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Review

Slip and leak in mitochondrial oxidative phosphorylation

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Key words: Oxidative phosphorylation; Slip; Leak; Membrane permeability; Stoichiometry change

Contents

Abbreviations: BSA, bovine serum albumin; L_H, effective proton conductance of the mitochondrial inner membrane; DCCD, *N,N'*-dicyclohexylcarbodiimide; E_h, redox potential at a defined pH with respect to a standard hydrogen electrode; ΔE_h, difference in redox potential between two carriers of reducing equivalents at a defined pH; FFA, free fatty acid; ΔG_p, phosphorylation potential, which is defined as $\Delta G_p = \Delta G^{0\prime} + RT\ln[ATP]/[ADP][P_i]$; H^+/ATP, number of protons taken up from the external medium by the F_0F_1-ATPase per ATP synthesized; H^+/O, number of protons released to the external phase per pair of electrons passed to oxygen; J_{H^+}, the net leak or current of protons through the mitochondrial inner membrane; Δp, protonmotive force across the mitochondrial inner membrane which is defined (in mV at 37 °C) as $\Delta p = \Delta\psi - 61.5\Delta pH$; ΔpH, proton concentration gradient across the mitochondrial inner membrane; P/O, number of ADP molecules phosphorylated per pair of electrons passed to oxygen; state 3, state of respiration when respiratory substrate and excess ADP are present; state 4, state of controlled respiration when respiratory substrate is present and added ADP has been phosphorylated; T_3, L-3,3',5-triiodothyronine; T_4, L-3,3',5,5'-triiodothyronine; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; TPMP, methyltriphenylphosphonium cation; $\Delta\psi$, electrical potential difference across the mitochondrial inner membrane.

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Summary

During oxidative phosphorylation by mammalian mitochondria part of the free energy stored in reduced substrates is dissipated and energy is released as heat. Here I review the mechanisms and the physiological significance of this phenomenon.

I. Introduction

The free energy necessary for mammalian metabolism is provided by reduced substrates. In mitochondria, oxidative phosphorylation transduces this free energy into a displacement from equilibrium of the ATP = ADP + P_i reaction. The free energy stored in this displacement from equilibrium (ΔG_p) is used by the cell to do chemical, osmotic and mechanical work. No process operates reversibly and therefore at all stages of metabolism free energy will be dissipated and energy will be lost as heat. In this review, I discuss the mechanisms and physiological significance of incomplete coupling in oxidative phosphorylation.

I-A. A definition of slip and leak

Low yields in oxidative phosphorylation result from incomplete transduction of the redox energy available in reduced substrates (ΔE_h) into a displacement from equilibrium of the ATP = ADP + P_i reaction (ΔG_p). Because of these, oxidation and phosphorylation are incompletely coupled. There are two types of partial coupling: slip and leak.

Consider how slip and leak operate during oxidative phosphorylation. According to the chemiosmotic hypothesis [1,2] ΔE_h is transduced into a Δp across the mitochondrial inner membrane. This Δp is then converted into a ΔG_p. The Δp across the mitochondrial inner membrane is formed by the movement of electrons down the respiratory chain, from carriers with low (reducing) values of E_h to those with high (oxidizing) values of E_h. This electron movement is coupled to proton translocation across the mitochondrial inner membrane. If the number of protons translocated across the membrane is decreased, and all other factors are unchanged, then a lesser fraction of the available ΔE_h will be converted to Δp and thus to ΔG_p: this is slip and will result in the dissipation of the free energy stored in ΔE_h and the release of energy as heat. The Δp across the mitochondrial inner membrane synthesizes ATP by driving protons down their electrochemical gradient, through the F_0F_1-ATPase. Some protons may bypass the F_0F_1-ATPase and go directly through the mitochondrial inner membrane without synthesizing ATP. This is leak and will result in the dissipation of some of the free energy stored in Δp.

The application of the theory of irreversible thermodynamics to slip and leak [3,4] is not considered here. This is beyond the scope of this review which concentrates on the experimental evidence for and mechanism of slip and leak, not their theoretical treatment. However, a full discussion of slip and leak is best done within a framework of irreversible thermodynamics [3,4]. The experimental evidence for the occurrence of slip and leak during oxidative phosphorylation is outlined in Section II. Leak is discussed in more detail in Section III and slip is further considered in Section IV. The physiological significance of both processes is discussed in Section V.

I-B. The mechanism of oxidative phosphorylation

To discuss the yield of oxidative phosphorylation a model of the process is necessary. It is now generally accepted that Mitchell's chemiosmotic coupling hypothesis [1,2] is the best available description of mitochondrial oxidative phosphorylation [5,6]. However, there is evidence that Mitchell's hypothesis may be incomplete [7,8]. This has led to the proposal of modifications of the chemiosmotic coupling hypothesis, such as localised coupling units [8] or direct interactions between proton pumps [9,10]. It is not yet clear if these (and other) proposals are an improvement on Mitchell's original hypothesis, or if the effects they seek to explain are experimental artifacts [7]. In addition, any future 'localised' model will have to be consistent with the evidence supporting 'delocalised' chemiosmotic coupling: it is therefore more likely to be a modification of Mitchell's hypothesis than a fundamentally new theory. In this review, all experiments and models are discussed within the framework of Mitchell's hypothesis; some of my conclusions may have to be altered if a modified version of chemiosmotic coupling gains acceptance.

II. Do slip and leak occur during oxidative phosphorylation?

As was pointed out in Section I, there are potentially two sources of low yield in oxidative phosphorylation: slip and leak. This prompts the question, "Which process occurs during oxidative phosphorylation?" Here I review the experimental evidence for slip and leak, and thus demonstrate that both processes occur during oxidative phosphorylation and that both are substantially increased at high value of Δp relative to low values.

II-A. The experimental evidence for slip and leak

Oxidation and phosphorylation can be uncoupled by the addition of specific reagents to mitochondria. According to the chemiosmotic coupling hypothesis, many of these uncouplers act by increasing the proton permeability of the mitochondrial inner membrane and

thus dissipating Δp [11,12]. by analogy with the effect of such uncouplers, the endogenous proton permeability of the mitochondrial inner membrane was also thought to cause partial uncoupling of oxidation and phosphorylation. This Δp-driven proton leak was thought to exist in state 4 and was considered to be the major control of state 4 respiration [2,13]. To demonstrate that the proton leak controlled state 4 respiration, Mitchell and Moyle [13] measured the conductance of the mitochondrial inner membrane at low values of Δp. They then multiplied this conductance by the state 4 value of Δp to estimate the proton leak through the mitochondrial inner membrane in state 4. They concluded that the respiration rate in state 4 was the rate of respiration required to balance the proton leak. As we shall see, the conductance measured at low values of Δp cannot be used to calculate the state 4 leak. However, this does show that, at the inception of the chemiosmotic coupling hypothesis, the leak of protons through the mitochondrial inner membrane was considered responsible for low yields in oxidative phosphorylation.

Early measurements of Δp [12,14–16] did not explicity discuss the leak of protons through the mitochondrial inner membrane; this was first done by Nicholls [17]. He showed that the relationship between respiration rate and Δp (in rat liver mitochondria respiring on succinate) was nonlinear when the respiration rate was gradually decreased by the inhibitor malonate. The relationship between respiration rate and Δp was linear up to substantial values of Δp. Above these values a given increase in respiration rate caused a far smaller increase in Δp than at lower values of Δp. This indicates that the transduction of ΔE_h into Δp was less complete at high values of Δp than at low values. Nicholls extended the proposal of Mitchell and Moyle [13] (that the respiration rate was controlled by the proton leak) to suggest that the nonlinearity of plots of Δp against respiration rate was caused by an increase in the proton conductance of the mitochondrial inner membrane at high values of Δp [17]. He proposed that the conductance of the mitochondrial inner membrane was constant at low values of Δp (the membrane behaved as an omhic conductor); and that at high values of Δp the conductance increased substantially (the membrane behaved as a nonomhic conductor). This change in conductance would allow large increases in respiration rate at high values of Δp, without substantially increasing Δp. This is because the permeable inner membrane would allow most of the protons pumped across it at high values of Δp to leak back through the membrane. Nicholls pointed out that a decrease in the stoichiometry of proton pumping by the mitochondrial respiratory chain (i.e., increased slip) at high values of Δp could also explain the nonlinearity. This would operate as follows: a decrease in stoichiometry at high values of Δp would allow the respiration rate to increase substantially without greatly inceasing Δp. Most of the respiration rate would not be used to translocate protons across the mitochondrial inner membrane and would therefore not increase Δp. Measurements of the stoichiometry of proton pumping by the mitochondrial respiratory chain at high values of Δp are technically demanding; therefore, it was difficult to distinguish between these two hypotheses.

Nonlinear plots of Δp against mitochondrial respiration rate have been found in a range of systems: in several varieties of mitochondria respiring on a range of redox donors and acceptors [18–26]; in submitochondrial particles [27] and in mitochondria within hepatocytes [28,29]. Plots of ΔG_p against respiration rate are also nonlinear; this eliminates possible artifacts, such as increases in ion cycling via valinomycin, which would set a limit to the value of $\Delta\psi$ and thus give artifactually curved plots of Δp against respiration rate [25]. Analogous nonlinear relationships have been demonstrated in related systems: in mitochondria hydrolysing ATP [18]; in thylakoid membranes [30]; in membrane vesicles prepared from *Paracoccus denitrificans* [31] and in intact cells and chromatophores of the photosynthetic bacterium, *Rhodobacter capsulatus* [32–35].

The nonlinearity of plots of Δp against respiration rate indicate that the transduction of ΔE_h into Δp is less complete at high values of Δp than at low values. These data are consistent with other experimental evidence indicating decreased yield at high values of Δp. The P/O ratio increases when mitochondria respiring on succinate are titrated with malonate [36]. This occurs because malonate (an inhibitor of respiration) decreases Δp and thus increases the yield of oxidative phosphorylation. More of the oxygen consumed is used to synthesize ATP, less is wasted by leakage or slippage and therefore the P/O ratio increases. Stimulating the rate of ATP turnover (by increasing the amount of hexokinase) increases the P/O ratio [37]. This occurs because the higher rates of ATP turnover decrease Δp and thus increase the degree of coupling of oxidation and phosphorylation. The heat produced per mole of oxygen consumed by mitochondria respiring on succinate was substantially less in state 3 than in state 4 [38]. This occurs because the state 3 value of Δp is less than that in state 4 and thus the fraction of ΔE_h dissipated is less in state 3 than in state 4. All of these results confirm a decrease in yield at high values of Δp. This disagrees with the proposal that the overall reaction of oxidative phosphorylation is close to equilibrium and that cytochrome oxidase is the only site at which there is substantial dissipation of free energy and loss of energy as heat (Ref. 39 and references quoted in Ref. 40). However, the above evidence for decreased yield at high levels of Δp does not indicate its mechanism: it could be by slip, leak or both.

In agreement with Nicholls' proposal [17] the proton conductance of the mitochondrial inner membrane (L_H) does increase at high values of Δp, i.e., the mitochondrial inner membrane behaves as a nonohmic conductor (Refs. 20–24, 41 and subsection III-A). However, it is uncertain if this change in conductance can fully account for the nonlinearity of plots of Δp against respiration rate. In experiments to test this point conflicting results have emerged. Brown and Brand [21] measured the proton leak through the mitochondrial inner membrane and the respiration rate at a range of values of Δp. By multiplying the respiration rate by the stoichiometry of proton pumping of the respiratory chain at low values of Δp under state 4 conditions they calculated the rate of proton efflux. In a steady state this could equal the proton leak, provided the stoichiometry at low values of Δp holds for higher values. Therefore, they had a direct and an indirect measurement of the proton leak as a function of Δp. They found that they two curves were in reasonable agreement. This suggests that the change in conductance at high values of Δp can account for the nonlinearity in plots of Δp against respiration rate. However, these authors no longer consider these experiments as strong evidence against slip [23]. In contrast, from essentially similar experiments, Zoratti et al. [19] found that at high values of Δp the measured leak as a function of Δp diverged substantially from the leak calculated from the respiration rate. They concluded that the stoichiometry of proton pumping must decrease (slip) at high values of Δp.

Other data can be interpreted to suggest a decrease in the stoichiometry of proton pumping, or slip, by the respiratory chain at high values of Δp. If the rate of respiration is multiplied by the stoichiometry of proton pumping, the result is the rate of proton efflux. In a steady state, the rate of proton efflux will equal the proton leak. Therefore, plots of Δp against the rate of proton efflux are also plots of Δp against the proton leak. This suggests that plots of Δp against the proton leak should be superimposable, irrespective of the respiratory span used, because they all represent the same property of the mitochondrial inner membrane. When different combinations of inhibitors and substrates were used, and an appropriate fixed stoichiometry of proton pumping for that respiratory span was assumed, the Δp against proton leak curves produced were superimposable at low values of Δp but they diverged at high values [18,42]. This indicates that the stoichiometries of proton pumping chosen may have been appropriate at low values of Δp, but that at high values they were not; i.e., the stoichiometry of proton pumping decreases at high values of Δp. Zoratti et al. [19] generated K^+ diffusion potentials greater than the state 4 $\Delta\psi$. This suggests that the maximal value of $\Delta\psi$ in state 4 is controlled by a decrease in the stoichiometry of proton pumping and not by the conductance properties of the mitochondrial inner membrane, which was capable of supporting a $\Delta\psi$ greater than that found in state 4.

All of these indicate that the stoichiometry of proton pumping by the respiratory chain decreases at high values of Δp (see also subsection IV-A). However, none of them is direct or conclusive. In experiments similar to those above, but explicitly designed to measure the change in stoichiometry at higher values of Δp Murphy and Brand [22–24] it was shown that the stoichiometry of proton pumping, by the respiratory span from succinate to oxygen, decreased as $\Delta\psi$ was increased [22,23]. Further experiments showed that this stoichiometry change was confined to cytochrome oxidase and that the stoichiometry of the cytochrome bc_1 complex was invariant as Δp was altered [24]. It has already been shown that the proton conductance of the mitochondrial inner membrane increases at high values of Δp, therefore leak will also contribute to the nonlinearity of plots of Δp against respiration rate. They calculated that the stoichiometry change and the change in conductance each contributed about 50% to the nonlinearity of $\Delta\psi$ against respiration rate curves in state 4 [23]. This calculation was identical to that of Zonatti et al. [19] except that a different stoichiometry was used. The results of Zoratti et al. [19] give a similar result if a H^+/O stoichiometry of 6 instead of 8 is used in their calculation [23].

The evidence for a change in proton conductance at high values of Δp is convincing. In contrast, much of the evidence for a stoichiometry change at high values of Δp is indirect and less conclusive. The only direct measurement of a change in stoichiometry was technically difficult [22–24] and artifact cannot be entirely eliminated, even though many controls were carried out [23]. However, one internal control of the direct measurement of stoichiometry does suggest that the stoichiometry change is genuine. When Murphy and Brand [24] measured the stoichiometry of cytochrome oxidase and the cytochrome bc_1 complex independently over a range of values of Δp they found that the stoichiometry of the cytochrome bc_1 complex was invariant, while that of cytochrome oxidase decreased. This suggests that their technique does not introduce an artifactual apparent stoichiometry change when Δp is varied.

Another explanation for the nonlinearity of Δp against respiration rate plots has been proposed, based on the heterogeneity of mitochondrial preparations [43]. A mitochondrial preparation will contain subpopulations of mitochondria with low coupling between oxidation and phosphorylation. If the amount of this subpopulation is significant then most of the respiration at high values of Δp will be due to the poorly coupled mitochondria. If the uncoupled subpopulation of mitochondria were more sensitive to the inhibitor than the coupled, the initial additions of inhibitor would

eliminate the uncoupled respiration. This would substantially decrease the rate of respiration but would have only a small effect of Δp, because the uncoupled mitochondria would have low values of Δp. Subsequent additions of inhibitor would eliminate the respiration due to the coupled population and thus cause a substantial decrease in both respiration rate and Δp. The overall effect of this would be to produce nonlinear plots of Δp against respiration rate. This explanation is not valid. Brown and Brand [21] pointed out that this explanation could account for malonate titrations (because uncoupled mitochondria are more sensitive to inhibition by malonate than are coupled mitochondria [42]) but that it cannot explain antimycin titrations (antimycin is an equally potent inhibititor of coupled and uncoupled mitochondrial respiration [42]). Plots of respiration rate against Δp are nonlinear when either antimycin or malonate is used as an inhibitor. Zoratti et al. [19] estimated the percentage of damaged mitochondria in their preparations and concluded that it was insufficient to account for the nonlinearity of Δp against respiration rate plots. Nonlinear relationships between Δp and respiration rate are also found when then the respiration rate is increased by adding substrates [24,25]. If the nonlinearity of such curves is also due to the heterogeneity of the mitochondrial preparations, then the different subpopulations of mitochondria would have to have different sensitivities to the same substrate and the sensitivity would have to change with Δp. It is unlikely that an effect such as this would produce curves similar to those caused by the putative different sensitivity to inhibitors. When the $H^+/2e$ stoichiometry of the cytochrome bc_1 complex was estimated during a malonate titration it was found to be invariant [24]. This is incompatible with the change in the H^+/O stoichiometry from succinate to oxygen [23] being due to an uncoupled subpopulation of mitochondria. In summary, the balance of evidence suggests that this hypothesis is unlikely to be true.

A third possible explanation of nonlinear Δp against respiration rate curves, based on local proton circuits, has been proposed [8]. As outlined in subsection I-B, no clear consensus has yet emerged about such local proton circuits. Therefore, for the remainder of this review I will discuss the possible explanations of nonlinear Δp against respiration rate curves within a 'delocalised' chemiosmotic coupling framework. However, it should be borne in mind that localised proton circuits may be partially responsible for these phenomena.

II-B. Summary: both slip and non-ohmic leak occur during oxidative phosphorylation

At low values of Δp, both the stoichiometry of proton pumping and the conductance of the mitochondrial inner membrane are constant. The yield of oxidative phosphorylation at low values of Δp is substantially more than that in state 4. Any incomplete coupling under these conditions will be due to leak of protons through the mitochondrial inner membrane and also to inefficiencies in cytochrome oxidase and, to a lesser extent, of the other proton pumps (see subsection IV-D). At high values of Δp the conductance of the mitochondrial inner membrane increases substantially increasing the Δp-driven proton leak through the membrane (see subsection III-A). In addition, the balance of evidence suggests that the stoichiometry of proton pumping by the respiratory chain decreases. At maximal values of Δp (state 4) both of these processes contribute about 50% to the rate of respiration [23]. The change in the stoichiometry of proton pumping is discussed in detail in Section IV. The proton leak through the mitochondrial membrane and the change in conductance at high values of Δp are discussed in Section III. The degree of slip and leak occurring in vivo may be substantially less than that which occurs in state 4: their magnitude and physiological significance are discussed in Section V.

III. The nature of leak: ion permeability properties of the mitochondrial inner membrane

In this section the ion permeability properties of the mitochondrial inner membrane are reviewed. The Δp dependence of proton movement through the membrane, the mechanism of this movement and of the movement of other ions are all discussed. In addition, factors which can alter the permeability properties of the mitochondrial inner membrane are considered.

III-A. The proton conductance of the mitochondrial inner membrane

The leak (or current) of protons through the mitochondrial inner membrane (J_{H^+}) will be driven by the protonmotive force (Δp) just as a current of electrons is driven by a voltage difference. If Ohm's law is used to relate Δp and the leak, then the proportionality factor between them (L_H) is the effective proton conductance of the mitochondrial inner membrane [2,6].

$$J_{H^+} = L_H \cdot \Delta p \qquad (1)$$

The usual units of L_H are nmol $H^+ \cdot min^{-1}$ (mg mitochondrial protein)$^{-1} \cdot mV^{-1}$. This leak of protons has two components: proton movement in one direction and hydroxyl ion movement in the other. The movement of a proton from one phase to another is thermodynamically equivalent to the movement of a hydroxyl ion in the opposite direction, but these processes will have different mechanisms. Movement of either (or both) species may occur. In addition, proton/hydroxyl

transport across the membrane may occur by several modes: by movement through the lipid bilayer itself, on the lipid/protein interface of membrane spanning proteins or by movement on specific carriers. All of these processes are included in the effective proton conductance. In addition, L_H is not a constant but is a function of Δp and may have a different dependence on the components of Δp, therefore

$$L_H = f(\Delta\psi, pH_{in}, pH_{out}) \quad (2)$$

The first attempt to quantify the proton leak of the mitochondrial inner membrane was by Mitchell and Moyle [13]. Their measurements were carried out on rat-liver mitochondria in the presence of valinomycin and high external K^+ to abolish $\Delta\psi$. A small amount of acid was then added to the mitochondrial suspension, and the decay of this pH pulse was followed. These experiments were carried out at pH values close to 7.2 and the change in pH produced by the acid pulses were small, to avoid damaging the mitochondria. The driving force for the uptake of protons was a Δp comprising entirely of ΔpH. The value of L_H they obtained for a ΔpH-driven proton leak was 0.11 nmol $H^+ \cdot min^{-1} \cdot mg^{-1} \cdot mV^{-1}$. This value was assumed to be invariant as the value and composition of Δp were altered.

Nicholls [17] extended the work of Mitchell and Moyle [13] by suggesting that L_H was a function of Δp. This is discussed in subsection II-A.

Krishnamoorthy and Hinkle [20] were the first to directly measure the L_H of the mitochondrial inner membrane at a range of Δp values. They demonstrated a nonlinear relationship between $\Delta\psi$ and the leak of protons across the mitochondrial inner membrane. This indicates that L_H increases at high values of $\Delta\psi$. In addition, they found a nonlinear relationship between $\Delta\psi$ and proton leak and a linear relationship between leak and ΔpH in liposomes. To measure the leak they added valinomycin to K^+-containing liposomes or mitochondria suspended in low-K^+ media. This induced a K^+ diffusion potential which caused the uptake of protons by the mitochondria or liposomes. The initial uptake of protons was measured by following the pH changes of the external medium. The magnitude of the K^+ diffusion potentials were varied by different concentrations of K^+ in the external medium. Most other potential proton exchanges were inhibited, so the leak they measured was largely due to the movement of protons through the membrane. The ΔpH-driven leak was a linear function of ΔpH in vesicles composed of soybean lipids or of mitochondrial lipids. To vary the value of ΔpH they set the internal pH of the vesicles at 5 and altered the pH of the external medium from 5 to 9.5.

O'Shea et al. [41] carried out experiments similar to those of Krishnamoorthy and Hinkle [20]. They confirmed that the relationship between proton leak and $\Delta\psi$ was nonlinear in mitochondria and liposomes and that the relationship between leak and ΔpH was linear in liposomes. They also showed that the relationship between ΔpH and proton leak was linear in mitochondria, for pH gradients up to 2.5–3 pH units. A plot of leak against ΔpH was superimposable on a plot of leak against $\Delta\psi$ up to about 120 mV/2 pH units: above this value the $\Delta\psi$ curve diverged sharply from the ΔpH curve. They also found that incorporating cytochrome oxidase into vesicles did not affect their conductance properties.

Measurements of the proton leak through the mitochondrial inner membrane at different values of Δp were also carried out by Brown and Brand [21]. Their experimental techniques were essentially similar to those of Krishnamoorthy and Hinkle [20] in that they set up a K^+ diffusion potential by adding valinomycin to mitochondria suspended in low K^+ media. However, instead of calculating $\Delta\psi$ from the assumed distribution of K^+, they measured it from the uptake of the lipophilic cation TPMP. They found a nonlinear relationship between $\Delta\psi$ and the leak of protons across the mitochondrial membrane.

Zoratti et al. [19] also measured the proton leak of the mitochondrial inner membrane as a function of $\Delta\psi$. They used the rate of K^+ efflux after addition of valinomycin to mitochondria in low K^+ media to estimate the proton leak. The K^+ diffusion potential was calibrated against the steady-state TPMP distribution. In about half of their experiments L_H increased at high values of $\Delta\psi$ but in the others it was invariant.

Murphy and Brand [22–24] used a technique similar to those mentioned above to measure the proton leak of the mitochondrial inner membrane as a function of $\Delta\psi$. However, in their experiments, mitochondria were respiring in a steady state at a range of accurately measured values of $\Delta\psi$. This is in contrast to the measurements of transient diffusion potentials in the other experiments and should result in a more accurate measurement of $\Delta\psi$. Addition of a respiratory inhibitor to mitochondria in such a steady state would be expected to cause a sharp drop in $\Delta\psi$. However, the influx of protons is balanced by an efflux of K^+ ions which effectively buffers $\Delta\psi$ (see Ref. 23 for a discussion of this). Therefore, addition of inhibition induced K^+ efflux (via valinomycin, which was present in the steady state); the initial rate of K^+ efflux was taken as equal to the steady-state proton leak. The plots of leak against $\Delta\psi$ were nonlinear. This experiment was repeated using a variety of electron donors and acceptors and in all cases gave similar plots of $\Delta\psi$ against leak. This indicates that the leak is a property of the membrane and does not depend on the particular respiratory span used.

In summary, most direct measurements of the con-

ductance of the mitochondrial inner membrane show that the proton leak increases as a nonlinear function of $\Delta\psi$. This implies that the L_H increases at high values of $\Delta\psi$. The shapes of the plots are in qualitative agreement, being linear at low values of $\Delta\psi$ and curving sharply at a threshold value of $\Delta\psi$; this is in agreement with Nicholls' proposal [17] that L_H is a constant at low values of Δp and that it increases at high values of Δp. From direct measurements of the leak as a function of $\Delta\psi$ [20,21,23,24,41] the threshold value of $\Delta\psi$ at which nonomhic conduction starts is about 100–120 mV. The maximal values of $\Delta\psi$ found in state 4 are scattered over a range, probably due to variations in the methods of measurement of $\Delta\psi$.

L_H is approximately a constant at low values of $\Delta\psi$ and its value in this omhic region can be estimated by two methods: by direct measurement of the leak as a function of Δp in the ohmic region, or by transforming the measurement of respiration rate against Δp in the ohmic region into a plot of Δp against proton leak. In the steady state, the rate of proton efflux will be equal to the proton leak. The rate of proton efflux is equal to the respiration rate multiplied by the stoichiometry of proton pumping, which will be constant in the omhic region. Mitchell and Moyle [13] obtained a value for L_H of 0.11 nmol $H^+ \cdot min^{-1} \cdot mg^{-1} \cdot mV^{-1}$ by direct measurement. There are no further direct measurements of L_H but the value can be estimated to be about 0.05–0.25 nmol $H^+ \cdot min^{-1} \cdot mg^{-1} \cdot mV^{-1}$ from the published data [20,23,41]. Nicholls [25] assumed a constant stoichiometry of 6 H^+/O for the respiratory span from succinate to oxygen to transform plots of Δp against respiration rate into plots of Δp against proton leak. From this he obtained a value for L_H of 0.7 nmol $H^+ \cdot min^{-1} \cdot mg^{-1} \cdot mV^{-1}$. This is the only published value but, by using Nicholls' method [25], L_H can be estimated to be in the range 0.3–0.7 nmol $H^+ \cdot min^{-1} \cdot mg^{-1} \cdot mV^{-1}$ from the published data [20,23]. Therefore, the value for L_H in the linear, or omhic, region of plots of Δp against respiration rate is about 0.05–0.7 nmol $H^+ \cdot min^{-1} \cdot mg^{-1} \cdot mV^{-1}$. The results are scattered over an order of magnitude: this is to be expected, bearing in mind the technical difficulty of the measurements and that different conditions, temperatures and types of mitochondrion were used.

In most of the above experiments the proton leak was measured as a function of Δp, where Δp was composed mainly of $\Delta\psi$. This is of relevance to mitochondria in vivo, where Δp is largly $\Delta\psi$ [44]. The leak through the mitochondrial inner membrane has a linear dependence on ΔpH [41]. However, this would not be relevant to mitochondria in vivo, where large values of ΔpH do not occur. Instead, investigation of relatively small changes in ΔpH when a substantial fraction of Δp is composed of $\Delta\psi$ would be more appropriate. Brown and Brand [21] investigated the effect on respiration rate of varying the contributions of $\Delta\psi$ and ΔpH to Δp. The proton leak was not measured directly; instead they determined the relationships between Δp and respiration rate when either the ΔpH or the $\Delta\psi$ component of Δp was changed while the other component was fixed. They found similar relationships between respiration rate and Δp. In another experiment, 15 mV was transferred from ΔpH to $\Delta\psi$ without changing the overall value of Δp: this had no effect on respiration rate. Because the state 4 respiration rate is largely controlled by the proton leak of the mitochondrial inner membrane [45] this suggests that ΔpH and $\Delta\psi$ have similar effects on leak. Nicholls [25] varied the relative contribution of $\Delta\psi$ and ΔpH to Δp and found that the state 4 Δp was similar, implying that the maximal value of Δp was limited by Δp itself and not its components. In conclusion, these experiments suggest that ΔpH and $\Delta\psi$ have similar effects on the proton leak when Δp is largely composed of $\Delta\psi$, but they are not conclusive: they have to be compared with the results of O'Shea et al. [41] which show that large values of $\Delta\psi$ and ΔpH have different effects on the leak.

The increase of L_H at high values of $\Delta\psi$ found for the mitochondrial inner membrane is also found in many other membrane systems: in liposomes prepared from soybean lipids or beef heart mitochondrial lipids [20]; in phospholipid vesicles [41]; in intact cells and chromatophores of *Rhodobacter capsulatus* [32–35] and in planer lipid membranes [46]. Similarly, the linear relationship between ΔpH and proton leak is also found in many other membrane systems: in liposomes prepared from soybean lipids or beef heart mitochondrial lipids [20]; in phospholipid vesicles [41]; in bacteriorhodopsin liposomes [47]; in phosphatidylcholine vesicles [48] and in the bacterium *Streptococcus lactis* [49]. Therefore, the leak behaviour of the mitochondrial inner membrane is that of a "typical" membrane: it has a nonlinear relationship between proton leak and $\Delta\psi$ with a threshold value of $\Delta\psi$ above which the proton conductance increases and a linear relationship between leak and ΔpH. There are some differences between the leak properties of the mitochondrial inner membrane and other lipid membranes which should be noted. The mitochondrial inner membrane has, in common with other biological membranes, a conductance about an order of magnitude higher than other lipid membranes at a given value of Δp [20,41,50,51]. This could be due to the high protein concentration of the membrane. This would increase the membrane's dielectric constant and thus decrease the energy of a charge passing through the membrane making it easier for protons to pass across the membrane [20,42]. Alternatively, the protein could provide defects in the lipid bilayer, or provide specific

proton channels and thus increase the conductance of the membrane [51].

III-B. The mechanism of proton movement through the mitochondrial inner membrane

The proton permeability characteristics of the mitochondrial inner membrane were outlined in the previous section. Here, the mechanisms of proton movement through the membrane and of the change of the membrane's proton conductance at high values of $\Delta\psi$ are considered.

The mechanism of proton transport through lipid membranes is not well understood. This transport may be by a variety of modes of both proton and hydroxyl movement [46]. The proton conductance of lipid bilayers is about 10^6-fold greater than their conductance to other ions [46,50]. This suggests that the mechanism of proton/hydroxyl movement through a lipid bilayer is qualitatively different from that of other ions and is probably not by simple diffusion through the membrane [46]. Models of the transport of protons through lipid bilayers have been proposed: Nichols and Deamer [50] and Nagle and Tristram-Nagle [53] suggested that protons could move across a lipid bilayer via membrane-spanning hydrogen-bonded chains (HBCs) of water. This model is based on the Grotius mechanism of proton conduction in ice and water [54]. However, the water present in a lipid bilayer is probably too dispersed to form long-lived chains [46], but transient membrane-spanning HBCs could form by collision of shorter chains. Proton transport by a HBC might be substantially altered by the membrane's proteins, which could stabilize HBCs of water on their surfaces and/or incorporate amino acids into the HBC [53]. This is similar to the proton-wire idea of Nagle and Morowitz [55] in which HBCs within proteins conduct protons. In summary, the mechanism of proton conduction through the mitochondrial membrane is uncertain. There may be several modes of proton (and/or hydroxyl) movement through the membrane: through the lipid bilayer via HBCs, through HBCs on the surfaces of proteins or through HBCs containing amino acids. All these processes could operate simultaneously and their contributions to the overall proton leak could alter as the magnitude and composition of Δp varied.

Until the mechanism of proton movement through the mitochondrial inner membrane is known, any discussion of the mechanism of the membrane's conductance change at high values of $\Delta\psi$ is speculative. However, some work on the effect of high values of $\Delta\psi$ on the mitochondrial inner membrane is of relevance. Corda et al. [56] demonstrated that the formation of a diffusion potential in liposomes increased the microviscosity of their membranes, as measured by fluorescence polarization. This change in the microviscosity was a nonlinear function of $\Delta\psi$. O'Shea et al. [57] confirmed this work and extended it to the mitochondrial inner membrane. They found that the mitochondrial inner membrane's microviscosity increased nonlinearly as $\Delta\psi$ was increased, but not as ΔpH was increased [57]. These plots of microviscosity against $\Delta\psi$ were qualitatively similar to plots of respiration rate against $\Delta\psi$, and both plots change from linear to nonlinear relationships at similar values of $\Delta\psi$. Therefore it seems probable that the increase in L_H is related to this change in the state of the membrane at high values of $\Delta\psi$. In addition, high values of ΔpH, which do not increase the proton conductance of the mitochondrial inner membrane [41], also do not increase its microviscosity [57]; this suggests that the change in membrane structure is related to the conductance change. Brierley et al. [58] and Brown and Brand [21] found that high values of $\Delta\psi$ dramatically increased the mitochondrial inner membrane's permeability to a variety of nonphysiological cations. This change in permeability occurred at similar values of $\Delta\psi$ as the increase in L_H. This increase in the nonspecific permeability of the membrane suggests that the membrane has been altered by high values of $\Delta\psi$.

The most probable alteration in the mitochondrial inner membrane to occur at high values of $\Delta\psi$ is some type of electrical breakdown. Although the maximal membrane potential across the mitochondrial inner membrane is only about 180–200 mV, because the width of the mitochondrial membrane is 7–8 nm [59], the field strength across the membrane will be $2–3 \cdot 10^5$ $V \cdot cm^{-1}$. This is sufficient to cause dielectric breakdown [60]. Dielectric breakdown of many lipid bilayers occurs at $\Delta\psi$ values of about 200 mV [61] and similar potentials cause lipid vesicles to become permeable to sucrose and monovalent cations [60,62]. The reversible electrical breakdown of lipid bilayers has been reviewed by Zimmermann [63–65]. Reversible breakdown of lipid bilayers can be induced in cell membranes by exposure of cell suspensions, or of giant cells impaled on electrodes, to d.c. pulses of about 1 V for a short time (ns to μs): longer times lead to irreversible breakdown. This may be related to the breakdown in the mitochondrial inner membrane which is reversible but which occurs at lower, constant potentials. Long exposure to a voltage of 200 mV leads to electrical breakdown in phospholipid vesicles [62,63]. The reversible breakdown of the membrane is not fully understood [62,64,65]. A model (a development of Crowley's [67]) has been proposed based on a decrease of the membrane's width due to electrocompression leading to the formation of hydrophilic pores [60,62–65]. However, there are probably many other changes occurring in the membrane at high values of $\Delta\psi$ [64,65]. Related to this [60,62–65] it has been suggested that the polar headgroups of phospholipids (which are dipoles aligned parallel to the mem-

brane's surface [62,67]) reorient on application of an electrical potential; in addition, the applied potential will probably decrease the width of the membrane and lead to increased permeability [68]. In vivo this breakdown will probably occur at the lipid/protein interfaces; this will reseal more slowly than breakdown in a pure lipid membrane [63–65] and may lead to hysteresis-type effects in vivo.

The current–voltage curves for carrier-mediated ion transport through lipid bilayers are nonlinear (references quoted in Ref. 20). This nonlinearity has been modelled. Hall et al. [69] treated the membrane as a trapezoidal potential energy barrier whose shape varied with the applied potential: this approach generated nonlinear current–voltage curves in good agreement with experiments on lipid bilayers. This approach also gives a reasonable fit to experiment for the proton permeability of mitochondrial and liposome membranes [20]. Therefore, the increase in membrane conductance at high values of Δp may be due to alterations in the potential barrier across the membrane as well as to changes in the membrane itself. In addition, simple kinetic treatment of the passage of protons through the membrane will also tend to give a non-omhic current–voltage curve.

In conclusion, the increase in the membrane's conductance at high values of $\Delta\psi$ is partly explained by alterations in the potential energy barrier across the membrane at high values of $\Delta\psi$. In addition, high values of $\Delta\psi$ change the membrane in a number of ways which lead to its electrical breakdown by an undefined mechanism. This electrical breakdown can be interpreted as the formation of inverted hydrophilic pores which facilitate the movement of electrolytes across the membrane. At state 4 values of Δp these 'pores' have started to form and are in part responsible for the increased L_H and permeability to nonphysiological cations under these conditions.

III-C. Leaks caused by other ion movements

In addition to protons passing through the mitochondrial membrane there will be net movement and cycling of protons and other ions via ion carriers. In this subsection, I consider the mechanism and significance of these other leak pathways.

As predicted by the chemiosmotic hypothesis [1,2], there are a range of metabolite and ion carriers in the mitochondrial inner membrane. The occurrence and properties of metabolite carriers have been reviewed [70–72]. These carriers connect the mitochondrial matrix and cytoplasmic metabolite pools for processes such as Kreb's cycle, fatty acid oxidation, the urea cycle and fatty acid synthesis. They also connect the matrix and cytoplasmic ATP, ADP and P_i pools. In addition to the metabolite carriers, there are carriers for ions such as Ca^{2+}, K^+ and Na^+. These control the mitochondria's ionic and osmotic properties. The operation of both metabolite and ion carriers can cause leaks. They do this by allowing the net dissipation of Δp. This is distinct from the mechanisms of the carriers themselves, which may entail slip.

The mitochondrial inner membrane contains an electrophoretic Ca^{2+} uniporter which catalyses the $\Delta\psi$-driven accumulation of Ca^{2+} in the mitochondrial matrix [73–75]. There is also a separate Ca^{2+} efflux pathway from the mitochondria which exchanges Ca^{2+} for protons in the liver and Ca^{2+} for Na^+ in the heart [73,74]. Both exchangers are electroneutral [76,77] (but see references quoted in Refs. 76 and 77, which claim they are electrogenic). The Ca^{2+}/nNa^+ exchanger in the heart is effectively a Ca^{2+}/nH^+ exchanger because of the rapid Na^+/H^+ exchange in that tissue. The net result of these two Ca^{2+} carriers is a constant cycling of Ca^{2+}: in a steady state Ca^{2+} will be extruded from the mitochondria in exchange for protons and will then be taken up again by the Ca^{2+} uniporter [73,75]. This will lead to a constant concentration of Ca^{2+} in the mitochondrial matrix and cytoplasm and the partial dissipation of Δp. This Ca^{2+} cycling probably benefits the organism by allowing control of the matrix free $[Ca^{2+}]$ and thus the activity of some Ca^{2+}-sensitive matrix dehydrogenases [78,79]. When Ca^{2+} cycling is inhibited the state 4 respiration rate decreases, indicating that part of state 4 respiration is used to drive Ca^{2+} cycling [80,81]. This dissipation due to calcium cycling can account for 15–30% of state 4 respiration in isolated mitochondria [80,81].

There is an electroneutral H^+/K^+ exchanger in the inner mitochondrial membrane [82–86]. It is generally of very low activity, but it can be stimulated by a variety of factors such as swelling of the mitochondria or depletion of matrix Mg^{2+} [87]. The mitochondrial inner membrane is usually almost impermeable to K^+; however, at high values of Δp a voltage-gated K^+ uniport activity can be induced [21,86,88,89]. This K^+ uniport activity is probably a nonspecific leak (Refs. 21, 83, 86, 90 and subsection III-B). If the K^+ uniporter and the K^+/H^+ exchange operated simultaneously the resultant K^+ cycling would dissipate Δp in the same ways as Ca^{2+} cycling. However, the activity of the K^+/H^+ exchanger is generally too low to be of significance. Addition of quinine, an inhibitor of the K^+/H^+ exchanger [91], does not decrease the respiration rate of mitochondria in state 4 (Murphy, M.P. and Brand, M.D., unpublished data): if there was substantial cycling of K^+ in state 4, the addition of quinine would inhibit state 4 respiration.

The Na^+ uniport activity of the mitochondrial membrane may be the same voltage-gated nonspecific leak as was seen for K^+ and other cations [21,83]; however, it is possible that the pathways of K^+ and Na^+ uptake

are different [83]. In addition, there is a Na^+/H^+ exchanger [14] distinct from the K^+/H^+ exchanger [83,91]. It is more active than the K^+/H^+ exchanger but is still of low activity unless induced [87]. Therefore, there is a potential for Δp dissipation by Na^+ cycling, but there is no evidence that this is significant in vivo.

Many mitochondrial inner membrane metabolite carriers transport protons in symport or antiport with metabolites. However, the activity of the majority of such carriers does not result in the net movement of protons. This is because the proton taken up with the substrate is usually carried out by the product [71,92]. Therefore there is no net movement of protons by the overall process. In contrast to these, the glutamate/aspartate carrier is electrophoretic and its operation results in the uptake of one proton. This carrier is part of the malate/aspartate shuttle which is discussed in subsection IV-C.

The other metabolite carriers directly involved in oxidative phosphorylation are the adenine nucleotide carrier and the phosphate carrier. In the presence of an applied $\Delta\psi$ the adenine nucleotide carrier catalyses the electrophoretic exchange of ATP^{4-} for ADP^{3-} [93,94]. The P_i carrier transport P_i in symport with H^+ [95]. Therefore, during ATP synthesis these carriers will take up one proton, in addition to the 2 or 3 protons required for ATP synthesis by the ATPase, per ATP translocated to the cytoplasm [96–98]. The free energy expended by the movement of this extra proton is conserved in the cytoplasmic ΔG_p, which is greater than the matrix ΔG_p. Therefore, the operation of these two carriers does not result in leak. However, if the adenine nucleotide carrier had variable stoichiometry it could act as a leak pathway.

In summary, the majority of steady-state ion and metabolite movements do not result in leak. The one well established leak is due to the cycling of Ca^{2+}. These is potential for cycling of K^+ and Na^+ under some conditions but there is no evidence that these processes are significant in vivo. The only metabolite carrier which causes leak is the electrophoretic glutamate/aspartate carrier, which is part of the malate/aspartate shuttle.

III-D. Short-term changes in membrane conductance

Short-term changes in the leak of protons through the mitochondrial inner membrane could be advantageous to an organism. They could adapt the organism to short term environmental changes by optimising heat production or the rate of ATP synthesis. Alternatively, alterations in the leak may be useful in allowing the organism to optimise the efficiency of coupling of oxidation and phosphorylation [119,201]. Changing the concentration of a substance (such as a hormone or a factor controlled by a hormone) which acted as an uncoupler [99,100] would alter the leak.

One endogenous compound which uncouples isolated mitochondria is free fatty acid (FFA) [101–106]. Free fatty acids have also been shown to uncouple adipocytes [107,108], lymphocytes [109,110] and hepatocytes in the absence of albumin [111,112]. However, because of the high concentration of plasma albumin, uncoupling is unlikely in vivo [45]. The effect of FFAs and fatty acyl-CoAs on mitochondrial parameters has been reviewed by Wojtzak [113]: FFAs and fatty acyl CoAs increase the permeability of the mitochondrial inner membrane to monovalent cations (but only in the absence of Mg^{2+}) and, at higher concentrations, FFAs cause 'classic' uncoupling.

FFAs may have other effects in addition to uncoupling. Rottenberg and Steiner-Mordach [114] found that FFAs uncouple reverse electron transport and phosphorylation in submitochondrial particles. However, they found that FFAs do not decrease Δp or inhibit ATP synthesis by an artificially imposed Δp. Rottenberg and Hashimoto [115] showed that FFAs stimulate state 4 respiration and ATPase activity in mitochondria but that FFAs did not decrease Δp. This effect is similar to those of general anaesthetics [116]. Rottenberg suggested that FFAs (and general anaesthetics) are a different class of uncouplers which he calls decouplers. He proposed that decouplers interfere with the transfer of protons from redox pumps to the F_0F_1-ATPase [116]. However, Luvisetto et al. [117] found that FFAs did decrease Δp under similar conditions to those of Rottenberg and Hashimoto [115]. They proposed that FFAs act as classical uncouplers and that in addition they act as intrinsic uncouplers of the proton pumps [118]. In summary, FFAs can act as uncouplers, but they may also have other effects on oxidative phosphorylation which have yet to be clarified. Soboll and Stucki [119] suggested that these properties of FFAs are used by the organism to modulate oxidative phosphorylation. They found that FFAs decreased the coupling of oxidative phosphorylation in perfused liver. They suggested that the organism controlled the concentration of FFAs, and thus the degree of coupling of oxidative phosphorylation, in order to optimize the efficiency of oxidative phosphorylation [119]. This modulation of oxidative phosphorylation by FFAs could be by classical uncoupling or by inducing slip in the proton pumps of the respiratory chain.

High concentrations of the thyroid hormone T_4 uncouples oxidative phosphorylation [120–123]. Hoch and Lipmann [122] proposed that this was how thyroid hormones increased the basal metabolic rate in vivo. However, as was pointed out by Guernsey and Edelman [124], the in vivo concentrations of thyroid hormones are too low to cause uncoupling. Therefore the in vivo effects of the thyroid hormones (T_3 and T_4) on respiration are not due to uncoupling, although this may occur in extreme hyperthyroid states.

The short-term regulation of the leak of protons through the mitochondrial inner membrane could also be effected by a hormonally controlled proton or hydroxyl channel. Such a leak protein, thermogenin, is found in the inner membrane of BAT mitochondria (see subsection III-F). However, neither thermogenin nor a protein with an analogous function has been found in other tissues.

In summary, FFAs are the only known candidate for the short-term modulation of the proton leak through the mitochondrial inner membrane. The metabolic significance of this process is uncertain.

III-E. Long-term changes in membrane conductance

Changing the leak of the mitochondrial inner membrane would alter the yield of oxidative phosphorylation. In subsection III-D, short-term effects of this nature were considered. Long-term alterations of the membrane's conductance could also be important. Such changes could be achieved by altering the membrane's lipid or protein composition. This could be altered by hormones, or could change with development or dietary status. There will also be differences in the lipid composition and protein content of mitochondria from different tissues. These changes in composition could alter the proton conductance of the mitochondrial membrane: the conductance of the membrane of *Rhodobacter capsulatus* changes in response to alterations in metabolite supply [125]. In this subsection I consider the long-term changes in leak brought about by alterations of the membrane's composition.

The lipid composition of the mitochondrial membrane has been reviewed by Daum [126]. The membrane's lipid composition and their degree of saturation changes in response to ageing, diet and hormonal status. Thyroid hormones [127–129] and growth hormones [130] alter the degree of saturation of membrane lipids. Thyroid hormones also alter the area of the mitochondrial inner membrane [131]. Similarly, the protein content of the mitochondrial inner membrane alters in response to many factors, in particular, thyroid hormones (references quoted in Ref. 45) and growth hormone [132]. Possibly, the amount and type of proteins will affect the leak of protons through the membrane (see subsection III-A).

Long-term changes in leak have been studied in mitochondria from rats with altered thyroid status. Hafner et al. [133] found that, for a given respiration rate, Δp was increased in mitochondria from hypothyroid rats relative to mitochondria from euthyroid rats. This suggests that the leak (or possibly the slip) is decreased in mitochondria from hypothyroid rats. Verhoeven et al. [134] found that the proton leak of the mitochondrial inner membrane in state 4 was decreased in mitochondria from hypothyroid rats with respect to mitochondria from hypothyroid rats treated with T_3 for 24 h prior to death. However, this effect could be due to differences in the state 4 values of Δp, which were not measured, and the conductance could have been unchanged. These effects are probably related to the many alterations in the inner membrane of mitochondria from hypothyroid rats when compared with normal rats. In mitochondria from hypothyroid rats, Hulbert et al. [127] found that lipid unsaturation was increased, the temperature of the membrane phase transition decreased and the activation energy of succinate dehydrogenase was increased when compared with mitochondria from euthyroid rats. All these parameters returned to normal values on treatment of the hypothyroid rat with T3 for 12 h prior to death [128,129].

In summary, the composition of the mitochondrial inner membrane changes with conditions, and these changes probably affect the degree of leak and thus the efficiency of oxidative phosphorylation: however, not much is known about how the membrane's composition affects its conductance.

III-F. The specialised leak of brown adipose tissue mitochondria

There is one leak in the mitochondrial inner membrane whose mechanism, control and function is well understood. That is the leak through the protein thermogenin in brown adipose tissue (BAT) mitochondria. This leak has been comprehensively reviewed [135–137] and here the main points from those reviews are summarised.

BAT mitochondria are important in thermogenesis, especially in neonatal and cold-adapted mammals. Thermogenesis by brown adipose tissue is controlled by the sympathetic nervous system, via noradrenaline. Noradrenaline interacts with β-adrenergic receptors on the cell surface, stimulating cAMP production via adenylate cyclase. The cAMP then interacts with a protein kinase to activate a lipase thus releasing FFAs. These FFAs are oxidized by the mitochondria to produce heat, not ΔG_p. This occurs because the FFAs, in addition to acting as a substrate, switch on a leak in the mitochondrial inner membrane. They do this by binding to a protein (thermogenin) in the mitochondrial inner membrane. The binding of FFAs to thermogenin converts it into an effective proton leak through the mitochondrial inner membrane. This leak is probably an hydroxyl channel [136,137], but the net effect would be the same if it were a proton channel. This dissipates any Δp formed by the oxidation of fatty acids and releases energy as heat. Thermogenin's hydroxyl channel is normally inhibited by the binding of cytoplasmic purine nucleotides; the binding of FFAs overcomes this inhibition by displacing the nucleotides. The L_H of the BAT mitochondrial inner membrane is 0.7 nmol $H^+ \cdot min^{-1} \cdot$

$mg^{-1} \cdot mV^{-1}$ in the absence of FFAs; in their presence it increases to 16 nmol $H^+ \cdot min^{-1} \cdot mg^{-1} \cdot mV^{-1}$ [25]. Thus, switching on thermogenin's leak increases the conductance of the inner mitochondrial membrane by over 20-fold.

In summary, this tissue has evolved a highly specialised, hormonally controlled, leak to generate heat as required. Neither thermogenin, nor a protein with an analogous function, has been found in other tissues. Therefore, it seems that this method for controlling the leak is confined to a highly specialised tissue and is not utilised by other types of mitochondria.

III-G. Summary

The Δp-driven leak of protons through the mitochondrial inner membrane is qualitatively similar to the leak through other lipid bilayers. The conductance of the mitochondrial inner membrane is a constant at low values of $\Delta\psi$ and it increases substantially at high values of $\Delta\psi$. This is of relevance to the in vivo situation where Δp is largely composed of $\Delta\psi$. When Δp is composed solely of ΔpH the conductance does not increase at high values of ΔpH as it does at high values of $\Delta\psi$. The mitochondrial inner membrane's conductance is higher than that of pure lipid bilayers, probably because of its high protein content. In addition to the leak of protons through the membrane there are leaks due to ion cycling. Probably the only significant leak is that due to Ca^{2+} cycling. The leak of protons through the mitochondrial inner membrane can, in principle, be modified by factors such as FFAs. The leak will also change in response to long-term changes in the lipid and protein composition of the membrane. In BAT mitochondria there is a highly specialised leak, due to the protein thermogenin, which is not found in any other tissue.

IV. The nature of slip: stoichiometry change in mitochondrial proton pumps

Changes in the stoichiometry of proton pumping by the mitochondrial proton pumps (i.e. changes in the mechanistic stoichiometry) can be achieved in two ways: by a large value of Δp, or by exposure of the proton pumps to certain compounds or conditions. In the latter case stoichiometry changes are usually shown by experiments (such as oxygen pulse experiments) carried out at negligible values of Δp. In this section, I consider both ways of altering the stoichiometry of proton pumping, effective changes in the stoichiometry by alterations in the pathway of electron flow to oxygen (i.e., changes in the phenomenological stoichiometry) and the occurrence of slip in related systems.

IV-A. Changes in the stoichiometry of proton pumping at high values of Δp

Nicholls' experiments [17] (see subsection II-A) were the first that could be interpreted as evidence for a change in the proton pumping stoichiometry of the respiratory chain at high values of Δp.

The first explicit proposal of slip at high values of Δp was by Pietrobon et al. [42] which is discussed in subsection II-A. Pietrobon et al. [18] measured Δp as a function of the rate of proton efflux due to respiration or ATP hydrolysis. Invariant stoichiometries of the respiratory chain and of the ATPase were assumed and the rates of proton efflux were calculated by multiplying the rate of respiration, or ATP hydrolysis, by these stoichiometries. The rates of proton efflux were varied by the inhibitors antimycin or oligomycin. These plots were superimposable at low values of Δp but they diverged at higher values and the respiratory chain gave a higher maximal value of Δp than the ATPase. The divergence of these curves at high values of Δp indicates that slip occurs when Δp is substantial. The minimal interpretation of this experiment is that the ATPase slips when it is hydrolysis ATP at high values of Δp: it does not prove that the proton pumps of the respiratory chain slip. In contrast, Nicholls [17] found that ATP hydrolysis and state 4 respiration generated similar maximal values of Δp. This is the expected result if the maximal value of Δp is limited by the conductance properties of the mitochondrial inner membrane. Pietrobon et al. [18] noted this, but pointed out that their experiments had been done on the same mitochondrial preparation, and are therefore comparable, while those of Nicholls were on different mitochondrial preparations. Zorratti et al. [19], from experiments discussed in subsection II-A, suggest that the proton pumps slip at high values of Δp. In contrast, in experiments essentially similar to those of Zoratti et al. [19], Brown and Brand [21] found no evidence for slip (see subsection II-A). However, the experiments of Brown and Brand [21] involved an extrapolation of the TPMP-electrode trace which may not be accurate [23].

Zorratti et al. [19] generated a K^+ diffusion potential greater than the state 4 $\Delta\psi$. This was done by adding valinomycin to mitochondria in state 4 suspended in a low KCl medium. $\Delta\psi$ was measured in state 4 by the distribution of TPMP; addition of valinomycin caused further TPMP uptake indicating that the K^+ diffusion potential was greater than the state 4 $\Delta\psi$. This implies that the state 4 $\Delta\psi$ is controlled by slip and not by the proton leak properties of the membrane because the membrane can support a $\Delta\psi$ greater than that occurring in state 4; the state 4 $\Delta\psi$ is not limited by the properties of the mitochondrial membrane. However, this observation is not necessarily a proof of slip. Curves of Δp against proton efflux are not completely flat at

high values of Δp, therefore addition of valinomycin may lead to a rate of charge efflux (driven by the K^+ concentration gradient) greater than the rate of proton efflux in state 4 and thus increase $\Delta\psi$.

Murphy and Brand measured the proton pumping stoichiometry of the respiratory chain as a function of $\Delta\psi$ [22,23]. The method they used was based on the steady-state technique of Al-Shawi and Brand [138] and it its discussed in subsection III-A. In these experiments [22–24] they showed that increases $\Delta\psi$ decreased the stoichiometry of proton pumping by the redox span from succinate to oxygen. These measurements were technically difficult and, even though many controls were carried out, artifact cannot be completely ruled out. However, when this technique was applied to cytochrome oxidase and the cytochrome bc_1 complex individually, the stoichiometry of cytochrome oxidase varied as $\Delta\psi$ was increased, while that of the cytochrome bc_1 complex did not. This suggests that the change in stoichiometry seen for cytochrome oxidase is genuine and is not due to some unspecified artifact of the method of measurement. Such a stoichiometry decrease at high values of Δp for cytochrome oxidase in vesicles has been proposed previously [139].

Other measurements of the stoichiometry of the cytochrome bc_1 complex have been made at high values of Δp. The measurements of Brown and Brand [140] agree with those of Murphy and Brand [24] that the stoichiometry at high values of Δp is similar to that at low values of Δp. Similarly, the measurement of the stoichiometry of the cytochrome bc_1 complex from the photosynthetic bacteria *Rhodobacter capsulatus* during cyclic electron flow in invariant at values of Δp up to 150 mV [33]. However, the stoichiometry of the cytochrome bc_1 complex in lipid vesicles was decreased under conditions of substantial (but unquantified) ΔpH [141]. The effect of ΔpH on the stoichiometry may be different from that of $\Delta\psi$.

An earlier measurement of the H^+/O stoichiometry of the respiratory chain at a range of values of Δp showed it to be invariant [142]. This apparently disagrees with the results of Murphy and Brand [22,23]. However, the values of Δp in those experiments had been overestimated and were in fact too low to cause slip [22,23].

In conclusion, it seems probable that there is a genuine slip in the proton pumps at high values of Δp and that this slip occurs in cytochrome oxidase, not in the cytochrome bc_1 complex. Work by Brown and Brand [143] suggests that the stoichiometry of the NADH-ubiquinone oxidoreductase is the same at high values of Δp as at low. The evidence for a change in the stoichiometry of proton pumping is not as conclusive as that for a change in the conductance of the mitochondrial inner membrane at high values of Δp.

IV-B. Stoichiometry changes brought about by external factors

The exposure of mitochondrial proton pumps to certain conditions or compounds alters their proton pumping stoichiometries. These compounds or conditions are not usually of physiological relevance. However, they may help elucidate the mechanism of the stoichiometry change at high values of Δp or the mechanism of proton pumping in general.

One factor which alters the measured stoichiometry of proton pumping by the mitochondrial respiratory chain is dicyclohexylcarbodiimide (DCCD). It is a non-specific protein reagent which binds covalently to glutamate and lysine residues [144]. DCCD treatment of mitochondria, or of mitochondrial complexes in vesicles, decreases the stoichiometry of proton pumping of a range of complexes: cytochrome oxidase [145,146], the F_0F_1-ATPase [147,148], the nicotinamide nucleotide transhydrogenase [149] and the cytochrome bc_1 complex (Refs. 150, 151, 152 and references in Ref. 153). The mechanism of this stoichiometry change is unknown, but the covalent binding of DCCD to the protein complex is necessary. In cytochrome oxidase DCCD has its effect on the stoichiometry by binding to a specific glutamate residue in subunit III of that complex [146]. DCCD may act by decreasing the number of protons released to the external phase and thus apparently decrease the stoichiometry when measured by kinetic techniques although the mechanistic stoichiometry was unaltered. Brand et al. [153] found that DCCD decreased the stoichiometry of the cytochrome bc_1 complex when measured by a kinetic method (oxygen pulse) but that it was unchanged when measured by a thermodynamic method.

Other factors have DCCD-like effects on the stoichiometries of proton pumping. Lam et al. [154] found that fluorescamine (which labels primary amines) inhibited proton ejection to a greater extent than respiration during an initial rates stoichiometry measurement. This suggests that fluorescamine decreases the stoichiometry of proton pumping. Tu et al. [155] showed that fluorescamine decreased the measured stoichiometry of both the cytochrome bc_1 complex and cytochrome oxidase when their stoichiometries were determined independently. The concentration of external Mg^{2+} has been shown to affect the stoichiometry of proton pumping by the mitochondrial respiratory chain as measured by an oxygen pulse technique [156], but it does not affect the stoichiometries of complexes determined independently, which suggests that this effect may be complicated.

The removal of subunit III from cytochrome oxidase decreases its stoichiometry of proton pumping [157–162]. DCCD binds to subunit III of cytochrome oxidase

[146] and DCCD's effect on the stoichiometry of proton pumping may be related to the stoichiometry decrease on removal of subunit III. Finel and Wikstrom [163] decreased the stoichiometry of cytochrome oxidase without removing subunit III. Their treatment caused the complex, which is normally a dimer, to form monomers. They suggested that dimeric cytochrome oxidase is necessary for proton pumping. This is a development of Wikstrom's earlier proposal that cytochrome oxidase operates as a dimer [164,165]. This agrees with a similar proposal by Sone and Kosako [166]. Finel and Wikstrom [163] further suggested that the removal of subunit III decreased the stoichiometry by preventing the dimerization of cytochrome oxidase and that the binding of DCCD to subunit III also decreases the stoichiometry by preventing dimerization. This elegant hypothesis has yet to be verified. It could be related to the stoichiometry change seen in cytochrome oxidase at high values of Δp: this would occur if the degree of dimerization of cytochrome oxidase was a function of Δp [24]. This mechanism could not extend to all classes of cytochrome oxidase because cytochrome oxidases from shark [167,168] and *Paracoccus denitrificans* [169] are monomeric and both actively translocate protons [170,171]. Heating cytochrome oxidase to 43–45 °C also decreases its stoichiometry [172]. This may be related to the stoichiometry decrease on removal of subunit III.

Crespo-Armas and Mowbray [173] suggested that a thyroid hormone (T_3) can directly alter the stoichiometry of proton pumping of the mitochondrial respiratory chain. They reported that mitochondria from hypothyroid rats have a lower H^+/O stoichiometry than mitochondria from euthyroid rats. In addition, they reported that in vivo administration of T_3 to hypothyroid rats 15 min prior to death increased the H^+/O stoichiometry to normal levels. This work has been severely criticised by Hafner and Brand [174] and there is now no strong evidence for an effect of thyroid hormones on the proton-pumping stoichiometry of the respiratory chain.

It has been proposed that compounds such as uncouplers and free fatty acids can act as intrinsic uncouplers of proton pumps [23,42,117,118,175,176]. An intrinsic uncoupler would act by somehow interfering with the operation of the proton pump and thus altering its stoichiometry. The mechanism by which this would be done is as yet uncertain, but it is probably by decoupling electron transfer and proton translocation. So far the evidence supporting the action of intrinsic uncouplers is inconclusive but it is possible that they could represent an in vivo mechanism for the modulation of stoichiometry [119].

In summary, there are no known physiological effectors which alter the stoichiometry of proton pumping by the respiratory chain in vivo. There are a number of artificial effectors which can alter the stoichiometry and the mechanism of these changes may be related to the mechanism of stoichiometry change at high values of Δp in vivo.

IV-C. Effective changes in the stoichiometry of proton pumping by altering the pathway of electron flow to oxygen

So far, only changes in the stoichiometry of proton pumping by individual complexes have been considered. The effective stoichiometry of the respiratory chain can also be changed by altering the pathway of electron flow down the chain, even though the stoichiometries of individual complexes are unchanged. One example, from plant mitochondria, illustrates how such stoichiometry changes could occur. In the respiratory chain of the Arum lilly (*Arum maculatum*), electrons can pass directly from the Q-pool to oxygen via an alternative oxidase which does not pump protons [177]. Therefore the electrons bypass the cytochrome bc_1 complex and cytochrome oxidase and none of the redox energy available in the Q-pool is conserved. Instead, heat is produced which volatilizes insect attractants and thus ensures pollination. Similar alterations in the pathway of electron flow to oxygen in the respiratory chain would result in an effective stoichiometry change.

The oxidation of cytoplasmic NADH can be induced in rats by a 15 min exposure to cold [178]. This process, which is sensitive to cyanide but insensitive to amytal and antimycin, is due to cytochrome b_5, a mitochondrial outer membrane enzyme. This enzyme oxidizes cytoplasmic NADH, via a flavoprotein, and passes the electrons to cytochrome *c*. The desorption of cytochrome *c* from the inner mitochondrial membrane was proposed to initiate the oxidation of cytoplasmic NADH [178]. This would allow the cytochrome *c* to come in contact with cytochrome b_5 on the mitochondrial outer membrane. The operation of this process will decrease the effective stoichiometry of proton pumping for the oxidation of cytoplasmic NADH and will thus increase heat production.

α-Gycerophosphate dehydrogenase is present on the outer face of the mitochondrial inner membrane. It passes electrons from the cytoplasmic NADH pool to the mitochondrial Q-pool. This pathway of electron flow decreases the overall stoichiometry of proton pumping for the oxidation of NADH because it bypasses the NADH-ubiquinone oxidoreductase. In insect flight muscle this pathway oxidizes all the glycolytically produced NADH. Treatment of euthyroid rats with desiccated thyroid [179] increases the amount of this enzyme in liver by about 20-fold with smaller increases in other tissues. However, in euthyroid rats [70] it is the amount of substrate, not enzyme, which limits the activity of this pathway, even though the enzyme is present in very low amounts. This enzyme's activity is sensitive to $[Ca^{2+}]$ in the in vivo range [78] and therefore its activity could be modulated.

The malate/aspartate shuttle transfers electrons from the cytoplasmic NADH pool to the mitochondrial NADH pool [70]. Because the E_h of the matrix pool is lower than that of the cytoplasmic pool, an energy input is required to drive this process. This energy is provided by the uptake of a proton by the glutamate/aspartate carrier. Therefore, the uphill transport of electrons is driven by Δp. This proton uptake decreases the stoichiometry of cytoplasmic NADH oxidation when this process is significant.

IV-D. Summary

The balance of evidence suggests that the stoichiometry of proton pumping by the mitochondrial respiratory chain decreases at high values of Δp. This stoichiometry change is confined to cytochrome oxidase. The stoichiometry can also be decreased by particular substances or treatments. In addition, altering the pathway of electron flow through the respiratory chains changes its effective stoichiometry.

Slip has been reported in analogous systems. The stoichiometry of the chloroplast bf complex has been proposed to decrease its stoichiometry of proton pumping at high light intensities (presumably, because of the high values of Δp under these conditions) [180–182]. However, the evidence for this stoichiometry change has been criticised [183]. In bacteriorhodopsin slip occurs at high values of Δp [184–189]. The H^+/ATP stoichiometry of the plasma membrane proton pump of *Neurospora crassa*, which is normally 1, increases to 2 when the energy supply is restricted [190].

In state 4 the values of ΔE_h across the NADH-ubiquinone oxidoreductase and the cytochrome bc_1 complexes are close to equilibrium with Δp [39,143]. In contrast, even in state 4 there is a substantial disequilibrium across cytochrome oxidase [44,143,191]. The net disequilibrium, or driving force, is about 250–300 mV per pair of electrons passing through the complex [44,191]. This disequilibrium was calculated using a H^+/O stoichiometry of 2, [44,191] which is probably applicable at low values of Δp [192–194]. Therefore, in the absence of slip, 25–30% of the ΔE_h across cytochrome oxidase would be dissipated and not converted to Δp. If slip does occur (as seems likely) then the inefficiency of cytochrome oxidase is substantially greater. Similar inefficiencies will occur in the other proton pumps when they operate away from equilibrium and will occur at all values of Δp. However, because the other pumps operate closer to equilibrium than cytochrome oxidase, their inefficiencies will be less.

The mechanism of slip is uncertain and will remain so until the mechanism of proton pumping itself is determined. Even so, speculative models of slip, such as the necessity of dimerization for proton pumping by cytochrome oxidase [163] can be profitably investigated. The proposed mechanism of slip for bacteriorhodopsin may also be of relevance. Two photocycles have been proposed for this pump, one of which pumps protons and one which does not [184,185,195]. High values of Δp are proposed to shift the complex from its pumping to its non-pumping cycle. A similar mechanism of slip in cytochrome oxidase has been proposed [40]. The slip discussed here is a 'redox slip': i.e., electrons pass through the complex (or ATP is hydrolysed) without pumping protons. An alternative type of slip, 'proton slip', has been proposed [18,19,42,117,118,196]. This occurs when proton pass back through the complex itself without causing reverse electron transport (or ATP synthesis). These proton slips would be similar to leak. There is no evidence for the occurrence of proton slip and its proponents believe redox slip to be of greater significance [42,196].

V. The physiological significance of slip and leak in oxidative phosphorylation

The leak of protons through the mitochondrial inner membrane occurs at all values of Δp, but it only causes a substantial decrease in yield at high values of Δp, when the conductance of the mitochondrial inner membrane increases. Similarly, slip (due to inefficiencies in cytochrome oxidase and, to a lesser extent, of other complexes) occurs at all values of Δp, but it too only becomes significant at high values of Δp. The existence of inefficiencies such as slip and leak prompts the question, "are slip and leak just wasteful byproducts of oxidative phosphorylation, or do they have a useful function?". In a few cases, such as leak in brown adipose tissue mitochondria and slip in the Arum lilly, their physiological role is evident. However, slip and leak cannot usually be assigned an obvious physiological role.

Under physiological conditions mitochondria operate between states 3 and 4 and have a $\Delta\psi$ of about 140–170 mV [44,45]. Therefore, from Murphy and Brand [23], the degree of slip and leak in vivo is substantially less than maximal. Further evidence that slip and leak in vivo is submaximal is that the value of Δp generated by NADH-linked substrates is insufficient to cause significant slip and leak [25,27]. In addition, supplying durohydroquinone to hepatocytes respiring on endogenous substrates increases respiration rate and Δp [28,29,197]. Durohydroquinone, which feeds electrons directly into the Q-pool bypassing the NADH-ubiquinone oxidoreductase [197], can thus increase Δp and respiration rate substantially above the values for NADH-linked substrates. These suggest that in vivo, where much of respiration passes through the NADH pool, slip and leak are less than maximal. Oxidative phosphorylation could be set to operate at submaximal values of Δp to

optimize its yield by avoiding the limitations of the membrane and the pumps. Slip and leak may be difficult to avoid at high values of Δp. Slip may exist at high values of Δp, not because it is useful but because of the difficulty of 'designing' a redox pump to avoid slip [40]. Brown and Brand [21] pointed out that the high conductance of the mitochondrial inner membrane at substantial values of Δp necessitates a large stoichiometry of proton pumping by the respiratory chain to conserve the available redox energy.

Oxidative phosphorylation may operate at submaximal slip and leak to avoid excessive dissipation of ΔE_h. This could be done by limiting the substrate supply [45]. However, there may be other advantages to the organism, beyond increased yield in having a leak or slip at high values of Δp and operating oxidative phosphorylation below this region of low yield. This would allow the respiration rate to be increased increased with little increase in Δp. Possible functions for this can be suggested: it could allow the buffering of Δp (and thus ΔG_p) and the distribution of ions such as Ca^{2+} against changes in the rate of respiration. This could be important in the homeostasis of the cell's environment. In addition, it could allow respiration rates to be increased independently of ΔG_p: for example to run β-oxidation rapidly to produce large amounts of ketone bodies. Such a phenomenon occurs when FFAs are added to hepatocytes or perfused liver. Oxygen consumption is increased but without a concomitant increase in the rat of ATP production (for a review see Ref. 45). This is not due to uncoupling by the FFAs and could be due, in part, to the increased substrate supply increasing Δp and thus increasing slip and leak. However, this effect is probably largely due to a cytosolic ΔG_p-gated ATPase [45]. Similarly, Ca^{2+} activation of dehydrogenases [78,79] could also increase substrate supply resulting in an increased Δp and thus increase thermogenesis by slip/leak or by a cytoplasmic ATPase; this would allow rapid hormonal control of thermogenesis [45].

The in vivo degree of slip and leak may itself be of advantage to the organism, even if it is never increased to maximal values. Chance and Williams [198] pointed out that there may be advantages in running oxidative phosphorylation at less than full yield to optimize other factors, such as the rate of ATP production. Slip and leak are effectively futile cycles. Futile cycles are thought to have two functions: theromgenesis and to allow better regulation of a process [199,200]. In Ca^{2+} cycling across the mitochondrial inner membrane (subsection III-C) the dissipation of Δp is the price paid to allow rapid regulation of the Ca^{2+} distribution. This allows hormonally induced changes in cytoplasmic Ca^{2+} to modulate respiration [78,79]. Similarly, slip and leak may exist to optimise or control oxidative phosphorylation or related processes. It has been suggested that slip and leak exist to optimise the efficiency of coupling of oxidation and phosphorylation [201] and that the degree of slip and leak can be altered by the concentration of FFAs [119]. This suggestion arises from the theories of non-equilibrium thermodynamics. It would allow slip and leak to vary in order to optimise the efficiency of coupling at maximal economic output.

It has been tactily assumed that slip and leak are wasteful processes which have to be minimised or, at best, are a price to be paid for improved regulation of other processes. However, a futile cycle's other function, thermogenesis, could also be sufficient justification for slip and leak. In short, there may be no advantage in optimizing oxidative phosphorylation, because the product of slip and leak (heat) is also important to the organism. Thermogenesis in mammals originates from dissipation of free energy throughout metabolism, especially in oxidative phosphorylation and in other processes, such as ion leaks through the plasma membrane, which result in the net nonproductive hydrolysis of ATP [202,203]. The dissipation of free energy at all steps of oxidative phosphorylation has been investigated [204]. It was found that about 35–50% of the redox energy available in reduced substrates is converted to ΔG_p and that the rest was released as heat. Roughly equal losses of free energy as heat occurred at each stage of oxidative phosphorylation. This is in reasonable agreement with Prusiner et al. [205] who found that about 25% of ΔE_h is transduced into ΔG_p with the rest being dissipated. This contrasts with those who propose that the overall reaction of oxidative phosphorylation is close to equilibrium, apart from the cytochrome oxidase reaction, and that up to 75% of ΔE_h is conserved as ΔG_p (Ref. 39 and references quoted in Ref. 40). Therefore, the dissipation of free energy in oxidative phosphorylation is a major source of heat. It is worth noting that the dissipation of $\Delta\psi$ will produce heat but that the dissipation of ΔpH will not. As heat is essential for mammals to maintain their temperatures there may have been no strong evolutionary pressure to increase the yield of oxidative phosphorylation by eliminating slip and leak. This refers to the basal heat production of oxidative phosphorylation. Operating oxidative phosphorylation in the the region of high slip and leak would be an effective mechanism for inducible extra thermogenesis.

In summary, mitochondria in vivo operate just below maximal leak and slip. In a few special cases, slip and leak can be seen to have a useful function. However, under most conditions there is no clear function for slip and leak although possible functions can be postulated. It may be that slip and leak are minimised to optimise the yield of oxidative phosphorylation by avoiding substantial wastage of ΔE_h. Alternatively, slip and leak may have a useful function under physiological conditions or oxidative phosphorylation may occasionally switch to conditions of high slip and leak for particular

functions. One interesting possibility is that slip and leak exist to optimise the efficiency of coupling of oxidation and phosphorylation at maximal economic output. Thermogenesis by slip and leak in mammalian oxidative phosphorylation may be sufficiently important that there has been no strong evolutionary pressure to increase the yield of oxidative phosphorylation.

VI. Concusion

In this review I have outlined the evidence for slip and leak in oxidative phosphorylation by mammalian mitochondria. In addition, the mechanisms and physiological significance of these processes were investigated. Definite answers to questions concerning the mechanism and significance of slip and leak are not yet possible. However, it should now be evident that a full understanding of the dissipation of free energy in oxidative phosphorylation is essential for a complete description of cellular metabolism.

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