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The Electromechanochemical Model of Mitochondrial Structure and Function

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The gross configurational changes which mitochondria undergo during energization and deenergization were the experimental foundation stones for the conformational model which we first proposed in 1967.[1–3] Out of these studies emerged the notion of the energized state of the transducing unit and the notion of coupling the relaxation of the energized unit either to the synthesis of ATP or to active transport.[4] Implicit in the conformational model is the conservation of energy in a metastable state[4, 5] of the transducing unit rather than in a high-energy covalent intermediate.

Three major tasks faced us in the further development and extension of the conformational model: (1) to define the nature of the metastable energized state; (2) to define the mitochondrial structures implicated in the generation and relaxation of the energized state; and (3) to deduce how these structures could serve as the molecular instruments for implementing the physical principles underlying energy transduction. The model of mitochondrial structure and function to be described in the present communication embodies the essence of our current views on these questions.

Before developing the model systematically, a few general comments about our efforts at model building might be appropriate. The model is rooted in the experimentally established structural attributes of the basic mitochondrial systems, namely the transducing units, the membranes, and the protein network systems in the two mitochondrial spaces. The present model is a product of a long process of fitting experiment with theory. The structural features of the mitochondrion have been our invariant guide to the selection of the different physical possibilities. But the model in its present form goes beyond the experiments on which it was based. Experiment provided the basis for the initial, inductive, developmental phase and theory provided the basis for the final, deductive, emergent phase.

A working model of mitochondrial structure and function fulfills

three major roles: (1) it organizes and unifies the known phenomena of mitochondrial coupling on the basis of a few principles; (2) it can lead to the prediction of new phenomena; and (3) it sets a new standard for progress. A more acceptable model must at least rationalize the same wide range of phenomena rationalized by our model and encompass the same structural and functional parameters, only more accurately. Our view is that no general, all-encompassing model of mitochondrial structure and function can be constructed which does not mirror accurately the most fundamental attributes of energy transduction. The very generality of the model provides the most powerful support for its validity in principle.

Finally we would like to submit that biological problems of great magnitude such as the structure of DNA,[6] the structure of the membrane[7, 8] or the mechanism of muscular contraction[9] can only be approached via model building. The one-experiment-one-conclusion approach is inapplicable to problems of such dimension. But while experiment alone is clearly insufficient for meaningful progress, experiment in conjunction with model building can provide a combination equal to the requirements for solving problems of great complexity.

I. *The Basic Tenets of the Model*

A. *Electromechanochemical coupling*

The central concept in the development of the conformational model has been the "energized state"[4, 5] of the inner membrane repeating unit in which the component proteins assume metastable conformations. The discharge of these energized, metastable conformations was assumed to be coupled to work performances of the mitochondrion. In analyzing the nature of the energized state of the repeating unit, we came to the conclusion that the repeating unit in the energized state can be characterized by three fundamental properties—chemical, mechanical, and electrical. Chemical because the repeating units themselves are sites of bond formation or bond breaking (catalysis); mechanical because energization induces conformational rearrangements of the component proteins and leads to mechanical strain; and finally electrical not only because fixed charges are integral parts of protein structures in general but also because of the postulated existence of uncompensated charges in the interior of the energy transducing unit during energization. These conceptually distinct parameters are so intimately intertwined within the membranous macromolecular framework that it is no longer possible to separate the chemical, mechanical and electrical components involved in conformational changes accompanying energy transductions. Just as the impossibility of separating the

electrical and chemical parts in the energetics of ion transports in solution led Guggenheim[10] to invoke the concept of an "electrochemical" potential, so our theoretical considerations require invoking an "electromechanochemical" potential. In addition to chemical activities, Coulombic charges and electric field intensities which appear in the mathematical expression for the electrochemical potential,[11] the quantitative expression for the electromechanochemical potential would involve information about the microscopic structure of the macromolecular energy transducing unit in the energized state.

In terms of this new concept, the ground and energized states of the repeating unit differ not only in respect to the content of electromechanochemical potential energy* but also in respect to the relative contributions of the three component potential energy forms to the total potential energy of the repeating units. The electrical and mechanical contributions are predominant in the energized state whereas the chemical contribution is predominant in the ground state. The metastable character of the energized state may be ascribed to the fact that the repeating unit becomes kinetically unstable when the system is strained electrically and mechanically.

Implicit in the argument is the assumption that the three different forms of potential energy are readily interconvertible within the repeating unit. Man-made energy transducing devices are known which illustrate the interconvertability of these three forms of energy. Fuel cells and batteries, for instance, are structurally designed to achieve the conversion of chemical potential energy into electrical potential energy. Electrolysis cells facilitate the opposite energy conversion, namely the conversion of electrical potential energy into chemical potential energy. These devices may be considered as examples of two-component transducers because they only involve two different types of potential energies (in this respect, we are not regarding thermal energy as potential energy). As an example of a three-component energy transducer, we may consider a mechanical spring connected on both ends to two spherical masses with opposite electrical charges. At the equilibrium state, the net forces acting on the two spheres will be zero because the electrostatic force will be equal and opposite to the mechanical force of the spring. In this resting state, the total potential energy of the system can be expressed as the sum of the mechanical and electrical potential energies.

*At the time when the manuscript was submitted, we were not certain whether the "potential energy" of the system in the sense we were using it could be considered equivalent to "free energy." Largely as the result of discussions with Dr. J. H. Young, we have concluded that the two terms are equivalent in the context of the present communication. Wherever the phrase "potential energy" is found in the text, the reader may assume that the concept of "free energy" is applicable. We are defining chemical potential energy as the free energy associated with changes in the electronic state of valence electrons.

However, if the system is perturbed by stretching the spring and then releasing it, the system will oscillate around the equilibrium position. The total potential energy of the system is now expressible in terms of three component energy forms—the electrical, mechanical, and kinetic energies (the electromechanical system). It can be readily shown that during any period of oscillation of the electromechanical system, the three different forms of energy undergo interconversion in accordance with the law of conservation of energy. These simple examples reveal the important observation that interconversions of different forms of potential energy are the inevitable consequences of the structural attributes of energy transducing devices.

Although there is no established precedent for a three-component energy transducer capable of interconverting the electrical, mechanical and chemical potential energies, we are postulating that the mitochondrion is structurally capable of performing this function. If we express the total potential energy of the repeating unit as ψ and the electrical, mechanical and chemical components of the potential energy as E, C and M respectively, we can write

$$\psi_g = E_g + M_g + C_g \tag{1}$$

and

$$\psi_e = E_e + M_e + C_e \tag{2}$$

where subscript g refers to the ground state and subscript e to the energized state. The potential energy difference between the two states is then

$$\Delta\psi = \psi_e - \psi_g = (E_e - E_g) + (M_e - M_g) + (C_e - C_g) \tag{3}$$

If the transition of the repeating unit from the ground state to the energized state is isoenergetic, equation (3) becomes

$$(E_e - E_g) + (M_e - M_g) + (C_e - C_g) = 0 \tag{4}$$

which shows that the electrical, mechanical and chemical potential energies are interconvertible. When the repeating unit undergoes a transition from the ground state to the energized state by virtue of a spontaneous chemical reaction, the chemical potential energy decreases, and the quantity $(C_g - C_e)$ becomes more positive. This means, according to equation (4), that the electrical potential energy difference $(E_g - E_e)$ and/or the mechanical potential energy difference $(M_g - M_e)$ must be negative. In other words, the chemically induced energized state of the repeating unit possesses higher electrical and/or mechanical potential energies than does the ground state. Thus, we can associate an electric field and conformational strain with the energized state.

The phenomenon of interconversion of the electrical, mechanical and chemical potential energies will be referred to as "electromechanochemical coupling". The enzyme system capable of catalyzing such energy conversion will be called the "electromechanochemical coupling system". It is our fundamental postulate that the repeating unit of the inner mitochondrial membrane is an electromechanochemical coupling system.

B. *The Mechanistic Principles of Mitochondrial Energy Transduction*

In the previous section, we have postulated that the mitochondrion is an electromechanochemical energy transducer in the sense that the structural attributes of the organelle enable facile interconversion of the electrical, mechanical and chemical potential energies. Since, the mitochondrion contains many thousands of repeating units,[4] we will define the smallest operational unit capable of catalyzing energy conversion as the "supermolecule" of mitochondrial energy transduction. We wish to distinguish the supermolecule from the classical tripartite repeating unit which was originally defined more or less as a structural unit and not as an operational unit.[12] Unlike the tripartite repeating unit which consists of the headpiece, the stalk and the basepiece, the structure of the supermolecule must be variable depending on the nature of work performance executed by the mitochondrion. Although the detailed macromolecular structure of the supermolecule for a particular work performance is still unknown, we can nevertheless recognize two major structural components of the energy transducing unit: (1) the component participating in the energization process (the energization structural component); and (2) the component involved in the deenergization process (the deenergization structural component). Clearly, the basepieces (in which the electron transfer complexes are localized[13]) as well as the headpieces (in which the ATPase is localized[13]) can be identified as the energization structural components. It is the identity of the deenergization structural component of the supermolecule which determines the nature of the work performance—the headpiece for oxidative phosphorylation,[14] transprotonase for active transport, the transhydrogenase for energized transhydrogenation, and the basepiece for reversed electron flow. It is interesting to note that both the headpiece and the basepiece can act either as the energization structural component or as the deenergization structural component of the supermolecule.

Having described the overall structural requirements for mitochondrial energy transduction, we are now in a position to inquire into the possible mechanism by which the supermolecule implements the principle of electromechanochemical coupling. We are of the

opinion that whatever the mechanistic principles operating for a particular work function, the same principles must be applicable to all other work functions of the mitochondrion. On the basis of the biochemical and structural data presently available on oxidative phosphorylation and also on the basis of theoretical investigations in our and other laboratories, we have been led to the following conclusions: (1) the unit of mitochondrial energy transduction is a supermolecule in which electromechanochemical potential energy is manipulated; (2) when the supermolecule is energized either by substrate oxidation or by ATP hydrolysis, there is a vectorial charge separation in the energization structural component of the supermolecule—a consequence either of the separation of electrons and protons in the basepiece, or of the rearrangement of the structural dipoles in the headpiece (see below); (3) the energization of the supermolecule is the consequence of the enzyme-catalyzed conversion of the chemical potential energy of the bound substrate into the potential energy of the supermolecule (including the bound substrates) which is now predominantly in the electromechanical form; (4) the local electric field created as the result of the energization process (substrate oxidation or ATP hydrolysis) *induces* a complementary electric field in the deenergization structural components probably through an electric field effect; (5) the electrostatic interaction between the electrically polarized energization and deenergization structural components leads to a stabilization of the energized supermolecule (the metastable state); (6) when the polarized deenergization structural component is *depolarized* by a chemical reaction in the active site, the supermolecule is deenergized and relaxes to the ground state with concomitant conversion of the predominantly electromechanical potential energy either to kinetically stable molecules [ATP or TPNH] or to kinetically labile species with high electrochemical activities (for example accumulated ions); and finally (7) the local conformational perturbations at the active site where the chemical reactions actually take place, trigger the depolarization process.

The essential features of the above conclusions may be schematically represented as in Fig. 1. The system under consideration is a supermolecule (depicted as a regular rectangle or a ballooned-out rectangle) in which are contained the reduced electron carrier AH_2, the oxidized electron carrier B, and bound ADP and P_i in the ground state. When AH_2 is oxidized by B, the chemical potential energy decreases with concomitant increase in the electrical and mechanical potential energy of the "strained" supermolecule (depicted as a ballooned-out rectangle). We postulate that the strain in the metastable supermolecule is primarily due to a chemically induced charge

separation. The electric field generated in the local region of the oxidoreductive active site is potentially available for electrostatic interaction with any charged groups or polarizable groups within the supermolecule. However, under the conditions of oxidative phosphorylation, the deenergization structural component binding ADP and P_i (i.e., the headpiece) is preferentially polarized. Now, the initiation P–O bond formation between ADP and P_i triggers the depolarization of the supermolecule with the conversion of the predominantly electromechanical potential energy inherent in the metastable supermolecule into the chemical potential energy of ATP.

Again, it is instructive to compare the present model with well known energy converters such as the hydrogen–oxygen fuel cell.[15] In this cell, the anode is electrically polarized by the oxidation reaction which converts hydrogen molecules into protons. The electron flow from the anode to the cathode now induces an electrical polarization of the latter electrode, which is in turn depolarized by the reduction

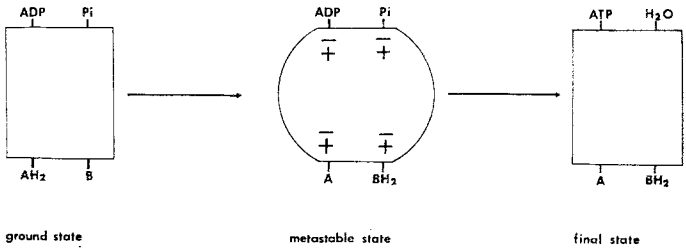

Figure 1. Schematic representation of the electromechanochemical model of oxidative phosphorylation.

of oxygen molecules into hydroxide ions.[16] In a sense, the anode–cathode system is analogous to the supermolecule in our model, with the important difference that whereas the electrode system is virtually free from charge-induced mechanical deformations, the supermolecule is extremely sensitive to charge-induced conformational deformations as schematically shown in Fig. 1. Just as we can regard the hydrogen molecule as a polarizer and the oxygen molecule as a depolarizer, we may consider the substrates of oxidoreduction as polarizers and the combination of ADP and P_i as a depolarizer. As is common with any analogy, there are many differences between the supermolecule and a fuel cell. In addition to the difference in structural rigidity between the two energy transducers already pointed out above, there are a few more points of difference: (1) both of the chemical reactions involved in polarization and depolarization of electrodes in fuel cells are oxidoreduction reactions, whereas in the supermolecule only the reaction in the basepiece and not the reaction

in the headpiece is an oxidoreduction reaction; (2) both the polarization and the depolarization reactions are energetically down-hill in fuel cells, whereas in the supermolecule the polarization reaction is exergonic and the depolarization is endergonic; and finally (3) the induced polarization in fuel cells is mediated by actual movement of electrons from the anode to the cathode through a metallic conductor whereas in the supermolecule the induced polarization is achieved by an electric field effect without involving any movement of electrons between the two polarizable structural components.

We have considered in some detail the basic notions embodied in the electromechanochemical model of mitochondrial structure and function. It is our view that the mechanistic principles deduced from oxidative phosphorylation apply to all other energized processes. Accordingly, we may represent the work performances of the mitochondrion in terms of the following equations:

a. *Oxidative phosphorylation*

$$\begin{pmatrix}\text{Polarized}\\ \text{ATPase}\end{pmatrix} + \text{ADP} + \text{P}_\text{i} \rightarrow \begin{pmatrix}\text{Depolarized}\\ \text{ATPase}\end{pmatrix} + \text{ATP} + \text{H}_2\text{O} \quad (5)$$

b. *Active transport*

$$\begin{pmatrix}\text{Polarized}\\ \text{transprotonase}\end{pmatrix} + \text{H}^+_{\text{in}} + \text{K}^+_{\text{out}} \longrightarrow \begin{pmatrix}\text{Depolarized}\\ \text{transprotonase}\end{pmatrix} + \text{H}^+_{\text{out}} + \text{K}^+_{\text{in}} \quad (6)$$

$$\text{H}^+_{\text{out}} + \text{Ac}^-_{\text{out}} \longrightarrow \text{H}^+_{\text{in}} + \text{Ac}^-_{\text{in}} \quad (7)$$

c. *Energized transhydrogenation*

$$\begin{pmatrix}\text{Polarized}\\ \text{transhydrogenase}\end{pmatrix} + \text{DPNH} + \text{TPN}^+ \rightarrow \begin{pmatrix}\text{Depolarized}\\ \text{transhydrogenase}\end{pmatrix} + \text{DPN}^+ + \text{TPNH} \quad (8)$$

C. *Charge Separation and the Generation of Membrane Potential*

When an electron transfer complex is reduced by its appropriate substrate, simultaneously three events take place: (1) the complex becomes energized; (2) a pair of electrons is transferred to the electron transfer chain[4]; and (3) a membrane potential is generated.[17] These three events are clearly interrelated but the nature of this interrelationship has hitherto been an enigma. Two developments were crucial in deducing the nature of the interrelationship. First, the

electron transfer complex by virtue of being an intrinsic repeating unit of the inner membrane most likely has a double-tiered structure (four proteins in each of the two tiers).[18] Second, Complex IV of the electron transfer chain can be resolved into two fractions equal in weight—one containing the oxidation–reduction components of the complex and the other devoid of these components.[19] From the splitting of the complex into two qualitatively different halves, we have deduced that in one of the two tiers, a set of four proteins is concerned with the transfer of electrons while in the other of the two tiers, a set of four proteins is concerned with the transfer of protons

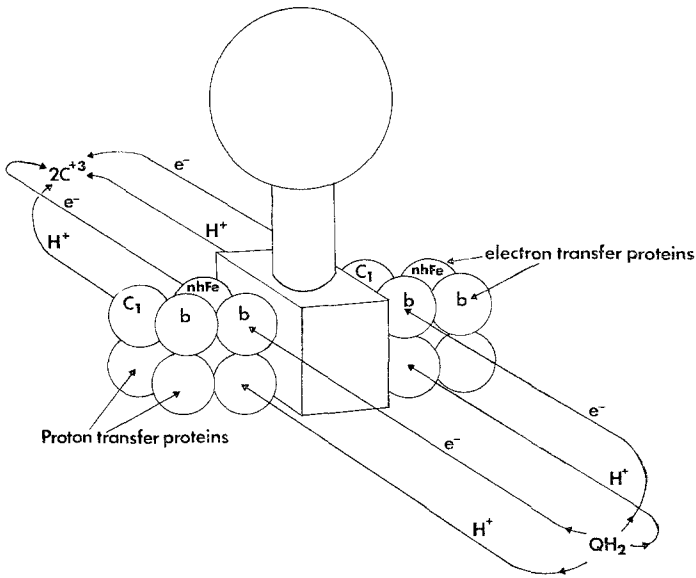

Figure 2. Electron and proton transfer routes through Complex III.

(Fig. 2). The substrate in effect transfers a molecule of H_2 to the complex. In this process, a pair of electrons ends up in the proteins of one tier of the complex (the electron transfer proteins) and a pair of protons end up in the proteins of the other tier of the complex (the proton transfer proteins).

The separation of charge which accompanies the reduction of the complex depends upon a simultaneous conformational transition the exact nature of which remains to be determined. If the electrons and protons were in cavities in the dead center of their respective proteins the distance of charge separation would be about 30 Å.

It is known that the electron can be localized within a deep channel in oxidoreduction proteins such as cytochrome *c*.[20] The electron that enters one of the empty 3d orbitals of heme iron[21] upon reduction can

be treated as an uncompensated charge. We are postulating that the uncompensated charge of a proton can also be localized on a specific acceptor group within a deep channel in the proteins of the "proton transfer chain". The electron and proton are buried deep within the protein interior and are thus isolated from the aqueous milieu which surrounds the membrane. Penetration by the solvent into the protected internal cavities in which the uncompensated charges are localized would lead to the erosion of the electric potential.

The inner membrane when energized develops a membrane potential, positive on the intracristal side and negative on the matrix side.[17] Therefore, it is necessary for us to postulate that the electron transfer proteins of a complex are in the tier on the matrix side whereas the proton transfer proteins are in the tier on the intracristal side as shown in Fig. 2. In the interaction of DPNH with Complex I, or of reduced coenzyme Q with Complex III, or of reduced cytochrome *c* with Complex IV,[22] we could conceive of these interactions as the equivalent of the abstraction of 2 hydrogen atoms from the substrate and transfer of these hydrogen atoms to the complex. It is to be noted that no distinction is made between substrates such as Q_{H_2} which provide these 2 hydrogen atoms directly or substrates such as DPNH and cytochrome *c* which must be protonated as a preliminary to hydrogen abstraction. The point at issue is that a proton and an electron together are abstracted from the substrate by the appropriate dehydrogenating enzyme and that they are fed into respective acceptor sites in the proteins arranged in a double-tiered pattern—one set accepting the negative charge, and the other set accepting the positive charge. Thus, we view the charge separation in the electron transfer chain as the direct consequence of the catalysis of oxidoreduction reaction in the inner membrane. In this way, we avoid the necessity for invoking any hypothetical "proton pump" driven by substrate oxidation in order to achieve charge separation.

D. *Structure of the ATPase Coupling Unit*

The studies of Tzagoloff[23] suggest that the ATPase coupling unit consists of a headpiece which is attached by a cylindrical stalk to a structure in the membrane, as yet unidentified. There is apparently a set of associated proteins in the membrane (molecular weight of about 100,000[23]) in which the stalk is anchored. Let us describe the combination of the stalk sector and this membrane-anchoring structure as the "stalk complex" (see Fig. 3).

Two component elements of the mitochondrial energy transducing unit are well characterized morphologically, namely the headpiece (90 Å sphere) and the stalk (30 × 50 Å cylinder).[12] Experiments with ETP_H[24] have provided evidence that the headpiece may assume two

distinct geometric relations with respect to the inner membrane depending on the coupling capability of ETP_H and on the presence or absence of ATP in the medium. In other words, the headpieces in tightly coupled ETP_H have been found to be on or partially in the membrane continuum in the presence of ATP (the apposed configuration; see Fig. 3a), whereas under the same experimental condition the headpieces in uncoupled submitochondrial particles project away from the membrane surface and are connected to the membrane by the 50 Å stalk sector (the extended configuration; see Fig. 3b). In the apposed configuration, the stalk is probably pushed deep into the membrane while in the extended configuration, the

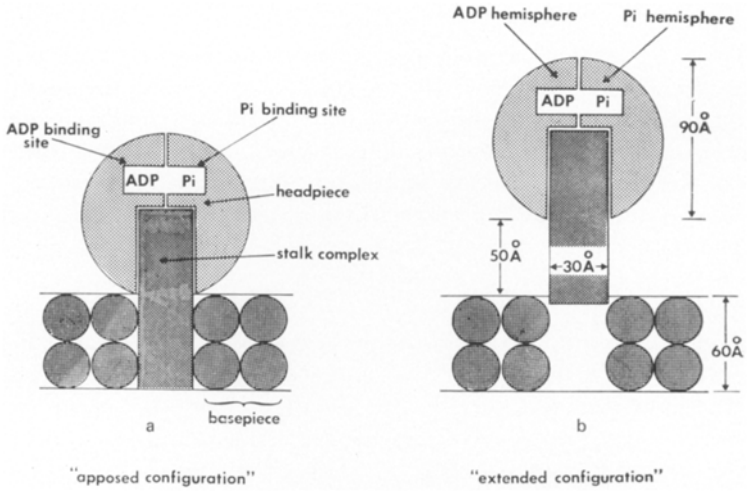

Figure 3. Postulated internal structure of the ATPase coupling unit and its relation to the electron transfer complexes.

stalk is pulled out of the membrane as shown in Fig. 3. Since the extended configuration is characteristic of uncoupled mitochondria or submitochondrial particles and the apposed configuration is associated with phosphorylating ETP_H,[24] we have deduced that the apposed configuration of the headpiece and stalk is a prerequisite for oxidative phosphorylation and active transport in mitochondria. Coupling requires the insertion of the stalk into the membrane continuum and the contact of the headpiece and membrane. Whenever these conditions are not fulfilled as in the orthodox configuration,* then electron transfer or ATP hydrolysis cannot be coupled to any work performance.

* It has not been generally appreciated that in mitochondria the headpiece-stalk projections are visualized only in the orthodox configuration.[12] The headpiece, but not the stalk, can be visualized in the aggregated and twisted configurations.

The evidence that supports the above structural interpretations may be summarized as follows. The ATPase coupling unit has been isolated as an oligomycin-sensitive complex from both beef heart and yeast mitochondria.[23,25] The unit interacts with phospholipids to form membranous arrays the periodicity of which is identical with the periodicity of headpieces in the intact mitochondrial membrane.[13] Under coupling conditions, the headpieces, but not the stalk, can be visualized by positive staining in the cristae of mitochondria.[26] However, in the orthodox configuration, both the stalk and the headpieces are visualized by negative staining and both project from the membrane.[12] Finally, as alluded to above, the headpiece in ETP_H extends away from the membrane via the stalk when ETP_H is uncoupled.[24]

We may consider the stalk as a dielectric for effective transmission of the electric field from the electron transfer complex to the headpiece of the ATPase coupling unit. When the stalk is extended from the membrane as in the orthodox configuration, the means for transmission of the electric field may be lacking. This would account for the loss of coupling capability when the inner membrane is stabilized in the orthodox configuration.[27]

E. *Induction of an Electric Field in the ATPase Coupling Unit*

To simplify visualization of the process by which an electric field is generated in the headpiece, we may consider the headpiece as a sphere divided longitudinally (perpendicular to the plane of the membrane) into two hemispheres, each corresponding to a multimeric unit of globular proteins (Fig. 3). The cavity into which the stalk complex is inserted is located between the two hemispheres. Furthermore, the active sites for ATP synthesis and hydrolysis are assumed to spread over the two hemispheres. ADP and P_i probably bind to separate hemispheres ("ADP hemisphere" and "P_i hemisphere" in Fig. 3). It is known that ATP hydrolysis leads to the generation of a membrane potential.[17] In principle there appear to be three possible ways of generating a membrane potential linked to ATP hydrolysis: (1) ATP hydrolysis drives a proton pump as in the chemiosmotic hypothesis[28]; (2) the conformational changes associated with ATP hydrolysis in the headpiece propagate through the stalk to the basepiece which undergoes a conformational transition leading to a membrane potential[29]; and (3) ATP hydrolysis conformationally polarizes the headpiece, and the electric field of the headpiece induces a polarization of the basepiece. For theoretical reasons (see above), we have chosen the third alternative as the preferred mechanism for generating a membrane potential. In our view, the electric field effect is singularly suited for coupling the ATP hydrolysis

event to the polarization of the electron transfer chain over a relatively long distance (the center to center distance between the headpiece and the basepiece is about 70 Å[12]).

Since it is unlikely that ATP hydrolysis itself will lead to any net charge separation at the active site, we have deduced that ATP hydrolysis must be coupled to the perturbation of structural dipoles as a means for generating an electric field. To fulfill this role, the dipoles must be arranged on a plane parallel to the inner membrane surface (the *xy*-plane), so that in the unperturbed state of the headpiece there is no net dipole moment in the direction perpendicular to

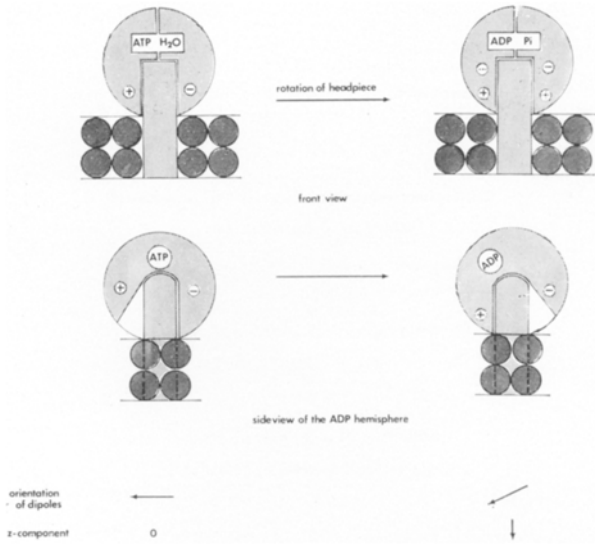

Figure 4. Polarization of the headpiece by ATP hydrolysis.

the plane of the inner membrane (the *z*-direction; see Fig. 4). When ATP hydrolysis takes place, the accompanying conformational perturbation compels the headpiece to undergo a state transition. In the perturbed state, the pair of structural dipoles are forced out of the planar arrangement and a net dipole movement in the *z*-direction results. This is schematically shown in Fig. 4 where the ATP-induced state transition of the headpiece is represented as an anti-clockwise rotation of the hemispheres. The important steps involved in linking ATP hydrolysis to the generation of the electric field in the headpiece are summarized in Fig. 5.

The conformational transition of the headpiece from the unperturbed to the perturbed state may be induced either by ATP hydrolysis as described above or by electron transfer in the basepiece. In the

latter case, the electric field associated with the basepiece acts on the structural dipoles of the headpiece (charge–dipole interaction).

We have not specified the polarity of the immediate environment that surrounds the structural dipoles in Fig. 4. If the environment of the dipoles is hydrophobic, there would be a relatively strong dipole–dipole interaction even before the headpiece is conformationally perturbed, leading to an electromechanochemically strained headpiece. This electromechanochemical strain of the headpiece in the ground state is neither necessary from the point of view of our model

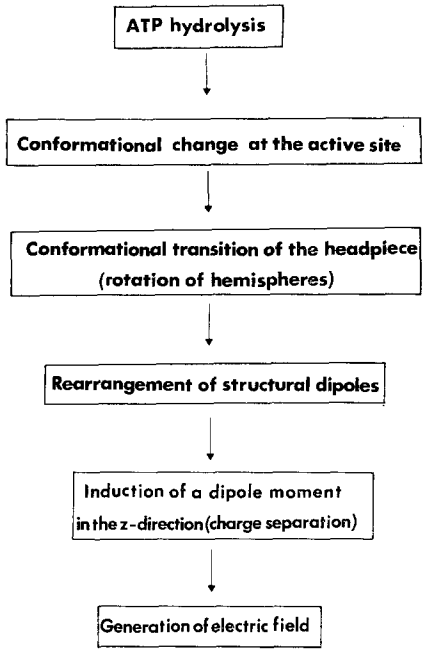

Figure 5. Induction of an electric field by ATP hydrolysis in the headpiece.

nor likely in view of the fact that protein systems (except cytochromes) rarely accommodate uncompensated charges in their hydrophobic interior under normal conditions. This consideration led us to entertain the notion that the dielectric environment of the structural dipoles undergoes a decrease in polarity simultaneously with the headpiece transition from the ground to the perturbed state; the polar environment of the dipoles would attenuate the electrostatic interaction between the dipoles in the ground state, but the decreased polarity of the dipole environment in the perturbed state would still induce a relatively strong electric field in the z-direction. Thus we postulate that the conformational perturbation of the headpiece involves not only the geometric rearrangement of the structural

dipoles but also associated variations in the dielectric constant of the dipole environment in order to diminish the dipole–dipole interaction in the ground state and to enhance the intensity of the induced electric field in the perturbed state of the headpiece. Such a conformationally controlled variation of the polarity of protein microenvironment has been suggested by X-ray studies of the structure of cytochrome *c*.[30]

We have invoked above a full-blown fixed charge system in the interior of the headpiece. This does not mean that the surface charge redistribution and the changes in the chemical and mechanical states of the headpiece will not attenuate the effective magnitude of the fixed charges. For our purpose, all that is required is the presence of a fixed charge system which exerts a net electric field in the *z*-direction outside the headpiece when the headpiece undergoes conformational perturbation.

F. *Electric Field Effects*

The electric field in the inner membrane is established within a multimeric protein complex about 60 Å in thickness.[31] It is generated by the separation of two protons and two electrons per coupling site—the protons being localized near the intracristal side and the electrons toward the matrix side. The energy required to separate electrons and protons derived from the substrate molecule and to transfer these separated charges to the respective acceptor sites is obviously of paramount importance. A precondition for the acceptability of the mechanism we have proposed for generating a membrane potential is that the energy must be less than the energy derivable from the oxidoreduction reaction which takes place within a single coupling site. Unfortunately, the exact value of the energy required for charge separation in the inner membrane cannot be determined in the absence of more accurate structural and chemical information than is presently available. However, an order-of-magnitude estimation can be made by considering the major steps involved in the charge separation. There are five such steps: (1) breaking of the carbon–hydrogen bond of the substrate; (2) ionization of the abstracted hydrogen atom into an electron and a proton; (3) separation of the electron and proton from the initial intercharge distance after ionization (say 2 Å) to the final intercharge distance of about 30 Å; (4) chemical interactions of the electron and proton with their respective acceptor groups; and finally (5) the conformational rearrangements of polypeptide chains accompanying charge separation. The net energy change due to the charge separation in an electron transfer complex is then the algebraic sum of all the energies produced or consumed by the above elementary steps. It is interesting

to note here that the energy required to carry out step (3) above (purely electrostatic work involved in increasing the intercharge distance from 2 to 30 Å) is about 15 Kcal/mole assuming a dielectric constant of 10. This energy is at least an order of magnitude smaller than the other energy terms. The important point to be emphasized is the fact that the electrostatic work required to separate an electron and a proton to a desired distance is not prohibitively large but is within the range of the energy derivable by oxidoreduction in the electron transfer complex.

The next item of importance is the strength of the electric field generated by charge separation in the basepiece. If we assume that the intercharge distance between electron and proton is 30 Å and the average dielectric constant of the medium separating the charges is 10, the electric field intensity at a point 70 Å away from the center of the basepiece is about 3×10^4 volts/cm. This electric field intensity is of the same order of magnitude as is known to induce conformational transitions in DNA molecules[32] and as is experimentally measured across the thylakoid membrane in chloroplast.[33] We are assuming that this electric field at the headpiece is of the right order of magnitude to drive the synthesis of ATP by direct union of ADP and P_i.[34]

The electric field generated in the electron transfer complexes has multiple effects on the component systems in the mitochondrion. Let us consider three such effects: (1) the asymmetric perturbations of the proteins in the inner membrane; (2) the perturbation of the matrix system; and (3) the induction of an electric polarization of the headpiece.

The electric field asymmetrically perturbs the relation of the bimodal proteins to the water–lipid interface at which these proteins are poised. If on one side of the membrane (the matrix side) the proteins sink below the interface due to the membrane electric field, then on the other side (the intracristal side) the proteins must rise above the interface due to the vectorial nature inherent in an electric field. If on the intracristal side, groups previously buried are exposed and ionize, leading to the generation of free protons, then on the matrix side, the opposite will take place, namely the burying of ionizable groups leading to the uptake of protons from the medium. Since the numbers of groups exposed on one side and buried on the other will be comparable, the net free energy change may be quite small even though the number of protons released or taken up is considerable.

The energized inner membrane is negatively polarized on the matrix side and positively polarized on the intracristal side.[17] The matrix network is enclosed between two cristae generally separated by relatively short distance, e.g. <300 Å in beef heart mitochondria. Thus

the bulk of matrix proteins may exist within short distances from the inner membrane, probably in the order of one Debye length.[35] The intense cathodic electric field on the membrane surfaces which bound the matrix space will, therefore, inevitably affect the state of matrix proteins; positively charged proteins will migrate toward the membrane surfaces (electrophoretic effect); electrically neutral proteins with high dielectric constant will move to a region of high field intensity (dielectrophoretic effect[36]); and the p*K*a's of the ionizable groups on protein surfaces will decrease (the second Wien effect[37]) causing pH changes in the matrix space. In addition, the state of matrix water may undergo pronounced changes due to the fact that the water molecule is dipolar.[38] Several authors have speculated on the possibility of cooperative configurational transitions of protein systems induced by electric field.[39] It is probable that similar cooperative, electrostatic interactions exist between the inner membrane and the matrix system.

The electric field in the energized electron transfer complex can induce a complementary field in the ATPase coupling unit under the conditions of oxidative phosphorylation (see below). Similarly the electric field in the energized ATPase coupling unit can induce a complementary field in the electron transfer complex under the conditions of ATP-driven reversed electron flow and active transport. This induction of complementary field provides a mechanism for partitioning potential energy within different sectors of essentially one supramolecular unit (the electron transfer complex plus the ATPase coupling unit).

G. *De facto Unit of Mitochondrial Control*

The control of mitochondrial coupling depends upon the interplay of three basic systems: the inner membrane, the protein network systems in the matrix and the intracristal spaces. These three systems are so closely intertwined structurally and functionally that they can be considered as a single operational unit which we describe as the *de facto* unit of mitochondrial control. The matrix network can exist in a dispersed or condensed state[40]; and in the condensed state the matrix can exist either in a form with high ion binding capability or in a form with low ion binding capability. There are thus at least three alternative states of the matrix network system. The proteins in the intracristal space in general appear electron microscopically transparent. However, under certain conditions the intracristal space proteins can assume a lattice structure.[41] This again suggests that the intracristal network can also exist in a dispersed or condensed state. The matrix system is highly concentrated in the mitochondrion (between 10 and 50 grams protein per 100 ml of matrix fluid) and accounts for the bulk

of the solubilizable protein of the mitochondrion. Moreover, the work of Hackenbrock clearly shows that the matrix proteins have a high degree of structural organization.[40]

The inner membrane contains two closely associated but independent repeat structures—the electron transfer complexes[22] and the ATPase coupling unit.[13,25] There is a 1:1 correspondence between the number of ATPase coupling units and electron transfer complexes. There are three alternative conformational states of the ATPase coupling unit: (1) the state in which the proteins of both the headpiece and stalk are in the membrane (fused regions of the coalesced configuration); (2) the state in which the headpiece projects from the membrane but the stalk is buried in the membrane (the twisted and paired configurations*); and finally (3) the state in which both the headpiece and stalk project from the membrane (the orthodox configuration).[12] The inner membrane can exist in at least four different states—nonenergized or energized with the headpiece-stalk unit projecting from the membrane (the orthodox configuration), nonenergized with the proteins of the headpiece-stalk unit buried in the membrane (fused regions of the coalesced configuration), and energized with the headpiece projecting from and the stalk in the membrane (the paired or the twisted configuration).

The configuration of the mitochondrion as visualized electron microscopically reflects the relationship of the three interdigitating systems and preponderantly the relationship of the inner membrane to the matrix system. Each of the four configurational states of the mitochondrion (orthodox, twisted, paired and coalesced) represents a given state of the matrix, a given state of the intracristal system and a given state of the inner membrane. These interrelationships are different for each of the four configurational states. Thus in a particular configuration such as the twisted, we can specify that the matrix system is condensed, the intracristal system is dispersed, the inner membrane has a helical twist, and the headpiece sector of the ATPase coupling unit is projecting into the matrix space while the stalk is embedded in the membrane.

There is a feedback relationship between the three mitochondrial systems. The state of the matrix affects the state of the inner membrane and the state of the intracristal system. The energization of the inner

* The configuration previously described as "aggregated" embraces two similar configurations—the "coalesced" which obtains under nonenergizing conditions in 0·25 M sucrose (the NEagg configuration according to Penniston *et al.*[1]), and the "paired" configuration which obtains under energizing conditions (the Eagg configuration according to Penniston *et al.*[1]). The purpose of introducing the new terms "coalesced" and "paired" is to describe the inner membrane configuration in purely morphological terms devoid of any reference to the energy state of the mitochondrion. This is necessary because of recent findings that the so-called "energized" configurations (e.g. E or ET) could be induced by nonenergized means.[45]

membrane affects the state of the matrix system which in turn affects the conformation of the inner membrane, etc. We are thus dealing with a delicately poised set of systems which collectively respond to changes in the ionic composition of the medium, to energization or deenergization, to osmotic pressures and to reagents which affect any one of the three interdigitating systems.

When the mitochondrion is exposed to energizing conditions in presence of the reagents required for oxidative phosphorylation, the *de facto* unit undergoes a rearrangement which leads to the establishment of a steady-state configuration (a mixture of the paired and coalesced configurations). When energized in presence of reagents that are required for active transport of K^+, it assumes yet another coupling configuration (the twisted configuration[42]). Thus for each coupling mode there is a unique configurational state of the *de facto* unit which defines the states of the three basic systems in relation to one another and ultimately the state of the supermolecule (see above). These unique configurations are maintained as long as energizing conditions are present. When energizing conditions are no longer available, the configuration of the *de facto* unit returns to the configuration that obtained prior to energization. Energization is therefore one of the crucial determinants of the coupling configuration. Many reagents are known (e.g. fluorescein mercuric acetate[43]) which can prevent configurational adjustment of the *de facto* unit and thereby interdict the induction of the configuration required for either oxidative phosphorylation or active transport.

The *de facto* unit can undergo ion-induced configurational rearrangements which are electron microscopically indistinguishable from those induced by energizing conditions.[44–47] Such nonenergized rearrangements of mitochondrial configurations are, however, stable and are not influenced by the reagents that block energized configurational changes. A major control feature of mitochondria is thus the regulation of the configurational adjustment of the *de facto* unit. Ions such as Ca^{2+} and Mg^{2+} play key roles in this regulation.[27]

Central to the notion of the *de facto* unit is the recognition that the macromolecular systems (the matrix, inner membrane and intracristal proteins) assume closely interacting relationships because of the fact that they are compelled to exist in a relatively small space enclosed by the outer membrane. Unlike the space outside the outer membrane, the intramitochondrial space is highly heterogeneous in material composition as well as in local electrostatic environment. Since it is clear that the configuration of the *de facto* unit will determine the state of the macromolecular systems inside the mitochondrion, it would follow that the configuration of the *de facto* unit also determines the conformational state of the supermolecule and hence the nature of

mitochondrial work performance. In our view, therefore, the mechanism by which mitochondrial function is controlled is inextricably tied into the general causal sequence shown in Fig. 6. We may regard the *de facto* unit as the operational unit for mitochondrial control and the supermolecule as the operational unit for energy transduction.

The basic structural difference between ETP_H and intact mitochondria is that ETP_H lacks one of the two membranes (the outer membrane), one of the two spaces (the matrix space) and one of the two protein networks (the matrix network).

While ETP_H is incomplete in control functions, it nevertheless is capable of performing energy transductions—a token that ETP_H resembles more a collection of supermolecules. From the enzymological point of view, therefore, the intact mitochondrion represents an operational unit complete in terms of control as well as of energy transduction, whereas ETP_H represents a functional unit which is

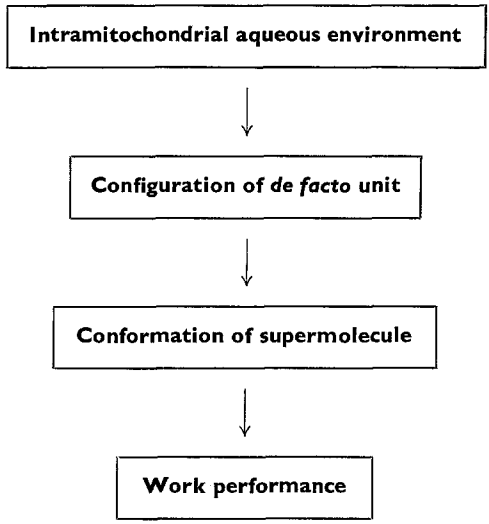

Figure 6. General causal sequence involved in mitochondrial control.

capable only of certain energy transductions with incomplete control capability.

It cannot be a happenstance that the various sets of ions which play a predominant role in determining configuration are the very ions which are required for the different work functions, e.g. $P_i + ADP$, $DPN^+ + TPNH$, $Ca^{2+} + P_i$, $K^+ +$ weak acid anions. By this tactic, the *de facto* unit responds to the ions required for a particular work function by undergoing a transition to a configuration which is uniquely required for the exercise of that work function.

H. *Octet and Bimodal Themes for the Construction of the Electron Transfer Complexes and the Headpiece of the ATPase Complex*

The membrane model proposed by Vanderkooi and Green[7, 18] is based on two basic assumptions: (1) the membrane proteins (intrinsic proteins[49]) are globular; and (2) the surface properties of these intrinsic proteins are bimodal. Recently, Vanderkooi and Capaldi have shown that the intrinsic membrane proteins have polarities well below those of the majority of soluble proteins,[49] which is in line with the second assumption. Given the validity of these two assumptions, the simplest membrane model that is compatible with experimental facts would be the double-tiered model of Vanderkooi *et al.*[18] There is suggestive evidence that the active site of ATP synthesis in the headpiece as well as the active sites of oxidoreduction in the basepiece is (are) located within the hydrophobic interior of these units. Since

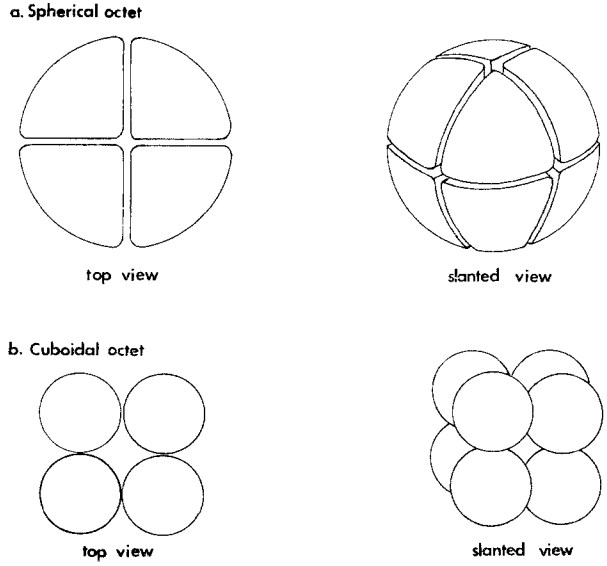

Figure 7. The octet theme for the construction of the headpiece and the electron transfer complexes.

the substrates for these active sites are either electrically charged molecules or potential charge generators, it is logical to postulate that these active sites consist of polar cavities located within the hydrophobic interior of globular protein systems, which are connected to the external medium through substrate-specific channels. Again, the simplest geometric arrangement of globular proteins which will afford the central cavity and substrate channels appears to be the octet arrangement (see Figs. 7 and 8).

The two transducing centers—the electron transfer complex which is part of the inner membrane, and the headpiece of the ATPase coupling unit which is a projection from the membrane—are probably built on the same constructional themes, namely the octet and bimodal themes. Let us first consider the structure of the electron transfer complex which is the easier of the two to visualize. There are four bimodal proteins on one tier apposing four bimodal proteins on the opposite tier of a double-tiered structure. Each set of four proteins will be arranged two in one tier and two in a tier immediately behind (Fig. 7). Top and bottom surfaces of the proteins of the octet will be polar whereas the interior surfaces of these proteins will be nonpolar. There is pairing of the hydrophobic surfaces of the proteins on the upper tier with the hydrophobic surfaces of the proteins on the lower tier. Moreover, there is also lateral juxtaposition of the hydrophobic

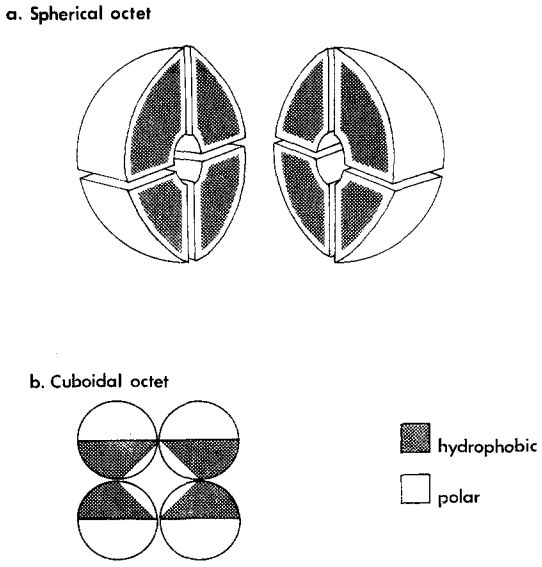

Figure 8. Polar and nonpolar patches in the octets.

surfaces of the proteins on the same tier. Thus, each protein makes hydrophobic links with its two neighboring proteins on the same tier. It is these multiple hydrophobic associations which endow the complex with a high degree of impenetrability to water. The complex is presumed to enclose a polar cavity which is in the hydrophobic center of the octet (Fig. 8b). This cavity in the interior is accessible from the exterior of the octet by selective channels into which reductant and/or oxidant can enter and leave. The stability of the polar region in the central cavity is achieved by the packing of the

eight proteins in such a fashion that each protein contributes a polar "patch" to the wall of the cavity. The summation by appropriate packing of the eight polar "patches" leads to the formation of a polar lining for the cavity. A cavity formed in this fashion could be stable if polar residues in the hydrophobic interior of an octet are so constructed that they could not reach a more polar environment. The channels which lead into the central cavity from the exterior surface of the octet can be lined with either polar or nonpolar residues. In Complex III, there would be a nonpolar channel for coenzyme Q and a polar channel for cytochrome *c*; in Complex I, a polar channel for DPN^+ and a nonpolar channel for coenzyme Q, etc. The channels are selective for the specific reductant or oxidant. The polar channels would exit on the polar surface of the octet and the nonpolar channels would exit on the hydrophobic face of the octet. The same thermodynamic considerations that have been invoked for predicting the stability of the polar cavity would apply with equal force to the stability of the polar channels in hydrophobic regions of the octet.

The octet premise for the structure of the electron transfer complexes is compatible with the data on the molecular weights of the complexes[50] and the molecular weights of the individual proteins.[22] The assumption of eight proteins to each complex is yet to be proven but it corresponds to the simplest fit. The bimodal principle of membrane construction may be a general principle applicable to all biological membranes.[51] The concept of an internal cavity with channels leading into the cavity has been well established for multimeric units such as hemoglobin[52] and for monomeric units such as cytochrome *c*.[20]

If we bear in mind that the headpiece is surrounded by the aqueous phase, then the octet and bimodal premises would require that the bimodal proteins in the headpiece be rotated 45° with respect to the orientation of the bimodal proteins in the membrane (Fig. 8a). That is to say, the polar surface of the proteins would form the external surface of the headpiece whereas the nonpolar surface of the same proteins would be oriented interiorly. The same double tier arrangement would apply to the headpiece octet as for the electron transfer complex, and the concept of a central polar cavity with feeder channels would also apply. The active groups of the headpiece, i.e., the groups concerned with synthesis or hydrolysis of ATP are assumed to be localized in an interior cavity. Since ADP and P_i would probably be bound to the active sites by more than one ligand, it would appear that each of several globular proteins in the octet should contribute to the active sites. Hence no one protein alone of the headpiece would show ATPase activity in agreement with experimental findings.[53]

The evidence for the bimodality of the headpiece proteins rests on the polarity and solubility properties of these proteins as well as on the fact that the headpiece can be collapsed into the membrane as evidenced by the existence of fused regions of cristal membrane in the coalesced configuration. The assignment of eight molecules per headpiece is an approximation based on available data.

II. *Description of the Model*

The model which will be developed in this section is based on a set of physical principles and on the structure of the systems involved in mitochondrial transduction. It is our view that within the framework of these principles and this structural information, all the necessary ingredients are present for a complete description of mitochondrial coupling. The particular model we are presenting is a model and not necessarily *the* model. But *the* model will be, we suspect, a variation of the themes implicit in the present model. Thus the emphasis should be placed primarily on the strategic aspects of the model rather than the exact details.

A. *Oxidative Phosphorylation*

We have already considered some of the principles which underlie the mechanism of the coupling of electron transfer to the synthesis of ATP. Before we discuss oxidative phosphorylation in detail, it will be helpful to present a scheme for visualizing the mechanism of oxidative phosphorylation as we formulate it. Figure 9 depicts three major steps involved in oxidative phosphorylation at coupling site 2. The energy transducing unit is composed of the headpiece, the stalk complex and the electron transfer complexes. The substrates include reduced Coenzyme Q (QH_2), oxidized cytochrome *c* (c^{3+}), ADP and P_i. When QH_2 is oxidized by Complex III, the abstracted hydrogen atoms are separated into electrons and protons which are stabilized in the two tiers of the globular proteins as described earlier (Fig. 2). The electric field generated by the charge separation in the inner membrane simultaneously polarizes the pair of dipoles embedded in the interior of the headpiece giving rise to a metastable state of the energy transducing unit (Figure 9b). We postulate that the chemical potential energy decrease accompanying the oxidation of QH_2 is conserved predominantly as electrical (charge separation) and mechanical (mechanical strain) potential energies of a metastable state which has a finite life time. In the metastable intermediate state, ADP and P_i are bound to respective binding sites in such a way that one of the terminal oxygen atoms of ADP is in a precise orientation with respect to the phosphorus atom of inorganic phosphate

favoring the formation of the P–O bond between ADP and P_i by the direct-union mechanism discussed by Korman and McLick.[34, 54] It is conceivable that in the metastable state both ADP and P_i are chemically activated via conformationally induced increase in the negative charge on the ADP oxygen atom and similarly induced increase in the positive charge on the phosphorus atom of P_i so that the activation energy barrier for the P–O bond formation is minimized. According to Korman and McLick,[54] the initially formed pentacovalent

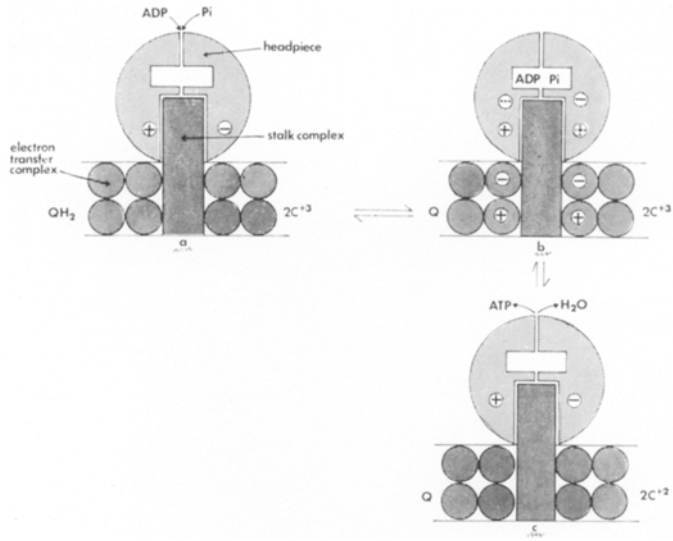

Figure 9. Mechanism of oxidative phosphorylation at coupling site 2.

phosphorus intermediate undergoes pseudorotation followed by dehydration before ATP synthesis is completed. Regardless of the precise molecular mechanism involved, it is likely that both the phosphorylation of ADP and the subsequent dehydration reaction proceed during the life time of the metastable state. If the chemical potential energy of ATP + H_2O is greater than that of ADP + P_i, the law of conservation of energy requires that ATP synthesis be accompanied by decreases in the electrical and mechanical potential energies of the enzymic system. In other words, as the covalent bond is formed between ADP and P_i, the supermolecule must relax electromechanically so that electrons and protons which were immobilized in the metastable state can now flow into the next mobile carrier and the original conformation can be restored (Fig. 9c).

An important observation to be noted here is that the electron transfer reaction is inhibited by the metastable state of the repeating unit (respiratory control). The charge-separated state of the electron transfer complexes are electrostatically stabilized by the polarized headpiece. The electron transfer in the basepiece can only proceed when the headpiece is depolarized via ATP formation. The conformational changes induced by the P–O bond formation between ADP and P_i act as a triggering mechanism for the relaxation of the headpiece and hence the collapse of the membrane potential. This triggering mechanism is not too surprising if we remember that the conformational changes at the active site *physically* affect the conformational state of the headpiece, and the headpiece and the electron

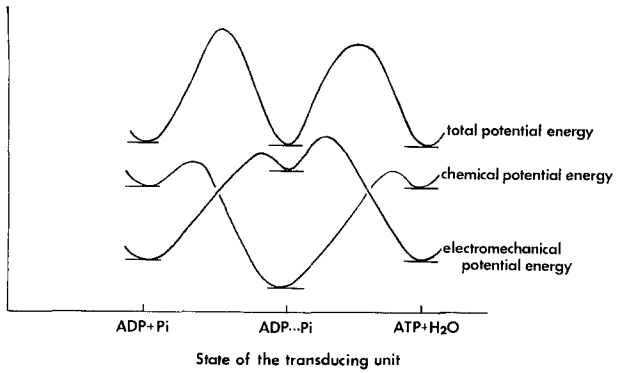

Figure 10. Potential energy diagram for oxidative phosphorylation.

transfer complexes are intimately linked by the electrostatic field effect.

If we assume that the oxidative phosphorylation reaction proceeds with 100% thermodynamic efficiency, the various types of potential energies of the energy transducing unit undergo fluctuations as shown in Fig. 10. It is clear from this diagram that the chemical potential energy is converted predominantly into the electrical and mechanical potential energies in the metastable state and this electromechanical potential energy is reconverted into predominantly chemical potential energy at the end of ATP synthesis. The difference between the initial and the final states of the energy transducing unit is that in the initial state the chemical potential energy resides mainly in the substrates of oxidoreduction whereas in the final state the chemical potential energy is accumulated, so to speak, in ATP. The interconversion of the different forms of potential energies during oxidative phosphorylation is summarized in Fig. 11. The sequence of potential energy changes chemical → electromechanical → chemi-

cal, represents in simplest form the principle of electromechanochemical coupling discussed earlier.

It is interesting to compare the salient features of the present model with the chemiosmotic hypothesis of P. Mitchell.[28] In the chemiosmotic model the membrane potential is generated secondarily as the result of separation of protons "out into" the intracristal space, and of hydroxide ions "out into" the matrix space, by maneuvering the movements of electron and hydrogen-atom carriers in the inner membrane. In the electromechanochemical model, the membrane

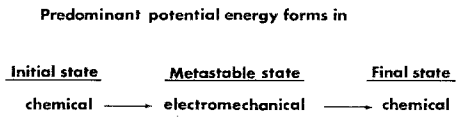

Figure 11. Variation of potential energy forms during oxidative phosphorylation.

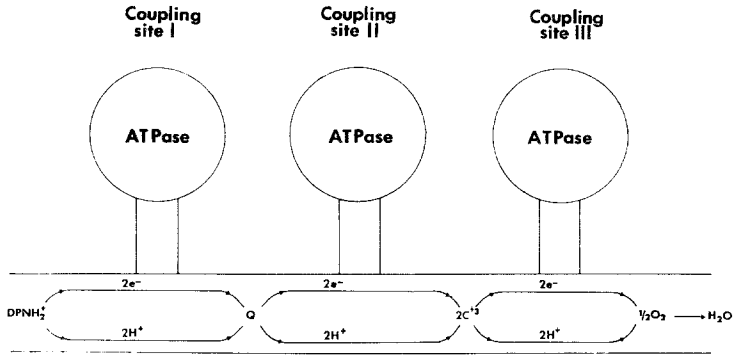

Figure 12. Arrangement of the proton and electron transfer pathways in the inner membrane.

potential is generated as a direct consequence of the chemistry of the oxidoreduction in the electron transport chain. We thus ascribe the charge-separating capability of the electron transport system to the localization of electron carriers in one tier of the double-tiered inner membrane and "proton carriers" in the other tier. Another important difference is that in the electromechanochemical model the protons separated from an oxidizable substrate are never allowed to leave the inner-membrane phase but are constrained to move within the hydrophobic environment (Figs. 12 and 13) thereby conserving the free energy of the oxidoreduction reaction within the inner membrane. Since there will be extensive conformational rearrangements of proteins in the vicinity of separated charges in the inner membrane,

the pH of the external medium and the matrix space will be influenced by the conformational changes which are induced by the "internal" charge separation in the electron transport chain.

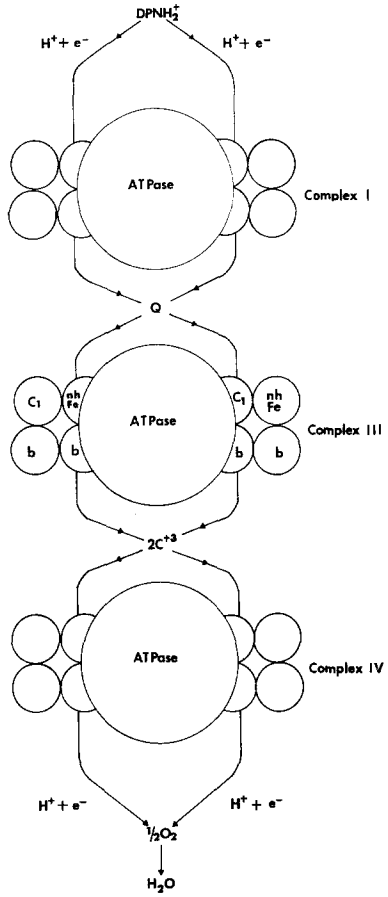

Figure 13. Surface view of the electron and proton transfer pathways in the inner membrane.

The electromechanochemical model of oxidative phosphorylation as presented above provides a unique approach to the mode of action of inhibitors and uncouplers.[55] There are four major steps involved in oxidative phosphorylation as evident in Fig. 14: (1) electron transfer; (2) electrical polarization of the basepiece (separation of e^- and H^+); (3) electrical polarization of the headpiece; and finally (4) bond formation between ADP and P_i. Any reagent or condition that prevent one or more of the above four steps can uncouple or inhibit

oxidative phosphorylation. Thus, rotenone,[56] antimycin A[57] and cyanide[58] inhibit electron transport by interacting with Complexes I, III and IV respectively. Lipid-soluble organic acids such as 2,4-dinitrophenol (DNP)[59] and *m*-chlorocarbonyl cyanide phenylhydrazone (*m*-ClCCP)[60] may uncouple oxidative phosphorylation by

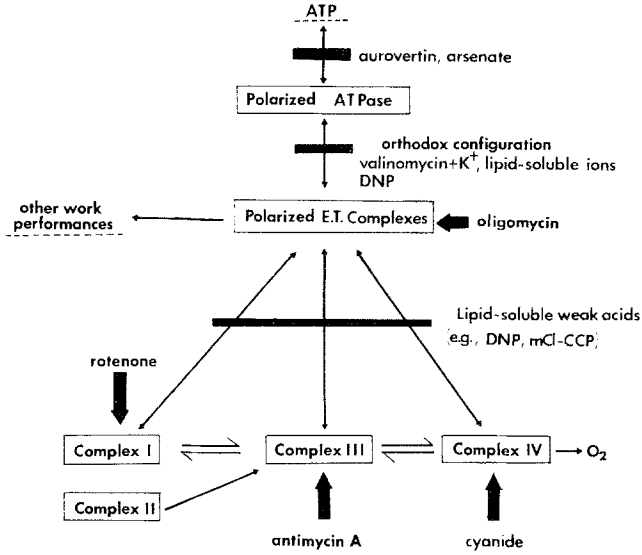

Figure 14. Proposed scheme for the sites of action of inhibitors and uncouplers of oxidative phosphorylation.

protonating the negative charge center and providing negative counter ions (conjugate bases) to the positive charge center in the inner membrane, thereby effectively depolarizing the basepiece. Since the basepiece is now disengaged in respect to electrostatic interaction with the headpiece, electron transfer in the basepiece is no longer inhibited by the electrical polarization of the headpiece and hence the electron transfer rate will be enhanced. Dinitrophenol can also act at the level of the headpiece by serving as the source of protons and dinitrophenoxide anion which can not only attenuate the electrostatic interaction between the headpiece and the basepiece but also prevent the electrostatic attraction between the two structural dipoles when the headpiece is in the perturbed state (Fig. 4). The elimination of the strong attraction between these two dipoles in the perturbed state will relieve the ATPase active site of the conformational constraints and will facilitate hydrolysis of ATP. This may account for the observation that dinitrophenol stimulates the ATPase activity of F_1 preparations.[61] Furthermore, if dinitrophenol acts at the level of the electron transport chain (the basepiece) with a greater

affinity than at the level of the headpiece, one would expect that an insufficient amount of dinitrophenol could selectively uncouple oxidative phosphorylation without inducing the ATPase activity of the headpiece, in agreement with experimental findings.[62] It is clear from these discussions that the postulated existence of a pair of structural dipoles within the interior of the headpiece is completely in line with the dinitrophenol phenomena. In fact we are inclined to suggest that the dinitrophenol data strongly support our postulate about the internal structure of the headpiece (Fig. 4).

According to the present model, it is possible to uncouple oxidative phosphorylation by preventing the polarized basepiece from inducing the polarization of the headpiece, or *vice versa*. In the orthodox configuration, the headpiece and the basepiece are separated from each other 50 Å further than in the paired configuration by the intervening stalk sector.[12,24] This increase in the distance between the headpiece and the basepiece will weaken the electrostatic interaction between them to such an extent that electrostatic coupling is abolished. The same result could be achieved by introducing highly polarizable molecules such as water in a critical region between the headpiece and the basepiece. Therefore, various conditions (aging, swelling[63]) and reagents ($CaCl_2$,[27] thyroxine[64]) which induce the orthodox configurations could uncouple oxidative phosphorylation either by their effect on the ultrastructural organization of the energy transducing unit or by their ability to introduce a highly polarizable dielectric between the headpiece and the basepiece.

Since oligomycin inhibits the ATPase activity not only of tightly coupled mitochondria (i.e., ATP synthetase) but also of dinitrophenol-treated or arsenate-treated mitochondria,[65] it is most likely that this antibiotic prevents both P–O bond-breaking during ATP hydrolysis and P–O bond-formation during ATP synthesis by preventing the rotation of the two hemispheres in the headpiece (Fig. 4). Oligomycin may effectuate this result by acting as a sort of "hydrophobic glue" between the two hemispheres, thereby increasing the rotational energy barrier of the hemispheres. In the absence of oligomycin, the electrostatic attraction between the two headpiece dipoles presumably serves as a rotational barrier. The rotational barrier due to the structural dipoles but not the barrier imposed by oligomycin can be removed by the depolarizer, dinitrophenol.

The headpiece when detached from the inner membrane (F_1) acquires oligomycin-insensitive ATPase activity.[66] This observation raises two important questions: (1) why does isolation of the headpiece induce ATPase activity? and (2) why is the ATPase activity of F_1 insensitive to oligomycin? A simple explanation that can provide an answer to both of these questions is suggested by our model of the

headpiece. It appears that in tightly coupled mitochondria there exists a hydrophobic region in the headpiece across which the two structural dipoles interact electrostatically and that this region is protected from the aqueous milieu outside the headpiece by the presence of the stalk sector. Therefore, as long as the stalk sector is attached to the headpiece, the hydrophobic region which is required for binding of oligomycin is available. Bound oligomycin then can prevent the quarternary structural changes (the rotation of the hemispheres) in the headpiece, and thereby inhibit the ATPase activity. On the other hand, if the stalk sector is removed from the headpiece, the oligomycin-binding site (or cavity) may be exposed to a more polar environment; oligomycin can no longer bind and thereby inhibit ATPase activity. There is a second sequella of removing the stalk sector from the headpiece. The removal of the stalk sector may enable a medium of high dielectric constant to intervene between the two structural dipoles in the perturbed state of the headpiece and thereby eliminate the electrostatic barrier to the rotation of the hemispheres. Thus the ATPase activity of F_1 is enhanced.

There have been several schemes proposed based on the chemical intermediate hypothesis[67,68] or the chemiosmotic model[69] which attempt to explain the large body of experimental observations relating to uncouplers and inhibitors of oxidative phosphorylation. Unfortunately, all of the classical schemes that are available now are based on two or more "high-energy intermediates" such as $X \sim I$ or $X \sim P$ whose existence in mitochondrial systems has never been established experimentally.[70] Unlike these models, the scheme we have presented is based on experimentally established structural facts and biochemical states of the structures involved. The only postulate which needs experimental proof is the notion of the structural dipoles within the headpiece. Given the validity of this postulate, our scheme appears to be capable of accommodating the major portion of the data on uncouplers and inhibitors without invoking any unproven intermediates.

B. *Active Transport*

Most of the published data on mitochondrial active transport can be readily explained if we assume the existence of an enzyme system in the inner membrane which can catalyze a transmembrane proton movement. We will hereafter refer to this postulated enzyme system as "transprotonase." The idea that the transmembrane proton movement is fundamental in mitochondrial active transport was first elucidated by P. Mitchell in his chemiosmotic hypothesis[71] and was subsequently incorporated into the conformational model of active

transport by Young, Blondin and Green.[72] It will be shown in the following section that although the electromechanochemical (EMC) model of active transport is formally indistinguishable from the chemiosmotic and the conformational models, the EMC model incorporates the basic structural features of biological membranes and in addition provides what we believe to be a more viable mechanistic linkage between the electron transfer chain (or the ATPase) and the active transport apparatus.

The principle of electromechanochemical energy transduction as formulated in the present paper and the molecular principle of biological membrane construction proposed by Vanderkooi and Green[7,18,51] provide a basis for deducing some of the essential structural and functional features of the transprotonase.

The transprotonase is most likely not a single protein but rather a set of intrinsic proteins.[49] This deduction is reasonable if one takes into account the distance ($\sim$ 60Å) across which the enzyme system transports protons. The electron transfer complexes which transport electrons over a similar distance are known to be composed of multiple proteins.[22] Since the transprotonase serves as the depolarizing structural component of the supermolecule for active transport, the enzyme system must be structured so as to undergo a precisely coordinated polarization–depolarization cycle coupled to the electron transfer reaction or to ATP hydrolysis. When the transprotonase is uncoupled from these polarizing reactions, however, the enzyme system must serve simply as a device for passively equilibrating protons across the inner membrane, since it is known that the inner membrane is permeable to protons to a limited extent under nonenergizing conditions.[73] Thus, when the transprotonase is coupled to a polarizing structural component it catalyzes a unidirectional flow of protons across the inner membrane (from the matrix to the intracristal side), and when the transprotonase is not coupled to any polarizing structural component, it facilitates the transmembrane equilibration of protons. We will speak of the coupled mode of transprotonase reaction as the "energized mode" and the uncoupled mode as the "nonenergized mode." Finally, we deduced from considerations of symmetry that one transprotonase complex transports two protons per turnover.

In Fig. 15a, a schematic diagram is shown in which the salient features of the transprotonase as described above are depicted. The structural details are perforce of secondary significance. There may be numerous ways of visualizing the same set of basic ingredients. We will represent the transprotonase as an octet of intrinsic proteins arranged in a double tier pattern in the phospholipid bilayer. In the interest of clarity the diagram in Fig. 15a shows only one-half of the transprotonase; the electron transfer complexes associated with

transprotonase are omitted from the diagram. Each globular protein in the transprotonase complex is assumed to contribute one polar end of a dipole. In the ground state (i.e., the non-energized state), the permanent dipoles are arranged in a perfectly compensating configuration so that there is no net dipole moment associated with the octet. Because of the symmetric structural features, the transprotonase is capable of translocating protons in either direction across the inner membrane depending on the direction of the pH gradient. However upon charge separation in the electron transfer complexes (see the electrical signs in Fig. 15b), the resultant electric field imparts directionality to the proton flow catalyzed by the transprotonase. This

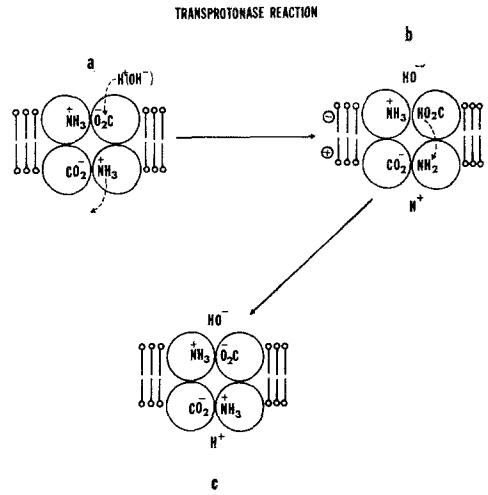

Figure 15. Mechanism of mitochondrial active transport.

directionality is imposed on the transprotonase probably through the field-induced asymmetric ionization of the polar groups within the octet. More specifically, the charge separation in the electron transfer complexes induces a complementary dipole moment in each of the two quartets of the transprotonase by protonating a basic group on the matrix side of the quartet and by deprotonating an acidic group on the intracristal side of the quartet as shown in Fig. 15b. For convenience we will refer to this phenomenon as "the field-induced protonation–deprotonation reaction." As the result of this field-induced protonation–deprotonation reaction, there is generated a pH differential (ΔpH) between the two compartments separated by the inner membrane (alkaline in the matrix space and acidic in the intracristal space). Most of the free energy derived from substrate oxidation in the case of electron transfer-driven active transport is still stored within

the polarized supermolecule (the electron transfer complex + the transprotonase) in the form of electromechanochemical free energy. It is this energy which is available for driving the electrogenic proton movement across the inner membrane unidirectionally. The precise molecular mechanism involved in the transmembrane proton migration is unknown at the present time, but it is almost certain that the proton movement is mediated by a series of bond-forming and bond-breaking chemical reactions (i.e., a series of transprotonation reactions) involving a set of amino acid residues which form what may be called the "transmembrane proton relay system." We may represent one of such elementary transprotonation reactions as follows:

$$H - \overset{\oplus}{X}_i + X_{i+1} \rightarrow X_i + H - \overset{\oplus}{X}_{i+1} \qquad (9)$$

where X_i and X_{i+1} represent two adjacent proton-stabilizing amino acid residues of the transmembrane proton relay system. The subscript i varies from 1 to $(n - 1)$ where n is the number of the proton-stabilizing amino acid residues constituting the relay system. If we assume that as the result of Reaction (9), a proton is translocated across the inner membrane say by 10 Å, there would be at most six proton-stabilizing residues per relay system, or $n = 6$.

It is assumed that regardless of whether the transprotonase is operating in the energized mode or in the nonenergized mode each elementary transprotonation reaction is accompanied by a compensating movement of membrane-permeable cations and/or anions across the inner membrane in appropriate directions so as to prevent any significant development of separated charges. In the absence of such compensating ion movement the transmembrane proton movement is probably suppressed.

When the transprotonase operates in the nonenergized mode, the net free energy change accompanying the transmembrane proton movement may be close to zero given the compensating charge migration so as to prevent charge separation (i.e., $\sum_i \Delta Y_i = O$). However, when the transprotonase operates in the energized mode, the unidirectional proton flow becomes an endergonic process even if accompanied by compensating charge movement and the corresponding free energy change will be positive (i.e., $\sum_i \Delta Y_i > O$). Therefore, under energizing conditions, Reaction (9) is to active transport what the reaction between ADP and P_i is to oxidative phosphorylation. Just as oxidative phosphorylation involves the conversion of electromechanochemical free energy into the chemical free energy of the P—O bond in ATP, so the transprotonation reaction involves the transduction of electromechanochemical free energy into the electrochemical free energy of the $H—\overset{\oplus}{X}_{i+1}$ bond according to Equation (9).

In this way it can be shown that both oxidative phosphorylation and energized transprotonation reaction are expressions of a common principle, namely the principle of electromechanochemical energy transduction.

Having described the fundamental features of the EMC model of active transport, we are now in a position to discuss specific examples. The mechanism of active transport of potassium acetate is shown in Fig. 16. The supermolecule involved in this particular work performance is composed of the electron transfer complex and transprotonase, both located in the inner membrane. The transprotonase is assumed to possess two acidic groups on the intracristal side and two basic groups on the matrix side. As electrons and protons are

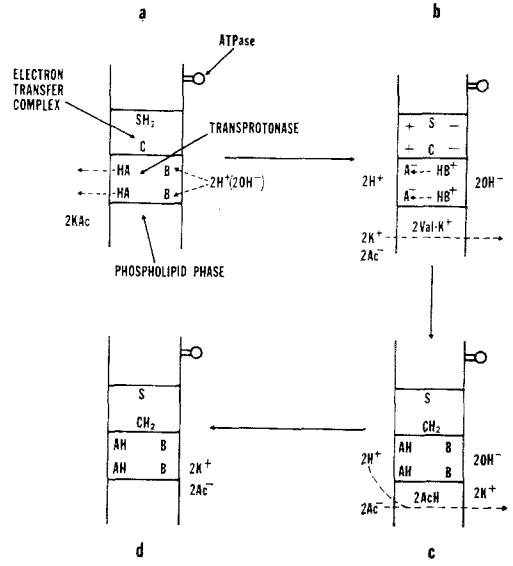

Figure 16. Depolarization of the metastable state induced by ion binding.

separated in the electron transfer complex, the ensuing electric field induces the non-electrogenic protonation and deprotonation reactions as shown in Fig. 16b leading to the generation of ΔpH. The transmembrane migration of protons can occur only when there is concomitant depolarization of the supermolecule. Valinomycin permits the rapid equilibration of K^+ in response to the proton movement. At the completion of the movement of protons across the inner membrane, two protons have left the matrix space in exchange for two potassium ions. The resulting ΔpH then drives the inward flow of acetate ion. The net result is that two molecules of K^+Ac^- are accumulated in each cycle of oxidoreduction involving the generalized substrate SH_2 and the generalized mobile electron carrier C. Our

formulation predicts a $K^+/2e^-$ ratio of two per coupling site. The experimentally measured $K^+/2e^-$ ratio appears to vary widely.[74] This variability of $K^+/2e^-$ ratio may be due in part to the non-ideal behaviour of the K^+-specific electrode and to the possible uncertainty about the rate of oxygen uptake owing to the slowness of response of the oxygen electrode. We predict that when due corrections are applied, the observed $K^+/2e^-$ ratio should approach the theoretical value of two.

In the absence of weak acid anions, the H^+/K^+ ratio should be about unity according to our formulation. The experimental data agree closely with this theoretical ratio (75).

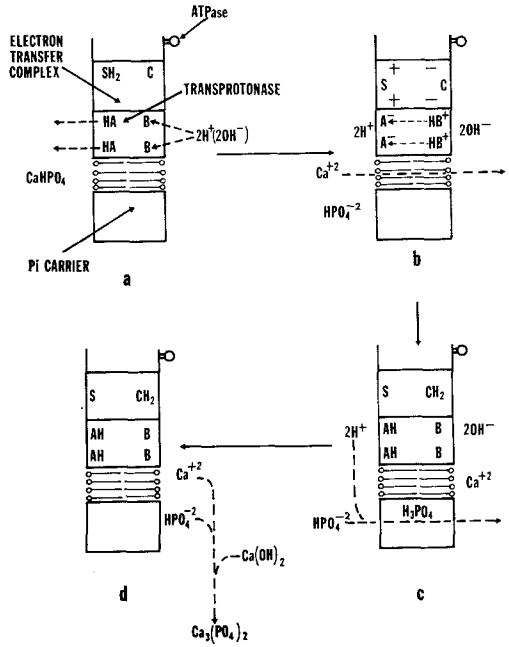

Figure 17. Generation of membrane potential by ATP hydrolysis.

The active transport of tricalcium phosphate $[Ca_3(PO_4)_2]$ can be accounted for in a similar manner as presented in Fig. 17. In this case, the calcium ion permeation is facilitated probably by an endogenous calcium ionophore while the permeation of inorganic phosphate is effectuated by the well-established phosphate carrier.[76,77] As developed in Fig. 17, charge separation in the electron transfer complex induces a ΔpH and polarizes the supermolecule. Concomitant with the depolarization of the supermolecule one calcium ion is translocated into the matrix space. If the influx of P_i driven by the pH gradient is slower than the rate of calcium ion uptake, the matrix space will be

maintained alkaline throughout the duration of active transport. When a sufficient concentration of Ca^{++} and P_i accumulate in the alkaline matrix space, a spontaneous reaction between $CaHPO_4$ and $Ca(OH)_2$ will take place to give the $Ca_3(PO_4)_2$ precipitate and water (see Fig. 17d). Again the $Ca^+/2e^-$ ratio per coupling site predicted by the present formulation is unity in contrast to some of the higher values reported in the literature.[78] It is quite probable that $Ca^+/2e^-$ ratios higher than one are due to the non-energized binding of Ca^{++} to mitochondrial membrane surfaces. In the absence of permeant anions, the present scheme predicts a value of 2 for the H^+/Ca^{++} ratio.

Montal, Chance and Lee (79) reported the interesting observation that submitochondrial particles can take up both H^+ and K^+ to the extent of about 10 nmoles per mg protein in an energy-linked fashion in the absence of any added ionophore. The direction in which protons flow is normal in the sense that it is the direction predicted by most of the available models of active transport (the chemiosmotic,[71] the conformational[72] and the EMC models). However, the potassium movement is abnormal because it is in the direction opposite to the direction determined by the H^+/K^+ exchange diffusion process. The EMC model suggests a simple explanation for this apparent dilemma. As described above, charge separation in the electron transfer complexes generates a ΔpH via the field-induced protonation–deprotonation reaction, leading to an acidification of the intravesicular aqueous phase, and an alkalinization of the extravesicular phase. A part of the intravesicular free protons can then exchange with external K^+ ions—a process mediated by transprotonase in the non-energized mode and the endogenous potassium ionophore. Due to the non-identity of the two phases involved, the number of protons released into the intravesicular phase would probably be less than the number of protons taken up by the external surface of the vesicle. If this were the case, the complete exchange of the internal protons for K^+ would lead to the apparently simultaneous uptake of H^+ and K^+. On the other hand, if the number of protons released and taken up are identical, incomplete replacement of internal protons with external potassium ions would account for the same result. The essence of the present explanation is that the abnormal direction of the K^+ ion movement is due to the fact that the K^+ movement is driven not by the electromechanochemical free energy of the polarized supermolecule but by the ΔpH alone.

By now there is ample evidence that various substrate anions permeate the inner membrane through specific carrier systems analogous to the phosphate carrier.[76,77] We have incorporated these carrier systems into our EMC model. There appears to be at least two general

carrier systems in the inner membrane—one for dicarboxylate anions and another for tricarboxylate anions as depicted in Fig. 18. It is to be noted that these carboxylate anions are assumed to pass through the respective carrier systems as un-ionized neutral species in either direction. That is, these carrier systems may be regarded as passive equilibrating systems for appropriate anions. There is no evidence that these anion carriers can be directly coupled to the primary energy source. That is to say, these carriers cannot serve as depolarizing structural components. Because of the non-electrogenic nature of the

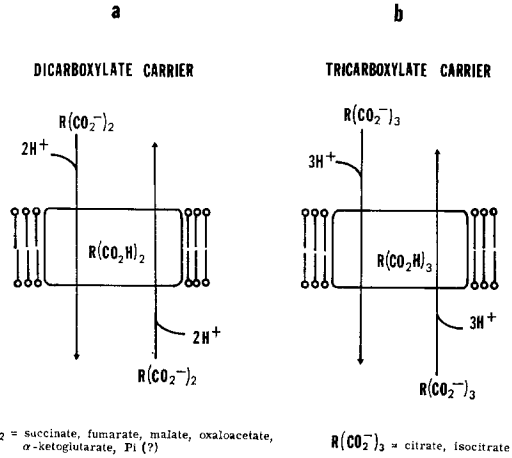

Figure 18. Generation of membrane potential by substrate oxidation.

translocation of these anions, the direction of the movement of these ions will be determined only by the electrochemical activities of H^+ and carboxylate anions. Consequently the transmembrane equilibration of substrate anions would be highly sensitive to the ΔpH across the inner membrane, and, conversely, the ΔpH would be influenced by the concentration gradient of these ions. As the result of the pH dependency of the transmembrane anion equilibration, various anion movements can be chemically linked.

A change in the electrochemical activity of any one of the numerous chemical species (i.e., H^+ and anions), therefore, would affect the equilibrating tendencies of all the other species. However, due to the kinetic parameters involved, it is conceivable that only a selected few of the ionic species would respond to a given electrochemical perturbation. This may underlie the phenomena of the numerous anion–anion antiports often reported in the literature.[80]

C. *Energized Transhydrogenation*

Mitochondrial transhydrogenase is an inner membrane system which during isolation is found to be associated with Complex I

(DPNH—Coenzyme Q reductase).[81] It may be considered as a basepiece of the inner membrane. Transhydrogenase catalyzes the transfer of the equivalent of a hydride ion from DPNH to TPN^+. Under energizing conditions, the equilibrium of the transhydrogenation reaction (9) shifts far to the right, the equilibrium constant for the reaction being approximately 500[82]:

$$DPNH + TPN^+ \rightleftharpoons DPN^+ + TPNH \qquad (9)$$

This phenomenon of energized transhydrogenation has been difficult to rationalize, since the redox couples DPN^+/DPNH and TPN^+/TPNH are known to be isoenergetic.[83] The energy transduction inherent in energized transhydrogenation represents yet another variation of mitochondrial coupling which any satisfactory general model of mitochondrial structure and function must rationalize. As we have indicated earlier, the electromechanochemical principle which satisfactorily rationalized the phenomena of oxidative phosphorylation and active transport also provides a useful mechanistic framework for accommodating the structural and biochemical data available on energized transhydrogenation.[84]

Let us first consider the components of the supermolecule involved in this work performance. The minimum requirement for the supermolecule implicated in transhydrogenation is met by a combination of transhydrogenase and one of the complexes of the electron transfer chain in the case of oxidoreductive transhydrogenation. This conclusion is based on the fact that decapitated submitochondrial particles are sufficient to catalyze energized transhydrogenation.[85] In ATP-dependent transhydrogenation, the supermolecule consists of the headpiece, the electron transfer chain and the transhydrogenase and we are assuming that these three entities are arranged as shown schematically in Fig. 19.

The transhydrogenase portion of the supermolecule is assumed to be constructed according to the Vanderkooi–Green membrane model (see Fig. 20[7,18,51]). The following postulates are required to account for the energized[86] as well as the nonenergized[17] transhydrogenation reaction within the framework of the electromechanochemical energy transduction principle: (1) there are two active sites per molecule of the transhydrogenase, one specific for the DPN^+/DPNH couple and the other specific for the TPN^+/TPNH couple; (2) the flavin coenzyme for the DPN^+/DPNH couple (F_D) is located in the intracristal tier of the DPN^+/DPNH couple (F_D) is located in the intracristal tier of the transprotonase and the flavin coenzyme for the TPN^+/TPNH couple (F_T) is located in the matrix tier of the transprotonase; (3) there exists a pair of globular proteins which are tightly associated with the F_D and F_T proteins and provide a proton transfer route accompanying the

hydride transfer between the coenzymes F_D and F_1 (see the right-hand tier of the quartet in Fig. 20); (4) the chemical reactions between the pyridine nucleotides and their respective flavin moieties are rapid and hence the rate-limiting step may involve either one or both of the two intramembrane charge migration processes—the hydride and the proton transfer reactions.

A possible mechanism for the energized transhydrogenation is presented in Fig. 20. In consequence of charge separation in the electron transfer complexes (see the encircled electrical signs in Fig. 20b), the transprotonase undergoes an electrical polarization via the hydride shift from DPNH to F_D and the binding of TPN^+ to F_T on the one hand and the intramembrane proton transfer from CO_2H to NH_2 on the other as indicated by the dotted arrows in Fig. 20a. The polarized supermolecule at this point can drive the energy-requiring hydride

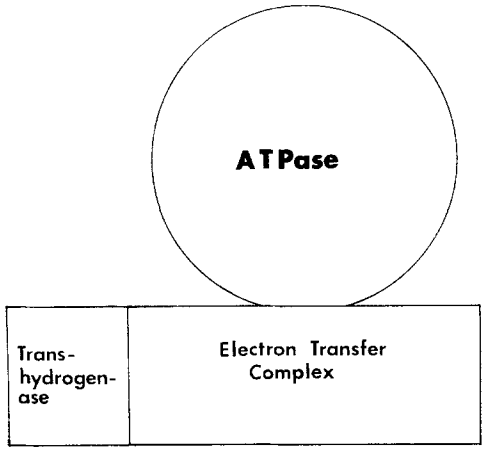

Figure 19. The supermolecule for energized transhydrogenase.

transfer from F_D to F_T. At the completion of this hydride transfer, the supermolecule becomes depolarized and TPNH and H^+ dissociate from the transhydrogenase leading to the state depicted in Fig. 20c. The net result is the conversion of the system DPNH + TPN^+ into the system DPN^+ + TPNH and the creation of ΔpH, acidic on the matrix side and alkaline on the intracristal side. The ATP hydrolysis reaction can achieve the same result because of the fact that the ATPase can induce the polarization of the electron transfer complexes. The predicted pH changes have been observed experimentally by Mitchell and Moyle.[87]

Under non-energizing conditions, the transhydrogenase acts independently of the electron transfer complexes and equilibrates H^- and H^+ across the enzyme system in response to the thermodynamic

driving force imposed by Reaction (9). If the rate of the proton migration through the transprotonase is slower than the rate of the hydride migration, the non-energized transhydrogenation will lead to the intramembrane separation of H^+ and H^- and to a membrane potential —minus on the matrix side and plus on the intracristal side when the concentration of (DPNH + TPN^+) is greater than the concentration of (DPN^+ + TPNH), and the opposite polarity (i.e., plus on the matrix side and minus on the intracristal side) when the relative magnitude of the concentrations are reversed.

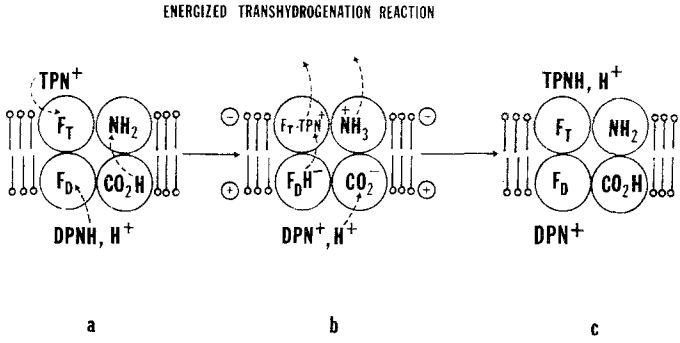

Figure 20. Postulated macromolecular structure of mitochondrial transhydrogenase.

Other Extensions of the Model

We have deliberately excluded from the present communication consideration of some mitochondrial phenomena not because the model is incapable of rationalizing these phenomena, but rather because there are limits to what can be developed satisfactorily in a single communication. Elsewhere we intend to consider pseudo-energized swelling and energized contraction. There are also a variety of observations which need explaining and which were not considered in the present paper. How the solubilization of succinic and DPNH dehydrogenases can be fitted into the framework of the octet principle; how the enzymes which carry out the citric and fatty acid cycles are integrated within the matrix system; how charged molecules like P_i, ADP and ATP penetrate the mitochondrial membranes; and finally how our postulated localization of the electron transfer chain on the matrix side of the inner membrane can be rationalized with the inaccessibility of the membrane on the matrix side to cytochrome *c*. None of these phenomena have posed insuperable problems for the model as we shall develop elsewhere.

Acknowledgements

In the development of the electromechanochemical model of energy transduction, we have drawn heavily from the insights of others. To Peter Mitchell we owe our first appreciation of the fundamental concept that the membrane is the instrument of transduction—a concept which is the foundation stone of our own approach. To V. P. Skulachev we are indebted for his incisive proof that an electric potential is generated when mitochondria are energized, and to H. T. Witt we are equally indebted for his important demonstration that the energized state of chloroplasts is characterized by an electric potential. To Lars Ernster we are indebted for his clarification of the localization of the citric cycle enzymes in the matrix space and for his discovery of energized transhydrogenation as a third coupling capability of energized mitochondria. A. L. Lehninger has for many years championed and developed the mechanochemical theme of energy transduction and his views have greatly influenced our own thinking about transduction mechanisms. The pioneer and systematic studies of E. Racker and his group on the identification of the headpiece as the site of ATPase activity have opened for us the door to the exploration of the ATPase coupling unit. The studies of W. Stein on the structure of glucose permease and of R. E. Dickerson on the x-ray crystallography of cytochrome *c* provided our introduction to the internal structure of multimeric and monomeric proteins. Several of our present and former colleagues have made independent and crucial contributions to the thinking which underlies our present model. E. F. Korman and J. McLick introduced us to the possibility of the synthesis of ATP by the direct union of ADP and P_i given the necessary conformational changes in the ATPase coupling unit. John Young was our guide to the notion of the metastable energized state and to the central role of the electric potential in active transport. Among the experimentalists in our laboratory, the work of George Blondin on mitochondrial ionophores and on the role of ion binding in active transport, the studies of Rod Capaldi and Jennie Smoly on the chemical capabilities of the matrix system, the resolution by Alan Senior of the headpiece into its component proteins, the pioneering contributions of A. Tzagoloff, D. MacLennan and K. Kopaczyk to the studies of the oligomycin-sensitive ATPase, the discovery by G. Vanderkooi of the principle of membrane construction, the superb electron microscopic work of J. Asai and T. Wakabayashi, the configurational studies of R. Harris, D. Allmann, O. Hatase and J. Penniston, the brilliant resolution of the electron transfer chain into the four complexes by Y. Hatefi, and the systematic study of the active transport of divalent metal ions and inorganic phosphate by G. Brierley, all have provided much of the experimental foundations for the structural framework of the electromechanochemical model. We acknowledge with gratitude the advice given to us over the many years by Harold Baum. It was primarily the work of his group which changed the course of our thinking about the mechanism of energy transduction.

Finally we are deeply indebted to Dr. H. Yu, Department of Chemistry and Dr. L. Shohet, Department of Electrical Engineering, both of the University of Wisconsin in Madison, for their advice in matters relating to the effect of the electric field on macromolecular systems.

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