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# Beyond the Chemiosmotic Theory: Analysis of Key Fundamental Aspects of Energy Coupling in Oxidative Phosphorylation in the Light of a Torsional Mechanism of Energy Transduction and ATP Synthesis—Invited Review Part 1

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**Abstract** In Part 1 of this invited article, we consider the fundamental aspects of energy coupling in oxidative phosphorylation. The central concepts of the chemiosmotic theory are re-examined and the major problems with its experimental verification are analyzed and reassessed from first principles. Several of its assumptions and interpretations (with regard, for instance, to consideration of the membrane as an inert barrier, the occurrence of energy transduction at thermodynamic equilibrium, the completely delocalized nature of the protonmotive force, and the notion of indirect coupling) are shown to be questionable. Important biological implications of this analysis for molecular mechanisms of biological energy transduction are enumerated. A fresh molecular mechanism of the uncoupling of oxidative phosphorylation by classical weak acid anion uncouplers and an adequate explanation for the existence of uncoupler-resistant mutants (which until now has remained a mystery) has been proposed based on novel insights arising from a new torsional mechanism of energy transduction and ATP synthesis.

**Keywords** Bioenergetics · $F_1F_O$-ATP synthase · Oxidative Phosphorylation · Photosynthesis and photophosphorylation · Mitochondria · Energy transduction · Chemiosmotic theory · Torsional mechanism · Coupling · Membrane and ion transport · Electrogenic · Electroneutral · Valinomycin · Uncoupler · Unified theory of ATP synthesis and hydrolysis

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## Introduction

The fundamental process of oxidative phosphorylation in mitochondria constitutes the core of bioenergetics. The quest for a comprehensive understanding of the link between energy-generating and energy-utilizing processes in the cell has inspired an immense amount of research. Yet a complete understanding of the subtle and complex problems in biological energy coupling and the detailed elucidation of the molecular mechanism of the functioning of the $F_1F_O$-ATP synthase and their counterpart elements in photosynthetic energy transduction and photophosphorylation have defied even the intense research efforts of numerous individuals and groups (Lipmann 1941; Davies and Krebs 1952; Slater 1953; Pullman et al. 1960; Williams 1961; Mitchell 1961; Williams 1962; the group of Lehninger (Lehninger 1964; Reynafarje et al. 1982); Mitchell 1966; Chance and Mela 1966; Pressman et al. 1967; Morowitz 1978; Kell 1979; Williams 1979; Green 1981; the group of Slayman (Hansen et al. 1981); Slater 1987; Boyer 1993; the group of Walker (Stock et al. 1999; Menz et al. 2001); Senior et al. 2002; the group of Pedersen (Bianchet et al. 1998; Chen et al. 2004); Junge et al. 2009).

In the 1970s, after considerable debate and controversy, the chemiosmotic theory emerged as the mechanism generally accepted ("more by erosion of the opposition," in the words of Prebble (2002)). Williams, however,

launched vigorous opposition to the theory, which he contended was based on false premises and postulated more localized models of coupling (Williams 1961; Williams 1962; Williams 1979). He independently developed the idea that respiratory chains produce protons by charge separation and that these protons are coupled to phosphorylation (Williams 1961; Williams 1962). He ascribed the coupling to anhydrous protons localized within the hydrophobic matrix of the membrane, i.e., in his mechanism, the protons responsible for ATP synthesis are intramembrane and are delivered to the $F_1F_O$-ATP synthase without crossing the coupling membrane. Comprehensive critiques of the chemiosmotic theory were also published by others (Green 1981; Slater 1987). Therefore, it must be stressed that the acceptance of the chemiosmotic theory was by no means universal, and currently a considerable amount of experimental data is found to be incompatible with the theory (Nath 2003). Indeed there are a large number of new mechanistic issues and searching questions that have not been answered satisfactorily by chemiosmosis nor by any of the other older mechanisms and theories.

During the past 15 years, the current author has formulated and developed in detail an alternative approach that has become known as the torsional mechanism of energy transduction and ATP synthesis (Nath 1994; Nath 1997; Nath 1998; Rohatgi et al. 1998; Nath et al. 1999; Nath et al. 2000; Nath and Jain 2000; Jain and Nath 2000; Jain and Nath 2001; Nath and Jain 2002; Nath 2002; Nath 2003; Jain et al. 2004; Nath 2004; Nath 2006; Nath 2008; Nath and Nath 2009). The original proposal and its logical development has been shown to resolve the fundamental issues in biological energy transduction discussed above and has offered new ways of analyzing and interpreting the plethora of experimental data. Recently, using a novel systems biology/engineering approach, the rationale has been advanced as a powerful unifying concept in bioenergetics and motility through the formulation in detail of the unified theory of ATP synthesis and hydrolysis (Nath 2004; Nath 2006; Nath 2008; Nath and Nath 2009).

On the tenth anniversary of the realization of the torsional mechanism (Nath et al. 1999), we propose, in Part 1 of this invited review, to summarize the central concepts of chemiosmosis and discuss major problems with its experimental verification. These have been analyzed and reassessed from first principles to identify their important biological implications. A fresh molecular mechanism of the action of the classical anionic uncouplers of oxidative phosphorylation has been proposed based on novel insights arising from the torsional mechanism and the unified theory. Since the article deals with the very topics epitomized by the title of this journal, we expect that it would be of great relevance and interest to the readers of the journal.

## The central physiological-cum-biochemical coupling concept of chemiosmosis

According to the chemiosmotic theory, the flux of electrons through the electron transport chain leads to the generation of a *delocalized* electrical potential ($\Delta\varphi$) by uncompensated, electrogenic translocation of protons from one bulk aqueous phase to the other across the energy-transducing membrane (Mitchell 1961; Mitchell 1966). This general principle, called "coupling by proticity", was hypothesized to energize bulk aqueous media on either side, such that this "protonmotive power" could be used by other complexes inserted in the membrane, such as $F_1F_O$-ATP synthase (Mitchell 1979). This protonmotive force, $\Delta p$, was expressed in electrical potential units (mV) by the equation

$$\Delta p = \Delta\varphi - 2.303RT\ \Delta pH/F \quad (1)$$

where R is the universal gas constant, T the absolute temperature and F the value of Faraday's constant.

In mitochondria, the major part of the $\Delta p$ was presumed to correspond to a delocalized electrical potential across the inner mitochondrial membrane. The protonmotive force was postulated to be maintained because of the low permeability of the inner mitochondrial membrane to ions. The membrane itself was conceived by chemiosmosis to act simply as an "insulator" between the energized aqueous media on either side and not to participate in conformational changes or energy transduction. Further, in the theory, uncouplers of oxidative phosphorylation were thought to act as proton conductors that achieve their uncoupling action by dissipating the protonmotive gradient but do not interact specifically with components of the ATP synthase.

Thus a major tenet of the theory is that the sum of the interchangeable $\Delta\varphi$ and $\Delta pH$ components across bulk aqueous phases generated in mitochondria by oxidation-reduction reactions provide an adequate $\Delta p$ (Eq. 1) and act as the sole driving force of phosphorylation, i.e. through *indirect* coupling mediated by $\Delta\varphi$ or $\Delta p$. Several reports have appeared in the literature that the putative driving force, $\Delta p$, is inadequate in magnitude to perform this role (Slater et al. 1973; Deutsch et al. 1979) and that ATP synthesis occurs at physiological rates in several biological systems at low values of $\Delta p$ (Ferguson and Sorgato 1982; Westerhoff et al. 1984; Slater 1987; Tupper and Tedeschi 1969). The original proponents of the theory have addressed this key issue only once (Mitchell and Moyle 1969), even though this is of fundamental importance and has major implications for molecular mechanisms of biological energy transduction. Their cryptic calculations, reported in a single Table, have not been seriously tested or derived from first principles. Do the calculations and the

interpretations resulting from them withstand careful scrutiny?

## Problems with the experimental verification of chemiosmosis and reassessment of the results from first principles

In their original work (1969), $K^+$-depleted mitochondria were energized by pulses of oxygen in the presence of respiratory substrate (e.g., 3-hydroxybutyrate) at various $\Delta$pH values. The $\Delta\varphi$ across the inner mitochondrial membrane was calculated in the presence of the ionophore, valinomycin, from the distribution of $K^+$ across the membrane. Interestingly, external potassium concentrations spanning three orders of magnitude (14.9 μM to 10.4 mM) were employed, and each entry in the Table (p. 480, Mitchell and Moyle 1969) was obtained for a different external $K^+$ concentration selected within this range. For each $\Delta$pH value, the delocalized $\Delta\varphi$ value in mV was calculated by use of the Nernst equation,

$$\Delta\varphi = 59\ \log([K^{+\omega}{}_I]/[K^{+\omega}{}_O]) \quad (2)$$

where subscripts I and O refer to the inside and outside potassium concentrations, and $\omega$ to the concentration prevailing finally in each compartment after transport. Note that the concentration terms within square brackets on the right hand side of Eq. 2 denote the independent variables, while the left hand side gives the value of the calculated, dependent variable.

In the experiments of Mitchell and Moyle (1969) shown in their Figs. 5–7, initiation of metabolism leads to an efflux of $H^+$ until the system reaches a steady state, and the $H^+$ efflux is matched by a $K^+$ influx of the same magnitude. Here the columns in the Table of Mitchell and Moyle (1969) are derived from the basics by staying close to the paper's approach and without making any other assumptions, which has not been done before. This is possible, as shown below, even though the exact values in the experimental trials were not reported. This endeavor will enable us to truly understand the entire calculation process of the delocalized $\Delta\varphi$, identify any errors, lacunae or fallacies in the process, and comprehend how the torsional mechanism of energy transduction and ATP synthesis overcomes these. Following the estimates made, the inner phase is taken to have a volume of 0.4 ml $g^{-1}$ protein, i.e., for a basis of 1 mg protein, to occupy $0.4\times10^{-3}$ml water per mg protein. The outer phase varies in protein concentration from 6.7 mg $ml^{-1}$ to 7.3 mg $ml^{-1}$ in the experiments. Taking the higher value of 7.3 mg $ml^{-1}$ used in the experiments related to the entries in the Table, and correcting for the dilute solution concentration, we obtain a volume ratio (out/in) of 340. Hence the concentration factor inside versus outside measures 340 and a concentration of a species in the outer compartment has to be multiplied by this factor to obtain its correct concentration in the inner compartment.

The above analysis gives the concentration factor required to calculate the concentration of a species ($K^+$) in the inner phase/compartment from the measured decrease in concentration of $K^+$ in the outer phase/compartment due to its uptake by mitochondria upon energization of an anaerobic mitochondrial suspension by respiratory pulses. The range of values of $pK_O{}^0$ and $pK_O{}^\omega$ for the four cases [(i)–(iv)] considered by Mitchell and Moyle (1969) are tabulated in Table 1. The original notation, where O refers to the outer compartment, 0 to the initial condition and $\omega$ to the final (State 4) condition is adopted. We can now readily calculate $\Delta K_O{}^+$ as the difference between the mean initial concentration of potassium in the outer compartment ($K_O{}^{+0}$) and the mean final concentration of potassium in the outer compartment ($K_O{}^{+\omega}$). These values are tabulated in Table 2. Using the $\Delta K_O{}^+$ value and the calculated concentration factor, an estimate of the final concentration of potassium in the inner compartment can now be readily made (Table 2). Knowing both $K_O{}^{+0}$ and $K_O{}^{+\omega}$, the value of the delocalized membrane potential, $\Delta\varphi$, is calculated from the Nernst equation and is shown in Table 2 for each of the four cases. The values tabulated in the original paper (in which the details were missing) are also presented alongside for comparison.

Calculation of the values in Table 2 was made using the mean of the experimental values of $pK_O{}^0$ and $pK_O{}^\omega$, as discussed above. For cases (iii) and (iv), the estimate of the delocalized electrical potential in Table 2 matches the values obtained in Mitchell and Moyle (1969) perfectly. It is possible to match the values in that paper more closely for cases (i) and (ii) by selecting concentrations of $K^+$ within the $pK_O$ range given in Table 1. For instance, selecting the extreme values of $pK_O{}^0$ and $pK_O{}^\omega$ of 4.01 and 4.825 and 4.01 and 4.585 in cases (i) and (ii) respectively yields a delocalized $\Delta\varphi$ value of 193.2 mV and 175.3 mV for case (i) and case (ii), respectively. Hence knowledge of the exact readings of $pK_O{}^0$ and $pK_O{}^\omega$ in the experimental trials within the range tabulated in Table 1 enables us to

**Table 1** Range of initial and final $K^+$ ion concentrations in the outer bulk phase in the experiments of Mitchell and Moyle (1969)

| Case | $pK_O{}^0$ | $pK_O{}^\omega$ |
|---|---|---|
| (i) | 4.010–4.050 | 4.660–4.825 |
| (ii) | 4.010–4.190 | 4.360–4.585 |
| (iii) | 3.200–3.296 | 3.440–3.500 |
| (iv) | 1.982–1.988 | 2.014–2.020 |

**Table 2** Calculated values of concentrations of $K^+$ in the outer and inner bulk phases/compartments and delocalized $\Delta\phi$ from first principles and its comparison with the $\Delta\phi$ values of Mitchell and Moyle (1969)

| Case | $K_O^{+0}$ [M] | $K_O^{+\omega}$ [M] | $\Delta K_O^+$ [M] | $K_I^{+\omega}$ [mM] | $\Delta\phi$ (this work) (mV) | $\Delta\phi$ (Mitchell and Moyle (1969)) (mV) |
|---|---|---|---|---|---|---|
| (i) | $9.33\times10^{-5}$ | $1.81\times10^{-5}$ | $7.52\times10^{-5}$ | 25.57 | 185.9 | 199±4 |
| (ii) | $7.94\times10^{-5}$ | $3.37\times10^{-5}$ | $4.57\times10^{-5}$ | 15.54 | 157.2 | 171±6 |
| (iii) | $5.65\times10^{-4}$ | $3.39\times10^{-4}$ | $2.26\times10^{-4}$ | 76.84 | 139.0 | 139±3 |
| (iv) | $1.03\times10^{-2}$ | $9.62\times10^{-3}$ | $7.35\times10^{-4}$ | 249.90 | 83.5 | 83±3 |

reach as close as possible to the calculated $\Delta\varphi$ of Mitchell and Moyle (1969).

## Biological implications of the reassessed data

As discussed above, in the early experiments, $K^+$-depleted mitochondria maintained anaerobically in the presence of oxidation substrate and valinomycin at various external potassium concentrations are activated by the introduction of oxygen. In the experiment, an efflux of $H^+$ is balanced by an influx of $K^+$ and a steady state is rapidly reached. A delocalized membrane potential has been calculated from the ratios of the internal and external $K^+$ concentrations by use of the Nernst equation, on the assumption that the $K^+$ distributes at electrochemical equilibrium. Under the conditions of the experiment, the $K_{in}^+$ concentrations increases with time while the $K_{out}^+$ concentration decreases with time more weakly due to the larger outside volume during the unsteady state part of the uptake. Subsequently, a steady state level of uptake is reached but now the calculated value of the delocalized field becomes a function of $K^+$ ion concentrations. Since the calculated $K_{in}^+$ concentration changes only by an order of magnitude from 15.5 mM to 249.9 mM (Table 2), while the independent variable, $K_{out}^+$ has been chosen in the experiment to vary over three orders of magnitude (from 14.9 μM to 10.4 mM), it is the value of the external potassium concentration that exerts the maximum control on the calculated value of the delocalized electrical potential. This explains why the assumption of a constant inside potassium concentration of 150 mM made by Tedeschi (2005) did not alter the calculations drastically, even though this was not the exact value under the experimental conditions, as clearly seen from Table 2. Thus, the larger the value of the external potassium concentration selected, the higher is the potassium concentration that remains outside at the end of the transport, and the smaller is the value of the calculated delocalized $\Delta\varphi$ (Table 2). Thus, $\Delta\varphi$ is a strong function of $K_{out}^+$ concentration as plotted exactly in Fig. 1. It varies approximately as $\ln[1/K_{out}^+]$, if the variation of inside potassium concentration is neglected, and it is possible to obtain whatever value of $\Delta\varphi$ that one wishes by appropriate selection of the external potassium concentration, which is an independent variable in the experiments, along with the valinomycin concentration, and hence is readily under the control of the experimentalist. The lower the value of $\Delta\varphi$ that one requires, the higher the value of the $K_{out}^+$ concentration that one should choose, and having fixed a certain value of $\Delta$pH, it is possible to obtain a $\Delta\varphi$ that when added to the $\Delta$pH will yield a total $\Delta$p of 240 mV. Hence the results ($\Delta\varphi$) reported in the Table (Mitchell and Moyle 1969) have to be interpreted with great caution.

As shown above, it is possible to select data values of the external potassium concentrations ($K_{out}^+$) in such an experiment that will provide the value of $\Delta\varphi$ that is required by the experimentalist. In fact, one can obtain any value of $\Delta\varphi$ that one pleases by appropriate selection of the independent variables. In a nutshell, mathematically speaking, this is due to the fact that Eq. 2 has *infinite solutions*. The particular solution is dependent on the value chosen for the external potassium concentration ($K_{out}^+$) and the valinomycin concentration. Furthermore, in the experiments discussed above, the steady state internal concentration and the final external concentration of potassium is dependent on the rate of $K^+$ influx, which in turn is a function of the rate of efflux of protons by the primary redox $H^+$ pump, i.e. it is condition-dependent, e.g. on the $K_{out}^+$ and valinomycin concentration. This fact is inconsistent with the crucial assumption of equilibrium in relation to $K^+$ made by the

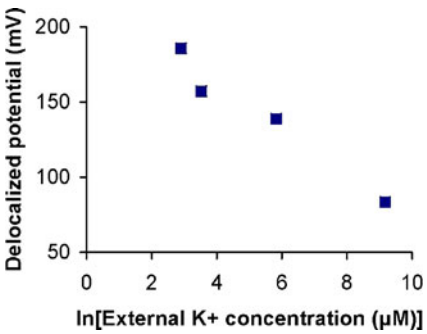


**Fig. 1** The calculated values of the delocalized electrical potential as a function of the natural logarithm of the mean potassium concentration (μM) in the bulk aqueous medium of rat liver mitochondria incubated under the experimental conditions of Mitchell and Moyle (1969)

chemiosmotic theory and required by the use of the Nernst equation in chemiosmosis.

The above concept, that in the mitochondrial experiments in question the potassium ion reaches a nonequilibrium steady state other than the equilibrium assumed by the chemiosmotic theory, is also in accordance with other data, such as the valinomycin concentration dependence of the ion movements (Rottenberg and Solomon 1969; Massari and Azzone 1970; Azzone and Massari 1971; Massari et al. 1972; Massari and Pozzan 1976). Here we only discuss aspects that have not been presented by us previously (Nath 2003). The data show that the measured steady state concentration ratio $K_{in}^{+}/K_{out}^{+}$ is a function of the valinomycin concentration. This is inconsistent with the chemiosmotic theory because, in the equilibrium formulation, the $K^+$ distribution should reach the same steady state level, irrespective of the valinomycin concentration, i.e. the $K_{in}^{+}/K_{out}^{+}$ ratio should be completely independent of the amount of potassium transport or leakage Unfortunately, this is not borne out by the data cited above (Rottenberg and Solomon 1969; Massari and Azzone 1970; Azzone and Massari 1971; Massari et al. 1972; Massari and Pozzan 1976), in contradiction with the data in Fig. 6 of Mitchell and Moyle (1969). A possible explanation of the discrepancy could be that in the higher concentration range of valinomycin used, the binding sites for valinomycin reach a saturation, as expected from adsorption and surface science principles (Nath and Shishodia 1993; Nath 1999; Nath 2003) applied to aqueous-organic interfaces, while the data of the Azzone group were recorded at lower valinomycin concentrations than those employed by Mitchell and Moyle (1969), conditions at which the binding sites for valinomycin did not saturate. In any case, the arguments advanced by Mitchell and Moyle in their 1969 work to justify their assumption of equilibrium are not water-tight, and do not have the capacity to explain datasets recorded by other investigators (Rottenberg and Solomon 1969; Massari and Azzone 1970; Azzone and Massari 1971; Massari et al. 1972; Massari and Pozzan 1976).

## Molecular mechanism of uncouplers in oxidative phosphorylation

A key prediction of the chemiosmotic theory is that uncouplers of oxidative phosphorylation are proton conductors that interact nonspecifically with the lipid component of the biomembrane and dissipate the protonmotive force. However, a large body of experimental data, not all of which can be covered in this article, is contradictory to the above simplistic explanation. For instance, it has been shown conclusively that the uncoupling action of various compounds at different pH values bears no relationship with their ability to enhance the conductivity of artificial lipid bilayers (Wilson et al. 1971). Similarly, the uncoupling ability of the well-studied classical uncouplers of oxidative phosphorylation, 2,4-dinitrophenol and trinitrophenol have been shown not to correlate with their effect on proton conductivity of submitochondrial particles (Hanstein and Hatefi 1974a). Hence the proposal that uncouplers simply act as proton carriers and increase $H^+$ conductivity is not a suitable explanation for their effect in uncoupling phosphorylation from oxidation. Moreover, there is sufficient evidence that uncouplers interact with protein components of mitochondria and that these binding sites are functionally involved in the act of uncoupling (Hanstein and Hatefi 1974b).

How does the torsional mechanism of energy transduction and ATP synthesis explain the uncoupling of oxidative phosphorylation by weak acid anions? The action of such classical uncouplers of oxidative phosphorylation has been explained within the framework of the torsional mechanism by a fresh, completely different rationale, and uncoupling mechanisms of oxidative phosphorylation are far more complex and subtle than currently believed, as depicted in Fig. 2 (Nath 2004; Nath 2008). According to the torsional mechanism, uncouplers act at a specific proteolipid binding site in the inner mitochondrial membrane and interfere with the establishment of the high-energy conformational (metastable) state of the c-subunit in the $F_O$ portion of ATP synthase (Nath 2002). An uncoupling anion ($U^-$) enters through the anion access channel, i.e., *separately* from the proton which enters through its own $H^+$ half-access channels, and

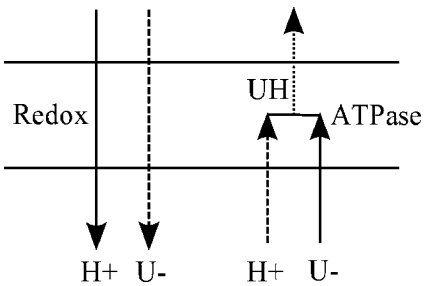


**Fig. 2** Uncoupling mechanisms in oxidative phosphorylation according to the torsional mechanism. Uncoupler anion ($U^-$) competes with substrate anion ($A^-$) for entry into the anion access channel in the a-subunit in $F_O$. The uncoupling process involves entry of $U^-$ and $H^+$ as distinct species through their respective specific, regulated access channels, their recombination (UH) in the vicinity of the proton and anion binding sites in the membrane due to the lipid solubility of the uncoupler $U^-$, their exit as a single, electrically neutral UH species (thereby interfering with the physiological temporal sequence of ion movements and *disrupting* the provision of energy to $F_1F_O$ by ~50%), dissociation of UH into $U^-$ and $H^+$ in the aqueous phase of the mitochondrial matrix, and pumping of the dissociated $U^-$ and $H^+$ separately in sequence by the redox complexes back across the membrane

*competes* with substrate anion ($A^-$) for entry, but because of its lipid solubility, approaches close to the proton to form the neutral UH in the vicinity of the $A^-/U^-$ and $H^+$ membrane binding sites and now moves across the membrane as UH, thereby dissipating the energy of the nonequilibrium conformational state and disrupting energy transduction. It dissociates back to the uncoupling anion and proton in the exit aqueous phase which are both pumped back by the redox complexes, thus uncoupling oxidation from ATP synthesis (Fig. 2). It is thus possible for either the first step of entry of the anionic form of the uncoupler into the mitochondrion, or the second step of passive outward diffusion of the uncharged species to be the rate-limiting step of the uncoupling process. Hence, such a molecular mechanism can readily explain both uncoupling data consistent with chemiosmosis, and also data summarized above that cannot be explained within the chemiosmotic framework (Fig. 2). Therefore, the new mechanism of uncoupling action can be considered an important step forward in the evolution of our knowledge of oxidative phosphorylation.

## Uncoupler-resistant mutants

Uncouplers are toxic to organisms and if they exert their effects by merely conducting protons across the lipid bilayer then we would not expect to find uncoupler-resistant mutants, as this would destroy their so-called "protonmotive force" essential to life. From our biological knowledge, every organism possesses a lipid bilayer membrane, and possession and retention of such a bilayer will always permit these uncouplers, in the chemiosmotic explanation, to act as protonophores and ferry protons across. However, uncoupler-resistant mutants do exist and have been isolated in bacteria (e.g. from *Escherichia coli and Bacillus megaterium*). The explanation for resistance to uncouplers in such mutant bacteria has remained a mystery.

Decker and Lang (1978) have isolated and studied the properties of mutants of *Bacillus megaterium* with uncoupler-resistant ATP synthesis. They have carefully characterized these mutants and have evaluated their membrane bioenergetic parameters (Decker and Lang (1978)). They observed retention of ATP synthesis in the absence of any significant bulk $\Delta pH$ and $\Delta\varphi$ in these mutants. These findings have been verified and extended by Guffanti et al. (1981). Griffiths et al. (1972) have also reported the isolation of uncoupler-resistant mitochondria in yeast mutants. Further, their subsequent finding of valinomycin-resistant mitochondrial mutants (Griffiths et al. 1974) also refutes the simple postulate of a nonspecific interaction of valinomycin with the lipid component of the biomembrane. According to the torsional mechanism, mutated specific uncoupler-binding sites existing in the energy-transducing membrane are no longer able to bind uncoupler or binding has changed in such a way that oxidative phosphorylation remains unaffected, and ATP synthesis is therefore uncoupler-resistant.

## Conclusions

Certain key, fundamental aspects of energy coupling in oxidative phosphorylation have been analyzed from first principles. There has been a problem concerning the nature and magnitude of the ion gradients in oxidative phosphorylation that has existed since the original report of the experiments designed to measure the delocalized electrical potential across bulk aqueous phases (Mitchell and Moyle 1969). This work has been quoted almost without question for more than 40 years. Here these experiments have been dissected and analyzed in minute detail. It has been shown that the calculated values of the delocalized electrical potential, $\Delta\varphi$, in the original publication are a function of the external potassium and valinomycin concentrations employed in the experiments. Hence the equation used to calculate $\Delta\varphi$ has been shown not to possess a unique solution, a property that places limitations on the approach adopted by chemiosmosis. It has been concluded from our analysis that re-interpretation of the nature of the ion gradients/electrical potential is necessary.

It has also been shown that previous theories are inconsistent with an immense amount of experimental data. These include data on the action of the classical uncouplers of oxidative phosphorylation such as 2,4-dinitrophenol, and the isolation of uncoupler-resistant mutants of oxidative phosphorylation by several workers in the field. These phenomena have been shown to be readily explained within the new framework (Fig. 2). This has been considered an important step forward in the evolution of our knowledge of ATP mechanism.

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