The current–voltage relationships of liposomes and mitochondria

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1. Current–voltage relationships were determined for various membrane systems. We show that phospholipid and mitochondrial membranes exhibit linear relations between H^+ flux and pH gradients. 2. These membranes, however, exhibited non-linear relationships when the applied voltage was a membrane potential. The current–voltage relationship approximated to an exponential function. 3. This relationship was found to be linearized when the membranes were treated with an electrogenic proton ionophore. 4. The incorporation of cytochrome *c* oxidase (EC 1.9.3.1) was found to have no effect on the current–voltage characteristics of the phospholipid membranes. 5. When a membrane potential of more than 140 mV was imposed across vesicular and mitochondrial membranes, they exhibited reversible di-electric breakdown. This phenomenon was correlated with the requirement of a permeant ion for the experimental demonstration of proton translocation by so-called 'proton pumps'.

The chemiosmotic hypothesis (Mitchell, 1968, 1981) has successfully predicted that bioenergetic organelles and bacteria are relatively impermeable to ions in general and protons in particular. Surprisingly, however, very few studies have been made of the latter aspect of this most fundamental postulate, namely the membrane semi-permeability to protons. The most comprehensive study of the proton permeability of mitochondria has been carried out by Mitchell & Moyle (1967). They showed that the membranes of normally prepared mitochondria are relatively impermeable to protons. Furthermore, it was also concluded that the rate of the net proton flux depends linearly on the magnitude of the imposed proton gradient, within the range of pH gradients employed (0–0.1 pH unit). Mitochondrial membranes, therefore, apparently behaved according to Ohm's law. This view was shown to be also the case for phospho-

Abbreviations used: CCCP, carbonyl cyanide *p*-chloromethoxyphenylhydrazone; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; pyranine, 8-hydroxypyrene-1,3,6-trisulphonate; PLVs, phospholipid vesicles; COVs, cytochrome oxidase vesicles.

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lipid (soya-bean phosphatidylcholine) membranes (Kell & Morris, 1980). In the latter, it was shown that the phosphatidylcholine vesicles behaved ohmically when a pH gradient across the membrane of more than 4 units was engineered. Analogous studies, made by Arents *et al.* (1981), led to a similar conclusion, although the imposed pH gradients were much smaller.

More recently, Jackson (1982) has measured the current–voltage relationships of bacterial chromatophores. Jackson (1982) elegantly demonstrated that the rate of ion flux into bacterial chromatophores (the current) does not exhibit a linear dependence on the membrane potential (the voltage), that is, a non-ohmic relationship. The latter findings are more in accord with the long-held view that a non-ohmic current–voltage relationship is a normal property of both artificial and natural membranes if a critical voltage is exceeded (see, for a review, Haydon, 1970). It is also worthwhile, at this point, to remember that neurophysiologists have long known of non-ohmic current–voltage relationships operating in nervous and muscle tissue (Hodgkin & Huxley, 1952).

It remains, from the above considerations, unclear as to the relationship between the electrochemical potential gradient and the rate of its equilibration, for membranes associated with bioenergetic functions, e.g. mitochondria. Furthermore, the artificial membrane systems used to study reconstituted membrane proteins have not

been characterized with respect to their current-voltage responses. Unfortunately, these are not trivial points. The unequivocal demonstration of either ohmic or non-ohmic behaviour with native biological membranes and/or reconstituted systems are quite relevant for the interpretation of experimental studies of oxidative and photosynthetic phosphorylation.

Most of the studies described above have been made with small pH jumps. Furthermore, none of the above-mentioned studies, with the exception of the experiments performed by Jackson (1982), included any consideration of an electrical potential difference driving proton uptake or efflux. As the membrane potential is possibly one of the most important parameters associated with energy coupling, some knowledge of the electrical current-voltage characteristics of the membranes would be useful. The present study, therefore, attempts to shed light on the effect of physiological membrane potentials and pH gradients on the membrane conductivity.

Reagents and preparations

Biochemical reagents were of the highest purity commercially available. Pyranine was obtained from the Eastman-Kodak Chemical Co.

Crude asolectin was purified by the method of Kagawa & Racker (1971), but with an additional step of stirring the crude asolectin with Al_2O_3 to bind peroxy compounds, which we have found to be a contaminant of commercially available crude lipid preparations. This step was followed by filtration to remove the Al_2O_3 together with the contaminating peroxides.

Cytochrome oxidase was purified by the method of Yu *et al.* (1975). Cytochrome oxidase protein was determined by application of the modified Lowry procedure (Wang & Smith, 1975) in which EDTA is used to mask interference of the detergent.

Rat-liver mitochondria were prepared by the method of Chappell & Hansford (1972). Mitochondrial protein content was assayed by using the biuret method (Gornall *et al.*, 1949).

The phospholipid vesicles (PLVs) and the cytochrome oxidase vesicles (COVs) were prepared by the so-called 'cholate-dialysis' procedure (see, e.g., Casey *et al.*, 1979). The advantage of such a method allows a medium of predetermined composition to be incorporated into the vesicle interior.

The starting materials for the vesicle preparations consisted of the following: 120mg of purified asolectin, 0.1 mM-KCl, 96 mM-choline chloride, 30mg of potassium cholate, 5 mM-Hepes, 0.5 mM-pyranine and 0.1 mM-EDTA, made up to 3.0 ml.

The pH of the mixture was adjusted to 6.80. The general procedure of Casey *et al.* (1979) was slightly modified to include the following step: after sonification the microdisperse suspension of lipid was centrifuged, at full speed for 2 min, in an Eppendorf bench centrifuge to precipitate any particles of titanium which may have sheared off the sonifier probe. All the remaining steps were essentially as described by Casey *et al.* (1979).

The first dialysis step was carried out against 100 vol. of the sonification medium, excluding the cholate. The two remaining dialysis steps were each carried out against 200 vol. of 0.1 mM-KCl/96 mM-choline chloride/5 mM-Hepes, pH 6.8.

Preparations were also made of COVs and PLVs containing SO_4^{2-} as the major anion. In these cases the vesicles had an internal ion composition of 0.1 mM-KCl, 64 mM- Li_2SO_4 and 5 mM-lithium Hepes. The dialysis media, therefore, were also adjusted accordingly.

There was no difference between the preparative procedure of the COVs with respect to the PLVs, except for the addition of purified freshly thawed cytochrome oxidase to a final concentration of 7.5 μM -haem *a* after the lipid had been sonicated and centrifuged.

All the dialysis steps were carried out at 4°C. Care was taken to protect the preparations from light by binding aluminium foil around the dialysis vessels.

The PLVs and the COVs were prepared in the presence of 0.1 mM-KCl and thus were assumed to contain 0.1 mM-KCl.

For experimental measurements, suspensions of COVs, PLVs or mitochondria were suspended in media containing various concentrations of K^+ (up to 110 mM).

The intra- and extra-mitochondrial K^+ concentrations were measured by atomic-absorption spectrophotometry.

Use was made of the pH-sensitive membrane-impermeant fluorescence probe pyranine (Clement & Gould, 1981). It was therefore possible to measure quickly and accurately the intravesicular pH by observing changes of pyranine fluorescence. The excitation wavelength was 460 nm. The emission wavelength was 510 nm.

In the case of the mitochondrial experiments, no pyranine was used.

Extravesicular and extramitochondrial pH changes were measured by using a combined pH electrode.

The experiments were carried out in a Perkin-Elmer spectrofluorimeter equipped with a stirring device. The reaction volume was 2.5 ml.

Periodically the pH electrode was etched with HF to increase the stability and response time. The half-maximum response time was typically less

than 1 s. The pH electrode was accurate to within 0.005 pH unit.

Voltage changes of the combined measure and reference pH electrode, as a result of pH changes, were amplified and displayed by using a Radiometer (Copenhagen) digital pH-meter, the pre-amplified output of which was further amplified and displayed on a two-channel chart recorder. A similar arrangement facilitated the output of the fluorimeter to be amplified and recorded.

The preparations and protocol for the experimental demonstration of proton translocation by cytochrome oxidase vesicles were as previously described (Casey *et al.*, 1979).

Experimental protocols and results

In addition, to the investigation of the current-voltage relationships of phospholipid vesicles and mitochondria, it was considered worthwhile to investigate the effects (if any) of a reconstituted membrane protein such as cytochrome oxidase (EC 1.9.3.1) on the permeability properties of the membrane. The protocol for the preparation of the COVs was identical with that for the PLV preparation (see the Reagents and preparations section).

The composite Fig. 1 indicates how the results shown in Figs. 2 and 3 were obtained. Mitochondria or vesicles were suspended in media containing different known K^+ concentrations. With the addition of valinomycin to the suspension, a pre-determined membrane potential was developed. This is due to the electrogenic equilibration of the K^+ gradients across the vesicular/mitochondrial membranes catalysed by valinomycin (Henderson *et al.*, 1969). At this point other ions move in an attempt to compensate, and ultimately equilibrate the membrane potential. We considered the most likely ion to equilibrate the induced potential difference to be the proton. Thus the intra- and extra-vesicular pH was measured. By using intravesicular pyranine fluorescence as a pH probe, it was possible to measure the exchange of K^+ for protons as the protons electrically compensate the K^+ loss from the external phase. Although the pyranine fluorescence response was found to be linear over a range of about 1 pH unit, it was not possible to measure pH changes, with pyranine, of the order of 2 units. This limitation, however, did not hinder the use of pyranine for our purposes. This was considered a safe conclusion, as only initial rates were measured during the course of these experiments (see Fig. 1). Under these circumstances the pyranine response was both very rapid and linear. Furthermore, a pH electrode was used to measure the appearance of the protons in the external medium. The results obtained with pyranine and the pH-electrode results were found to be self-consistent.

Fig. 1. Measurement of intra- and extra-vesicular pH

The above trace indicates the general response of intravesicular pyranine fluorescence to intravesicular pH changes caused by extravesicular pH jumps or the induction of a membrane potential. The exciting wavelength was 460 nm and the emission (measuring) wavelength was 510 nm. The initial rate of change of the fluorescence was measured after the system was perturbed in a variety of different ways. This involved inducing acid or alkaline pH jumps and membrane potentials of different magnitudes and polarities (for example, at the arrow in the above trace, valinomycin was added to a suspension of PLVs with a low internal K^+ concentration suspended in a medium of a high K^+ concentration; a downward deflection of the trace indicates increasing intravesicular pH). The extravesicular pH initial rate of decay to equilibrium was also measured with a pH electrode. This facility allowed the measurement of both the intra- and extra-vesicular pH changes and under all circumstances they were found to be self-consistent.

It was considered feasible, however, that other ions, e.g. Cl^- , could move, which would obviously complicate any possible interpretations. Such phenomena were, however, discounted when control experiments (results not shown) were carried out with SO_4^{2-} as an impermeant anion. As the results were identical in both cases, it was concluded that protons were the only ions moving, initially to equilibrate the membrane potential.

The usual direction of the potassium gradients across the vesicular membrane was where the extravesicular $[K^+]$ was higher. With the addition of valinomycin, protons would tend to move, therefore, from the internal phase to external phase to compensate for the electrogenic K^+ influx.

Experiments were also performed (results not shown) with the K^+ gradients across the vesicular membrane of opposite polarity. The results obtained were very similar to the results shown in the Figures, although it was not possible to induce the largest membrane potentials.

In addition to the measurements of electrically induced pH changes, pH jumps were also initiated. This allowed a direct comparison of the two driving forces for pH equilibration.

The maximum pH gradient imposed in the pH-jump experiments was 2.8 pH units. Under these circumstances the current-voltage relationship was linear, although the observations of Kell & Morris (1980) indicate that linearity still exists with much larger pH gradients.

The pH-jump experiments were also repeated with SO_4^{2-} as the major anion (results not shown). The results of these experiments were identical with those obtained with Cl^- .

Similar experiments to those with the phospholipid vesicles were performed with mitochondria; however, no pyranine was included and proton movements were measured solely with a pH electrode.

Membrane potentials were generated across the mitochondrial membrane in an analogous way to that of the COVs and PLVs, that is, use was made of the endogenous intramitochondrial K^+ concentration, which was of the order of 110 mM, depending on the mitochondrial preparation.

Fig. 2. Current-voltage relationships of COVs and PLVs

The current (rate of proton influx/efflux to or from the vesicles) at different membrane potentials (voltage) were determined as in Fig. 1. Vesicles, either PLVs (■) or COVs (●) with low internal K^+ concentrations were suspended in iso-osmotic media of different K^+ concentrations up to 100 mM. Iso-osmolarity was maintained with choline chloride. Membrane potentials were induced by the addition of $0.9 \mu\text{M}$ ethanolic valinomycin. Acid or alkaline pH gradients (□) were imposed by the addition of quantities of 0.1 M-HCl or 0.1 M-NaOH respectively. Under no circumstances was the ambient pH allowed to go above pH 9.0 or below pH 4.5. The reaction volume was 2.5 ml and included 4 mg of phospholipid as PLVs or as COVs. The ambient temperature was 27°C .

To obtain the experimental situation for the mitochondria analogous to that in the vesicle system, mitochondria must be inhibited with cyanide (or the experiments may be performed anaerobically). Furthermore, oligomycin and EGTA were included to discount any 'artefactual' proton production/consumption catalysed by the $\text{F}_1\text{-ATPase}$ and Ca^{2+} movements respectively, which would have complicated the measurements.

When specific membrane potentials are imposed across the vesicular or mitochondrial membrane, and the initial rates of proton movement measured (as in Fig. 1), then current-voltage plots may be constructed as in Fig. 2 (for vesicles) and Fig. 3 (for mitochondria). The current that initially collapsed the membrane potential (the voltage) was considered to consist only of aqueous protons.

When considering the current-voltage relationships, whereupon the voltage was a pH gradient, two further conditions were satisfied. The first was the prevention of a diffusion potential developing. Valinomycin was therefore present before the initiation of the pH jump. As valinomycin was also present during the course of the electrical measure-

Fig. 3. Current-voltage relationships of rat liver mitochondria

The experimental conditions were as follows: rat liver mitochondria (2 mg/ml), 2 mM-Hepes, 2 mM-cyanide, 0.5 mM-EGTA and $1.0 \mu\text{M}$ -oligomycin. Experiments were carried out at 27°C . Various media were composed of choline chloride/KCl mixtures that were iso-osmotic with the intramitochondrial space such that with the addition of $1.0 \mu\text{M}$ ethanolic valinomycin a known membrane potential would be developed (●). The intramitochondrial K^+ concentrations varied between 100 and 114 mM. Experiments were also performed with media supplemented with $0.5 \mu\text{M}$ -CCCP (■). Acid or alkaline pH gradients (mV) were imposed (□) by respective additions of quantities of 0.1 M-HCl or 0.1 M-NaOH .

ments, the experiments were directly comparable. Furthermore, the acid and alkaline pH jumps gave identical results. This would not be the case if a diffusion potential were developed, since the potentials would have different polarities. Secondly, changes in surface potential may affect the conductance properties of the membrane. The surface-potential effects, however, may be discounted for two reasons. Firstly, the ionic strength is relatively high in the two compartments. Secondly, the buffering capacity of the phospholipid was relatively constant with pH. Both the latter conditions imply that surface-potential effects were not a cause of any artefacts. It must be pointed out, however, that there will be a differential surface-potential effect on the imposition of a membrane potential. This effect was considered to be part of the conduction mechanism and therefore was not corrected for.

In addition to the above-described experiments, studies were also performed in the presence of an uncoupler (Fig. 4). The experiments were carried out exactly as described above, but the reaction medium was supplemented with the electrogenic proton-ionophore, CCCP. A complete current-voltage determination was then carried out for different concentrations of uncoupler (results not shown). The effect of the presence of the uncoupler was to inaugurate an ohmic current-voltage response. With each increment in the CCCP concentration the current-voltage response of the membranes further approached linearity. Fig. 4 indicates the final CCCP concentration used. Moreover, a similar effect was observed (results not shown) if the optimal valinomycin concentration was greatly exceeded, i.e. when the valinomycin/lipid ratio was of the order used by Arents *et*

al. (1981). Similar experiments were performed with mitochondria, with similar results (Fig. 3).

Permeability coefficient calculations

Table 1 shows the calculated proton/hydroxide [$P(\text{net})$] permeability coefficients of the various systems studied. They were calculated assuming a mean packing area in the outer bilayer per asolectin molecule of 0.74 nm^2 ($74 \text{ \AA}^2$). The area of the mitochondrion was calculated by using the values given by Mitchell & Moyle (1967).

The $P(\text{net})$ values for mitochondria and phospholipid vesicles obtained in the present work may be compared with those obtained by other groups (see Table 1). It was possible to estimate, from the data given by Mitchell & Moyle (1967), that the $P(\text{net})$ value for rat liver mitochondria is approx. 10^{-3} cm/s . This value is in good agreement with the value of $0.8 \times 10^{-3} \text{ cm/s}$ calculated from our measurements. The $P(\text{net})$ values for the asolectin vesicles both with and without the inserted cytochrome *c* oxidase can be compared with the values given by Nichols & Deamer (1980). It was again also possible to estimate a $P(\text{net})$ value from the data given by Kell & Morris (1980). It is noteworthy that all the $P(\text{net})$ values are relatively high when compared with the coefficients of other ions (Nichols & Deamer, 1980). The slight disagreements (*cf.* Nichols & Deamer, 1980) may be attributed to the different types of lipid used for the vesicle preparations.

It is interesting that the insertion of cytochrome *c* oxidase into the phospholipid membrane (see Fig. 2) does not appear to have any effect on the membrane ionic permeability. It would seem, therefore, that COVs are a good model system for vectorial studies of cytochrome oxidase.

The $P(\text{net})$ values obtained for the electrically induced pH gradients are quite similar (up to about 130 mV) to those obtained with the pH-jump experiments. With imposed voltages of 130–150 mV, however, the membrane apparently began to become much more permeable to protons. At even higher voltages the membrane exhibited a short-circuit response and the protonic conductance increased many-fold. This behaviour is reminiscent of the current-voltage responses of a class of semiconductors used in electronic-circuit theory known as Zener diodes.

The significance of the non-ohmic current-voltage relationship to studies of electrogenic membrane proteins

Figs. 2–4 show that the permeability to protons dramatically increases if the membrane potential exceeds about 150 mV. Such potentials, if allowed to be developed, would seriously interfere with studies of the vectorial properties of reconstituted

Fig. 4. Effect of a proton ionophore (CCCP) on the current-voltage relationship of PLVs

The experimental conditions were as described in Fig. 2 (●). In addition, experiments were also performed in the presence of $1.0 \mu\text{M}$ -CCCP (■).

Table 1. *Permeability coefficients of the various systems studied*

Values were calculated by assuming a mean packing area in the outer bilayer of phospholipid vesicles of 0.74 nm^2 ($74 \text{ \AA}^2$). The area of the mitochondrion was as given by Mitchell & Moyle (1967).

Membrane system	Permeability coefficient [$P(\text{net})$] (cm/s)	Reference
Soya-bean asolectin	3.0×10^{-4}	The present study
Mitochondria	0.8×10^{-3}	The present study
Egg phosphatidylcholine	1.7×10^{-4}	Deamer & Nichols (1983)
Mitochondria	$1.0 \times 10^{-3}\dagger$	Mitchell & Moyle (1967)
Soya-bean phosphatidylcholine	$1.0 \times 10^{-4}\dagger$	Kell & Morris (1980)
Soya-bean asolectin	$3.0 \times 10^{-4*}$	The present study

* Calculated from proton movements as a result of the induction of a membrane potential of less than 130 mV.

† Calculated from the results given in the paper.

electrogenic proteins. For example, the presence of a large membrane potential would seriously hamper the measurement of the number of protons pumped by cytochrome oxidase during limited turnover studies (see, e.g., Casey *et al.*, 1979). The vectorial process would thus be underestimated, owing to the very rapid return of the pumped protons to the vesicular interior.

Fig. 5 shows that there is a general requirement, as pointed out by Mitchell (1968), for some permeant ion to compensate for the electrical potential difference generated by a proton-pumping enzyme such as cytochrome oxidase if the stoichiometry of the reaction is to be measured.

With the addition of ferrocyanochrome *c* to an aerobic suspension of COVs, in the presence of a permeant ion, there was a rapid acidification of the extravesicular medium (Fig. 5*a*, curve 2). This phenomenon may be attributed to a net proton translocation across the membrane, associated with the cytochrome oxidase reaction (Casey *et al.*, 1979). In this case, K^+ catalysed by valinomycin were used as a charge-compensation system, although in principle any permeant charged species may be used. The number of protons pumped per electron transported usually approaches 1.0 in such experiments.

If, however, there is no adequate charge compensation, the pH becomes alkaline at a rate greater than, or comparable with the uncoupled rate (see Fig. 5*b*). The pH change then rapidly reflects the net alkalization reaction of the consumption of one proton per ferrocyanochrome *c* molecule oxidized (also shown by the uncoupled reaction in Fig. 5*a*, curve 1).

It would be instructive to consider what is responsible for this phenomenon. If one assumes that COVs (and coupling membranes in general) behave as spherical capacitors, then the voltage generated across the membrane as a consequence of the vectorial reactions may be calculated. For

Fig. 5. Requirement of a permeant ion for the demonstration of proton translocation by reconstituted COVs

Experiments were performed in the following media: (b) COVs (4 mg of lipid/ml) were suspended in 75 mM-choline chloride/25 mM-KCl/50 μM -Phenol Red, at pH 7.4 and 25°C. (a): 1, Supplemented with 0.5 nM-valinomycin and 0.5 μM -CCCP; 2, supplemented with 0.5 nM-valinomycin. Ferrocyanochrome *c* was added (at the arrows) sufficient for one turnover of each cytochrome oxidase molecule present in an active orientation. The time required for the half-time of the decays were: (a) 1, 0.8 s; (a) 2, 5.8 min; (b), 1.1 s. The traces show Phenol Red absorbance at 556.5–504.5 nm. A downward deflection of the trace indicates extravesicular acidification. The uncoupled (a)1 reaction indicates the consumption of 1 proton per added electron as ferrocyanochrome *c*. Further details may be found in Casey *et al.* (1979).

the calculation we assume two charges to be abstracted from the vesicular interior per ferrocytochrome *c* molecule oxidized. The calculation also assumes that in the absence of a permeant ion there is no initial charge compensation. The capacitance is taken as $1.0 \mu\text{F}/\text{cm}^2$ (Pethig, 1979) and to be invariant. The vesicular sizes and areas are the same as those used in the calculations of the $P(\text{net})$ values.

The calculation yielded a value for the generated membrane potential of about 300–350 mV for one turnover of the enzyme ensemble. It is clear that if such a potential or, indeed, even half this value, were to be developed, then the short circuit region of the current-voltage relationship would be entered. We would predict, therefore, that this would manifest itself as a rapid alkalization on addition of ferrocytochrome *c* sufficient for one turnover per oxidase molecule. Fig. 5(b) shows that this phenomenon was indeed observed. The rate of alkalization is about the same as the uncoupled rate shown in Fig. 5(a), curve 1. Fig. 5(b) also indicates that protons are responsible for the charge-compensation phenomena. Protons appear to be much more permeable than other ions, although in principle other ions should also be able to collapse the membrane potential. The pH relaxation rate should, therefore, also reflect the rate of attainment of equilibrium of any other displaced ions. It does not, so one may conclude that no displacement has taken place.

It is clear from the above considerations that care must always be taken to dissipate any membrane potential when making studies of the kind described above.

Discussion

The proton electrochemical potential difference (divided by the Faraday constant), the so-called 'protonmotive force' (Δp), between two phases is normally considered to consist of two components (Mitchell, 1968): an electrical quantity, the membrane potential, and a chemical quantity, the pH gradient. Although there is a thermodynamic equivalence between the two components, there is not an equivalence of their respective effects on the membrane conductivity to protons.

It is clear from the data shown in Figs. 2 and 3 that only in the case of pH gradients do membranes exhibit a linear 'ohmic' response to the rate of net proton flux with respect to the magnitude of the pH gradient across the membrane.

In the cases of an electrical gradient driving the proton flux, there is not a linear current-voltage relationship. In fact the relationship may be described by an exponential function of the form of eqn. (1):

$$I = k_1 (e^{k_2(v)} - 1) \quad (1)$$

Where I is the current, v is the voltage, and k_1 and k_2 are constants. There is, however, an approach to linearity over certain regions of the current-voltage plots.

It is interesting that eqn. (1) may also describe (curve-fitting not shown) the current-voltage relationships of bacterial chromatophores measured by Jackson (1982). The conductance constants obtained when fitting eqn. (1) to Jackson's (1982) results are similar to those of the present work, although the significance of such a quantification is not at present relevant.

The non-ohmic current-voltage relationship of membranes observed under our experimental conditions could be linearized if the vesicle or mitochondrial suspensions were treated with the electrogenic proton ionophore CCCP, as shown in Fig. 4. This phenomenon was also observed by Jackson (1982) under different experimental conditions. The effect of an electrogenic ionophore on the observed current-voltage relationship was simply predicted from the observations made with the pH-jump experiments. The effect of the combination of the proton- and K^+ -ionophores would catalyse the non-electrogenic exchange of K^+ for protons across the membrane. Such a combination is thus equivalent to a pH jump, although most of the protons would be carried by the ionophore. The conduction mechanism, therefore, is probably quite different.

The results outlined above are particularly significant with respect to biological energy-transduction processes. The $P(\text{net})$ calculations indicate that energy-conserving membranes are fairly permeable to protons; however, because of the very small concentrations of protons ($0.1 \mu\text{M}$) in cellular fluids, the actual rates of permeation are small. The efficiency of energy conversion, therefore, is not grievously affected.

The exponential current-voltage relationships suggests an ability by which such membranes may regulate either the conductance or the voltage across the membrane. Such a mechanism would allow variations in the flux across the membrane at the same voltage, or, alternatively, would allow a variation in the voltage at a constant flux. Presumably, membrane proteins have evolved to initiate more rapid fluxes of either or both condition(s). since the main purpose of the membrane must be insulatory. The consequence of the current-voltage relationship, therefore, will be a limitation of the maximum voltages that the integral membrane proteins experience.

The exponential current-voltage relationships we have described has some bearing on the current debate concerning the coupling mechanism of oxidative phosphorylation (Kell & Morris, 1981; Mitchell, 1981). It has been shown by Nicholls

(1974) that a linear relationship does not exist between respiration (current) and Δp (voltage). It was suggested by Kell & Morris (1981) that there should be no relationship between these two parameters. Nicholls' (1974) observation, therefore, was interpreted by Kell & Morris (1981) as casting doubt on the Chemiosmotic Hypothesis and supporting their own point of view. Nicholls (1974), however, pointed out at the time that the membrane conductance may have changed as a function of the Δp , thus the complex relationship could be accommodated by the chemiosmotic viewpoint. This hypothesis was, unfortunately, not further tested. Our work, therefore, provides additional support for the suggestion of Nicholls (1974). The expected relationship between respiration and Δp is therefore exponential, provided that the membrane conductance is not further interfered with, for example, by doping with uncouplers or initiating phosphorylation.

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