion barrier to protons. Both structurally and energetically it constitutes the coupling system

itself, by virtue of the barriers to proton passage across the membrane/solution interfaces, and as such is the site of energy storage.

Before considering experimental evidence in favour of such a formulation I think it worthwhile to review some concepts concerning electricity, electrodics and proticity, for very fruitful analogies may be drawn between the processes of electrochemistry and biological energy transduction by proticity (cf. Refs. 20, 64).

IIIA-2. Electricity and proticity, and the importance of interfaces. Electricity is the flow of electrons from regions of higher electrical potential to regions of lower electrical potential. In an analogous fashion (Table I), proticity is the flow of protons from regions of high protonic potential to regions of lower protonic potential (cf. 65, 65a, 65b). Now neither electrical nor protonic potentials may be defined in absolute terms, but must either be related to an arbitrary reference point of zero potential or expressed as a difference in potential between the point of interest and another point in space. The electrical potential difference between two points is given by:

$$\Delta G_e = -zF\Delta V \tag{1}$$

where ΔG_e is the electrical potential energy difference, in kcal $\cdot$ mol^{-1}, between the two points, z is the number of charges on an electron (-1) and ΔV is the potential difference in volts between the two points; F is the Faraday constant (23.08 kcal $\cdot$ V^{-1}).

Similarly, the electrochemical protonic potential difference between two points in space is given by [24]:

$$\Delta\tilde{\mu}_{H^+} = \Delta\psi - 2.303\frac{RT}{F}\Delta\text{pH} \tag{2}$$

TABLE I

ELECTRICAL CONCEPTS AND THEIR PROTIC EQUIVALENTS

	Electrical term	Protic term
Flow of current	Electricity	Proticity
Thermodynamic cause of current flow	Electromotive force (emf)	Protonmotive force (pmf)
Interface between energy source and aqueous solution for the purpose of performing chemical work	Electrode	Protode
Free energy	Electrical potential difference $\Delta G = -zF\Delta V$	Protonic potential difference $\Delta\tilde{\mu}_{H^+} = \Delta\psi - 2.303\,RT\Delta\text{pH}/F$
Aqueous conducting solution	Electrolyte	Buffer solution
Current-carrying entity in aqueous solution	Ions	Protons
Current-carrying entity in external circuit	Electrons	Protons *or* 'H'
Site of 'high' potential	Positive ions	Acidic moieties (pK < pH)
Site of 'low' potential	Negative ions	Basic moieties (pK > pH)
Storage of energy	Capacitance	Buffering power plus electrical capacitance

where $\Delta\tilde{\mu}_{H^+}$ is the electrochemical potential difference of protons (in volts), $\Delta\psi$ is the electrical potential difference in volts and ΔpH is the pH difference in pH units. R and T have their usual thermodynamic significance, and the factor 2.303 RT/F has a value of approximately 60 mV at 30°C. Mitchell (e.g. Ref. 23) has used the symbol Δp for the electrochemical proton gradient between two bulk phases separated by an energy-transducing membrane. In this review I shall reserve the Δp symbol for this specific (transmembrane bulk phase) meaning, and use the symbol $\Delta\tilde{\mu}_{H^+}$ in more general terms to signify an electrochemical difference of protons between any two regions. The particular regions will be identified by a superscript, using the phase of lower protonic potential as a reference point. Thus for the two-dimensional protic circuit of Fig. 1, with which much of the present discussion will be concerned, the symbol $\Delta\tilde{\mu}_{H^+}^{SL\text{-}R}$ refers to the electrochemical protonic potential difference between the surface phase adjacent to phase L and the bulk aqueous phase R.

Now since our interest in proticity lies in its ability to be coupled reversibly to the performance of useful chemical work, a comparison between electricity and proticity should necessarily begin with a consideration of the manner and mechanism by which electricity can be coupled to the performance of useful chemical work. Therefore I shall begin this comparison of electricity and proticity by the consideration of certain interfacial electrochemical phenomena, for a lively, lucid and more rigorous derivation of which the reader is referred to Bockris and Reddy [2].

If we consider an aqueous solution of chemical entities, such as a 10 mM hydrochloric acid solution, together with a source of electrical energy, work can be done by the latter on the former only through the intercession of electrodes. An electrode may be defined

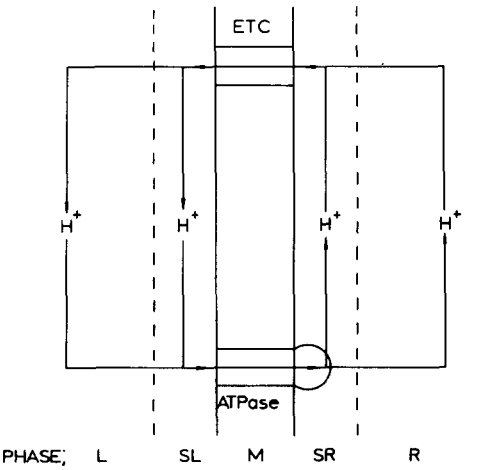
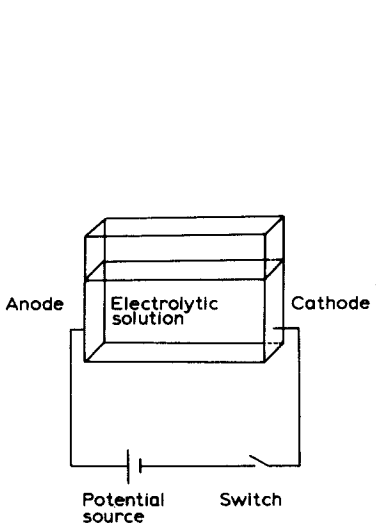


Fig. 1. A typical protic circuit. The diagram represents a phospholipid membrane M phase separating two aqueous phases L and R. Interphases SL and SR exist between the M phase and the two bulk phases L and R, respectively. The M phase contains a protonmotive electron transport complex (ETC) and a protonmotive ATPase of the appropriate polarities, and the radial and lateral flow of the proton current, i.e., a two-dimensional proton current flow, between them is indicated by the arrows. Most of the discussion in this review will be concerned with such a two-dimensional protic circuit, and it will be argued that in real systems the bulk of the proton current flow is confined within the S phases. The electrical equivalent of this circuit, and the resultant one-dimensional protonic potential profile across the membrane phase, are given in Fig. 9.

for the present purposes as an interface between a source of electrical energy and an aqueous solution, at which the electrical energy may be used to carry out useful chemical work. In order for continuing chemical work to be done, however, two electrodes are required, to serve as a connection between the two poles of the electrical energy source. Thus we may consider the system shown in Fig. 2, which represents a source of electrical energy, the battery, with its negative pole connected to a plane-parallel Pt electrode (cathode) and its positive pole connected to a plane-parallel Ag electrode (anode), the two electrodes being immersed in a solution of acidified water. As soon as the switch is turned on (Fig. 2) an electrical field is set up, given by the potential difference across the solution divided by the distance between the electrodes. The electrical potential difference takes the form shown in Fig. 3. The system is not at equilibrium, and in an attempt to restore equilibrium positive ions move toward the cathode and negative ions toward the anode. Such a tendency towards gross charge separation means that electroneutrality is upset in the bulk of the solution; the separated charge causing the lack of electroneutrality tends to set up its own electric field, of an opposite polarity to that of the applied field. If the two fields were to become equal in magnitude the net result would be that the solution was an ideal capacitor, and no chemical work would have been done. However, in the circuit described by Fig. 2 there are two types of current-carrying entity; within the metal wires of the external circuit current is carried by electrons, whereas in the aqueous solution the current is carried by hydronium, H_3O^+, and hydroxyl, OH^-, ions. A steady flow of current round the circuit can be maintained only if there is a change of charge carrier at the electrode/electrolyte interface. The transfer of electrons from the ions of the solution to the external circuit results in chemical changes (in the valence states of the ions), and under such conditions the flow of current continues in



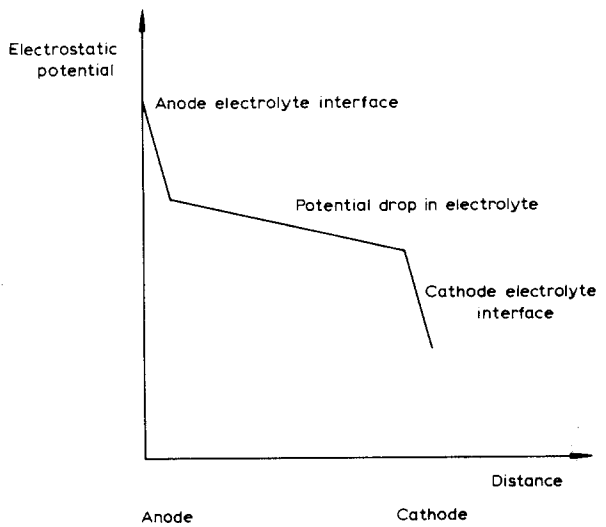


Fig. 2. An electrolytic circuit. The minimal configuration for an electrolytic cell. For discussion, see text. The variation in electrical potential around the circuit is given in Fig. 3.

Fig. 3. Potential-distance diagram for the system of Fig. 2. The diagram illustrates the potential variation in the circuit shown in Fig. 2. It is of particular interest that the major potential drops occur across the electrode/electrolyte interfaces. The reasons for this are discussed in the text.

response to the applied potential and useful chemical work is done. What has happened to the energy stored in the battery? Neglecting losses in the wires of the external circuit and in the internal resistance of the battery, two sites of energy dissipation, or the doing of work, may be identified. Work ('*iR* work') has been done in moving the ions from the bulk of the solution to the electrode/electrolyte interfaces, and work has been done, necessarily associated with chemical changes, in causing electron flow between the ions of the solution and the wires of the external circuit.

Two particularly important points emerge from Fig. 3, therefore. The potential drop across the electrode/electrolyte interface is much greater than that across the electrolyte solution, and furthermore is the only potential drop directly associated with the performance of useful chemical work. The study of electrified interfaces, and of their molecular properties, is called electrodics. In the definition by Bockris and Reddy [2], "Electrodics concerns the region between an electronic and an ionic conductor and the transfer of electric charge across it." Now electron transport phosphorylation concerns the generation of electrochemical proton gradients across a lipophilic membrane containing electronic conductors, separating two aqueous phases containing ionic conductors. I therefore consider it a properly electrodic study. It may be noted that, in the electrodic view, an intact vesicular structure is not required (nor is excluded) for efficient protonic coupling.

The model for biomembrane energy transduction by proticity, which I shall be putting forward in more explicit detail in the following sections, contains a major departure from the more traditional chemiosmotic view of these processes. This departure is the suggestion that there is a significant change in protonic potential across the interfaces between energy-transducing biomembranes and the adjacent aqueous phases. I will therefore describe certain salient features of electrodic theory and practice that I believe are of relevance to the present considerations.

IIIA-3. Electrodics: the mechanistic kinetics of electrode processes. Consider again the electrical circuit of Fig. 2. The initial change at the cathode is:

$$M_{Pt} + e^- + H^+ \rightarrow M...H(\rightarrowtail M + \tfrac{1}{2}H_2) \tag{3}$$

and at the anode:

$$M_{Ag} + OH^- \rightarrow M...OH + e^-(\rightarrowtail M + \tfrac{1}{4}O_2 + \tfrac{1}{2}H_2O) \tag{4}$$

Now the sole action of the electromotive force in bringing about these chemical changes is due to its effect in charging the electrode surfaces relative to the bulk phase. If we consider the cathode, the electrical potential drop may be split into three distinct regions, and takes the form shown in Fig. 4. In media of high ionic strength, the Gouy-Chapman region of diffuse charge is relatively insignificant and may be conveniently omitted from consideration for the present purposes. The important events take place within the Stern-Grahame layer, which extends for 1 nm or so from the electrode surface.

The constitution of the Stern-Grahame layer, consisting of inner and outer Helmholtz planes, is given in Fig. 5. The inner Helmholtz plane is populated by contact-absorbed water molecules, whose dipole orientations, induced by the negative charge on the electrode surface, are as given in Fig. 5. Adjacent to the inner Helmholtz plane is a layer of solvated protons, consisting of molecules of the general formula $(H_{2n+1}O_n)^+$ together with other solvated cations (cf. Ref. 65c for a similar analysis of biological membranes). In understanding the reason(s) for the dramatic potential drop across the Stern-Grahame layer we must ultimately consider those molecular forces acting on the hydronium ions in the outer Helmholtz plane which cause them to donate their excess protons to the

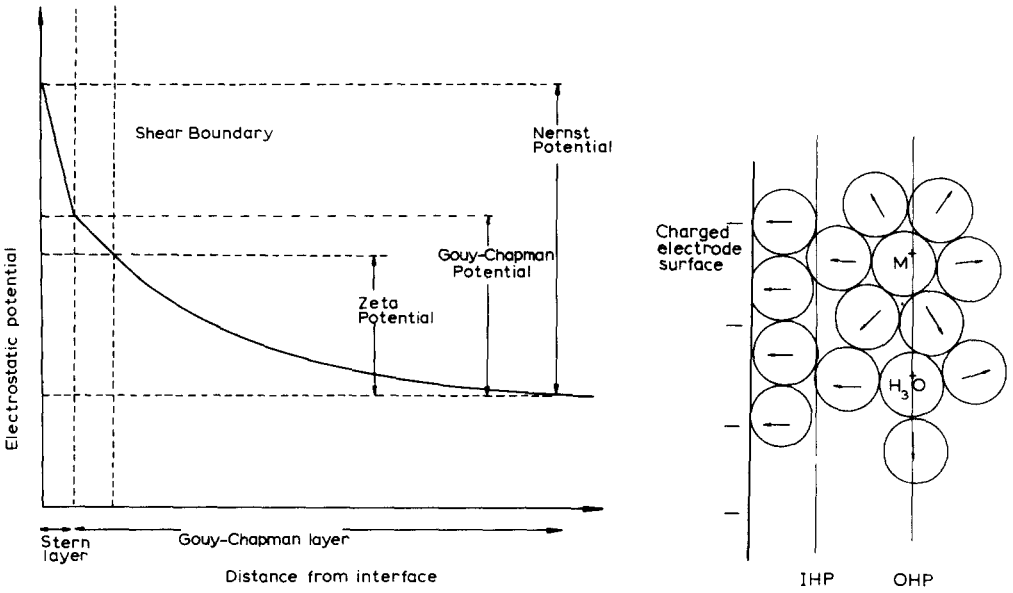


Fig. 4. A graphical representation of an electrical double layer (after Jain [66]). Different regions in the interphase between a bulk aqueous phase and a charged surface, which may be distinguished by their physical properties. The Stern layer (see also Fig. 5) extends for a few tenths of a nanometre from the surface, while the extent of the Gouy-Chapman region, also known as the region of diffuse charge, may extend for 10–50 nm at low ionic strengths, but at high ionic strengths (as in the figure) it is severely reduced. The Nernst potential, or phase boundary potential, is equal to the total interfacial potential. The shear boundary separates the free water from the water of hydration, and the potential at this point is called the zeta potential; it may be obtained from electrokinetic measurements.

Fig. 5. The Stern-Grahame layer adjacent to a charged surface. A schematic illustration of the constitution of the Stern-Grahame layer adjacent to a charged membrane in contact with an aqueous solution. The symbol ⊖ represents a water molecule with the arrow indicating the positive pole of the water dipole. M^+ and H_3O^+ represent alkali metal cations and hydronium ions respectively, and the position of the inner (IHP) and outer (OHP) Helmholtz planes is indicated by thin lines. Thus the IHP is found at the boundary of the primary layer of adsorbed water molecules, while the OHP delineates the midpoints of the primary layer of solvated ions adjacent to the IHP.

charged electrode. The free energy and potential profiles across the Stern layer under the circumstances of the reaction under consideration during the steady state are given in Fig. 6. The factor β (see Fig. 6) is an extremely important one in electrodics (see, for example, Refs. 2 and 59), and to understand its significance the Butler-Volmer equation will be introduced.

When no net current is flowing, the forward reaction of Eqn. 3, the electronation of protons, is taking place at a rate equal and opposite to that of the back reaction, the de-electronation of hydrogen atoms. Under such conditions, therefore (no applied field), we may write a rate equation for the electronation current density, $\vec{i}$, and the de-electronation current density, $\overleftarrow{i}$. These values are equal and opposite in the absence of a field; they are then called the equilibrium exchange current density, i_0. Thus,

$$i_0 = \vec{i} = -\overleftarrow{i} \tag{5}$$

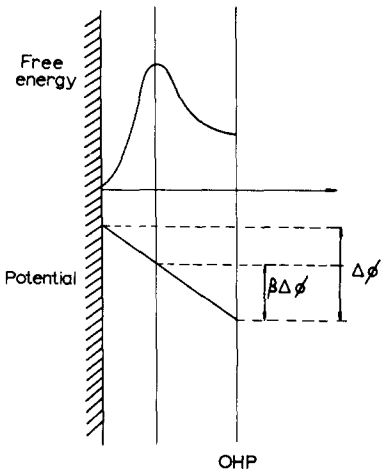


Fig. 6. The free energy and potential profiles across the interface between a charged electrode and an electrolytic solution. Only the Stern layer, represented by the inner Helmholtz plane and outer Helmholtz plane (OHP) as in the previous figure, is considered. The electrical work necessary to activate the ion is determined by the potential difference across which the ion has to be moved to reach the top of the free energy vs. distance relation. The factor, β, defining this work, is called the symmetry factor.

where

$$\vec{i} = F\vec{k}_e C_A \exp(-\beta F \Delta\phi/RT) \tag{6}$$

and

$$\overleftarrow{i} = F\overleftarrow{k}_e C_D \exp((1-\beta)\,\Delta\phi F/RT) \tag{7}$$

β is the symmetry factor of Fig. 6, C_A and C_D are the concentrations of electron acceptor (protons) in the aqueous phase and the electron donor at the electrode surface (Pt), respectively. $\vec{k}_e$ and $\overleftarrow{k}_e$ are rate constants derived from the absolute theory of reaction rates [67–69] and are given by

$$\vec{k}_e = \frac{kT}{h} \exp(-\Delta G^{\circ\ddagger}/RT) \tag{8}$$

where k is Boltzmann's constant, h is Planck's constant and $\Delta G^{\circ\ddagger}$ is the standard free energy of activation. $\vec{k}_e$ and $\overleftarrow{k}_e$ have the dimensions s^{-1}.

When an ionmotive electrical field or 'overpotential' is applied across the interface, however, equilibrium is upset, there is a change in free energy across the interface and net current flows. The value of the net current density is given by the Butler-Volmer equation:

$$i = \overleftarrow{i} - \vec{i} = F\overleftarrow{k}_e C_D \exp((1-\beta)\,F\Delta\phi/RT) - F\vec{k}_e C_A \exp(-\beta\Delta\phi F/RT) \tag{9}$$

This equation may be more conveniently written in terms of the exchange current density i_0 and the current-producing field or overpotential:

$$i = i_0 [\exp((1-\beta)\,\eta F/RT) - \exp(-\beta\eta F/RT)] \tag{10}$$

If the symmetry factor β is equal to $\frac{1}{2}$, as is in fact frequently the case, the Butler-Volmer equation reduces to:

$$i = i_0 \sinh(\eta F/RT) \tag{11}$$

and the i versus η, or current vs. voltage, plot is of the form of a hyperbolic sine function. Two limiting cases of Eq. 11 are of interest. The first is one in which the overpotential is large (say, greater than 100 mV), and

$$\exp(F\eta/2RT) >> \exp(-F\eta/2RT)$$

such that Eqn. 10 reduces to:

$$i = i_0 \exp[(1-\beta) F\eta/RT] \tag{12}$$

and thus the current density increases exponentially with the overpotential. If the field is very small, however (say less than 10 mV), one can consider that $F\eta/2RT << 1$, and use the approximation $\sinh(F\eta/2RT) \approx F\eta/2RT$. The low-field approximation therefore reduces the Butler-Volmer equation to:

$$i = \frac{i_0 F\eta}{RT} \tag{13}$$

with the result that a linear relationship apparently exists between the driving overpotential and the current density. A typical plot of the current vs. voltage relations of an inorganic electrode, a Tafel plot, is shown in Fig. 7. Its significance will be realised when we consider the relationship between the rate of performance of chemical work by a transmembrane protonic potential gradient and the size of that gradient. As I have indicated above, the contribution of any rate terms in the diffuse charge region has been neglected in this treatment; the reason for this will be indicated in the section on zeta potentials.

IIIA-4. A summary of electrodics. Three salient points emerged from a consideration

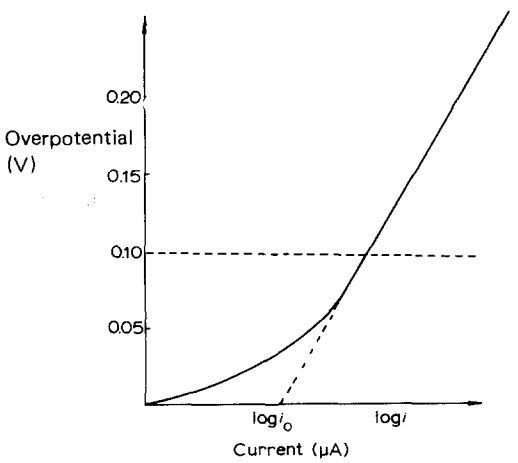


Fig. 7. Tafel plot. A typical Tafel line for a one-electron-transfer electrode reaction is given, showing the exponential relationship at high overpotentials, which makes the relation between η and log i linear.

of the structure of the electrode/electrolyte interface and its influence on the kinetics of electrode processes. The first was that the movement of protons between the outer Helmholtz plane and the electrode surface was associated with the only transformation of electrical into chemical energy (Figs. 2, 3), and the second was that the large (electrical) potential drop across this region could be viewed in terms of the energy required to break the H-O bonds in H_3O^+ ions and carry them over the 'hump' of the activation energy barrier (Fig. 6). The third was the resultant relationship of voltage to current (i.e., of $\Delta\tilde{\mu}_{H^+}$ to the rate of performance of chemical work such as ATP synthesis). We shall consider later one or two other aspects of electrodics, and their relevance to biomembrane energy transduction by proticity, in particular the notion of the protonic capacitance of energy-transducing membranes. However, it is first appropriate to consider in some detail the nature of proton transport and proticity.

IIIB. Proticity at electrified interfaces

IIIB-1. Interphases and the kinetic control of the flow of proticity. Nearly 20 years ago, Williams [70] (cf. Ref. 17), independently of Mitchell, surmised that localised proton gradients might be involved in electron transport phosphorylation, and drew particular attention to the energetics of protons within the phospholipid membrane phase. As noted above, Williams has more recently laid emphasis upon the concept of a kinetic control of proton current flow [17,18]. Certainly, a reduction of the volume occupied by the functional proton current, which volume might be affected by the conditions of incubation, would neatly account for all those problems associated with the magnitude and variability of the protonmotive force that I have discussed earlier. Whilst postulates 1–5 (above) concerning the possible functional proton current during electron transport phosphorylation are in broad agreement with certain of the proposals of Williams [17,18], I have chosen to lay particular emphasis on events at the membrane/solution interfaces in this process. Based on a plethora of experimental approaches, a large number of previous authors have also emphasised the importance of the membrane surface and its electrical charges during electron transport phosphorylation and other energy-linked membrane processes (e.g. Refs. 71–90), and it is within this context that I now present this framework for the role of the membrane/solution interphases in exercising an important control on the proton current pathway during electron transport phosphorylation.

Fig. 8 shows a fluid mosaic model of the structure of an energy-transducing membrane (after Packer [91]), containing randomly juxtaposed electron transport complexes and ATP synthase complexes embedded in a proton-impermeable phospholipid bilayer membrane. Fig. 9 indicates the electrical equivalent of the electrodic view of two protonmotive cells embedded in a phospholipid membrane (cf. Fig. 1). The 'batteries' represent protonmotive electron transport complex and ATPase, respectively. The wires (cf. Refs. 65 and 65a) and the smaller resistors indicate proton transport pathways that occur with low loss in free energy, whilst the larger resistances indicate the energy barrier that must be overcome in bringing protons from the bulk phases to the membrane surfaces, just as in the case of inorganic electrodes in Figs. 2, 3 and 6. The protonic resistance of the membrane phase is equated with the internal resistance of the batteries. The capacitances represent the protonic buffering powers of the different phases.

In the following, I shall be equating the interphase S phases, in which the major functional proton current is postulated to be carried, with the Stern-Grahame layer (cf. Figs. 4 and 5), and in order to defend this view it is necessary to review current ideas of the kinetics of proton transfer processes (e.g. Refs. 2 and 92–100).

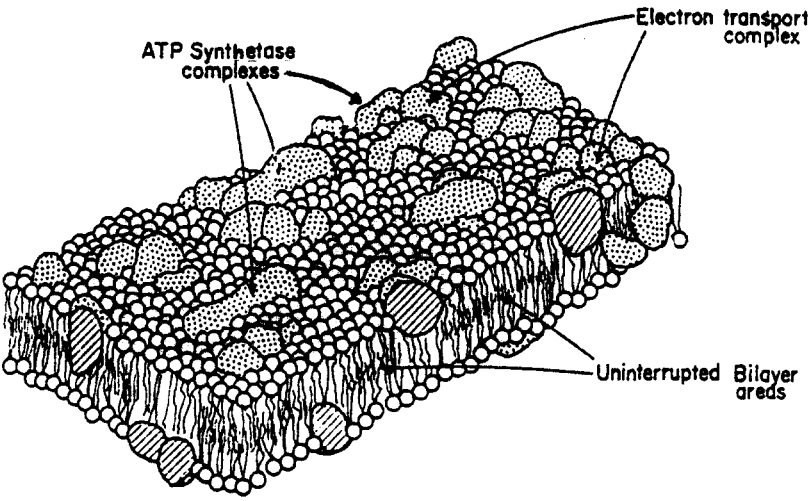


Fig. 8. A fluid mosaic model of the structure of an energy-transducing biomembrane. The diagram depicts the rather heterogeneous composition of an energy-transducing membrane as being variable between organised regions that have hydrophobic domains, probably in lipid bilayer configuration, and areas of membrane in which the hydrophobic domains are interrupted by proteins or lipoproteins. F_0F_1-ATPase enzymes are randomly juxtaposed with electron transport complexes.

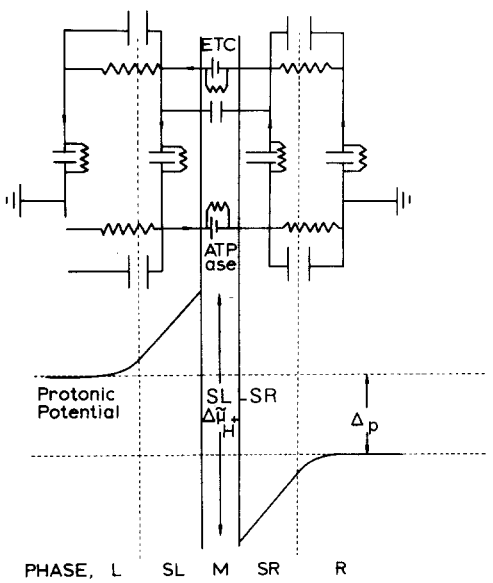


Fig. 9. A five-phase protic circuit. The upper half of the figure shows the electrical equivalent of the electrodic view of the five-phase protic circuit given in Fig. 1. The significance and possible magnitudes of the batteries, resistors and capacitors is given in the text. It differs from the chemiosmotic version in its inclusion of large interphase capacitances and a significant resistance to protic current flow between the interphases and their adjacent bulk aqueous phases. The lower half of the figure indicates the variation in protonic potential perpendicular to the plane of the membrane, and the relationship between Δp and $\Delta\tilde{\mu}_{H^+}^{SL\text{-}SR}$. The lower half of the figure is intended to serve only as an indication of the general trend of the protonic potential (i.e., assuming a lateral smearing of protonic potential) for the microscopic nature of the diagram, and the heterogeneous nature of energy-transducing membranes is not appropriately represented by macroscopic potential vs. distance relationships.

IIIB-2. The kinetics of proton transfer processes. As a direct result of its extremely small size, and thus of its very high charge density, the proton does not exist free in solution, but as the hydronium H_3O^+ ion. This hydronium ion may be further solvated (cf. Fig. 5) to give species of general formula $(H_{2n+1}O_n)^+$, where n is commonly equal to 4. These extra water molecules attached to a given hydronium ion are termed the secondary hydration sheath. In aqueous solution the rate-limiting step for the transfer of a proton from one water molecule to an adjacent water molecule is the reorientation time necessary for Grotthus transfer of the type shown in Fig. 10.

However, the mobility of protons in ice crystals exceeds even the proton mobility in aqueous solutions by one to two orders of magnitude. In this case the rate-limiting step for proton transfer is the non-classical quantum mechanical 'tunnelling' [95] under the activation energy barrier. The reason for this change in rate limitation is that in the case of ice the individual water molecules are both less concentrated and are 'structured' in a manner favourable to the rapid intermolecular transfer of protons. Thus any sort of favourable 'structuring' of water molecules will effect a specific kinetic control upon the pathway of a proton current.

There is one other type of situation which is responsible for affecting significantly the rate of proton transfer, and that is the presence of chains of acid or base centres (other than water molecules). The rate of transfer of protons between acid/base centres is given by the product of a rate constant and the concentration of acid/base centres. This rate constant is itself dependent upon the difference in pK between the donor and acceptor molecules [92]. Thus, as recently emphasised by Nagle and Morowitz [65], the nature and concentration of different acid/base centres will affect significantly the vectorial rate of a proton transfer process. Direct proof of this in the case of a protein has been given recently for the enzyme carbonic anhydrase c, in which it has been shown [101] that the rate of proton translocation to the active site of the enzyme, relative to that in carbonic anhydrase b, is greatly increased by the presence of a chain of acid/base centres on the enzyme surface, visualised by high-resolution proton NMR. It is therefore of great interest, in the context of the present model of proton transfer during electron transport phosphorylation, to enquire whether such a mechanism might operate during the latter process. Such systems have been called charge-relay systems [102].

In the discussion earlier in this review of the structure of the electrode/solution interface it was shown that an important role was played by water molecules adsorbed to the

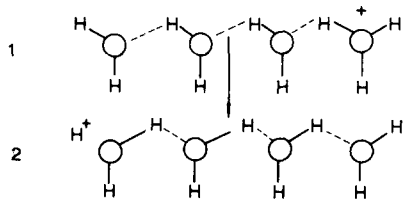


Fig. 10. Proton transfer in water by a Grotthus mechanism. In the initial state (1), three water molecules and a hydronium ion are lined up favourably for proton transfer along the chain by an essentially concerted mechanism. Such a transfer leads to state 2, and is accomplished in approximately 10^{-14} s. For another proton to be bound to the water molecule which was a hydronium ion in state 1, a rotational reorientation is necessary, and under normal conditions this takes place in approximately $2 \cdot 10^{-13}$ s. Such a Grotthus transfer mechanism accounts for the anomalously high mobility of the proton in aqueous solutions, and along chains of other hydrogen-bonded molecules.

electrode surface, and which constituted the inner Helmholtz plane. It was shown how the inner Helmholtz plane formed a kinetic barrier to proton uptake during current flow from the bulk phase. Additionally, their very 'structured' nature suggests that they must play a further key role in the context of a rapid channelling of the flow of proticity, in the sense that lateral flow, in contrast to the flow of protons to and from the bulk aqueous phases, would be encouraged by this type of structure. I shall therefore now consider very briefly the types of evidence that suggests that energy-transducing biological membranes do indeed have 'structured' water molecules adsorbed to their surfaces, just as do electrodes, and that the properties of such 'bound' water molecules are very different from those of the water molecules of the bulk aqueous phases. For a comprehensive overview of this topic the reader is referred to Drost-Hansen [103].

IIIB-3. 'Bound water' at biological interfaces. A large number of authors have drawn attention to the fact that biological membranes possess adsorbed or 'bound' water molecules which have considerably different physical properties from the water molecules of aqueous solutions (e.g. Refs. 103–115, cf. also Ref. 116). Evidence that such bound water molecules exist has come from two main types of experiment. The first concerns measurements of the physical properties of membranes, such as thermal phase transitions [103], infra-red [117] and magnetic resonance (see Refs. 103, 112) spectroscopic studies, and measurements of dielectric properties [118–120]. The other type of study involves measurements of the rate of diffusion of solutes across biological membranes under various conditions (see Ref. 103).

The purpose of the present section is not to review the enormous body of evidence that is consistent with the occurrence of 'bound' or 'structured' water at biological interfaces, but to draw attention to the importance that such a water structure would have for the relative kinetics of proton flow along the membrane surface and into the bulk aqueous phase during electron transport phosphorylation. The importance of 'bound' water molecules in crystalline proteins has been discussed more generally by Hagler and Moult [115] and Scanlon and Eisenberg [121]. Therefore, whilst Williams [17] emphasises the possible role of 'structured' water molecules in the F_0 part of the ATPase (see also Ref. 122), I should prefer to stress the role of structured water molecules in more general terms within the context of electron transport phosphorylation, namely as a medium, which possesses lower free energy (and entropy) than the bulk phase water, for the conduction of protons liberated by electron transport or ATP hydrolysis (cf. Ref. 117) along the surface of biological membranes.

Now ions can affect the degree of organisation of water such that dipole changes and changes in hydrogen bonding occur. The sequence of activity of some anions in decreasing the degree of organisation of water is:

$$BPh_4^- > SCN^- > ClO_4^- > I^- > Br^- > NO_3^- > Cl^- > OH^- > F^-$$

(see, for example, Ref. 66). The ions at the head of this series are known as chaotropic anions [123,124]. In line with the observation that the rate-limiting step in the transmembrane movement of many lipid-soluble ions is passage across the 'unstirred' interphase layers (e.g. Refs. 125–127), such chaotropic ions are most rapidly able to permeate biological membranes. Therefore, within this context of the ability of ions such as SCN^- to permeate biological membranes (e.g., Refs. 128, 129), it should be remembered that they are very effective in disrupting the structure of 'ice-like' water in the interphase S phases. Significantly, Yamamoto and Nishimura [111], in a study of the effect of temperature on the kinetics of proton movements in spinach thylakoid suspensions, stressed

the possible role of structured water molecules in determining the rate of dark efflux of protons into the outer aqueous phase following a period of illumination. Regrettably, current theories of the structure and electrical properties of water at the surfaces of energy-transducing membranes are insufficiently developed to permit quantitative comparison with experimentation. Whatever the nature of the mechanism by which electron transport-derived protons may be moved along the surface of biological membranes, such a mechanism would offer two important advantages: (a) a subtle and effective partitioning may be made of the energy generated by electron transport between ATP synthesis and other energy-requiring processes such as active substrate transport; (b) if the functional proton current involved in electron transport phosphorylation does pass along specific membrane-associated channels not in equilibrium with the bulk aqueous phase protonic potentials, there is no problem associated with the ability of marine or alkalophilic bacteria (see Refs. 26, 130) to exist in environments in which ATP synthesis would be thermodynamically impossible if a chemiosmotic type of system were operative.

It is appropriate at this juncture, therefore, to review experimental evidence that has been, or may be, interpreted to suggest that there is indeed a kinetic (or thermodynamic) diffusion barrier to proton flow between an energy-transducing membrane surface and the adjacent bulk aqueous phases under conditions approximating those in vivo.

IV. Evidence for a diffusion barrier to protons near the surface of energy-transducing membranes

Direct evidence for the existence of a proton-diffusion barrier at the outer surface of the thylakoid membrane, observed using the hydrophilic pH-indicating dye cresol red, has been presented by Junge and Ausländer [131] (see also Ref. 132). The rapid binding of protons to the surface of light-energy-transducing membranes, followed by their slower equilibration with a bulk aqueous phase, has also been indicated by the observations of Rumberg and Muhle [82], Kraayenhof [84], Nishi et al. [85], Ort et al. [133] and Masters and Mauzerall [133a].

IVA. The role of 'permeant' ions

The slow rate and extent of proton ejection into bulk phase L induced by the addition of a small pulse of oxygen to anaerobic suspensions of mitochondria [134] or bacteria (e.g. Refs. 135, 136) in the absence of permeant ions is well known. The ability of permeating ions such as K^+ *plus* valinomycin [134] or SCN^- [135] to increase the rate and extent of H^+ ejection by these systems has been explained on the basis of an electrophoretic migration of these ions acting to neutralise the thermodynamic back-pressure of electrical potential caused by the rapid build-up of a protonmotive force [24,27,134,135, 137]. However, this interpretation has been severely questioned on the basis of some measurements of bacterial $\rightarrow H^+/O$ ratios in the absence of permeant counterions, in particular the observation [136] that the addition of a second oxygen pulse immediately following the first is not subject to this back-pressure. There is abundant evidence from work with liposomes that thiocyanate [124], the valinomycin-K^+ complex [126,138–139a] (and cf. Refs. 140, 140a for valinomycin-based K^+-sensitive electrodes) and other 'permeant' ions (e.g. Refs. 125, 127, 140b) interact with phospholipid membranes to alter the surface potential of such membranes.

Now the rate-limiting step in valinomycin-mediated transmembrane ion conductance, from one bulk aqueous phase to another, at saturating valinomycin concentrations, is the

diffusion of the charged valinomycin-cation complex in the unstirred Stern layers adjacent to the membrane (e.g. Refs.126, 141; cf. Refs. 125, 142). Thus it is easy to see that protons liberated by electron transport at the surface of a charged membrane, and which are restricted from entering the bulk phase by a diffusion barrier in the form of an unstirred interphase, may enter the bulk phase if the diffusion barrier be lifted by an alteration of the surface potential (and hence protic resistance and capacitance) of the membrane. In this regard it might be expected that the electrophoretic mechanism of charge-neutralisation (at saturating ion concentrations) should operate equally satisfactorily with co- or countermigration of appropriately charged permeant ion. However, if surface effects in the interphase are of importance in causing stoichiometric proton ejection into the bulk aqueous phase (cf. Ref. 142a) then it need not be expected that saturating concentrations of (say) valinomycin · K^+ or thiocyanate would have the same effect as each other on the proton current pathway in the bulk aqueous phases. Such a lack of correspondence between the stoichiometry of H^+ liberation in the presence of the two types of permeant ion has indeed been noted [135,143]. This mechanism for an action in the surface layers of SCN^- in stimulating proton ejection is indicated in Fig. 11. Equally, in the electrodic view, *non-ionic* chaotropic contact-adsorbing species should act to increase the magnitude of experimentally determined $\rightarrow H^+/e^-$ ratios. Such a surface-phase mechanism is fully consistent with the observation that both buffering in the bulk aqueous phases and the presence of 'permeant' ions are required either to uncouple electron trans-

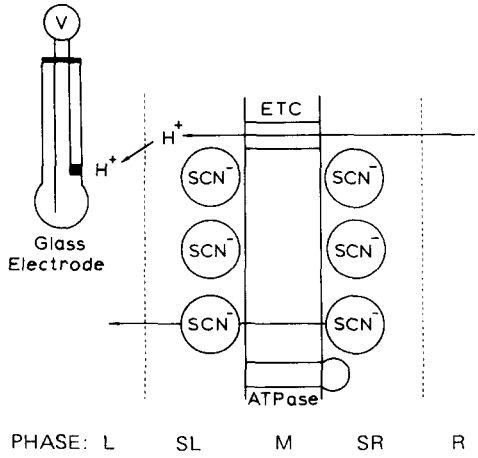


Fig. 11. Effect of contact-adsorption on H^+ current pathway. Dual mechanisms by which membrane-permeable ions act to stimulate proton ejection into bulk aqueous phase L during oxygen-pulse experiments, illustrated with the SCN^- ion. As well as an electrophoretic migration of SCN^- across the membrane, contact-adsorbed SCN^- ions decrease the capacitance and increase the resistance of the S phase, by substituting for the adsorbed water molecules of the inner Helmholtz plane. According to the calculation given in the text, under certain conditions approximately 90% of the stimulation of H^+ ejection into the bulk of phase L must be accounted for by the contact-adsorption mechanism, while approximately 10% may be ascribed to a charge-neutralising electrophoretic migration of the SCN^- ion. According to this electrodic view, therefore, all membrane-permeable ions, such as the K^+-valinomycin complex, will exhibit chaotropic behaviour, a fact in accordance with experimental observation. Additionally, according to the electrodic view, non-ionic contact-adsorbing species will also act to stimulate proton movements between the membrane system and the bulk aqueous phases. Other symbols as in Fig. 1.

port from phosphorylation (e.g. Refs. 128, 144, 145) or to drive phosphorylation during 'acid-bath' experiments (e.g. Refs. 39, 40, 145–147), since the 'permeant' ion would speed proton equilibration across the S phases in both directions (see Ref. 148). A further prediction stemming from this type of mechanism is that the stoichiometry of proton ejection, using any appropriate system, measured in the presence and absence of 'permeant' ions, should be influenced in a predictable manner by treatments affecting their surface charge. Appropriate systems might include bacteriorhodopsin-containing liposomes of different surface charge, or natural membranes whose fixed surface charge has been modified by compounds such as biguanides (see Refs. 78, 79, 149–152) or salicylates [153]. It is critically important to remember that protons are seen in the bulk phases only under *uncoupled* conditions, i.e. with K^+/valinomycin or SCN^- present.

Thus in electrodic terms, the action of membrane-permeable ions in stimulating proton ejection into the bulk aqueous phases is, by substituting for the adsorbed water molecules of the inner Helmholtz plane, to decrease the capacitance and to increase the resistance to lateral proton current flow, just as is found, capacititively, in electrode kinetics for such 'contact-adsorbed' ions (e.g. Refs. 2, 154, 155). The insufficiency of the electrophoretic mechanism to account for the stimulation by thiocyanate of H^+ ejection by *P. denitrificans* cells may be put on a more quantitative footing using the data of Scholes and Mitchell [135,136]. (Surprisingly, there appears to have been no study which has addressed itself directly to the question of whether the stoichiometries of 'extra' H^+ movement and co-/counter-ion movement as measured in the bulk phases with electrodes tally one with the other.) All relevant data are those cited by Scholes and Mitchell [135, 156] except for the following, which are unpublished observations of the present author: the $t_{1/2}$ for SCN^- uptake by inverted *P. denitrificans* vesicles, which was obtained using the ion-selective electrode method described by Kell et al. [44,129], was 45 s, and it is assumed that this holds true for SCN^- efflux from intact cells of the organism. Additionally, the volume enclosed by the cells, measured using the sucrose-impermeable space method described in Ref. 42 was 2.0 ± 0.2 ml per g dry weight.

Scholes and Mitchell [135] found an apparent $\rightarrow H^+/O$ ratio of 4 in the absence of SCN^-, which was increased to 7.5 when 17.5 mM SCN^- was present, at pH 6.0–6.1. According to the electrophoretic mechanism, therefore, the extra 3.5 H^+ ejected into the outer bulk aqueous phase per O reduced must be accompanied by a similar number of negatively charged SCN^- molecules during the time of reduction of the pulse of oxygen added. The conditions used by Scholes and Mitchell [135] were 20 mg dry weight of *Paracoccus* cells per 4 ml reaction mixture, and 23.5 ngatom O were injected. The uncoupled respiration rate was 1.5 ngatom $O \cdot mg^{-1}$ dry weight $\cdot s^{-1}$ [135,156]. Thus the respiratory burst induced by the pulse of O_2 lasted 0.784 s.

The pseudo-first order rate constant for transmembrane electrophoretic movement of SCN^- is given by (ln 2/45) $s^{-1} = 0.0154\ s^{-1}$. The actual rate of SCN^- movement across the membrane is given by $k \cdot [SCN^-]$, where k is the pseudo-first order rate constant. Therefore the rate of SCN^- movement is given by 0.0154×17.5 nmol $\cdot \mu l^{-1} \cdot s^{-1} = 0.27$ nmol $\cdot \mu l^{-1} \cdot s^{-1}$. The internal volume of the cells in the suspension was 40 μl, and thus the total SCN^- movement during the burst of respiratory activity $= 0.27 \times 40 \times 0.784 =$ 8.5 nmol.

Now the amount of oxygen added was 23.5 ngatom, and therefore the extra H^+ which must be 'neutralised' $= 3.5 \times 23.5 = 82.3$ nequiv. H^+. Thus, under the conditions described by Scholes and Mitchell [135], it would appear that an electrophoretic mechanism of charge-neutralisation may account for only 10% of the extra H^+ released. It is

concluded that a re-examination of the role of SCN^- and other 'permeant' ions in increasing the stoichiometry of H^+ ejection measured during pulses of electron transport activity may indeed be warranted, both in the case of *P. denitrificans* cells and more generally.

Further, were there to be found non-ionic compounds which acted to increase the $\rightarrow H^+/e^-$ ratio observed in pulse-type experiments, an electrophoretic mechanism of action, for these ions *and* for the more commonly used ionic 'permeant' chaotropes, would be excluded. There are indications that ethanol [157] or a general anaesthetic [157a] might be such a compound, and according to the electrodic view the effect of such compounds in increasing the apparent $\rightarrow H^+/e^-$ ratio as measured with a glass electrode, should be potentiated by membrane-impermeant buffers in the external aqueous phase.

IVB. Ion lipophilicity, membrane potentials and proton movements

An important and specific prediction stemming from the present formulation, therefore, is that both proton and ion movements should be affected dramatically by the concentration of contact-adsorbing 'permeant' ions in the interphase S phases. If the argument is restricted to vesicular systems in which electron transport or ATP hydrolysis causes the lumen of the vesicle to become acidic or positive relative to the medium, it is necessary to consider proton and anion *uptake*.

In such inverted systems it is well known (see, for example, Refs. 137, 145) that electron transport-induced proton uptake is stimulated both in rate and extent by 'permeating' anions, and this is explained, within the chemiosmotic framework, by an electrophoretic migration of the permeant ion tending to neutralise the membrane potential component of the protonmotive force, concomitantly replacing it with a steady-state pH gradient (e.g. Ref. 24). Two effects may be distinguished. At low concentrations of hydrophilic permeant ion (10–20 μM), electron transport- or ATP-induced ion uptake occurs to an extent governed by the Nernst potential, and the ions may be used as a probe for the Nernst potential (e.g. Refs. 32, 42). At much higher concentrations of hydrophilic permeant ion, however, (say greater than or equal to 10 mM), ion uptake causes a decrease in the Nernst potential to energetically insignificant values, together with a large stimulation of proton uptake and the formation of a pH gradient. If, under the present model, it is considered that these effects are largely mediated within the interphase S phases, however, the effects of hydrophilic and lipophilic permeant ions (the latter operationally defined as those which bind to biomembranes to a significant degree under unenergised conditions) may be distinguished, for the concentration of contact-adsorbed lipophilic ion in the S phases will be much greater than the concentration of hydrophilic ions in the S phases for a given added ion concentration, and it may be expected that lipophilic ions will be more effective both in stimulating proton uptake and in being themselves taken up in response to electron transport. I will restrict consideration here to the extent of *anion* uptake induced by electron transport, as a function of ion lipophilicity.

It was shown elsewhere [129] that the extent of anion uptake induced by NADH oxidation in submitochondrial particles was identical for the hydrophilic nitrate and thiocyanate ions. However, other, more lipophilic ions, which bind significantly to unenergised membranes, in particular the tetraphenylborate ion [158], would be expected to be taken up to a greater extent on energisation than the more hydrophilic nitrate and thiocyanate ions. Such behaviour has been reported for a number of lipophilic ions in submitochondrial particles by Azzone et al. [48]. Chloroplast thylakoids are of especial interest in this regard, for it is well known that the electrophoretic movement of Cl^- and

Mg^{2+} across the thylakoid membrane in response to electron transport (e.g. Ref. 159) results in the neutralisation of a transmembrane potential [160] and the expression of the protonmotive force solely in the form of a pH gradient (see, for example, Refs. 30, 161). However pea chloroplasts have been shown to take up the lipophilic phenyldicarbaundecaborane ion (PCB^-) in response to electron transport under steady-state conditions [162], consistent with the view, advanced here, that the electrical (and protonic) potential at the surface of the membrane is greater than that within the bulk phase L (cf. Fig. 9). In this regard it is of particular interest that the magnitude of the field-indicating carotenoid absorbance change (the 515 nm shift) is consistent with the idea that an electrical potential of approximately 100 mV exists across the pigment molecules during continuous illumination (see, for example, Refs. 30, 157, 161, 163, and further discussion in Section VI). It will be of interest, therefore, to test the predictions that stem from these findings: that tetraphenylborate but not thiocyanate should increase the steady-state extent of light-induced proton uptake by chloroplast thylakoids, and that a given (low) concentration of tetraphenylborate should enhance the light-induced proton uptake of *R. rubrum* chromatophores more than does the same concentration of thiocyanate.

IVC. An electrodic explanation of the mechanism of action of uncouplers

It is widely believed (but cf. Ref. 38) that weakly acidic lipophilic uncouplers of electron transport phosphorylation act by virtue of their ability to conduct protons across natural (e.g. Refs. 33, 156, 164) and synthetic (e.g. Refs. 35, 36, 66, 165) bilayer membranes. However, the rate of proton translocation catalysed by a variety of uncouplers is strongly dependent upon the surface potential of charged membranes. This has been noted, for example, in the case of dinitrophenol [34], 5,6-dichloro-2-trifluoromethyl benzimidazole [166] and carbonyl cyanide-*p*-trifluoromethoxy phenylhydrazone (FCCP) [35]. Bakker et al. [167] have also shown that the binding of a variety of uncouplers to both liposomes and mitochondria both affects and is affected by the surface potential and the bulk pH in a manner consistent with the predictions of double layer theory. Therefore whether or not there is a diffusion barrier to protons entering and leaving the bulk aqueous phases from the Stern layer as suggested in the present review, the potent ability [168,169] of uncoupler molecules to short-circuit a proton current between the S phases is to be expected. The mechanism is illustrated schematically in Fig. 12. The uncoupling activity of non-acidic molecules such as the phenylisothiocyanates [37] and pentachloronitrobenzene [170] would be ascribed, in the electrodic formulation, to a disruptive effect on the dipoles of the water molecules of the inner Helmholtz plane, and a chaotropic effect brought about by contact adsorption of uncoupler molecules (cf. Fig. 11). The ability of thiocyanate to reduce the uncoupling effectiveness of FCCP in *P. denitrificans* cells [135] was in fact ascribed to a 'space charge' effect, although it could perhaps as easily be explained by a lowering of the proton and uncoupler 'reservoir' within the S phases (cf. Figs. 1 and 9). Conventional explanations of the mechanism of uncoupling action of ionophores of the valinomycin and nigericin types (e.g. Refs. 24, 137, 141) are also as easily applicable to situations in which the functional proton current of electron transport phosphorylation occurs within the S phases as if it occurs within the two bulk aqueous phases. Therefore, although the seminal observation that one gramicidin molecule per thylakoid (see Refs. 157, 171) or one valinomycin molecule per chromatophore [172] are sufficient to give a certain degree of 'uncoupling' have been widely interpreted to indicate that the functional proton gradients of electron transport phosphorylation occur across the bulk aqueous phases, they are fully consistent with the pres-

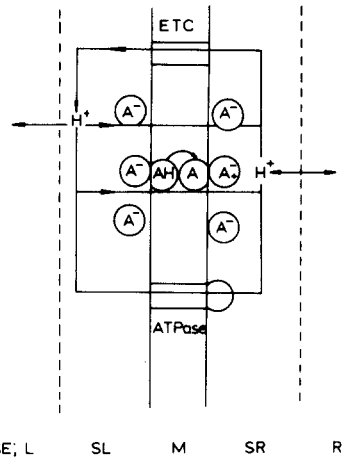


Fig. 12. An electrodic view of uncoupling. According to the electrodic view, weakly acidic uncouplers of oxidative phosphorylation exert two types of action. They conduct proton across the membrane phase itself, either by diffusion of the uncharged form of the uncoupler or by a relay mechanism, and they also contact-adsorb, and, like other chaotropic 'permeant' ions, speed the equilibration of protons between the S phases and the adjacent bulk aqueous phases. The relative effectiveness of the second type of uncoupling will be governed by the buffering power of the bulk aqueous phases. Only the second type of mechanism is possible with non-acidic uncouplers, which, according to the electrodic view, should stimulate apparent $\rightarrow H^+/e^-$ ratios by virtue of their contact-adsorbing activity. AH and A^- represent neutral and ionised uncoupler molecules, respectively.

sent five-phase formulation. Thus, although the protonic potentials in the S phases are greater than those of the adjacent bulk aqueous phases, the protonic potential throughout a given S phase *may* be essentially constant (Fig. 9), at least in these photosynthetic systems, and a single uncoupler molecule per vesicle may be expected to change it. However, we would note the following points with regard to these observations. Firstly, the protonophoric activity of gramicidin is due to the gramicidin *dimer* (e.g. Ref. 173), so that one gramicidin molecule per vesicle should not uncouple even a chemiosmotic system. Secondly, neither in the case of chloroplast thylakoids [157] nor of bacterial chromatophores [172] was *full* uncoupling observed when one ionophore molecule per vesicle was present; an equilibrium thermodynamic hypothesis of protonic coupling, such as the chemiosmotic hypothesis, would require full uncoupling under these conditions. Finally, we note that the recent demonstration by Ort [173a] of the relative effects of lipophilic and hydrophilic uncouplers on ATP synthesis in chloroplasts might have been predicted by the electrodic views expressed here.

IVD. Thermodynamic and kinetic measurements of proton movements in vesicular systems; the resolution of a controversy?

The disquieting problem of the lack of harmony between the $\rightarrow H^+/ATP$ ratio measured for submitochondrial particles by kinetic methods in the presence of 'charge-neutralising', membrane-permeable ions [174,175] ($\rightarrow H^+/ATP = 2$), and by thermodynamic comparison of Δp and ΔG_p [45,176,177] ($\rightarrow H^+/ATP \geqslant 3$) has been alluded to earlier [45]. Similar controversies exist for both chromatophores [12,42,47,178] and chloroplasts (e.g. Refs. 30, 161, 163, 179, 180). In each of these systems the ATPase faces the medium, and thus

TABLE II

THE EFFECT OF MEMBRANE-PERMEABLE IONS IN INCREASING APPARENT $\rightarrow H^+/ATP$ RATIOS

Estimates for the $\rightarrow H^+/ATP$ ratio (given to the nearest integer) by various authors using different systems. Although the situation in intact mitochondria is complicated by the operation of substrate carriers, the results from the other systems are fully consistent with the idea that the apparent $\rightarrow H^+/ATP$ ratios are smaller when measured during 'pulse' experiments in the presence of a high concentration of 'permeant' ions. Chloroplast thylakoids have not been included owing to the controversy concerning the existence of a steady-state $\Delta\psi$.

	'Charge-neutralising' ions present?	H^+/ATP	Ref.
Mitochondria	Yes	3–4	182
	No	4 *	183
	No	3 *	184
	No	3	185
Submitochondrial particles	Yes	2	174
	Yes	2	175
	No	3	45
	No	3	48
	No	3	177
	No	3	186
Chromatophores	Yes	2	47
	No	5–6	42

* Calculated.

there are no complications from energy-linked movements of adenine nucleotides [181] or of phosphate [182]. I would suggest (Table II) that the most economical resolution of this problem lies in the mechanism elaborated here: (a) that the main functional proton current of electron transport phosphorylation occurs not in the bulk aqueous phases L and R but in S phases within the double layer, and (b) that the addition of high concentrations of 'charge-neutralising' ions such as thiocyanate or valinomycin · K^+ changes the capacity of the S phases to carry protic current, with expulsion of protons into the bulk phase L. If such an explanation be correct it might be expected that correspondence be found between (a) the $\rightarrow H^+/ATP$ ratio calculated from the thermodynamic relationship between Δp and ΔG_p measured in the presence of high concentrations of charge-neutralising ions, and (b) the $\rightarrow H^+/ATP$ ratio calculated by dividing the $\rightarrow H^+/O$ ratio by the P/O ratio measured under such conditions. An 'inverted' system with an unbranched electron transport chain, and with a fully reversible ATPase, would be required to test this hypothesis, and it would appear that ATPase-inhibitor-protein-depleted submitochondrial particles constitute such a system. On the basis of the present analysis it would therefore be concluded that the true $\rightarrow H^+/ATP$ ratio of the mitochondrial ATPase is 2, and that that calculated by us previously using hydrophilic ion-distribution techniques for the determination of Δp [45] is an overestimation, with the functional proton current carried in the interphase region.

V. The measurement of 'surface' potentials

VA. Fluorescent probes

In the mechanism of proton current flow during electron transport phosphorylation

described here, emphasis has been placed on the effect of surface/solution interfaces on local electrochemical potentials, and it is of interest to know therefore how such surface potentials may be detected in energy-transducing membranes. In this section I shall review current ideas concerning the ability of fluorescent probe molecules to monitor local surface potentials.

VA-1. 1-Anilinonaphthalene-8-sulphonate. 1-Anilinonaphthalene-8-sulphonate (ANS) has been widely used as a reporter of the 'energised state' of energy-transducing membranes (e.g. Refs. 87, 187–189). It responds to the energisation of submitochondrial particles by an increase in binding to the particles, with no change in intrinsic fluorescence yield (at least with ATP-energisation), the bound form having a larger fluorescence than the free form (see Ref. 87). Although the molecular basis for the 'ANS response' remains unclear [87], it has been shown [74] that the optical response of anionic dyes depends on the membrane surface potential. ANS in particular [75,78,87,152,190,191] has been shown to report changes in the surface, or more particularly Stern [191] potentials. Although attempts have been made [178,192] to calibrate the ANS response in terms of a transmembrane electrical potential by means of K^+ · valinomycin diffusion potentials, it has been shown [87,192] that the kinetic characteristics of such diffusion potential-generated ANS responses are different from those induced by electron transport or by ATP hydrolysis. Of particular interest in this context is the suggestion [192] (see also Ref. 193) that ANS responds to potential energy gradients of a more localised nature than bulk phase transmembrane electrical potentials.

VA-2. 9-Amino acridine. The energy-linked fluorescence quenching of 9-amino acridine has also been used as a probe of membrane energisation, in chloroplast thylakoids [194], bacterial chromatophores [12,195,196] and submitochondrial particles [197]. It was originally proposed [194] that there was a direct correspondence between the interaction of 9-amino acridine with the thylakoid membrane and the 'bulk' transmembrane pH gradient measured from the distribution of hydrophilic amines. However, it is now believed that in many systems [81,84,88,198–200] the quenching of 9-amino acridine fluorescence arises from binding of the protonated form of the probe at a surface of the membrane. In this respect it is of interest that results presented earlier [43,45] showed that the pH gradients determined using the 9-amino acridine method were always approximately 1.5 pH units higher than those determined from the distribution of methylamine. A similar conclusion has been reached, following a more systematic analysis, by De Benedetti and Garlaschi [201]. It may be concluded that there is good evidence that the 'energised state' probed by both 9-amino acridine and ANS is intimately associated with the generation of electrical charges at the membrane surface, and with a change in Stern potential.

VB. Direct measurement of energy-linked changes in surface charge distribution

Fig. 4 indicates the electric charge profile between a charged membrane surface and an adjacent 'bulk' aqueous phase. Although this is based upon the Gouy-Chapman and Stern-Grahame theories, with their attendant approximations, it serves to illustrate how changes in the membrane surface charge distribution may be reflected at different points in space. In particular, the zeta potential, the electrical potential at the plane of shear, is a parameter which is directly accessible to experimental analysis using microelectrophoretic mobilities. Measurements of the zeta potential of energy-conserving biomembranes, and of its changes upon membrane energisation, have been reported by, for example, Davies et al. [202], Nobel and Mel [203], Kamo et al. [204], Aiuchi et al. [205], Quintanilha and

Packer [206] and Nakatani et al. [206a]. However, although changes in the zeta potential of energy-transducing membranes do indeed accompany membrane 'energisation', such changes are energetically very small, and it is for this reason that I have suggested that the majority of the functional proton current occurs within the Stern layer, i.e. (Fig. 4) within the plane of shear.

It may be concluded from the previous three sections, therefore, that although the fluorescent probe methods may be regarded as providing a sensitive monitor of membrane energisation as indicated by changes in Stern potential and membrane surface charge distribution, such changes are not readily apparent at the plane of shear. The development [74,207,208,208a] of fluorescent probes sensitive to pH changes at the membrane/solution interface would seem to offer important possibilities for testing electrodic views.

VC. The electrical and protonic capacitances of energy-transducing membranes

According to the chemiosmotic view of biological energy transduction, the sole function of the proton-impermeable phospholipid membrane is to separate the bulk aqueous phases across which the electrochemical proton gradient is generated. The electrical capacitance of the membrane phase itself constitutes the only part of the system across which a potential energy drop takes place. According to the electrodic view presented here, however, the electrical (or, more properly, protonic) capacitance of the system resides additionally, indeed particularly, in the S phases on either side of the membrane. Thus, as in the study of electrochemistry (e.g. Refs. 2, 62) analysis of the current vs. voltage relationships of the membrane, either in the steady state or during 'transients', may be used to gain an understanding of the nature of the electrical and protonic capacitors involved in electron transport phosphorylation. In particular, a consideration of the properties of a series array of capacitors allows a resolution of the 'Pacific Ocean' controversy, and it is to this topic that I now turn.

According to Mitchell, the lipoprotein membrane surrounded on either side by an aqueous phase may be regarded as a three-phase system, consisting of a lipophilic membrane M phase forming an osmotic barrier between two aqueous phases L and R, and it was for this reason that the term 'chemiosmosis' was conceived. On this basis, any proton moved 'across' the membrane must necessarily move from one bulk aqueous phase to another. This conception has been repeatedly criticised on theoretical grounds by Williams as involving an unacceptable loss of free energy if the buffering capacity in one or both aqueous phases be sufficiently large (e.g. Ref. 17), the so-called 'Pacific Ocean' effect. This criticism is rejected by Mitchell (e.g. Ref. 16), but in the following I shall indicate that, although functional proton currents do occur across the plane of the M phase, provided that the functional proton current under normal conditions is confined to the interphase regions, bacteria may indeed live in the Pacific Ocean [17] or in regions of extreme alkalinity (e.g. Refs. 26, 130) without a significant loss in free energy and despite the generation of transmembrane (but non-bulk phase) electrochemical proton gradients as an intermediate in energy coupling.

According to the chemiosmotic view (e.g. Refs. 16, 23) the 'differential buffering capacity' (B) of a chemiosmotic system is given by:

$$1/B = 1/B_o + 1/B_i ,$$

where B_o and B_i represent the external and internal phase buffering powers. Thus if *either* B_o or B_i is made infinitely large, although the differential buffering capacity of the system will be affected, the system will still be able to store a certain amount of protic

energy. (If one of them is made infinitely large the differential buffering capacity will equal the buffering power of the other aqueous phase.) In the context of the present formulation the protonic capacitance of the five-phase membrane system is ostensibly given similarly by:

$$1/C = 1/C_L + 1/C_M + 1/C_R + 1/C_{SL} + 1/C_{SR} ,$$

where the subscripts refer to the five phases described in Figs. 1 and 9. For mercury electrodes the (electrical, rather than protonic) capacitance of the double layer is approximately 17 $\mu F \cdot cm^{-2}$ [2] whilst the electrical capacitance of the M phase of biological membranes is approximately 1 $\mu F \cdot cm^{-2}$ [23,209,210]. However, even if the buffering power in *each* aqueous phase were made infinitely large, the *apparent* capacitance of the system, in the electrodic view, would be reduced to only 0.9 $\mu F \cdot cm^{-2}$ when measured in a plane perpendicular to the membrane. In other words the existence of large buffering capacities in either the bulk phases or in the S phases will have a negligible effect upon the apparent capacitance of the whole membrane system as measured in a plane perpendicular to the membrane, whilst the capacitative elements (buffering powers) experienced by the protic current flow between the electron transport complexes and the F_0F_1-ATPase complexes, occurring as they do in planes *parallel* to the membrane phase, would be rather large according to the present model. Only when the S phase capacitances are severely *reduced,* e.g. by the presence of contact-adsorbing ions [2,61,127], can the storage capacity of the membrane system be affected by the buffering power of the bulk aqueous phases. It is worth noting in this context that Young [76] has calculated surface (interphase) capacitances for the chloroplast thylakoid membrane of 140 $\mu F \cdot cm^{-2}$, whilst a value for the interphase capacitance of phospholipid bilayers of 50 $\mu F \cdot cm^{-2}$ is given by Feldberg and Delgado [209]. Therefore the suggestion [23] that translocation across the mitochondrial cristae membrane (capacitance 1 $\mu F \cdot cm^{-2}$) of 1 ng-ion H^+ per mg protein should lead to a transmembrane potential of 250 mV is not fully correct within the context of the present formulation, for the large interphase capacitances will have the effect of reducing the effective bulk transmembrane potential for a given quantity of charge removed from the intramitochondrial S phase. A similar analysis (ignoring interphase capacitances) has recently been given for chromatophores [210] and for bacteriorhodopsin-containing proteoliposomes [210a].

Such considerations as the foregoing lead to a method of distinguishing experimentally the properties of chemiosmotic and electrodic systems. The production of large buffering powers in the bulk aqueous phases L *and* R is expected to decouple a chemiosmotic system, but not an electrodic one; in order to uncouple the latter, it is necessary to have both buffering *and* the presence of chaotropic ions or molecules. Surprisingly, there does not seem to have been any study aimed at analysing the extent of uncoupling caused by buffering out both aqueous phases (but cf. Ref. 133).

The analysis presented here does, however, have important implications for the recent data of several authors concerning the nature and flow of proticity. Thus the inclusion of large interphase capacitances in membrane models: (i) offers an alternative to Na^+/K^+ gradients as a $\Delta\mu_{H^+}$ buffer [211]; (ii) offers a very reasonable explanation for the data of Ort et al. [133] (cf. Ref. 212) concerning the smallness of the effect of non-contact-adsorbing permeant buffers in inducing a significant lag in ATP synthesis by thylakoids: (iii) is fully consistent with the ability of thylakoids to synthesise ATP under direct electrical stimulation with no bulk phase proton movements [171,213] (and see Ref. 213a for bacteriorhodopsin); (iv) may be used to equate the 'energised state' of energy-transducing membranes

as detected by the use of fluorescent probe methods with the protonic charging of the interphase capacitances.

It is additionally appropriate to note here that the electrodic analogy developed thus far has, for reasons of simplicity, used the metal-electrolyte interface as a conceptual basis. It may well be [2,214,215] that the semiconductor/solution interface constitutes a better analogy. Further, it has so far been implicitly assumed that the interphase capacitances are voltage-independent, an assumption unlikely to accord with fact [2,44,46,89, 127,216,216a,216b]. However, a more detailed analysis of such questions does not seem appropriate at the present time.

As regards experimental approaches to such questions, I would draw attention finally to a study by Coster and Simons [217]. These authors used a Wayne-Kerr conductance bridge to study the capacitance changes in phospholipid bilayer membranes. Whilst they enjoined caution in the acceptance of the quantitative aspects of their work, they did show that there were indeed layers of water adjacent to the membrane surface with capacitance properties very different from those of bulk phase water (and cf. Refs. 113, 118–120).

VI. Some further approaches to the analysis of protic networks

VIA. Analysis in two dimensions

VIA-1. The current-voltage relationships of protonmotive circuits. In the introduction to electrodics presented earlier, particular emphasis was laid on the relationship between the potential across an electrode and the rate of current flow through it. It is appropriate therefore, in the context of the present formulation, to discuss the relationships observed between these parameters in systems catalysing electron transport phosphorylation.

It has been known for some time that the absorption spectrum of membrane carotenoids undergoes a red shift upon membrane 'energisation', which may be mimicked (at least partially) by ionic diffusion potentials [218]. It is now generally accepted that the 'carotenoid band shift' of chromatophores and the '515 nm shift' of thylakoids is indeed electrochromic in nature (e.g. Refs. 31, 137, 157, 161, 171, 220–224) and the extent and decay of the shifts have been used to provide a molecular voltmeter and ammeter in both chromatophores and in chloroplast thylakoids. We note with interest, however, evidence that the light-induced carotenoid shifts may indeed be monitoring non-bulk phase changes in electrical potential [161,224–227]. The purpose of this section, inter alia, is to draw attention to the similarity of the current vs. voltage plots obtained by this type of method and the Tafel plots of electrochemistry (cf. Fig. 7), to suggest that such a similarity may be interpreted to constitute further evidence for the electrodic analogy which I have drawn in this review, and to stress that such current vs. voltage plots provide an important tool for analysing the role of Stern layer protonic capacitances in biomembrane energy transduction.

In both the chemiosmotic and electrodic formulations the rate of electron transport, the rate of ATP synthesis and the rate of proton current flow (neglecting leaks across the membrane) are related by: rate of proton transport = rate of electron transport $x \rightarrow H^+/e^-$ = rate of ATP synthesis $x \rightarrow H^+/ATP$. For a protic circuit the rate of 'current' flow is equal to the rate of proton transport in the steady state, whilst the current-producing field or 'overpotential' may be equated with the protonmotive force *across the M phase.* Thus the protic equivalent of the Butler-Volmer equation (Eqns. 9, 10) is:

$$i_{H^+} = i_0 [\exp(1 - \beta') \Delta\tilde{\mu}_{H^+} F/RT - \exp(-\beta' \Delta\tilde{\mu}_{H^+} F/RT)] \quad (14)$$

where i_{H^+} is the rate of proton current flow and $\Delta\tilde{\mu}_{H^+}$ is the electrochemical proton gradient between phases SL and SR (cf. Fig. 9). i_0 is a threshold value of proton current flow necessary to induce a protonmotive force and coupled phosphorylation (cf. Refs. 157, 161, 163, 228–230). The significance of β' is the same as that of β in the Butler-Volmer equation (see above), where it has the effect of reducing the proportion of free energy which is available for doing useful chemical work (on a functional time-scale). For proton current flow *within the S phases* (or within the bulk phases) it is assumed that β' is vanishingly small, but for other proton diffusion pathways which may be considered there will be different values of β'. A case of particular interest within the context of present-day bioenergetics, the chemiosmotic hypothesis and the five-phase formulation outlined here, is the comparison between the protonmotive forces between the two bulk aqueous phases on either side of the membrane and the 'true' protonmotive force between the two interphases. In this case the relationship $\Delta p^{L\text{-}R} = \beta'' \Delta\tilde{\mu}_{H^+}^{SL\text{-}SR}$ holds, where β'' is a special value of β' reserved for this case. Based on observations with electrodes (see earlier) it may be predicted that $\Delta\tilde{\mu}_{H^+}^{SL\text{-}SR}$ and the *logarithm* of the rate of ATP synthesis (or of electron transport) will be proportional to each other for values of $\Delta\tilde{\mu}_{H^+}^{SL\text{-}SR}$ over 100 mV. Such a relationship may be invoked for chloroplast thylakoids, for example [163].

What are the current-voltage relationships when the protonmotive force is measured between the two bulk aqueous phases? The most complete study of this question has been that of Azzone et al. [48,231–233], who have used a hydrophilic ion-distribution method to examine the current-voltage relationships of the inner mitochondrial membrane. They found no relationship between the size of the protonmotive force and the rate of proton current flow, and concluded that a bulk-phase protonmotive force was not involved as an intermediate in ATP synthesis. This may be explained by substituting the value of

$$\beta'' = \Delta p / \Delta\tilde{\mu}_{H^+}^{(SL\text{-}SR)}$$

into Eqn. 14, giving:

$$i_{H^+} = i_0 [\exp(\Delta\tilde{\mu}_{H^+} - \Delta p)\, F/RT - \exp(-\Delta p\, F/RT)] \qquad (15)$$

Thus it is not to be expected, according to the electrodic view, that there should be a relationship between Δp and the rate of protic current flow, as Azzone et al. demonstrated [48,231–233] (and cf. Refs. 44–46, 234).

VIA-2. Recapitulation. We have so far considered possible kinetic and thermodynamic constraints operating on the passsage of vectorially directed protons during electron transport phosphorylation in one and two dimensions. Two salient modifications to the chemiosmotic hypothesis were suggested, namely an acknowledgement of the existence of large interphase buffering capacitances and the existence of a significant resistance to proton current flow between the S phases and the adjacent bulk aqueous phases. A re-examination of the role of 'charge-neutralising' ions in stimulating proton movements into these bulk phases was also suggested. Leaving aside the question of the molecular nature of these generators and consumers of proton electrochemical gradients, the electrodic proposals elaborated here do not in essence otherwise differ from Mitchell's chemiosmotic principles, and are a special case, amenable to experimental analysis, of Williams's more general principles of diffusion control. The chief significance of the present ideas is that, for experimental purposes, phosphorylating membrane vesicle preparations should be thought of in terms of (at least) five, rather than three, phases, and that the protonmotive forces measured across the membrane phase, between the two S phases, should be greater

than that measured across the two bulk aqueous phases. Although there may be difficulties of interpretation, it was suggested that certain of the techniques available for the measurement of protonmotive forces (e.g. those based on the distribution of hydrophilic ions) measured the bulk phase protonmotive force Δp, whilst others, such as certain fluorescent probe methods, the carotenoid band-shift method and the distribution of lipophilic ions, responded to events within the S phases.

It is now appropriate to extend the foregoing analysis to three dimensions, and to ask the question, "If the flow of proton current *is* under diffusion control, what are the pathways taken along the membrane surface?" In view of the problems mentioned above of the quantitation of various techniques for the measurement of the protonmotive force, it would seem that a more functional analysis is required to gain generally acceptable insights into this question.

VIB. How localised are the proton circuits within the S phases? An extension to three dimensions

The foregoing analysis of protonic potentials, based on analogy with the processes taking place at the surface of inorganic electrodes, has been largely confined to one dimension, namely that perpendicular to the membrane surface, and it has been assumed, for purposes of simplification, that the biomembrane surface is as homogeneous as that of inorganic electrodes, an assumption that is of course unrealistic (see Fig. 8, and cf. Ref. 18 for structural constraints on the flow of proticity). It is appropriate, therefore, to extend the foregoing analysis to three dimensions, and to consider to what extent the functional proton current of electron transport phosphorylation is localised. Although this section will largely be concerned with selected studies in which some type of protonmotive force has been measured, other types of functional study, such as the effects of colicins on membrane potentials and other energy-linked processes (e.g. Ref. 235), and the question of the competition of endergonic processes for the protonmotive force, offer important alternative analytical approaches to these questions.

VIB-1. Functional analyses. Several authors (e.g. Refs. 17, 18, 192, 193) have drawn attention to the possibility that the functional proton current between electron transport and ATP synthase enzymes may be 'localised' in the sense that the high-energy intermediate generated by the former is not equally distributed among all the latter. Within the framework of the electrodic view, and of theories stressing the importance of a kinetic diffusion control [17,18], this hypothesis seems very attractive, and offers a simple explanation for the puzzling finding [45,236] that even when the protonmotive force generated across submitochondrial particles by the oxidation of NADH or reduced TMPD were of similar magnitude, a phosphorylation potential was generated by only the former substrate. Thus, if the energy generated by the oxidation of reduced TMPD were distributed among all ATPase molecules on a given submitochondrial particle it would be expected that the magnitude of the protonmotive force alone should be the determinant of the phosphorylation potential generated if a bulk chemiosmotic mechanism were operative. If, however, the energy generated by respiration at the third 'site' were made available to only a restricted fraction of the ATPase enzymes on a given submitochondrial particle, it would be expected that the other ATPase molecules would be able to hydrolyse the ATP generated at the 'active' ATPases. Two testable predictions stem from this: (1) addition of ADP, glucose and hexokinase to submitochondrial particles respiring on reduced TMPD should not lower the time-averaged membrane potential, in contrast to what is observed in the case of NADH oxidation [45,237]; (2) inhibition of ATP hydrol-

ysis activity by the ATPase inhibitor protein or by adenylylimidodiphosphate should allow generation of a reasonably high phosphorylation potential. It should be noted that the submitochondrial particles used in the above work are devoid of ATPase inhibitor (see Ref. 45).

Apart from the above type of functional analysis, the use of single- or double-inhibitor titrations of the protonmotive force (or of another energy-linked parameter), which do not depend on the absolute magnitude of the measured protonmotive force, but only on the linearity of the measuring system (cf. Ref. 237), can offer significant analyses of the localisation of protonic coupling between electron transport complexes and ATPase enzymes in situ (e.g. Refs. 238–241). In all such analyses presented so far (see for example, Refs. 12, 44, 46, 48, 183, 231–233, 242–242b), it has been concluded that the degree of localisation in vivo is intermediate between the purely delocalised chemiosmotic model and models of the opposite extreme in which use of the protonic potential difference generated by a given electron transport complex is restricted to a single ATPase molecule. Similar functional approaches of this type may be seen in the experiments of Lee and Ernster [243] and of Grebanier and Jagendorf [244]. It seems reasonable that, in the steady state, the protonic potential throughout a given bulk aqueous phase is constant [23], and such 'localisation' effects are presumably mediated by chains of hydrogen bonds located at the membrane/solution interfaces.

Thus it may be concluded that a certain degree of localisation of the proton current between electron transport complexes and particular ATPase molecules does take place. However, it should be noted that the ability of the uncoupler SF 6847 [168] to exert a full uncoupling effect at a concentration of 0.2 molecules per respiratory chain suggests that such localisation cannot be complete.

VII. Is specific channelling a property shared by protons and other metabolites?

VIIA. Metabolite microcompartmentation: introduction

The mechanism of electron transport phosphorylation outlined here contrasts with the bulk phase chemiosmotic formulation elaborated by Mitchell in the sense that while chemiosmosis predicts an equilibration of the protons involved in electron transport phosphorylation with bulk phase protonic potentials, the present mechanism would suggest a specific channelling of protons along a multi-enzyme (membrane) surface. Such ideas concerning specific channelling by multienzyme complexes using more conventional substrates have also been evolved, and I believe that a comparison of the present ideas with those evolved to account for 'metabolite microcompartmentation' is worthwhile. The theoretical and experimental arguments which favour the occurrence of metabolite microcompartmentation will be reviewed, in the belief that similar approaches to the analysis of the present model of electron transport phosphorylation will prove fruitful.

VIIB. Metabolite microcompartmentation: theory

It is becoming increasingly clear that the cytoplasm of the living cell can not adequately be explained by simple solution chemistry. Atkinson [245] drew attention to the undesirability of large solution concentrations of metabolic intermediates in the living cell, and of the need to conserve solvent capacity. He suggested that relatively high enzyme : substrate ratios, together with low metabolite concentrations, would be an effective way of avoiding large changes in intracellular metabolite concentrations whilst preserving adequate metabolic fluxes. This analysis was lucidly extended by Sols and Marco [246], who showed, inter alia, that the number of intracompartmental molecules

of certain tricarboxylate and glycolytic intermediates are probably barely in excess of their protein binding-sites. Currently, a new paradigm is evolving (e.g. Refs. 104, 114, 247, 248) to meet the challenge of describing and explaining the organisation of the milieu intérieur of the living cell. In the belief that the physicochemical basis for these ideas is similar to that upon which the present model of electron transport phosphorylation must rest, I shall indicate some of the supposed advantages of metabolite microcompartmentation and 'channelling'.

The evolutionary, and hence [249] energetic, advantages of the maintenance of a high degree of spatial order in the cellular cytoplasm have recently been comprehensively reviewed by Welch [247,248]. The advantages inherent to all multienzyme complexes of increased efficiency and control potential have been repeatedly pointed out (e.g. Refs. 247, 250–252). Examples include tryptophan biosynthesis [247,252,253], fatty acid synthesis and oxidation [254,255], glycolysis [256] and the oxidation of pyruvate to acetyl CoA [250]. In each of these cases, metabolic intermediates generated during the synthesis of the end-product of the pathway remain protein-bound, and do not equilibrate with the bulk of the cellular compartment. Welch [247] has emphasised the possible extensiveness of this phenomenon, and it has been suggested [256] that the protein-bound nature of glycolytic and other low molecular weight metabolites is largely responsible for the finding (e.g. Refs. 110, 112, 257) that the apparent diffusion coefficients of these compounds within the cell are several orders of magnitude different from those for the same substance in aqueous solution. "Thus the use of multienzyme complexes with restricted diffusion paths may be forced on the cell by the need to achieve efficient function at very low free metabolite concentrations" [256].

The possible advantages of both soluble and membrane-bound multienzyme aggregates which 'channel' metabolites, compared with those which allow metabolite equilibration with the bulk intracellular pool include the following [252]:

"1. A decreased diffusion time for the intermediates concerned;

2. Competition with other pathways is minimised by keeping an intermediate in a limited microenvironment;

3. The restriction to a microenvironment of a few molecules of an intermediate can present an effectively high concentration to the next active site;

4. The protein-protein interactions of a complex may be necessary for control features of the system;

5. Unstable intermediates may be protected;

6. Specific environments of varying nature (hydrophobicity) can be created to enhance specific reactions."

The application of these heuristic principles to delineating and investigating the present model of protonic coupling in electron transport phosphorylation is thus analogous and obvious. It may be noted that the major 'competing reaction' of energised protons is their neutralisation by acids and bases of the appropriate pK. Therefore whereas Mitchell [23,258,259], in recognising the importance of the membrane in electron transport phosphorylation, stressed that this importance was in separating electro-osmotic gradients, reasoning based on the supposed advantages of multienzyme complexes (1–6 above) leads me to suggest that such membrane-bound systems possess an important and intrinsic additional advantage: that of allowing subtle control and channelling of the protons released by electron transport and consumed by ATP synthesis and other energy-requiring processes. It is also of interest to note the efficiency of localised coupling systems emphasised in a socioenergetic context [260,260a].

VIIC. Metabolite microcompartmentation: evidence

Turning to the evidence concerned with the demonstration of intracellular compartmentation in unicellular and other 'single-compartment' systems, I would begin by noting that it is based on essentially three types of experiment. The first type studies the specific rate of incorporation of an isotopically labelled metabolite into one or more metabolic pathways sharing a common intermediate which is supposed to exist in 'free' solution in the cellular compartment. The expected specific activity may be calculated for each intermediate as a function of time from the actual activity of label, the metabolic fluxes and the pool sizes, using standard methods. It is clear that the specific rate of incorporation into the pathway will vary if the endogenously synthesised intermediate does not equilibrate with the intracellular intermediate pool derived from the exogenous (labelled) source. Such an approach has often been used in studies of energy metabolism (e.g. Refs. 240, 261–267). In each of these studies (and see Refs. 247, 252) it was established that a single membrane-limited cellular compartment (e.g. cytosol, mitochondrion, intact unicell) could not account for the partitioning of label into the different fractions with the assumption of a homogeneously dispersed pool of metabolites. It is also possible to explain in a similar fashion the recent interesting but puzzling data of Stubbs et al. [268] on the basis of a 'microcompartmentation' or functional interaction between adenine nucleotide translocase enzymes and specific ureogenic and gluconeogenic enzymes (cf. Ref. 267). This type of 'competition' approach, using buffering of the bulk aqueous phases, has been used to study the proton current pathway in spinach chloroplast thylakoids [133], while the 'functional approach' surveyed above would also fall into this class of experiment (see, for example, Refs. 12, 231, 242).

The second type of experiment is based upon magnetic resonance studies of energy-transducing systems. Briefly, the nuclear magnetic resonance (NMR) spectrum of a particular atom reflects the molecular and cellular environment in which that atom resides, and both the sharpness and the transverse relaxation time(s) of the signal reflect the homogeneity of this environment (e.g. Ref. 269). Thus, for a given metabolite, it is possible to study, in a nondestructive manner, the microheterogeneity of a supposedly homogeneous metabolic pool. Such methods (e.g. Ref. 110) have been used to determine the extent of water structuring and alkali metal cation complex formation in living tissues. It has been claimed from such studies [110] that the idea of a homogeneous pool of metabolites within a given membrane-bound cellular compartment is no longer tenable. It is of particular interest in the present context that Lange et al. [270] (and cf. Ref. 270a) have used high-resolution proton NMR to establish that phosphatidylethanolamine micelles may channel protons rapidly along their surface without permitting their equilibration with the bulk phase solvent protons. The potential of NMR methods for analysing recalcitrant molecular problems of interest to bioenergeticists has been exemplified by a number of recent papers (for example, Refs. 86 and 271–277).

The third type of experiment is based upon the analysis of the rates of metabolite fluxes catalysed by carefully isolated enzyme aggregates compared with those catalysed by their component enzymes. In each case, the channelling of metabolites along the enzyme's surface, within the double layer [278], results in a much more rapid and efficient transformation of substrates. For examples of such analyses the reader is referred to the papers of Welch [247], Mowbray and Moses [256], Matchett [279] and Koch-Schmidt et al. [280].

Lastly, I would draw attention to the work of Zeuthen [280a], who has given a direct demonstration of microcompartmentation in *Necturus* gallbladder cells by the use of ion-sensitive microelectrodes.

I now conclude with a reinterpretation of some of our own findings, which I tried to interpret at the beginning of this review within the framework of the chemiosmotic theory, by reconsidering them within the electrodic theory set out above.

VIII. An electrodic explanation of some anomalous results given earlier

According to the electrodic formulation elaborated in the preceding sections, then, the functional protonic potential gradients of electron transport phosphorylation are associated with the membrane/solution interfaces, and protonic potential differences measured between the two bulk aqueous phases should not possess the energetic properties of a coupling intermediate between electron transport and ADP phosphorylation. The extent to which the anomalies we have observed during measurement of the bulk-phase protonmotive force in parallel with other reactions can be accommodated within an electrodic view will now be reviewed.

In the case of chromatophores from *R. rubrum* it was noted above that there was a significant discrepancy between the value of the $\rightarrow H^+$/ATP ratio calculated from the thermodynamic comparison of Δp and ΔG_p [42] and that obtained [47] using spectroscopic analysis of the effect of ADP on the decay of pH changes following single-turnover flashes of light. The latter measurements were carried out in the presence of a high concentration of 'charge-neutralising' ions such that it was to be expected that all protons which were moved vectorially as a result of electron transport left the bulk phase external to the chromatophores suspension. However, if the native proton current flow is largely or wholly confined to the interphase Stern layers adjacent to the chromatophore membrane, it would indeed be expected that under well-buffered conditions such as our own, lacking significant concentrations of membrane-permeable ions [42] and in which microscopic charge-neutralisation was not complete, there would be no correspondence between the value of the $\rightarrow H^+$/ATP ratio measured by the two types of method.

In the case of bovine heart submitochondrial particles, the $\rightarrow H^+$/ATP ratio of 3 calculated by comparing the Δp and ΔG_p values generated by NADH oxidation [45] was greater than that obtained by direct glass electrode measurements following the hydrolysis of a pulse of ATP [174,175]. This anomaly is explained economically by the suggestions outlined in the current model: that the functional proton current of electron transport phosphorylation occurs in the interphase close to the membrane surface, and that charge-neutralising ions (as present in the pulse methods) act to cause ejection of protons into the bulk aqueous phase, whilst comparison of ΔG_p with a Δp measured by assessing the distribution of permeant hydrophilic solutes leads to an erroneously high value for the 'true' $\rightarrow H^+$/ATP ratio.

Further, in the case of bovine heart submitochondrial particles [45], it was found that the value of Δp varied markedly with the type of incubation medium used, without a corresponding variation in ΔG_p. This behaviour is not expected if bulk phase ion gradients are important in determining the thermodynamic extent of ATP synthesis. If, however, the functional proton current is largely excluded from the bulk aqueous phases, the parameter measured by the distribution of hydrophilic solutes would not be expected to bear a constant relationship to the phosphorylation potential attained, for changes in the structure of the Gouy-Chapman layer, or of the total buffering power of the bulk aqueous phases, must alter seriously the bulk phase protonmotive force without necessarily affecting the protonmotive force across the membrane itself. An independence between the bulk phase protonmotive force and the phosphorylation potential generated by bovine

heart submitochondrial particles was also found in the case of proton movements generated by electron transfer in the terminal region of the electron transport chain. Even on those occasions in which the measured protonmotive force generated by TMPD-mediated ascorbate oxidation was as high as that generated by NADH oxidation, a significant phosphorylation potential was not generated. This observation is explained economically if the functional proton current of electron transport phosphorylation is carried not in the bulk aqueous phases but along relatively localised channels at the membrane surface. The extent of the slow leak(s) into the bulk phases, which is what is measured by the flow dialysis assay of the distribution of hydrophilic solutes, would be expected under varying conditions of electron transport, and could occur quite independently of the specific current of protons along the membrane surface, which was suggested in this review to be the functional intermediate between the oxidoreduction and hydrodehydration reactions of electron transport phosphorylation. (This latter phenomenon was also observed in the case of *P. denitrificans* vesicles [43].)

Further, 10 mM nitrate added to bovine heart submitochondrial particles did not cause a transformation of the membrane potential component of the protonmotive force generated by TMPD-mediated ascorbate oxidation into a pH gradient, although this concentration of the permeant nitrate ion was sufficient to cause the formation of a pH gradient when submitochondrial particles were oxidising NADH under similar incubation conditions [45]. This result is fully consistent with the postulate that a rather specific proton pathway, close to the membrane, is responsible for providing the intermediate driving force in ADP phosphorylation, and, as with the inability of TMPD-mediated ascorbate oxidation to generate a significant ΔG_p, suggests that the functional pathways are different for NADH and ascorbate/TMPD.

If the ratio of protons released by the oxidation of one NADH molecule to those released by the oxidation of one succinate molecule is 3 : 2 [23] and the rate of succinate oxidation to NADH oxidation in these particles is 0.7 : 1 [281], the rate of proton translocation when NADH is the substrate would be approximately twice that when succinate is the substrate. This ratio of the rates of proton translocation with the two substrates is parallelled by the relative rates of ATP synthesis driven by the oxidation of these substrates [281]. However, both the phosphorylation potential attained and the protonmotive force generated as a result of the oxidation of these substrates under static head conditions are similar with the two substrates. This result, which is not expected in terms of an equilibrium thermodynamic hypothesis, can again most easily be explained by the existence of specific proton pathways between electron transport and phosphorylation, but is not expected if a bulk phase protonmotive force be the link between electron transport and ADP phosphorylation in a reversible system [45,46].

The variability of Δp without a corresponding variation in ΔG_p was also observed in the case of *P. denitrificans* vesicles [43]. The ability of the nitrate ion in this system to decrease the protonmotive force, as measured across the bulk aqueous phases, to an undetectable level, whilst having no effect upon the phosphorylation potential generated can not be explained on thermodynamic grounds, even in an apparently 'irreversible' system [282], if a bulk phase protonmotive force be required for the synthesis of ATP. The ability of low concentrations of uncoupler to raise the apparent $\rightarrow H^+/ATP$ ratio has been observed by other authors in bacterial chromatophores [12] and in submitochondrial particles [48], whilst low uncoupler concentrations affected the rate of ATP synthesis without decreasing the protonmotive force in the *P. denitrificans* vesicles [49]. None of these observations are easily accommodated within the framework of a requirement for a bulk

phase transmembrane proton gradient as a functional intermediate between electron transport and ADP phosphorylation, but may be explained within the context of an electrodic view, for in the latter formulation no strict relationship is expected between bulk phase transmembrane proton gradients and either the rate or extent of ATP synthesis.

IX. Summarising remarks

In this review I have offered some speculations concerning molecular determinants of the pathway of the transmission of electrochemical proton gradients associated with energy-transducing biomembranes. It leans heavily upon the chemiosmotic and 'localised proton diffusion pathway' hypotheses developed by Mitchell and by Williams, and represents an attempt to bring together a number of controversial experimental observations which appear mutually inconsistent. The electrodic view, as I have called it, is 'chemiosmotic' in the sense that transmembrane-phase proton gradients are involved, but is not chemiosmotic in the sense that the main functional proton current is not carried in the bulk aqueous phases on either side of the membrane. Local interphase phenomena, based on generally accepted physical, chemical and electrochemical principles, have been stressed, and a number of predictions that are susceptible to experimental analysis have been presented. An analogy between localised protonic coupling and localised metabolite transformations has also been drawn.

Note added in proof (Received April 4th, 1979)

Since this review was written a number of other relevant papers which merit explicit citation have come, or have been brought, to my attention.

(1) Dimensionality. The important paper by Adam and Delbrück [283] discussed in Ref. 247, derives quantitative diffusion equations that show how, even in the absence of favourable field or medium effects, the rate of diffusion of molecular species from sources to sinks is much faster along an interface than through three dimension for systems the size of a cell. A similar conclusion for proton transfer through thick protein membranes is given by Zabusky and Deem [284]. In a most eloquent article, Mikulecky (Ref. 285 and references therein) has drawn attention to the great utility of the Network thermodynamic method in the analysis of bioelectrochemical circuits of the type presently under consideration.

(2) Kinetic, thermodynamic and capacitative properties of protonmotive systems. It has kindly been brought to my attention by Dr. P. Mitchell (personal communication) that my treatment in subsection VC may be misleading, since I do not define the protonic capacitance. For a given kinetically homogeneous phase containing an electrochemical potential of protons, adjacent to other such phases, we may define the protonic capacitance of the first phase:

$$C_X = \left(-\frac{\partial(\rightarrow H_x^+)}{\partial(\Delta\bar{\mu}_{H^+})_x}\right)$$

For the separation of thermodynamic and kinetic factors in the analysis of H^+ transfer between sources and sinks in protonmotive energy-coupling systems it will be useful to distinguish thermodynamic and temporal (kinetically competent) coupling pathways. Whilst the term 'uncoupling' is useful in describing the lack of such a pathway in the thermodynamic sense, it is likely to be obfuscatory in the latter case, and the term

'decoupling' is proposed, as it seems to express nicely the fact that time must elapse between a species leaving its source and arriving at its sink.

(3) Chloroplast thykaloids. Schuurmans et al. [286] have shown that the extrinsic probe Oxonol-VI detects electrical potential changes on the same timescale (10–20 ms) as covalently-bound acridine probes of surface potential, amounting, in the steady state, to 50 mV. Vinkler et al. [287] have shown that the initial formation of ATP during single-turnover flashes is *decoupled* from a bulk-phase proton gradient formation. In the book 'Light-Transducing Membranes' both Hope [288] and Chow et al. [289] have presented evidence of a similar nature, and which they have interpreted in a manner in broad agreement with the present proposals.

(4) Chromatophores. Two relevant papers on photophosphorylation by *R. rubrum* chromatophores have recently appeared. DelValle-Tascon et al. [290] have chosen a similar interpretation to that presented here to unify our own observations with their experiments. Bashford et al. [291] have suggested that $\rightarrow H^+/ATP$ is 2 on the basis of a comparison of the phosphorylation potential with the protonmotive force under static head conditions. They calibrated the response of the probe Oxonol-VI by comparison with the carotenoid band-shift obtained under similar conditions, which itself was calibrated using potassium diffusion potentials. In view of the arguments presented in Ref. 226 and in the present article, it is concluded that their value for the $\rightarrow H^+/ATP$ ratio, by comparison with that obtained by us, is most easily harmonised within the framework of the general proposals outlined here.

(5) Oscillatory phenomena. Should there be doubt that there is not equilibrium during charge transfer across interfaces in working electrochemical systems, it is germane to draw attention to the well-known oscillations in interfacial charge (K^+ and H^+) transfer exhibited by mitochondria under appropriate incubation conditions (see, for example, Ref. 292). Such oscillatory reactions may occur only in systems far from equilibrium.

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