REVIEW LETTER

ELECTRIC FIELDS IN COUPLING MEMBRANES

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1. Introduction

Formation of an electric field coupling electron transfer and phosphorylation in biological membranes was postulated by Mitchell several years ago [1, 2]. Mitchell suggested that the energy released on electron transfer or ATP hydrolysis can be converted into the form of an electric potential difference across the coupling membrane. This postulate was supported recently by several independent lines of evidence. Some of them, related primarily to the energy-dependent movement of H^+ and natural cations through the coupling membranes, were reviewed by Mitchell [2, 3] and Greville [4]. The present paper summarizes the results of the study of the same problem by using synthetic ionized compounds readily penetrating across lipid membranes.

2. Experimental proof of electric field generation in coupling membranes

If an electric potential difference exists between two compartments separated by a membrane, an unequal distribution of penetrating ions across the membrane must arise, cations being accumulated in the negatively charged compartment, anions in the positively charged one. Distribution of any ions capable of penetrating the membrane must be affected by an electric field oriented across it. By measuring transmembrane ion flows which can be defined as "electrophoresis in membrane" it is possible to detect an electric potential difference across the membrane and to determine the direction of the field. To be able to conclude that an ion movement measured is caused by the electric field, one ought to be sure that this process meets the main requirements of ion electrophoresis: (i) the electrophoretic ion transport through the membrane must be non-specific to the penetrating ion structure, (ii) cations and anions must move in opposite directions. To minimize the interference of non-electrophoretic ion transport processes which could be due to the operation of ion-transporting enzymatic systems ("translocases") it is desirable to use synthetic ionized compounds of different structures as artificial (foreign) ionophores, i.e. components which are unlikely to interact with a translocase adapted to the transport of an ion normally present in mitochondria. This approach was first tried in experiments with valinomycin. Moore and Pressman [5] showed that this antibiotic, when added to animal mitochondria, induces an energy-dependent K^+ movement. Valinomycin-induced K^+ influx was accompanied by the efflux of stoichiometric amounts of H^+. Later Chappell and Haarhoff [6], Lev and Buzhinsky [7], and Mueller and Rudin [8] reported that valinomycin greatly increases the K^+ permeability of artificial phospholipid membranes and micelles. The fact that in mitochondria valinomycin, an ionophore foreign for these organelles, initiates the accumulation of K^+ ions in exchange for H^+, was considered by Mitchell [2, 3] as an indication of the K^+ movement being supported by an electric field. Unfortunately, K^+ ions are normally present in mitochondria. Therefore it was difficult to rule out a possibility that the role of valinomycin is that of increasing accessibility of K^+ to a hypothetical "K^+ translocase" which could be localized in the mitochondrial membrane and separated from the extramitochondrial space by the hydrophobic phase of the membrane. This concept was developed by Pressman et al. [9–11], Lardy et al. [12], and Massari and Azzone [13].

To exclude such an alternative, a series of investigations with synthetic ionized compounds was carried out [14–17]. It was found that synthetic ions easily penetrating through artificial phospholipid membranes, such as phenyldicarbaundecaborane (PCB^-), tetraphenylboron and picrate anions, and *N,N*-dibenzyl *N,N*-dimethyl ammonium, tetrabutyl ammonium and triphenyl methyl phosphonium cations, can be moved across mitochondrial membranes in an energy-dependent fashion. Anion and cation flows were oppositely directed. The ability to penetrate phospholipid membranes proved to be the only requirement for the ion to be transported by such a mechanism. Treatment of mitochondria by some synthetic compounds inducing the permeability for certain normally non-penetrating ions, allowed these ions to be involved in energy-dependent transport. It was found, for example, that addition of di-(pentafluorophenyl)-mercury, the compound increasing the iodide permeability of phospholipid membranes, induces energy-dependent I^- movement across coupling membranes. The energy-dependent transport of the tetraethyl ammonium cation can be induced by PCB^- or tetraphenyl boron anions.

All these properties of ion transport through the mitochondrial membrane satisfy the requirements of an electrophoretic mechanism. The electric field in the mitochondrial membrane was found to be oriented so that the mitochondrial interior was charged negatively. Sonicated submitochondrial particles, having opposite membrane polarity to that of mitochondria, were characterized by reversed ion movement.

Measurements of the movement of penetrating ions across the mitochondrial membrane are evidently a direct and most reliable probe for a membrane potential in objects as minute as intracellular organelles. In this case the electric field, i.e. the ability to carry out the work of charge translocation, can be detected by simply measuring ion concentration changes in the extra-organelle solution. Ion transport can also be followed by measuring pH changes which always accompany energy-dependent ion movements across coupling membranes. The accumulation of synthetic cations in mitochondria was associated with H^+ efflux, accumulation of synthetic anions in sonicated particles with H^+ influx [14, 15]. Protonophorous uncouplers, such as dinitrophenol, reversed these pH changes.

In other experiments [18–20] it was found that abilities of different protonophores to uncouple oxidative phosphorylation correlate with their abilities to increase electric conductance in artificial phospholipid membranes, which also supports the membrane potential concept.

A potential difference across a membrane can also be revealed by studying some other parameters which respond in some way to the appearance of a membrane potential. Jackson and Crofts [21] measured carotenoid spectra in chromatophores of photosynthetic bacteria. They found that generation of a potential difference across chromatophore membranes on addition of K^+ *plus* valinomycin, or H^+ and a proton carrier, induced a characteristic shift in the spectra of chromatophore carotenoids. The same spectral changes were observed on the transition of chromatophores to the energized state. Treatments discharging the membrane potential inhibited the carotenoid responses induced both non-enzymatically (by K^+ or H^+ gradients) and enzymatically (by photo-induced electron transport or ATP hydrolysis). The authors concluded that there is a membrane potential, the "*plus*" being inside, when chromatophores are in the energized state.

The same approach was used in experiments with a fluorescent dye, anilinonaphthalene sulfonate (ANS^-). It was shown [22] that non-enzymatic generation of membrane potential, induced by K^+ or H^+ influxes in the presence of valinomycin or tetrachlorotrifluoromethylbenzimidazole (TTFB), respectively, was accompanied by an increase in the fluorescence of ANS^- both in mitochondria and submitochondrial particles. The efflux of cations resulted in a fluorescence decrease. Discharge of the membrane potential prevented these effects.

These observed relationships can be easily explained if one takes into account the properties of ANS^-. This dye only has a high fluorescence yield in a hydrophobic medium [23, 24]. Therefore, the fluorescence of ANS^- in a suspension of mitochondria or particles is due to the portion of the ANS^- pool associated with the mitochondrial (or particle) membrane. Changes of this pool depend almost entirely on variations in the ANS^- concentration in the solution inside the mitochondrion (or particle), if one deals with phenomena of the redistribution of ANS^- between extra- and intra-mitochondrial compartments. For instance, the efflux of the whole portion of ANS^- which was initially in the intramitochondrial water cannot increase

significantly the ANS^- concentration outside the mitochondria; extruded ANS^- will be diluted in the relatively large amount of extramitochondrial water. As a result, the ANS^- concentration will decrease more inside mitochondria than it will increase outside. Therefore, the concentration of ANS^- in the mitochondrial membrane and, hence, the intensity of the fluorescence, should decrease when the ANS^- concentration inside the mitochondria goes down. Correspondingly, accumulation of ANS^- inside mitochondria should increase fluorescence. The latter effect, apparently, takes place when the mitochondrial interior is positively charged on K^+ (or H^+) influx, resulting in ANS^- influx down the electrical gradient.

Recently, ANS^- fluorescence measurements were introduced as a probe for the energized state of the mitochondrial membrane. It was found [25–28] that energization of mitochondria decreased, while energization of sonicated particles increased the ANS^- fluorescence. Azzi [29] noted that mitochondria extruded, while particles took up ANS^-, in an energy-dependent fashion. The direction of the movement of a fluorescent cation, Auramine O, proved to be opposite to that of ANS^-. Neither fluorescence, nor concentration in the medium of a non-ionized ANS^- derivative (ANS amide), were influenced by changes in the energy level. It is very probable that the energy-dependent responses of ANS^- are due to the appearance of the membrane potential ("plus" inside particles and outside mitochondria). Apparently, the ANS^- fluorescence probe, like measurements of penetrating ion concentrations in the medium, can be used for detecting membrane potential in mitochondria and submitochondrial particles.

Tupper and Tedeschi [30–32] tried to measure the potential difference across the mitochondrial membrane using a microelectrode technique. This seems to be a very difficult approach because of the high resistance of the inner mitochondrial membrane (10^7–10^9 Ω cm^2; see [2, 20, 33]), and the high hydrostatic pressure in the mitochondrial interior (more than 3 atm. [34]). It is hardly possible to insert an electrode into a mitochondrion without causing both a decrease in the initially very high resistance and irreversible damage to the mitochondrial structure. It is not surprising, therefore, that the results of microelectrode measurements proved to be quite different from what one could expect from the available data. The resistance of the mitochondrial membrane measured by microelectrodes was found to be very low (a few ohms), and the potential about 10 mV, "plus" inside the mitochondrion. It is impossible to rule out that these data reflect the pecularity of the experimental system (insect muscle mitochondria in a medium of high viscosity were used). However, it seems more probable that the mitochondrial structure was violently damaged by the electrode. Another explanation is that the electrode was inserted into the intramembrane space and the data obtained referred to the outer membrane only.

In discussing methods for detecting the membrane potential it should be emphasized that such an approach as measurements of the distribution of weak acids and bases (see, e.g. [35]) cannot give conclusive information on the subject. The distribution of these compounds between mitochondria and the extramitochondrial solution can be affected by transmembrane diffusion of the uncharged forms and their subsequent dissociation inside the mitochondria where pH values can differ from those in the outer space. This could not be the case for the synthetic penetrating ions mentioned above; such compounds as PCB^- or dibenzyl dimethyl ammonium do not exist in the undissociated form at neutral pH.

Summarizing this section we can conclude that the concept of the membrane potential is supported by several independent lines of experimental evidence, such as measurements of (i) movement of synthetic penetrating ions; (ii) movement of natural ions in the presence of antibiotics or synthetic ionophores; (iii) effectivity of protonophores as uncouplers and current conductors; (iv) ANS^- fluorescence changes; and (v) spectral responses of carotenoids. The available data allow one to conclude that the existence of an electric potential difference across the coupling membranes is experimentally proved.

3. The mechanism of membrane potential generation

Measurements of PCB^- concentration and ANS^- fluorescence changes were found to be the most sensitive methods for detecting the membrane potential [17, 22]. Using these probes the mechanism of membrane potential generation in different experimental systems was studied. It was found [14–17, 36] that

membrane potentials can be produced by any membrane system competent in redox chain energy coupling (mitochondria, submitochondrial particles, subchloroplast particles, chromatophores of photosyntehtic bacteria, and particles of the cytoplasmic membrane of respiring bacteria were tested).

The potential-generating mechanism localized in mitochondrial and chromatophore membranes was analysed in detail. It was proved that electron transfer via any site of energy coupling and hydrolysis of ATP were able to produce membrane potentials [14–16]. It was also shown that oxidation of NADPH by NAD^+ both in submitochondrial particles and in *Rhodospirillum rubrum* chromatophores generates a membrane potential of the same sign as electron transfer via redox chain coupling sites and ATP hydrolysis. This observation confirmed Mitchell's idea that the so-called "energy-linked transhydrogenase" represents an additional (fourth) site of energy coupling in the redox chain.

The unique property of the transhydrogenase coupling site which distinguishes it from the other three is that there is no difference between the energy levels of the electron donor and acceptor (the redox potentials of NADPH/$NADP^+$ and NADH/NAD^+ differ by not more than 5 mV). Thus, both forward (NADPH → NAD^+) and reverse (NADH → $NADP^+$) electron transfer can be energy-releasing when the reaction substrates (NADPH and NAD^+ in the former, $NADP^+$ and NADH in the latter case) are in excess over the products. Reversed electron transfer via other energy-coupling sites is energy-consuming even when traces of the reaction products, whose redox potential is much more positive than that of the substrates, are present.

According to Mitchell [2], the mechanism of membrane potential generation is due to charge separation by the redox chain and ATPase, two independently operating enzymatic systems localized in the same membrane. The concept which is usually opposed to Mitchell's scheme, suggests the existence of a single enzymatic system specializing in the formation of a membrane potential: the so-called "proton pump" which utilises the energy of an intermediate of oxidative phosphorylation (X ∿ Y) for proton translocation [38]. Fig. 1 illustrates two concepts of membrane potential generation coupled with the transhydrogenase reaction in submitochondrial particles. According

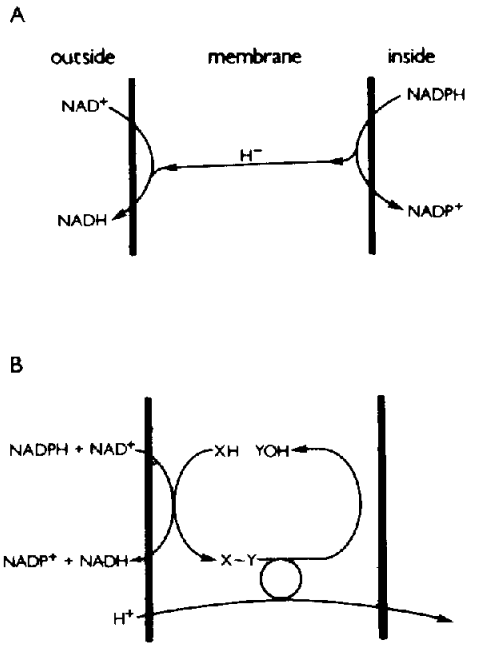

Fig. 1. Two versions of the mechanism of membrane potential generation coupled with transhydrogenase reaction. (A) Mitchell's scheme; (B) the X ∿ Y-hydrolysing proton pump scheme

to Mitchell's concept, (fig. 1A), membrane charging is a result of transmembrane movement of a charged component, say H^-, a process which does not require formation of X ∿ Y. The "proton pump" scheme, (fig. 1B), includes X ∿ Y formation as an obligatory step on the way to the membrane potential, which arises from H^+ influx coupled with X ∿ Y hydrolysis. In both schemes, reduction of NAD^+ by NADPH is coupled with charging of the membrane, the "plus" being inside the particles.

It is obvious that the transhydrogenase reaction running in the reverse direction (NADH → $NADP^+$) must generate a membrane potential of the opposite sign ("plus" outside the particles).

Mitchell and Moyle mentioned in one of their early works [37] that reduction of $NADP^+$ by NADH in particles was accompanied by some acidification of the mixture, while the forward electron transfer induced alkalinization.

We returned to this problem using PCB^- measurements as a sensitive probe for the membrane potential [39]. The result of a typical experiment is given in fig. 2. It is seen (exp. A) that the treatment of sonicated submitochondrial particles with NADPH and

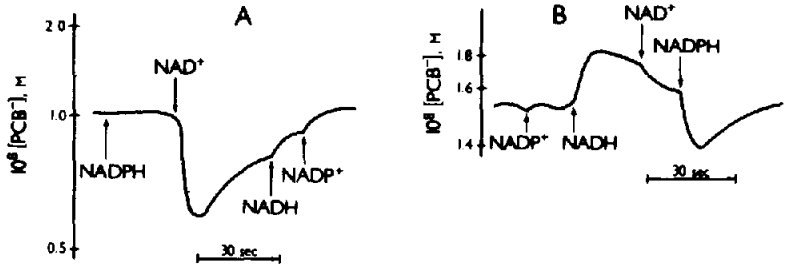

Fig. 2. The change in direction of ion flow due to reversal of the transhydrogenase reaction in submitochondrial particles [39]. *Ordinate*: concentration of the penetrating anion, phenyldicarbaundecaborane (PCB^-) in a solution containing: (A) 0.25 M sucrose, 0.05 M tris-HCl (pH 7.5), 2.6×10^{-6} M rotenone, 6.5×10^{-3} M NaCN, sonicated submitochondrial particles of beef heart (3.1 mg protein/ml); (B) 0.25 M sucrose, 0.05 M tris-HCl (pH 7.5), 1.3×10^{-6} M rotenone, 2×10^{-3} M KCN, sonicated particles (4.4 mg protein/ml). Concentrations of added $NADP^+$, NADH, NAD^+ and NADPH: 5×10^{-4} M.

NAD^+ (the substrates of the forward transhydrogenase reaction) results in the influx of PCB^- into the particles, testifying to the formation of a membrane potential ("plus" inside). Treatment with NADH and $NADP^+$ induces PCB^- efflux as if indicating generation of a membrane potential of the opposite sign (exp. B). It is also seen that addition of the reaction products ($NADP^+$ and NADH in the former, NADPH and NAD^+ in the latter case) up to the concentrations equal to those of the substrates returns the PCB^- concentrations to the initial levels. This important result supports the idea that the energy for PCB^- movement is supplied by the transhydrogenase reaction. In this case the excess of substrates over products is the only energy source, and production of energy must completely cease when concentrations of the substrates and products are equalized. In other experiments [39] it was found that protonophorous uncouplers prevent and reverse all transhydrogenase-linked responses of PCB^-. For example, addition of 1×10^{-7} M *p*-trifluoromethoxycarbonylcyanide phenylhydrazone (FCCP) prior to nicotinamide nucleotides did not influence the PCB^- concentration in the mixture with particles (demonstrating that particles are really de-energized), but prevented the (NADH + $NADP^+$)-dependent extrusion of PCB^-. Addition of FCCP after NADH + $NADP^+$ reversed their effect inducing PCB^- influx.

Thus, reversal of the transhydrogenase reaction causes changes in the direction of the energy-dependent PCB^- movements in the particle membrane, and, consequently, the change in the orientation of the electric field in the membrane.

It is of importance that the energy release in the transhydrogenase reaction can be regulated by changing the ratio of $[NAD^+][NADPH]/[NADH][NADP^+]$ in the incubation mixture. This property of the system may be used for discrimination between two alternative versions of the membrane potential-generating mechanism shown in fig. 1. These versions must differ one from the other in minimum values of the above ratio. In terms of scheme A, no threshold value of the substrate to product ratio can exist in the region of values > 1; some membrane potential should be formed in any case when the substrate concentrations are higher than the concentrations of products. In terms of scheme B, the membrane potential formation is possible only if the ratio is high enough to generate sufficient concentration of the high-energy intermediate ($X \sim Y$) to saturate the $X \sim Y$-hydrolysing enzyme enough to give the observed rate of reaction.

Measurements of the PCB^- responses showed that the membrane potential generation coupled with both the forward and the reverse transhydrogenase reactions can be detected when the substrate to product ratio is less than 10. Under these conditions the concentration of $X \sim Y$ should be very low, this observation making the idea of $X \sim Y$ involvement highly improbable. The fact that the membrane potential can be formed even if the excess of substrates over products is rather small, explains why the addition of the reaction products to bring their final concentrations equal to the initial concentrations of previously added substrates, not only reverses the effect of substrates but creates a membrane potential of the opposite polarity. This

phenomenon is demonstrated, for example, by fig. 2B. It is seen that addition of 0.5 mM NAD^+ + 0.5 mM NADPH to the particles incubated with 0.5 mM $NADP^+$ and 0.5 mM NADH results in the decrease of the PCB^- level below the initial one. In this case, the formation of small amounts of NAD^+ and NADPH in the reverse transhydrogenase reaction during 30 sec incubation proved to be enough to create a sufficient excess of the forward reaction substrate over products when 0.5 mM NAD^+ and NADPH were added. This result, being inconsistent with scheme B, indicates that transhydrogenase can generate a membrane potential without the participation of any high-energy intermediates, as originally postulated by Mitchell [2].

It is remarkable that Mitchell's idea of the membrane potential-generating redox chain was confirmed by studies of the transhydrogenase coupling site. In fact, it was the transhydrogenase step that seemed to be the most difficult to explain in terms of the chemiosmotic hypothesis. It was found [40] that the hydrogen atom carried by the energy-linked transhydrogenase is translocated from one nicotinamide nucleotide to another without H^+ being released into water. Consequently this process *per se*, even if directed across the membrane, cannot produce a transmembrane proton gradient. For this reason scheme A, fig. 1, must be in some way modified to explain the reaction mechanism. Mitchell [2] suggested that the transhydrogenase reaction might be primarily coupled with the movement of a metallic ion which can then be exchanged for H^+. We studied such a possibility but did not find any dependence of the transhydrogenase-linked potential generation process on the ionic composition of the incubation mixture.

Another possibility might be that H^+ ions are carried by a nicotinamide nucleotide being added not to the pyridine but to some other moiety of its molecule. For instance, phosphate groups of the nucleotide may be involved. This possibility is illustrated by fig. 3.

This scheme, highly speculative of course, describes the transhydrogenase-dependent generation of a membrane potential as a process including translocation of protonated and deprotonated forms of NADH in the membrane.

According to this scheme (stage 1), an oxidized nicotinamide adenine dinucleotide bearing one positive

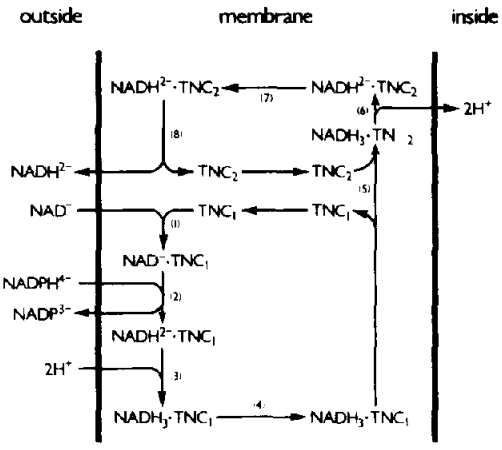

Fig. 3. A possible mechanism of H^+ transfer supported by the transhydrogenase reaction. TNC_1 and TNC_2, are two hypothetical transhydrogenase nicotinamide nucleotide carriers. NAD^-, $NADH^{2-}$, $NADP^{3-}$ and $NADPH^{4-}$, oxidized reduced forms of NAD and NADP, whose phosphate groups are ionized. $NADH_3$, reduced nicotinamide adenine dinucleotide, whose phosphate groups are neutralized by the addition of $2H^+$.

charge on the pyridine quarternary nitrogen and two negative charges on the ionized phosphate groups (NAD^-, net charge 1−) combines with a functional group of the transhydrogenase designated as TNC_1 (TNC, transhydrogenase nicotinamide nucleotide carrier). Bound NAD^- is reduced by $NADPH^{4-}$ on the outer surface of the submitochondrial particle membrane resulting in the formation of $NADP^{3-}$ and bound $NADH^{2-}$ (stage 2). It is proposed that the $NADH^{2-}$ formed can be released into the water outside the particles only after a series of transformations shown by stages 3–8. First of all, bound $NADH^{2-}$ is neutralized by addition of two H^+ ions to phosphate anions (formation of $NADH_3$, stage 3). Then the electroneutral complex $NADH_3$–TNC_1 is translocated to the other surface of the membrane (stage 4). There $NADH_3$ is transferred from TNC_1 to the second transhydrogenase nicotinamide nucleotide carrier (TNC_2, stage 5) with subsequent deprotonization of $NADH_3$ (stage 6). The negatively charged form of reduced nicotinamide adenine dinucleotide ($NADH^{2-}$) is translocated to the outer surface of the membrane generating an electric potential ("plus" inside particle, stage 7). Then the $NADH^{2-}$–TNC_2 complex dissociates into the $NADH^{2-}$ and TNC_2 (stage 8).

The mechanism of NADH translocation carried out by TNC_1 may be similar to that of the mitochondrial inorganic phosphate transfer system catalyzing the electroneutral symport of $H_2PO_4^-$ and H^+ while NADH translocation by TNC_2 could be compared with the electrogenic ATP–ADP exchange mechanism [41]. The scheme described is in agreement with experimental data showing (i) direct hydrogen transfer between nicotinamide nucleotides and (ii) accessibility of the transhydrogenase of the sonicated submitochondrial particles to both NAD and NADP.

One may think that the complexity of membrane potential generation in the transhydrogenase coupling site is primarily due to a peculiarity of the redox reaction, the transfer of two electrons and one proton. Such a reaction *per se* cannot be associated with H^+ gradient formation of $1H^+$: $1e^-$ stoichiometry.

The membrane potential-generating system may be organized much more simply when the substrate of the oxidation reaction furnishes electrons and protons in equal quantities. In such cases a pair of redox carriers, composed of one hydrogen carrier and one electron carrier, can produce an electrochemical potential difference in H^+ across the membrane. For this purpose it should be enough to place a hydrogen carrier (e.g. a flavin or CoQ) in proximity to one side of the mitochondrial membrane, and an electron carrier (e.g. a cytochrome or non-haem iron) close to the other side. There are many indications that flavins and cytochrome *c* are localized on different sides of the inner mitochondrial membrane (for reviews see [2, 4, 36]). As has been shown in this laboratory [42], PCB^- movement in mitochondria can be supported by electron transfer from succinate to ferricyanide. In the "inside-out" submitochondrial particles this redox couple proved to be ineffective. Similarly, the oxidation of ferrocyanide by oxygen initiates PCB^- transport in mitochondria but not in particles. On the contrary, the oxidation of NADH by fumarate was coupled with PCB^- movement in particles but not in mitochondria. In the same experiments it was found that penetrating reductants (succinate, reduced tetramethyl-*p*-phenylenediamine) and an oxidant (menadione) were equally effective in both mitochondria and particles. Thus, the flavin region of the potential-generating redox chain is accessible to non-penetrating oxidants (reductants) in particles and is inaccessible in mitochondria. The cytochrome *c* region is accessible in mitochondria, but not in particles. Comparing this result with those obtained by other methods one can conclude that reducing equivalents traverse the membrane when being carried from the flavin to the cytochrome *c* region of the redox chain. If it is electrons that are traversing the membrane, then the second coupling site of the redox chain can generate a membrane potential, like the transhydrogenase, with no high-energy intermediates being involved.

One can speculate that so simple a mechanism of membrane potential generation as the interaction of hydrogen and electron carriers arranged across the membrane, was evolved first. If this were the case, the complicated pattern of the transhydrogenase step might be thought of as an adaptation of this mechanism to the H^- transfer process whose chemistry strongly differs from that of the flavin–cytochrome interaction.

In any case, the observation that high energy compounds are not involved in the formation of the membrane potential, at least in the transhydrogenase coupling site, disproves the "proton pump" concept which is usually discussed as the alternative one to Mitchell's scheme of energy coupling. To confirm the chemiosmotic hypothesis of oxidative phosphorylation *in toto* it remains to be demonstrated that the redox chain components are not involved in the ATP-supported membrane potential generation process.

In fact, the available information is insufficient to rule out the possibility that charge transfer, directly coupled with operation of the redox carriers, is the only method of membrane potential generation in mitochondria. If this were so, ATP energy could be utilized for charging the membrane by a mechanism including reversed electron transfer along the membrane and forward electron transfer across the membrane. This scheme, which we have discussed [17, 36], requires the existense of two redox chains, one stretched along the membrane, participating in oxidative phosphorylation, and the other crossing the membrane and specializing in membrane potential generation.

The interrelation of the redox chain, $X \sim Y$, and membrane potential ($\Delta\psi$) in terms of Mitchell's scheme can be presented as:

$$\text{redox chain} \longleftrightarrow \Delta\psi \longleftrightarrow X \sim Y,$$

while in terms of the $X \sim Y$-hydrolysing proton pump

as:

$$\text{redox chain} \longleftrightarrow X{\sim}Y \longleftrightarrow \Delta\psi,$$

and in terms of the third scheme mentioned above as:

$$\Delta\psi \longleftrightarrow \text{redox chain} \longleftrightarrow X{\sim}Y.$$

Discrimination between the first of these schemes and the third seems to be the last barrier to overcome before accepting the chemiosmotic hypothesis *in toto*.

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