Biophysical Chemistry 255 (2019) 106271

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

Modern theory of energy coupling and ATP synthesis. Violation of Gauss's law by the chemiosmotic theory and validation of the two-ion theory

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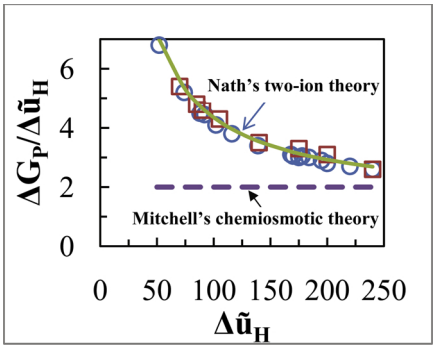
HIGHLIGHTS

- Electrostatic calculations made to justify a central tenet of Mitchell's chemiosmotic theory are scrutinized.
- It is proved from first principles that the above calculations violate Gauss's law.
- It is argued that theories of energy coupling that go beyond chemiosmosis are absolutely necessary.
- Nath's two-ion theory of energy coupling and ATP synthesis is validated and presented as a superior alternative.
- The modern theory is shown to be useful for understanding many other biological processes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Bioenergetics
Oxidative phosphorylation
Photosynthesis
Mitchell's chemiosmotic theory
Protonmotive force
Delocalized electrical potential
Local potential
Electrostatics and electroneutrality
Gauss's law
Maxwell's laws of electromagnetism
Nath's two-ion theory of energy coupling
Nath's torsional mechanism of energy transduction and ATP synthesis
Mitochondrial system length scales

ABSTRACT

Adenosine triphosphate (ATP) is the universal biological energy fuel, or nature's gasoline. The vast quantities of ATP required for sustenance of living processes in cells are synthesized by oxidative phosphorylation and photosynthesis. The chemiosmotic theory of energy coupling was proposed by Mitchell more than 50 years ago but has a contentious history. Part of the accumulated body of experimental evidence supports Mitchell's theory, and part of the evidence conflicts with the theory. Although Mitchell's theory was strongly criticized by several prominent scientists, the controversy was never resolved. Certain theoretical arguments and electrostatic calculations were originally made to justify the central tenet of the chemiosmotic theory of electrogenic proton transfer and violation of electrical neutrality in bulk aqueous phases by creation of a delocalized field. However, these calculations have not been scientifically scrutinized previously. Here it is proved from first principles that the original physical arguments and calculations made in support of steady state electrogenic ion transfer and chemiosmosis violate Gauss's law. Nath's two-ion theory of energy coupling in which the field is *local*, and ion translocation is dynamically electrogenic but overall electroneutral is shown to satisfactorily resolve the difficulties. Characterization of length scales in mitochondrial systems is shown to impose strong constraints on possible mechanisms of energy transduction. Some biological implications for energy coupling, transduction and ATP synthesis arising as a result of the above analysis are discussed. Examples of several other biological processes where the new theory is useful such as apoptosis, muscle contraction, the joint multisite regulation of oxidative phosphorylation and the Krebs cycle, and hindered protein aggregation arising from ATP's hydrotropic properties are outlined.

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https://doi.org/10.1016/j.bpc.2019.106271
Received 15 August 2019; Received in revised form 30 September 2019; Accepted 3 October 2019
Available online 17 October 2019

Nomenclature

a	distance (m)
A	area (m^2)
e	electronic charge (C)
E	electrical field strength (V m^{-1})
F	Faraday (C $mole^{-1}$)
ΔG	free energy change (kJ mol^{-1})
ΔH	enthalpy change (kJ mol^{-1})
k	proportionality constant between rate and driving force; Boltzmann constant (1.3807×10^{-23} J K^{-1})
l	inter-ionic length scale (m)
L	length (m)
n	pump stoichiometry
Δp	protonmotive force difference (mV)
ΔpH	pH difference (mV)
q	charge (C)
Q	charge (C)
r	radial distance (m); rate of ATP synthesis (mol s^{-1} mg $protein^{-1}$)
R	radius (m); universal gas constant (8.31446 J $mol^{-1}K^{-1}$)
T	temperature (K)
ΔU	internal energy change (kJ mol^{-1})
V	electrical potential (volts)
z	valence

Greek letters

Δ	difference
ε	permittivity (C^2 N^{-1} m^{-2})
λ	length scale (m)
$\mu^{\sim}$	electrochemical potential (kJ mol^{-1})
σ	charge density (C m^{-3})
φ	delocalized electrical potential (mV)
ψ	local electrical potential (mV)

Subscripts

0	standard state; vacuum
A	anion
C	cation
D	Debye
H	proton
i	component index
in	inside
O	oxidation
out	outside
P	phosphorylation or ATP synthesis
w	water

1. Introduction

The molecular mechanism by which energy is released, conserved, transduced, stored, and utilized in biological processes and how chemical reactions can be coupled are among the most fundamental questions in science. The vast quantities of the universal biological energy currency, adenosine triphosphate (ATP) needed by the cell to fuel its catabolic and anabolic reactions are synthesized by F_1F_O-ATP synthase enzyme in animal mitochondria, plant chloroplasts and bacteria by the fundamental processes of oxidative phosphorylation and photosynthesis. Almost all textbooks of biochemistry currently in print use Mitchell's chemiosmotic theory of energy coupling to explain the synthesis of ATP. A central tenet of the chemiosmotic theory is the *uncompensated*, electrogenic translocation of protons to cause a *delocalized* electrical potential, Δφ between two *bulk* aqueous phases across an inert, rigid and insulating membrane, and the coupling of two reactions (e.g. oxidation and phosphorylation) driven by the so-called "protonmotive force" (Δp), the sum of ΔpH and Δφ, with both components generated *solely* by protons (Eq. (1)) [1–3].

$$\Delta p = \Delta\varphi - \frac{2.303RT}{F}\Delta pH \tag{1}$$

Where, $\Delta pH = pH_{in} - pH_{out}$, and the delocalized potential difference, $\Delta\varphi = \varphi_{in} - \varphi_{out}$, with a reference potential of 0 mV taken for the external medium. Chemiosmosis is an *equilibrium* theory that postulates the establishment of an equilibrium protonmotive force by a redox proton pump that depends on the free energy of the driving reaction (oxidation in this case) and the number of protons, n_O translocated by the redox H^+ pump per oxygen atom from the inside bulk aqueous phase to the outside bulk aqueous phase. Thus, at equilibrium, according to the theory, the free energy change of oxidation, ΔG_O is exactly balanced by the protonmotive force, Δp, i.e.

$$\Delta G_O = n_O F\Delta p \tag{2}$$

These protons are translocated back from outside to inside at equilibrium through the access proton channels of the membrane-bound portion of ATP synthase with a stoichiometry of n_P protons per ATP, supposedly generating an equilibrium phosphorylation potential, ΔG_P, that is responsible for ATP synthesis (by a molecular mechanism that was never specified by chemiosmotic dogma) and is given by the equation

$$\Delta G_P = -n_P F\Delta p \tag{3}$$

It is hard to see the logic of translocating protons at equilibrium from the inside to the outside bulk aqueous phase, given that they are made to return to the inside bulk phase in order to shift the equilibrium of the ADP–ATP chemical reaction. Hence, in the author's opinion, the model is flawed in the overall sense itself. Some arguments and simplistic calculations to justify the chemiosmotic theory were originally made by its proponent; however, these arguments have escaped prior scientific scrutiny, and the calculations have not been verified previously.

Here, it is shown from first principles that the Mitchellian physical arguments and calculations made to support steady state electrogenic ion transfer and chemiosmosis [1–3] are erroneous and misleading, and violate Maxwell's laws of electromagnetism, and in particular, Gauss's law for electricity. This conclusion is substantiated by a detailed step-by-step derivation that devises a suitable reversible path from infinity (where electrical potential, V = 0) to the interior of the test sphere and mathematically calculates the potential, field, and work done by proper application of Gauss's law and use of the principles of calculus. The results prove compellingly that the electroneutrality of bulk aqueous phases cannot be violated to the very large and unprecedented extent postulated by the chemiosmotic theory and that the protonmotive force, created solely by the incessant, uncompensated translocation of protons does not exist.

2. Simplistic arguments made to justify the chemiosmotic theory

Mitchell considered [1,2] an electrically insulated spherical phase with a radius of 1 cm. Instead of satisfying the condition of electrical neutrality, this phase was taken to contain an excess of 10^{-10} mole of a monovalent ion. Using the expression for the electrical potential, V of a charged sphere of radius r *in vacuo*, $V = Q/(4\pi\varepsilon_0 r)$, he obtained $V = 0.96 \times 10^{-5}/(1.11 \times 10^{-10} \times 10^{-2}) = 0.86 \times 10^{7}$ V, a phenomenally large electrostatic potential. Next, he divided the sphere of

1 cm radius into a large number of small spheres (10^{12}) each of radius 1 μm, such that the total volume of these spheres was the same as the sphere of 1 cm radius (Fig. 1). Applying the same formula, he now obtained V = 0.96 × 10^{-5}×10^{-12} /(1.11 × 10^{-10}×10^{-6}) = 86 mV, a very small value [1,2]. He concluded, in a section entitled "The Fiction of Electrical Neutrality" that transfer of a single ion species between two bulk phases at different electric potentials is permissible and that electrical neutrality of a bulk aqueous phase can be readily violated in biological systems [1,2]. In his words, "Processes involving the transfer of a single ionic species between one phase and another at different electrical potential are permissible and have the same thermodynamic status as processes involving the transfer of a single uncharged molecular species" [1,2].

3. Falsity of the Mitchellian arguments for a conducting sphere

In an electrical problem involving spherical symmetry, we are not free to choose any radius coordinate arbitrarily (e.g. as r = 1 μm) and plug it into the standard electrical potential expression (as done by Mitchell), ignoring the spherical symmetry inherent in the physical situation (with r = 0 at the center of the large sphere, whatever its radius) (Fig. 1). Even more serious, for the case of the conducting sphere or shell, it was incorrect to divide charge q by the number of small spheres (10^{12}) and use this value as the charge in the electrical potential expression because nature simply does not behave in this way. In fact, according to Gauss's law, q will distribute itself on the surface in such a way that *all* points on the conductor — those on the surface as well as those inside — have the *same* electrical potential (Fig. 2) and not greatly less than its surface value as calculated by Mitchell. In other words, the field is zero everywhere inside the conductor (Fig. 2). The earlier calculations [1,2] therefore violate Gauss's law. A comparison of the results obtained by Mitchell and by the author is made in Table 1. It should be added that including a dielectric does not alter the difference in the results tabulated in Table 1. For ε = 80, the values in Table 1 will be smaller by a factor of 80, leading to an ~10^8 mV potential from Gauss's law, but a negligible, scarcely credible electrical potential value of only ~1 mV from Mitchell's calculation.

4. Exact calculations for a nonconducting sphere

Even if a nonconducting sphere is considered, the electrical potential (ψ) and electrical field (E) cannot be obtained in a simplistic way by blindly assuming r^{-1} and r^{-2} dependence respectively, but must be arrived at by devising a suitable reversible path from infinity (where V = 0) to the interior of the sphere and calculating the potential/field/work done by application of Gauss's law and the principles of calculus (not indulged in at all in the previous calculation [1,2]). A detailed step-by-step derivation from first principles below proves that ψ and E show a completely different behavior within the sphere as a function of r (ψ ∝ r^2, E ∝ r) (Fig. 3) from the earlier assumed dependence (ψ ∝ r^{-1}, E ∝ r^{-2}). In fact, the potential inside the large sphere can only exceed (or at most equal) the value of the potential at the surface and can *never be smaller* than the surface value as obtained previously [1,2]. Physically, this is because the work done in moving a test charge from infinity to the sphere interior can never be less than moving it to the surface of the sphere.

The potential V_B at an arbitrary point B is given by [4]

$$V_B = V_A - \int_{r_A}^{r_B} E dr \tag{4}$$

Setting $r_A = \infty$, $V_A = 0$, $r_B = a$, the radial coordinate of a point inside the sphere of radius R, and $V_B = V_a$,

$$V_a = -\int_{\infty}^{a} E dr = -\int_{\infty}^{R} E dr - \int_{R}^{a} E dr \tag{5}$$

For the first integral, application of Gauss's law [4] gives

$$4\pi r^2 \varepsilon_0 E = q \tag{6}$$

and the integral becomes

$$-\int_{\infty}^{R} E dr = -\int_{\infty}^{R} \frac{q dr}{4\pi\varepsilon_0 r^2} = \frac{q}{4\pi\varepsilon_0 R} \tag{7}$$

For the second integral, we can find E using Gauss's law

$$4\pi r^2 \varepsilon_0 E = q' \tag{8}$$

Where, q' is the charge enclosed within a radius r. Since q' is proportional to the volume, we obtain

$$q' = \frac{r^3}{R^3} q \tag{9}$$

$$\therefore E = \frac{r^3 q}{4\pi r^2 \varepsilon_0 R^3} = \frac{qr}{4\pi\varepsilon_0 R^3} \tag{10}$$

The second integral can now be readily evaluated as follows:

$$-\int_{R}^{a} E dr = \int_{a}^{R} \frac{qr dr}{4\pi\varepsilon_0 R^3} = \frac{q(R^2 - a^2)}{8\pi\varepsilon_0 R^3} \tag{11}$$

Therefore, the potential at any point 'a' within the nonconducting sphere is given by the expression

$$V_a = \frac{q}{8\pi\varepsilon_0 R^3}(R^2 - a^2) + \frac{q}{4\pi\varepsilon_0 R} = \frac{q}{8\pi\varepsilon_0 R^3}(3R^2 - a^2) \tag{12}$$

Inspection of the final result, Eq. (12), reveals quantitatively that the potential at any point 'a' inside the nonconducting sphere is always greater than the potential at the surface of the sphere (since R > a), and at most equals the potential at the surface of the sphere (for a = R).

Thus, it is concluded that the value of the electrical potential of only 86 mV at a point within the sphere (conducting or nonconducting) claimed in the original Mitchellian calculations [1,2] – which was astronomically lower than the value of the electrical potential at the surface of the sphere (86 × 10^8 mV) – was obtained by violating Gauss's law.

5. Perspective from quantum physics

Finally, if we were to believe Mitchell's view [1,2] and carry his argument of division of a single large sphere into an (arbitrary) large number of small spheres (of the same total volume as the volume of the

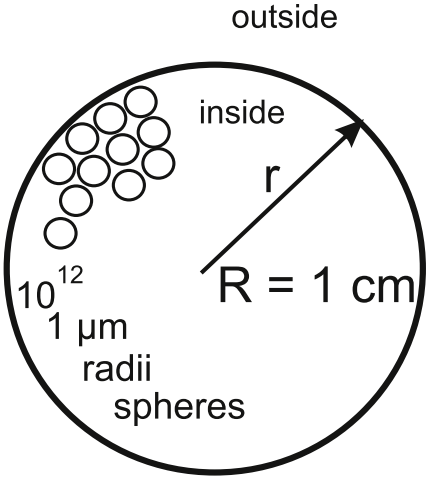

Fig. 1. Schematic diagram to represent the division of a sphere of radius R = 1 cm into 10^{12} small spheres each of radius 1 μm for calculation of the electrostatic potential by Mitchell [1,2]. The total volume of the small spheres equals the volume of the large sphere of radius 1 cm.

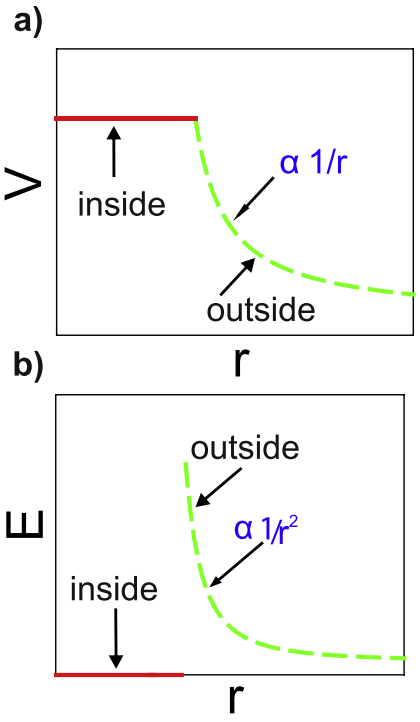

Fig. 2. (a) Electrical potential (V), and (b) field (E) as a function of radial coordinate, r for a conducting shell or sphere. (Red bold line: inside the sphere; Green dashed line: outside the sphere).

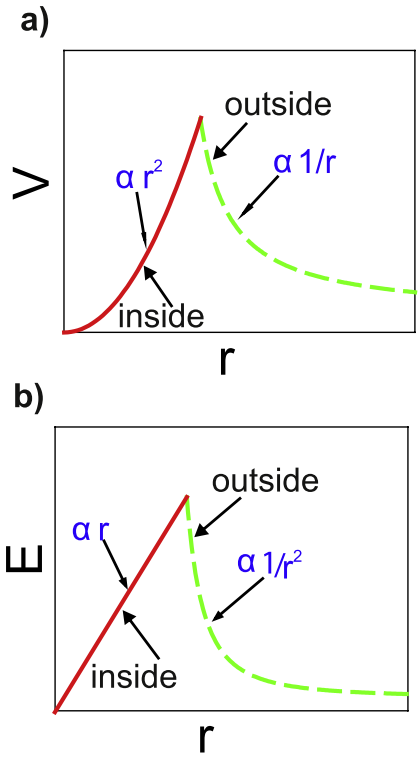

Fig. 3. (a) Electrical potential (V), and (b) field (E) as a function of radial coordinate, r for a nonconducting sphere. (Red bold line: inside the sphere; Green dashed line: outside the sphere).

Table 1
Comparison of the calculated values of the electrostatic potential, V for the conducting spherical case (R = 1 cm) depicted in Fig. 1 containing a small excess charge corresponding to 10^{-10} mole of a monovalent ion.

V by Mitchell's calculation (mV)	V from Gauss's law (mV)
86	86×10^8

large sphere, see Fig. 1) to its logical extent, we can obtain a charge on a small sphere less than the electronic charge, e (e.g. for $\sim 10^{15}$ or more spheres), and thereby violate all the laws of quantum physics. Thus, the above fundamental analysis reveals the catastrophic flaws and inconsistencies in chemiosmotic dogma [1–3] that have never previously been pointed out or even recognized.

The above difficulties are additional to the inconsistencies of chemiosmosis with experimental data that have been repeatedly pointed out previously [5–15]. The results prove compellingly that the protonmotive force, created by the incessant electrogenic translocation solely of protons, simply does not exist because electrical neutrality of bulk aqueous phases cannot be violated to the very large and unprecedented extent postulated by the chemiosmotic theory.

6. Novel molecular mechanism of energy coupling that resolves the above difficulties

A molecular mechanism of ATP synthesis/hydrolysis that is novel, unified and quantitative, and does not violate any of the known laws of nature is Nath's torsional mechanism of energy transduction and ATP synthesis which incorporates a two-ion theory of energy coupling [11–19]. In this mechanism, the driving forces for ATP synthesis, ΔpH and ΔpA (or ΔpH and $\Delta\psi_A$, $\Delta\psi_H$ and $\Delta\psi_A$, etc., depending on the stage of the conformational cycle and where one draws the boundary surface, because the driving force has to change form to act) are created by two *independent* sources, i.e., protons and membrane-permeable anions respectively and are thermodynamically intensive properties, related to the discrete, *ordered and sequential* elementary events of proton and *anion* translocations (binding and unbinding) through the F_O portion of the F_1F_O-ATP synthase at the a–c interface [11–19]. The mechanism is illustrated in Fig. 4. One of its achievements is the identification of the physiological anion involved in energy coupling and transduction in mitochondria and chloroplasts from a universal set of anionic substrates, metabolites and activators, following a ten-year experimental search [12–15].

Various aspects of the ATP torsional mechanism have also been discussed in research articles and books by other authors [20–28]. More importantly, from the standpoint of the present article, the Δψ in this mechanism is *local* and should not be confused with the delocalized Δφ of chemiosmosis across bulk aqueous phases. The mechanism is dynamically electrogenic but overall electroneutral (Fig. 4), and violation of electrical neutrality that occurs is only *transient*, and that too by the smallest quantity of charge (one quantum). Thus, unlike chemiosmosis, the new mechanism does not violate bulk electroneutrality. Moreover, the small deviation from electroneutrality is what determines the *order* of the elementary steps in the train of sequential transport processes. The above would then constitute the foundation of a central, inviolable design principle for coupling in biological energy transduction, and as a general principle for ordering of elementary biological processes in space and time.

It has been believed for decades that the delocalized Δφ is an indirect messenger and an obligatory intermediate mediating oxidation and phosphorylation. It is often stated that an equal and opposite flow of protons on the redox and ATP sides constitutes an electroneutral system. While this is true in an overall sense, we have argued that both redox and ATP sides are electroneutral separately, and that a delocalized Δφ does not exist [17]. Our arguments are based on the following facts. ATP synthesis has been experimentally demonstrated routinely with the enzyme molecule purified, and reconstituted into tight liposomes, which do not contain any redox complexes and offer no leakage pathways through the barrier of the lipid bilayer (Fig. 5). Therefore,

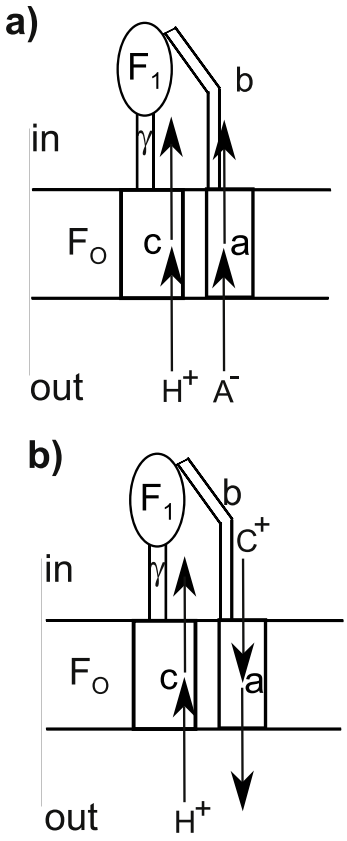

Fig. 4. Two-ion theory of biological energy coupling in Nath's torsional mechanism of energy transduction and ATP synthesis by F_1F_O-ATP synthase. Protons, H^+ and dicarboxylic acid anions, A^- (a) or Na^+/K^+ in the opposite direction, depicted as C^+ (b) translocate through the membrane-bound F_O portion of the enzyme, bind to their respective binding sites on the c-subunit/a-subunit respectively at the a–c interface in F_O, and cause c-oligomer rotation and conformational changes in the c-subunits by means of the $\Delta(\Delta\psi)$ induced by ion-protein interactions [11,14,17]. Protons bind and unbind within the electrostatic potential field created by anion/counter-cation translocation; in the diagrams, the discontinuity of the arrows within F_O represents the binding and unbinding of ions to/from their sites at the a–c interface. The energy of the discrete ion translocations is ultimately stored after a cascade of energy transformations as torsional energy in the central γ-subunit of the extramembrane F_1 portion of ATP synthase [11,13,19], thus explaining the name of the mechanism. The torsional energy is further transmitted to the β-catalytic sites in the F_1 portion of the enzyme and used to condense MgADP and inorganic phosphate Pi, and synthesize ATP by a novel catalytic cycle in F_1 [11,13,16].

either there is no electrical potential (Tedeschi's view) [9], or the electrical potential is created within the aqueous access channels of the ATP synthase enzyme itself, i.e. the Δψ is local (Nath's view) [17]. ATP synthesis is sustained for a long time in such a purified reconstituted system containing no complexes other than the synthase itself, and *there is no equivalent of the redox complexes to pump the protons back in such an experimental system*. The technology has been perfected over the years to enable even a single molecule of the ATP synthase to be embedded in the vesicle. If solely proton flow is postulated to occur in the system (Fig. 5a), as in Mitchell's theory, then the electroneutrality of inside and outside bulk aqueous phases will be violated. However, overall electrical neutrality of bulk aqueous phases is inviolable. Therefore, from inception, Nath's two-ion theory of energy coupling includes a flow of anions in the same direction as the protons (Fig. 5b), or an opposite flow of countercations such that overall electroneutrality of bulk aqueous media is maintained [11–15]. Recent high-resolution experiments reveal a flow of K^+ ions opposite to that of H^+ in the case of the thermophilic ATP synthase enzyme [29]. Such a system has also been

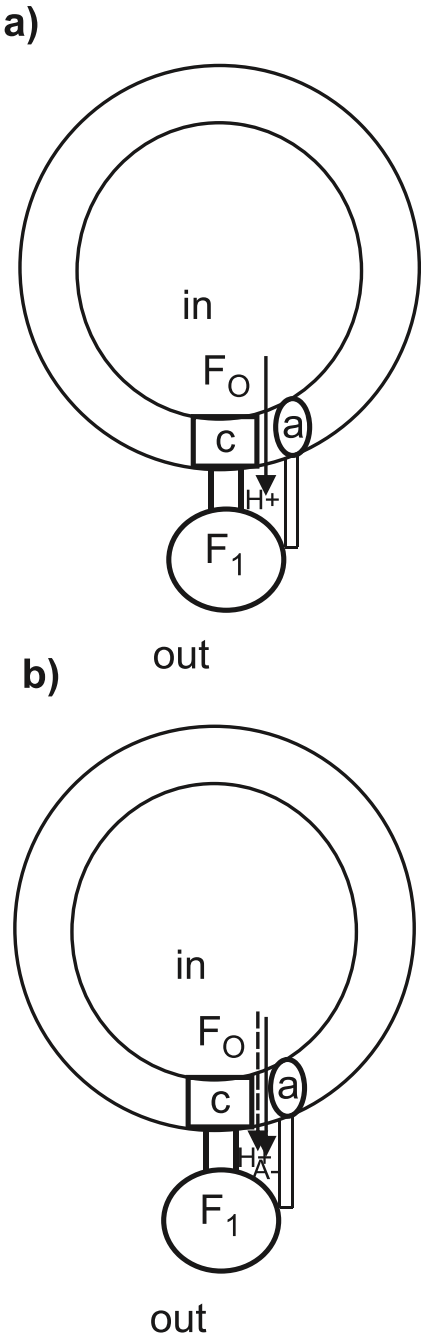

Fig. 5. Experimental model system of a purified ATP synthase molecule reconstituted into a tight liposome with aqueous access pathways at the a–c interface in the membrane-bound F_O-portion of the enzyme. Comparison of models of energy coupling based on a) Mitchell's chemiosmotic theory, and b) Nath's two-ion theory. The bold arrow represents the primary dicarboxylic acid monoanion translocation, while the dashed arrow shows the following proton translocation through the aqueous access channel.

quantitatively modeled from first principles [17].

The above analysis shows that the Δψ is local (i.e. localized inside the access half-channels of F_O) and that this local Δψ has no relationship with the delocalized Δφ presumed to exist across the membrane by the chemiosmotic theory. In fact, constricting the proton circuit to a local region close to the bilayer is not a solution, because how such a potential is efficiently collimated to the Asp/Glu binding site of the proton on the c-subunit has not been explained. Moreover, local events in the membrane are readily shown to be communicated to the bulk. Further, experimental probes target a large region adjacent to the nanolayer.

Hence we should not take refuge in the above view. In fact, what is needed is a mechanism that can communicate the acidification over large distances perpendicular to the membrane, and not remain confined to an ultra-thin layer adjacent to the membrane. Weak acids such as the dicarboxylic acids are ideal candidates for this purpose.

To summarize, the failure to include electroneutrality as a constraint in the reconstituted ATP synthase system (Fig. 5) has led to major misconceptions that have decelerated progress in the field. Further, it is not satisfactory to take refuge in the generally-believed view of charge imbalance at the membrane bilayer, which has not been experimentally validated. This is revealed by consideration of system scale sizes (Section 9), which is applicable to this case also. The novel alternative that the complexes are proton-dicarboxylic acid anion co-transporters, and that the local potentials are created by a dynamically electrogenic but overall electroneutral mode of ion transport solves the conundrum [11–19].

The proposal of dicarboxylic acid translocation across the energy-transducing membrane should be seen with an open mind and experimentally validated or disproved, rather than "shooting the messenger" at the outset itself. Our proposal should be seen in a positive light, especially given that it suggests new experiments to verify the hypothesis, and offers ways to widen our horizons. Some experimental evidence has been adduced for the above view in our previous works [12–15,18,35–37]; however, further experimentation by other groups would greatly help to clarify this important matter.

7. Mathematical equations for Nath's theory

The mathematical equations in Nath's two-ion theory of energy coupling and ATP synthesis are succinctly presented in this section. According to the theory, the overall driving force for ATP synthesis by the ATP synthase is proportional to the sum of the electrochemical ion gradients contributed by *two species of ions*, i.e. protons *and* counterions (either anions transported through symsequenceport or cations transported through antisequenceport), i.e.

$$d.f. = n_P(\Delta\tilde{\mu}_H + \Delta\tilde{\mu}_{A/C}) \tag{13}$$

Where, d.f. denotes the net driving force of ATP synthesis, subscript H denotes H^+, A^- stands for monoanion, C^+ for countercation, symbol / represents "either or". Since, according to the theory, electroneutrality must be maintained during the coupled process of anion/countercation and proton translocation/exchange, the number of discrete translocations of A^-/C^+ ($n_{A/C}$) and H^+ (n_H) must be equal. Let this number be represented by n_P. Thus, n_P is the number of discrete translocations of proton *and* counterion per molecule of ATP synthesized. The rate of ATP synthesis, r is proportional to the above driving force, with a proportionality constant k. Considering the case of photosynthetic and mitochondrial energy transduction, where the *physiological* co-ion whose translocation is coupled to proton translocation is a dicarboxylic acid monoanion [13–15,18], we have,

for the H^+ ion,

$$\tilde{\mu}_{H,in} = \mu_0' + RTln[H^+_{in}] + z_H F\psi_{in} \tag{14}$$

$$\tilde{\mu}_{H,out} = \mu_0' + RTln[H^+_{out}] + z_H F\psi_{out} \tag{15}$$

Where, μ_0' is taken as the common standard state chemical potential at the prevailing T, P. Hence, since valence $z_H = +1$,

$$\Delta\tilde{\mu}_H = \tilde{\mu}_{H,out} - \tilde{\mu}_{H,in} = RTln\frac{[H^+_{out}]}{[H^+_{in}]} + F(\psi_{out} - \psi_{in}) \tag{16}$$

Similarly for the A^- ion ($z_A = -1$),

$$\tilde{\mu}_{A,in} = \mu_0' + RTln[A^-_{in}] + z_A F\psi_{in} \tag{17}$$

$$\tilde{\mu}_{A,out} = \mu_0' + RTln[A^-_{out}] + z_A F\psi_{out} \tag{18}$$

$$\Delta\tilde{\mu}_A = \tilde{\mu}_{A,out} - \tilde{\mu}_{A,in} = RTln\frac{[A^-_{out}]}{[A^-_{in}]} - F(\psi_{out} - \psi_{in}) \tag{19}$$

The net driving force of ATP synthesis is given by

$$d.f. = n_P RT\left(ln\frac{[H^+_{out}]}{[H^+_{in}]} + ln\frac{[A^-_{out}]}{[A^-_{in}]}\right) \tag{20}$$

or,

$$d.f. = 2.303 n_P RT\left(log_{10}\frac{[H^+_{out}]}{[H^+_{in}]} + log_{10}\frac{[A^-_{out}]}{[A^-_{in}]}\right) \tag{21}$$

$$d.f. = 2.303 n_P RT\left[(pH_{in} - pH_{out}) + (pA_{in} - pA_{out})\right] \tag{22}$$

i.e.,

$$d.f. = 2.303 n_P RT(\Delta pH + \Delta pA) \tag{23}$$

Where, $\Delta pH = (pH_{in} - pH_{out})$ and $\Delta pA = (pA_{in} - pA_{out})$ are both positive definite.

The rate of ATP synthesis, r is given by

$$r = 2.303 k n_P RT(\Delta pH + \Delta pA) \tag{24}$$

8. A major difference between Mitchell's and Nath's theories

Mitchell's chemiosmotic theory is an equilibrium macroscopic thermodynamic theory based on a continuum charge distribution in which the uniform delocalized protonmotive force, Δp built up across the bulk aqueous phases solely by electrogenic H^+ translocation supplies free energy for the synthesis of ATP. On the other hand, in Nath's theory, *nonequilibrium* gradients of two ions, a proton and a dicarboxylic acid anion such as hydrogen succinate are built up and determine the *rate* of ion transport and ATP synthesis (as given by Eq. (24)). However, the theory is *molecular* in nature, and energy transduction and coupling result from *local* electrical potentials at the a–c interface in the membrane-bound F_O-portion of ATP synthase that arise from *discrete* anion and proton translocations that involve *microscopic* events. Thus the rate of ATP synthesis and the rate of ion transport is indeed determined by the corresponding ion concentration gradients, the ionic concentration ratios (Eq. (21)), or by the ΔpH and ΔpA (Eq. (24)). However, Nath's theory postulates that the energy for ATP synthesis is supplied by individual elementary translocations of anion and proton *discretely*, and the energetics of the process has no relationship with the kinetics, i.e. the rate of ion transport (see especially Section 7.2 in ref. [37]). In other words, the energy donated per ion translocated is the same irrespective of the rate of ion transport. Thus, the new theory takes into account the *discrete* character of the charges and the involvement in energy transduction of events that are *microscopic* in nature. Such a treatment is considered absolutely essential for an adequate description of energy transduction occurring at the scale of individual cristae within mitochondria. The failure of chemiosmosis arises partly from its lack of consideration of the local and discrete effects due to elementary ion translocation events in the aqueous access channels within the membrane-bound F_O portion of ATP synthase, and its focus only on osmotic driving forces across the membrane. These aspects will be further analyzed and the assumptions of the two theories will be checked by determination of the length scales of the mesoscopic system (see Section 9).

The above central difference between the two theories of energy transduction is based on whether the energy-transducing membranes are fuel cells [29] or nonequilibrium molecular energy machines [11,13,14,16]. Mitchell considered energy transduction by a chemiosmotic process to operate as a macroscopic fuel cell [29]. Nath designated the complexes mediating the energy coupling and transduction as nonequilibrium molecular machines [11,13,14,16] in which only the ΔH part of ΔG is convertible under isothermal conditions, i.e. the machines are primarily enthalpic, and the ΔS part of ΔG cannot be

transduced to useful work under isothermal conditions (See especially Section 4 of ref. 13].

This central difference between the two theories can be quantified. For example, Mitchell estimated a charge transfer of 0.8 μeq H^+/g protein in rat liver mitochondria [3] in order to build a delocalized Δφ of ~200 mV across bulk aqueous phases by the macroscopic process of chemiosmosis. Classical cytological data [30] and our own experiments reveal that 1 mg of mitochondrial protein contains, on the average, 7.2×10^9 mitochondria; hence the number of protons that need to be translocated electrogenically across the membrane in order to generate a delocalized electrical potential of 240 mV equals [$0.8 \times (240/200) \times 10^{-9} \times 6 \times 10^{23}$] / 7.2×10^9 = 80,000 H^+/mitochondrion. In other words, 8000 cycles of electron transfer by the respiratory chain are required to build-up the thermodynamic potential, Δp required for ATP synthesis by the chemiosmotic theory. Transfer of 2 e^- creates only a negligible delocalized potential in Mitchell's chemiosmotic fuel cell model, and does not lead to the requisite build-up of Δp, and as a consequence, does not result in ATP synthesis. On the other hand, in the case of an enthalpic molecular machine, the potential energy depends on a microscopic event, and the transfer of 2 e^- or translocation of one or a few H^+ or A^- leads to an energized state of a *single molecule* of the ATP synthase [16]. This stored internal energy/enthalpy (ΔU or ΔH) in the molecule is then used to synthesize ATP, as described previously [11–14].

The two concepts are depicted graphically in Fig. 6. In the case of the macroscopic fuel cell model of oxidative phosphorylation offered by Mitchell's chemiosmotic theory, transfer of a large number of electrons (~80,000) is required to create a protonmotive force, Δp that is compatible with the thermodynamic requirements imposed by the free energy of phosphorylation, ΔG_P (~60 kJ/mol) and is therefore competent to drive ATP synthesis. In the case of the concept of a Nathean molecular machine, transfer of only 2 e^- or translocation of only a few (approximately 3–4) ions through the user molecule (ATP synthase) is sufficient to generate a potential energy within the molecule that is compatible with the thermodynamic requirements imposed by the ΔG_P. These concepts were discussed during the formulation of "Nath's torsional mechanism of energy transduction and ATP synthesis" [16], and the author concluded that the central γ-subunit of the ATP synthase possesses a high potential energy because an internal energy, ΔU of ~60 kJ/mol is stored in the γ-subunit specifically as torsional energy (Fig. 6), equivalent to the thermodynamic requirements imposed by the phosphate potential, ΔG_P, and is therefore competent in ATP synthesis (for reviews, see refs. [11,14]). It should be added that the full name of the mechanism was not coined by the author but first appeared in books and articles written by other workers [24–27].

The two models are compared in the graphic of Fig. 6 and labeled "Mitchell's chemiosmotic theory" and "Nath's torsional mechanism" to highlight the differences. The interested reader may also take a look at Fig. 6 of ref. [37] that summarizes examples of other clear-cut differences between the predictions of the two theories of energy coupling and ATP synthesis for which experimental verification has been obtained.

9. Further comparison of the two theories: Implications of system length scales on the validity of the main assumptions

Almost no attention has been paid to the mitochondrial system length scales and their compatibility with the thermodynamic assumptions and the validity of the generally used continuum charge distribution models invoking a uniform delocalized potential, Δφ or protonmotive force, Δp. This can be analyzed by determining the Debye length, λ_D, which is a measure of the characteristic screening length in the bulk ionic media, and comparing it with the characteristic inter-ion distance in the ionic medium, l.

The Debye length, λ_D is given by the equation

$$\lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon_w kT}{2e\sigma}} \tag{25}$$

Where, σ is the charge density of the ions in Cm^{-3}, and since the ion translocation occurs in aqueous access channels, the dielectric constant of water is used. The characteristic inter-ion distance, l is given by

$$l = [\sigma/e]^{-1/3} \tag{26}$$

Chemiosmosis postulates a delocalized electrical potential across the inner mitochondrial membrane that faces and is in communication with a cytosolic solution of ionic strength of ~0.15 M. For such ionic media, l = 2.24 nm, and λ_D = 0.81 nm. Since $\lambda_D < l$, a consistent theory cannot be constructed using continuum models. In other words, the ions are too sparse to produce a screening effect within a Debye length, and therefore a discrete model is needed for an adequate description of such a mesoscopic system. Hence no charge separation is obtained beyond λ_D and in fact, use of the approximation of *quasi-neutrality* for the bulk phases, according to which the number densities of positive and negative charges are equal constitutes a superior and far more appropriate model. Thus, there can be no build-up of a delocalized field and macroscopic charge separation by electrogenic translocation of protons in such a meso-scale system.

Further, the osmotic contribution due to the chemical potential of chemiosmosis lacks meaning on the length scale corresponding to the fold of a single crista. For model tubular cristae [31], corresponding to a succinate concentration in the intracristal space of ~1 mM that yields the highest rates in our experiments [12], λ_D measures 9.5 nm. However, the characteristic length due to free H^+ at a pH of 5.0 in the intracristal space (that again has been shown to lead to maximal rates of ATP synthesis [18]) is > 55 nm. In fact, irrespective of the conditions in a crista, $\lambda_D < l$, and therefore we need a theory such as Nath's theory that considers discrete charges and local potentials, reflecting the fields of individual ions.

The local potential, Δψ in Nath's theory produced by the discrete binding/unbinding and translocation of an individual A^- / H^+ through aqueous access pathways in the F_O-sector of a single ATP synthase molecule can be calculated by the equation

$$\Delta\psi = \frac{FL}{\varepsilon_0 \varepsilon_w A} \tag{27}$$

Where A is taken as half the surface area of the cylindrical c-rotor that is interacting with the a-stator in F_O, and r is the radius of the c-rotor − a-stator complex from the center of the c-oligomer to the interface of the a-subunit (~3.5 nm) [32–34]. This yields a local potential due to each elementary act of translocation of a monoanion or a proton of ~115 mV, or due to an ion pair, of ~230 mV. Eight such translocations lead to the synthesis of 3 molecules of ATP at an energy expense of ~1840 meV. Thus, the cost of synthesizing one mole of ATP is ~59.5 kJ mol^{-1}, in agreement with the value arrived at from nonequilibrium thermodynamics and from mechanistic considerations [13].

In summary, based on the implications of system scale sizes, chemiosmosis fails to adequately treat mitochondrial energy transduction

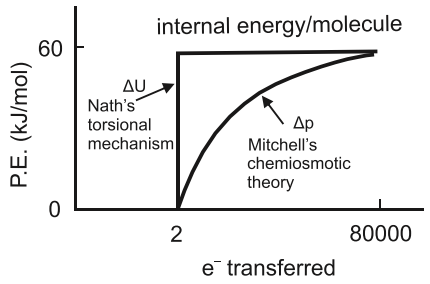

Fig. 6. Comparison of Mitchellian fuel cell vs. Nathean nonequilibrium molecular machine models of energy coupling.

processes that occur on the meso-scale, and is therefore not an appropriate theory. The need for a theory such as the one formulated by Nath in which local potentials and discrete charge translocations are considered [11–19] is seen in this light to be invaluable for characterizing the detailed properties of the mesoscopic system.

10. Application of Nath's theory to other fundamental processes

There are a number of other cases where the new theory will be useful, and some examples of application are given in this section.

10.1. Mitochondrial apoptosis

According to the theory, the exchange of ADP^{3-}/ATP^{4-} and binding of MgADP to the enzyme is the rate limiting step of ATP synthesis. Hence, in mitochondrial apoptosis, it would be logical if pro-apoptotic agents such as Bax induce a direct specific interaction with the adenine nucleotide translocase (ANT) that inhibits ADP^{3-}/ATP^{4-} exchange, as proposed [13]. This primary event of the action of Bax on ANT would subsequently trigger the release of cytochrome C and therefore is eventually responsible for further downstream events in the intrinsic apoptotic pathway. If this is the case, then cell life and cell death are two sides of a coin, and each can help understand the other in a better way.

10.2. Muscle contraction

Current proposals of energy transduction and force production in muscle contraction are limited to the involvement of the myosin II S-1 head domain. However, if the signal upon ATP hydrolysis in S-1 is transmitted as a wave of twist along the backbone of the myosin II molecule to the distant S-2 rod domain that is responsible for causing conformational changes in the S-2 coiled coil as proposed [19], then an interesting bidirectional communication can take place, and a unity of torsional-type mechanisms in different biological energy transduction devices would be on offer.

10.3. Regulation of oxidative phosphorylation and the Krebs cycle

Given the results in a recent analysis of the state 3 to state 4 transition [18], the signalling for increased redox activity, oxygen consumption, and increased turnover of the Krebs cycle in response to enhanced rate of ATP synthesis in mitochondria implies the need for joint, parallel "multisite activation and regulation" of the pathway. How is the signal for enhanced upsteam activity communicated upon increased ATP production downstream? This signalling can only be achieved by a molecule that is translocated back and forth from mitochondrial matrix to cristae and senses and reports the enhanced rate of ATP synthesis by binding to complexes on the redox side and to a component of the Krebs cycle, yet is itself not consumed in the pathway or transported out of mitochondria. Protons meet some of the above requirements, but they are not efficient signalling molecules in an alkaline pH 8 medium of the matrix, and therefore cannot be a candidate. Ideally, the candidate must be one of the regenerating substrates of the Krebs catalytic cycle, so that an increase in its concentration can increase the rate of the catalytic cycle. Further, the molecule should connect and be common to all the complexes involved in the pathway: the ATP synthasome, the redox complexes, and the Krebs cycle. It is proposed here that succinate is that elusive signalling molecule, and in fact, it is the only possibility that meets all the requirements listed above according to Nath's theory. Thus only a "multisite activation" by succinate binding can ensure tight regulation in oxidative phosphorylation along with maintenance of a constant steady state ratio of $NADH/NAD^+$ and constant steady state concentrations of ADP, ATP, Pi and other metabolites.

10.4. ATP as a biological hydrotope

Recently, in an interesting article [38], a hydrotropic role for ATP at cellular concentrations between 2 mM and 8 mM has been proposed. The new ATP theory helps offer a plausible explanation for the role of solubilization of hydrophobic molecules by ATP under these cellular conditions. Interestingly, in most molecular machines where MgATP is involved, binding of ATP to the motor and the action of the motor saturates between a concentration of 1 mM to 2 mM ATP, and in the Supplementary Information Section several examples from our own research are given along with a key summary Figure for each. Thus, for tri-site catalysis by F_1-ATPase, saturation of enzyme activity is obtained at 1 mM ATP (Supplementary Information, Fig. 1), while for actin motility in an *in vitro* assay, the sliding velocity reaches a plateau at 2 mM ATP (Supplementary Information, Fig. 2). In our recent publication on maximal uncoupling of ATP synthesis, the concentration of ATP employed was 2 mM [36]. All these examples of "active processes" require binding of MgATP to the molecular system for its action.

A corollary to the above is that beyond a concentration of 2 mM ATP, the binding and action of these motors would saturate. Increase in aqueous ATP concentrations beyond this limit should not adversely affect the performance of these molecular energy machines, and this can be experimentally tested. Hence it is perfectly reasonable that in the 2 mM to 8 mM range of ATP concentration, the ATP molecules are available for a second important function: to non-specifically solubilize hydrophobic proteins in aqueous solution and act as a biological hydrotope [38]. Hence the active processes involving ATP as a cellular currency are distinct from ATP's second role as a controller of material properties of the cell interior, and each function occurs in a different range of ATP concentration.

Thus we have shown in this section that the new theory has the power to offer a wealth of novel insights into a number of fundamental, closely-related biological processes and energy transductions.

11. Major biological implications

It has generally been believed that the product of the Faraday, F, and the so-called "protonmotive force" of the chemiosmotic theory is *equivalent* to the electrochemical potential difference, $\Delta\tilde{u}_H$, of thermodynamics and electrochemistry. Unfortunately, this is not true. The symbol ψ in the definition of the electrochemical potential $\tilde{\mu}_i$ stands for the *local* electrical potential due to the charged species i. It does not (and was never meant to) stand for or represent a *delocalized* electrical potential, which is an entirely different concept. It is only in the Mitchellian definition of the "protonmotive force" within the theory of chemiosmosis that the potential takes on a *delocalized role,* which is thermodynamically and mechanistically unfounded, and experimentally unsupported, because it assumes arbitrarily that such a primary, electrogenic translocation *solely* of protons takes place which is *uncompensated* by the movement of any other co-ion (e.g. membrane-permeable anions *in vivo)* or counter-ion *(*e.g. K^+ in the presence of valinomycin *in vitro)*, and also that a resulting summed-up, delocalized potential in the entire organelle arises *solely* from such a free and steady-state migration of the single ionic species i (e.g. protons) across biological membranes from one bulk aqueous phase to the other, thereby violating bulk electroneutrality. Therefore, it is suggested that the symbol ψ continue to be used to represent the *local* electrical potentials that are of interest to the torsional mechanism in order to differentiate it from the *delocalized* potentials of the chemiosmotic theory and to avoid confusion between these subtle but very different concepts. The delocalized electrical potential of chemiosmosis should be indicated by the symbol φ or E, as in the original literature [1–3].

Recently, another serious fundamental error was discovered in the governing equations of the chemiosmotic theory that revealed its unsound theoretical foundations; the error was rectified by the mathematical formulation of a two-ion theory of energy coupling [17]. In the

author's view, the aspects dealt with in this work constitute crucial and key elements whose lack of detailed consideration has held back the progress of research in this fundamental and important multi-disciplinary field. The fundamental results proved here and the design principles emerging from them have major implications for all of biological science. The new thinking should also have an impact on the teaching of the subject at all levels, on which a large, well-researched scientific literature already exists [39–45]. The concepts of biological energy have also been addressed in these pedagogical articles using a number of classroom examples and case studies. In particular, the difficulties of students of physics, chemistry, biology, and engineering in grasping energy concepts have been highlighted, and instructional methods to assist understanding have been discussed [43,40–45]. The design principles outlined here can also prove to be useful and profitable for the design, development and fabrication of micro and nano-devices in the field of nanotechnology.

12. Summary

The physical arguments and mathematical calculations originally made to justify the central tenet of Mitchell's chemiosmotic theory in a section entitled "Fiction of Electrical Neutrality" [1,2] has been subjected to scientific scrutiny. It has been concluded that these arguments are erroneous and misleading, and are inconsistent with the known universal laws of science. The falsity of the original calculations have been shown by a simple but rigorous derivation from first principles by devising a suitable reversible path from infinity (where electrical potential, V = 0) to the interior of the sphere and calculating the potential/field/work done by proper application of Gauss's law and use of the principles of calculus (not indulged in at all in the previous calculation [1,2]). It has been concluded that the electrical neutrality of bulk aqueous phases cannot be violated to the very large and unprecedented extent required to create the delocalized potential postulated by the chemiosmotic theory, and reveal the 'Fiction of the "Fiction of Electrical Neutrality"'. An alternative Nath's two-ion theory of energy coupling and ATP synthesis in which the field is local and bulk electroneutrality is not violated has been formulated in detail and shown to have the power to overcome the problems of the past. Analysis of system length scales in mitochondrial cristae has been shown to impose strong constraints on possible theories and mechanisms of biological energy transduction. The alternative theory has been applied to other related energy transductions and also shown to be useful for a deeper understanding of several biological processes. The new thinking contained in the modern theory of energy coupling and ATP synthesis has been concluded to offer great promise both for the progress of future research and for the teaching of energetics in physics, chemistry, biology, and interdisciplinary science and engineering curricula for students at all levels [39–45].

Declaration of Competing Interest

The author declares no conflict of interests.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bpc.2019.106271.

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