REDOX POTENTIALS OF THE CHARGE-PAIR MODEL OF BIOENERGETICS

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ABSTRACT

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By

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The theory of redox potentials in the charge-pair model of bioenergetics is developed for two specific versions, the original model and a new model. In both models the midpoint potentials are found to depend upon the phosphate potential, uncoupler concentration, and upon any other ion which may be coupled to the electron transfer process. The phosphate potential dependence of the original pair model is found to differ from experimental data, while the new pair model is generally, but not completely, in agreement. The new pair model also incorporates the "proton in the membrane" concept of Williams. The implications of these results for the pair theory and bioenergetics are discussed, and it is shown that features of both the reverse electron transport and the two cytochrome theories are incorporated in the pair model.

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I. INTRODUCTION

A. Statement of the Problem

For all of its complicated mechanisms and unknown details, the central problem of mitochondrial bioenergetics can be stated quite simply. In the mitochondrion the following two chemical reactions occur:

$$\text{I.A.1} \quad SH_2 + \tfrac{1}{2}O_2 = S + H_2O$$

$$\text{I.A.2} \quad Pi + ADP = ATP + H_2O$$

where SH_2 is a substrate, such as succinate. These two reactions are coupled together in a process termed oxidative phosphorylation, resulting in the transfer of energy from substrate to ATP (33). The central problems of bioenergetics is to explain how these two reactions are coupled together.

The object of this thesis is to apply one coupling theory, the paired moving charge theory, to the measurement of the midpoint potentials of certain electron carriers, and relate the results to the experimental data concerning shifts in these potentials. A secondary objective is to discuss these data in terms of other theories, and evaluate the relative merits of these theories. To do this, we must first discuss some background material

and set a frame of reference for the later discussions.

B. Introduction to Mitochondria

Mitochondria are located in the cytoplasm of aerobic eucaryotic cells. They have two membranes, referred to as the "inner" and "outer" membranes. The outer membrane encloses the organelle, while the inner membrane contains many inward folds called cristae. The material inside the inner membrane is called the matrix (23,33).

Located along the inner membrane are the electron transfer chains, which contain the electron carriers. Each chain is a complete unit, containing all the material needed to transfer electrons from substrate to oxygen. The electron carriers in the chain include flavin-linked dehydrogenases, which remove the electrons from the substrate and transfer them to some as yet unknown carrier; cytochromes, containing iron as part of a heme group; non-heme iron proteins, containing iron but not as part of a heme; and copper proteins(33).

On the inner surface of the inner membrane is a structure known as the F1 complex. It is the principal ATP-synthetase of the mitochondrion. As the electron flows through the electron transfer chain, the F1 complex is activated, and ATP is produced. Although the evidence is in some doubt, it appears that the F1 complex is also involved in ATP hydrolysis (23,33,53).

C. Introduction to Electron Transfer Chains

The term "electron transfer chain" dates back to the 20's, when it was believed there was a simple linear order to the sequence of oxidations and reductions (33,53). Today, it appears more probable that the electron-transferring molecules are arranged in four complexes, which facilitate the transfer of electrons from sources of low redox potential to sinks of high potential; the energy difference being transferred to the F1 complex (22,53,80). Thus, rather than the picture of a simple assembly line, with electrons as an input, H_2O an output, and ATP produced somewhere in between, we have a picture of four machines, with electrons as one of their raw materials, and energization of the F1 complex as the main product of three of them.

Complex I transfers electrons from NADH to ubiquinone, energizing F1 in the process. It contains FMN and a nonheme iron, among other electron carriers. Complex II transfers electrons from succinate to ubiquinone, but does not energize F1. It contains FAD and a nonheme iron, and probably other electron carriers. The next two complexes combine to transfer electrons from ubiquinone to oxygen, each energizing F1. Complex III contains the b and c cytochromes, and a non-heme iron. Complex IV is linked to complex III by a c cytochrome, and contains the a cytochromes and several non-heme coppers (33).

D. Introduction to Uncouplers

Before we discuss theories of energy coupling we will mention a few facts about uncoupling. Certain chemicals, such as 2,4 dinitrophenol, or FCCP, completely block ATP synthesis while enhancing electron transfer. This effect is called uncoupling. Other chemicals, such as oligomycin, block both phosphorylation and electron transport and are known as inhibitors. Still other drugs block only electron transport within a complex, such as rotenone in complex I, antimycin in III, and cyanide in IV (24, 52).

Until such time as a definite theory of energy coupling is known, uncoupling will necessarily remain a mystery. In the mean time, we can discuss mechanisms for the dissipation of energy, and temporarily ignore how this dissipation is mechanistically related to energy coupling. As might be expected, the nature of this dissipation is controversial.

Three models have been proposed to explain uncoupling. The oldest, inspired by the chemical theory, is that the uncoupler reacts with the chemical intermediate, appropriating the high-energy bond which was intended for ATP (9). Later, the chemiosmotic theory inspired a model in which the uncoupler collapses the transmembrane proton gradient, dissipating any energy stored therein (42). In yet another model the energy is converted into mechanical energy by the transport of the uncoupler across the membrane (3,25,61).

The evidence, both for and against these models, is indecisive. The strongest evidence supports the chemiosmotic model, and consists of the fact that the measured proton gradient is in fact reduced by uncoupling agents (24, 58). Furthermore, the correlation between a drug's effectiveness in increasing conductivity in artificial membranes, presumably by increased proton conduction, and in uncoupling, is high (21, 60).

However, it is not certain that these events are the primary step, rather than a secondary side effect, in the uncoupling process. Wilson has observed that the pH for optimum uncoupling does not coincide with the pH for optimum conductivity in artificial membranes, uncouples submitochondrial particles (24).

The chemical model explains these proton effects in terms of a "proton pump" driven by the high-energy intermediate (68). The uncoupler is postulated to react with this intermediate, thus shifting the equilibrium of the pump reactions. If this is true, then the pump reactions should be titratable, and indeed a titration effect has been found (40). Following up on this, Nicholls and Wenner have shown that this effect can be derived from a general kinetic analysis of uncoupling, which is independent of any specific mechanism (45). Consequently, nothing definite can be concluded.

Some studies have shown that complete uncoupling occurs at concentrations of less than one uncoupler molecule per

phosphorylation site (40, 59). This suggests that the transport model is incorrect. However, this argument overlooks the possibility that one uncoupler molecule may be transported through more than one phosphorylation site (4). We will return to this point in our discussion of the pair model.

E. Introduction to Energy Coupling Theories

Considering that the exact mechanism of energy coupling is unknown, there is a surprising amount known about oxidative phosphorylation, enough to put some severe constraints on possible theories. We can see what these constraints are by looking again at the overall reaction:

$$\text{I.E.1} \quad Pi + ADP = ATP + H_2O$$

In solution, at physiological pH's, this reaction would have the form:

$$\text{I.E.2} \quad Pi^{-} + ADP^{---} = ATP^{----} + H_2O$$

However, species such as ATP^{----} cannot exist in membranes; the species must be protonated (29). Since these protons are attached to oxygen, we can represent this symbolically by writing Pi-OH for Pi, and ADP-HO for ADP, where the H is the proton gained or lost in the reaction. For simplicity however, we will normally write just Pi, ADP, and ATP when there is no danger of confusion.

1. Nucleotide Transport

The fact that the form of the reactants in the mem-

brane must be different from their form in solution should alert us to the fact that there is a complicated mechanism involved in transporting the chemicals to the F1 complex. Two transport systems are known; one transports Pi into the F1 complex and the other exchanges an internal ATP for an external ADP. This means that reactant concentrations at the reaction site are carrier limited, rather than being diffusion limited as in typical chemical reactions. An exact model consequently will have the phosphorylation reaction dependent upon the carrier systems. However, we can assume as a first approximation that the carrier mechanisms and the phosphorylation mechanism are independent (13, 63, 64). Since the same substances are involved in both mechanisms, this may appear to be a drastic assumption. Its usefulness, however, has been well tested; this assumption has been made in virtually all energy coupling theories proposed to date (13).

The picture we shall use of these two systems, then, is the following. External nucleotides diffuse through the outer membrane of the mitochondrion. Next, the carrier systems transport the nucleotides into the matrix space. Finally, they diffuse to the F1 complex, where the phosphorylation reaction occurs.

While the mechanism of respiratory control is unknown, we do know some of the parameters involved. The most important one is the ADP concentration. When the ADP concentration is low, the respiratory rate is also

very low. Adding ADP causes the rate to increase, until all the ADP has been phosphorylated. Low ADP concentrations are referred to as State 4 mitochondria, while actively phosphorylating mitochondria are said to be in State 3, from a terminology proposed by Chance (9).

Besides ADP, ATP and Pi are also involved in respiratory control, although they are of lesser importance, and their role is not as easily described. It is convenient to have a single statistic relating these three terms, and the statistic commonly used is the term

$$\text{I.E.3} \quad P = (ATP)/(Pi)\cdot(ADP)$$

P is commonly called the "Phosphate potential", a bit of a misnomer (81), since it is actually only the logarithmic term in a possible potential expression, such as either the overall chemical potential of eq. I.E.1

$$\text{I.E.4} \quad \mu_{ATP} - \mu_{Pi} - \mu_{ADP} = \Delta G_p = \Delta G^{\circ}_p + RT \ln P$$

or the electrical potential in a coupled redox reaction.

The important point is to observe that unless the Pi and the ATP/ADP transport systems are in thermodynamic equilibrium with the F1 complex and the outside solution, the phosphate potential of the reaction environment and the external solution will not be identical. Since there is no <u>a priori</u> reason to assume that they are equal, and since some experimental evidence suggests that they are not equal (17, 56), any theoretical predictions based upon phosphate concentrations at the F1 complex cannot be expected to agree exactly with experimental measurements

made in solution.

2. Exchange Reactions

There is also a fair amount known about the intermediate steps in the phophorylation reaction. Isotope studies have shown that the following exchange reactions take place in the mitochondrion, where the labeled species have been marked with an asterisk:

I.E.5 $ATP + HO^*H = ADP + PO^*H$

I.E.6 $ADP\text{-}P + Pi^* = ADP\text{-}P + Pi$

I.E.7 $A^*DP + ADP\text{-}P = A^*DP\text{-}P + ADP$

I.E.8 $ADP\text{-}OP + H_2O^* = ADP\text{-}O^*P + H_2O$

I.E.9 $P\text{-}O^*H + HOH = P\text{-}OH + HO^*H$

The fact that these reactions are blocked by uncouplers or inhibitors is usually taken to mean that they are directly involved in the phosphorylation process (7, 31, 33). It will be seen that matters are actually a little more complicated than this.

Eq. I.E.5 indicates that the oxygen bridge between the last two phosphates in ATP comes from ADP and not from Pi. Eq. I.E.6 and I.E.7 are the individual steps in the overall reaction I.E.5. Eq. I.E.8 and I.E.9 are not as easily explained; they are less sensitive to uncouplers than I.E.5 and their kinetics are faster (7). Consequently they cannot be justified by simple reversal of oxidative phosphorylation; they must be explained

in terms of specific, rapid, intermediate steps in the overall reaction scheme. Thus, they can be used to check particular coupling models for completeness.

Eq. I.E.1 is written in an energetically unfavorable direction. We have explained that it is able to proceed as written because energy from the electron transfer chain drives it forward. The stoichiometry introduces another constraint upon possible energy coupling models, for the number of electrons transferred through a complex per ATP molecule synthesized is 2 and not 1. To further complicate matters, the electron carriers in the electron transfer chain all appear to transfer one electron at a time. Thus, in a given complex, the electron transfer step which energizes the F1 complex must either involve two carriers acting simultaneously, or, if only one carrier is involved, it must energize the F1 complex in $\frac{1}{2}$ step increments (23, 33). This $2e^-$/ATP ratio will be seen to be a most confounding fact.

3. Chemical Theory

Theories of coupling can be divided into two classes, chemical and non-chemical. Historically, the chemical theory came first, others being proposed as its shortcomings appeared. Nevertheless, the chemical theory has remained popular, possibly because its logical structure cannot be refuted by any empirical observation (1). This will become more apparent later on.

Lipmann, in 1946 (38) appears to have been the first to formulate the chemical theory. The names Slater, Chance, and Lehninger are the most prominent among those who have adopted it. The gist of the chemical hypothesis is that in the respiratory chain a "high energy" chemical intermediate is formed. This intermediate is the key ingredient in ATP production.

Before describing the chemical hypothesis we must describe some symbolism which we will use throughout this thesis. The usual way to write oxidized and reduced states of a chemical species A is by

$$A^{ox} \quad \& \quad A^{red}$$

or, if ionized, by their valence:

$$A^{+a} \quad \& \quad A^{+(a-1)}$$

The latter notation is suitable only for ions. In addition, neither notation provides the information really desired, namely, the electron balance. Therefore, we introduce the following notation for an oxidized and a reduced species:

$$A() \quad \& \quad A(e)$$

We can then write a reaction scheme for the chemical theory, exactly as it appears in a famous biochemistry text (33).

$$\text{I.E.10} \quad A(e) + I + B() = A()\text{-}I + B(e)$$

$$\text{I.E.11} \quad A()\text{-}I + E = A() + E\sim I$$

$$\text{I.E.12} \quad E\sim I + Pi = I + E\sim Pi$$

$$\text{I.E.13} \quad E\sim Pi + ADP = E + ATP$$

Here A & B are the relevant electron carriers, I is an intermediate, and E is the F1 ATPase.

This sample scheme illustrates the typical problems encountered in reconciling the partial reactions. The scheme, as written, indicates a stoichiometry of $1e^-$/ATP rather than 2. This means that eq. I.E.10 must be replaced by something like

I.E.14 $2A(e) + I + 2B() = 2A()-I + 2B()$

Unfortunately, this is a very unlikely reaction, as the A's are fixed in the membrane, and it is very improbable that they are precisely arranged so that one I can simultaneously bind with both A's. Consequently, a better try at improving eq. I.E.10 is:

I.E.15 $2A(e) + I + 2B() = 2A() + 2B(e) + I\sim$

which ignores the detailed mechanism, but still allows for some reasonable possibilities. Eq. I.E.11 must now be changed to

I.E.16 $I\sim + E = E\sim I$

and the rest can remain unchanged.

Now that we have the electrons balanced we can try to get the water exchanges correct. Eq. I.E.12 & I.E.13 are not written as involving protons. This is because in hydrolysis reactions in aqueous solution, H_2O as a reactant or product is often ignored, since the amount of water present does not affect the final equilibrium. The correct way to write I.E.12 and I.E.13 is:

I.E.17 $E-I + PiO^- + H^+ = I + E-POH$

I.E.18 $E-POH + ADPOH = E + ATP + H_2O$

I.E.9 may now be explained as the reversal of I.E.17 and I.E.18. Its relative sensitivity to uncouplers can be explained by postulating E-I or I to be the species affected by uncouplers. Its rapid kinetics can be explained by postulating I.E.15 or I.E.16 to be the rate determining step for oxidative phosphorylation.

No combination of these steps, however, can rationalize I.E.8. Comparison with I.E.5, the overall phosphorylation reaction, indicates that in I.E.8 the oxygen atom becomes attached to the wrong molecule, ADP instead of Pi. Consequently, I.E.8 cannot lie on the "main path" of oxidative phosphorylation; it must represent a side reaction (6, 31). Its significance for the reaction mechanism is that a proposed mechanism must allow for this side reaction.

The reason for developing the chemical theory the hard way was to show the importance of precisely defining the steps in an energy coupling scheme. Most of our arguments can be applied to theories other than the chemical. And having explored the pitfalls, we can survey other theories with more confidence.

The evidence for the chemical theory is indirect. First, this sort of mechanism is used for other biochemical phosphorylations. Second, the theory provides a reference frame which has led to the identification of other compounds and reactions, all of which are consistent with this model (31). The evidence against the chem-

ical theory is also indirect. In 30 years I has yet to be isolated, and every proposed candidate has proven to be something else.

4. Chemiosmotic Theory

The first serious challenger to the chemical hypothesis was the chemiosmotic hypothesis of Mitchell (41). Mitchell noted that isolated cytochromes cannot phosphorylate; indeed, an intact membrane appears to be necessary. He also noted that the overall oxidative phosphorylation stoichiometry indicates an overall transfer of protons. If the reaction components are suitably arranged in the membrane a proton gradient will result. Mitchell postulated that this gradient is responsible for oxidative phosphorylation.

Let us take another look at the endergonic component of oxidative phosphorylation:

I.E.1 $ADP + Pi = ATP + H_2O$

Obviously, if the H_2O concentration can be made small enough, the reaction will tend towards ATP production. Just as obviously, this is no mean feat in an aqueous environment. Mitchell has explained how the proton gradient could accomplish this task.

To begin with, Mitchell postulated that the active site of the F1 complex is hydrophobic. Without water present the equilibrium will drive the reaction into ATP production. Almost immediately, however, the water pro-

FIGURE 1

Diagram of the original chemiosmotic hypothesis, with the electron transport chain producing a proton gradient, and the phosphorylation reaction using this gradient as a source of energy.

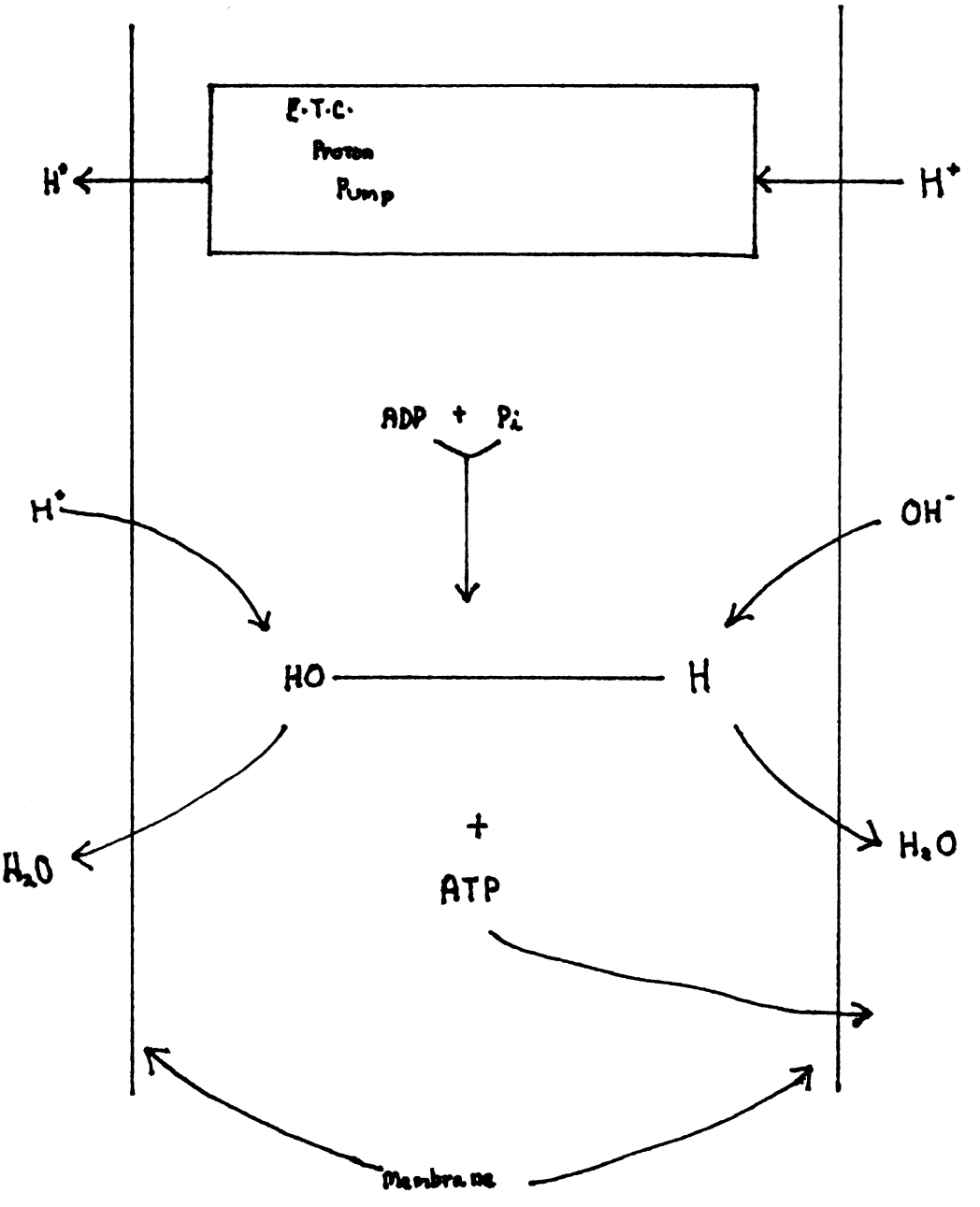


Figure 1

duced would stop the reaction, unless there is a mechanism for getting rid of it. This is where the proton gradient comes in.

The proton gradient will tend to drive protons across the membrane. The membrane is postulated to be so constructed that these protons pass near the active site, with the protons abstracting an OH^- from the water molecule (Figure 1). An OH^- from the other side of the membrane takes care of the rest of the water molecule. The water molecule is thus removed from the active site, which can now produce more ATP (34).

This, at least, was the original formulation. The novelty of the approach led to attempts to see if respiration did in fact produce a proton gradient. The result was that two protons are transferred per ATP, instead of the single proton which Fig. 1 predicts (43). Mitchell got the stoichiometry correct by introducing a new scheme, Fig. 2, where an intermediate molecule I has been introduced. I binds to another molecule X, and together they transport two protons and one oxygen molecule across the membrane. X and I formally play the same role in the chemiosmotic theory which the high-energy intermediate plays in the chemical theory, meaning that an experimental result in one theory can be immediately translated into the other (1).

There have been several other suggestions advanced to

FIGURE 2

Diagram of the revised chemiosmotic hypothesis.

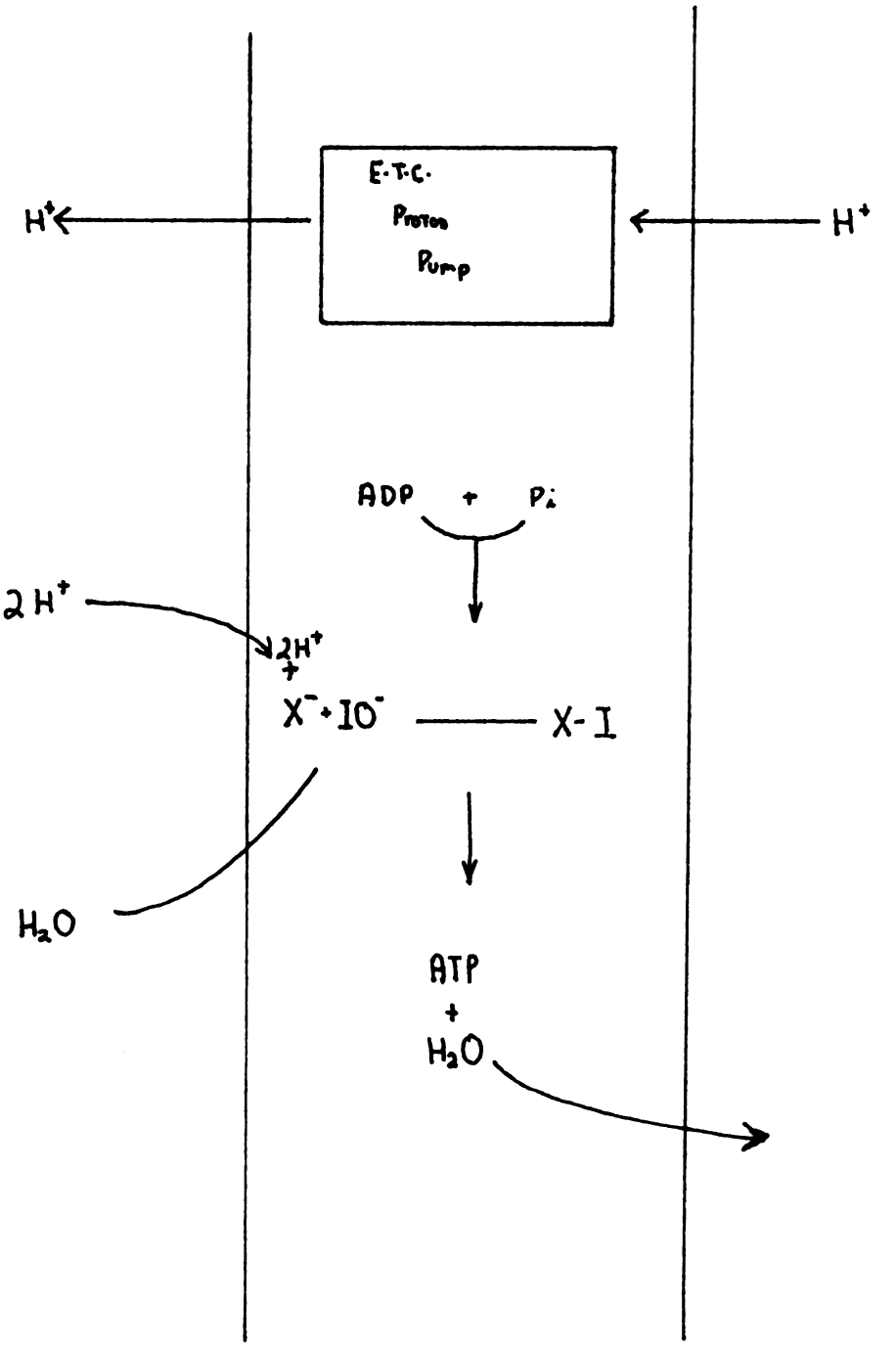


Figure 2

explain how a proton gradient may form ATP and H_2O from Pi and ADP. One suggestion is that two protons attack Pi^- to form species of the form POH_2^+, which then attack $ADPO^-$ (42). This is the only suggestion we are aware of which specifically incorporates the exchange reactions. Another, less specific proposal, is that a transmembrane potential somehow is involved; the sum of the energy in the proton gradient is often lumped together with the electrical energy and called the "proton motive force" (58).

Although the chemiosmotic theory has replaced the chemical theory as the favored theory among bioenergeticists, there has been enough evidence collected to indicate that the chemiosmotic hypothesis is not entirely correct. Kinnally and Tedeschi have estimated the proton motive force available under certain experimental conditions and concluded that it is not sufficient to drive the observed phosphorylation (30). And Good et. al. have done kinetic studies on chloroplasts and found that one obtains phosphorylation before the chloroplast has had time to build up a proton gradient (47).

5. Proton in the Membrane

Related to the chemiosmotic theory, and preceding it chronologically, is the "proton in the membrane" theory of Williams (72-74). Like the other theories, it has never been specified enough to be put in more than qualitative terms, but in those terms it is simple enough.

The theory is that any protons transferred by the electron transfer chain function by first accumulating in the F1 complex, where they form a highly acidic environment. Then the reaction I.E.1 can proceed easily, since it is well known that ester hydrolysis is catalyzed by acids, although for ATP the specific mechanism is uncertain (72).

6. Simple Pair Model

The pair theory originated with the observation that charges can exist in a membrane only when paired to an opposite charge (29). Denoting an electron by e^-, this means that we cannot talk about e^- by itself, but only as part of an e^--p^+ pair, where p^+ is a positive charge (Figure 3A). We can then diagram the transfer of an electron between two hypothetical cytochromes, X and Y, by Figure 3B.

The pairing is primarily electrostatic in nature, rather than being a chemical bond. As the pair is transferred, we postulate that the electron e^- passes from a region of low dielectric constant to one of high dielectric constant, with p^+ doing the opposite. The dielectric constant is, of course, a convenient macroscopic parameter of the internal membrane structure; an average over the short and long range interactions of the membrane with the pair.

Under these assumptions, the electrostatic energy of the pair resides mainly with the electron before the crossing and with the positive charge afterwards. In this man-

FIGURES 3A & 3B

Diagram of the basic features of the pair theory. X and Y are two hypothetical cytochromes. A) The pairing of two charges associated with a cytochrome. B) Transfer of the pair to a second cytochrome, with energy transfer.

/// region of low dielectric constant.

region of high dielectric constant.

ner energy may be transferred from the electron to the positive charge without having to postulate high energy intermediates (28).

Before moving on, let us compare the four theories to see what distinguishes them from one another. In the chemical theory, energy is stored in chemical bonds, and if the membrane-bound enzymes could be extracted, the reactions could proceed as well in solution as in the mitochondrion. In the chemiosmotic theory energy is stored in concentration gradients. The membrane is essential, but only to maintain the gradient. William's theory has the energy stored in local concentrations. The membrane is used to contain the concentration, much as a reaction vessel does. The pair theory has the energy stored in pairs, with the membrane being used to direct the motion of these pairs.

II. MITOCHONDRIAL MIDPOINT POTENTIALS

A. Introduction

While the ADP concentration is known to be a controlling element in respiratory control, the molecular mechanism remains a mystery. Late in the '60s several groups suggested that control was achieved through electron carriers in the chain changing their midpoint potentials (11, 15, 65, 66). Britton Chance's research group then attempted to measure the relationship between redox potentials and respiratory control. They discovered that the midpoint potentials of certain cytochromes were affected by ATP (10).

B. Experimental techniques

The measurement of midpoint potentials involves a number of steps, but is fairly straightforward (8, 20, 78, 80). First, the intact mitochondria, or submitochondrial particles, are extracted. Next, they are suspended in a suitable medium, buffered to maintain the pH, and placed in a reaction vessel designed for simultaneous measurement of spectra and potentials. To control the reaction rate, the measurements are usually performed anaerobically. To establish anaerobic conditions oxygen is removed by bubbling in agron. Finally, the appropriate redox mediators are added, and the

system is ready for measuring.

The concentrations of the oxidized and reduced species are measured spectroscopically. The redox potential is followed by inserting a platinum electrode and measuring its potential relative to a calomel electrode with a high impedance potentiometer.

It happens that, because the actural electron carrier is a metal atom embedded in a cytochrome which is itself embedded in a membrane, the electrode does not react with the cytochromes. Thus, the potential that is actually measured is that of a mediator molecule M, which is capable of reacting with both (12).

$$\begin{array}{ll} \text{at electrode:} & \mathrm{M(e)} \rightleftharpoons \mathrm{M()} + \mathrm{e^-} \\ \text{at cytochrome:} & \mathrm{A(e)} + \mathrm{M()} \rightleftharpoons \mathrm{A()} + \mathrm{M(e)} \\ \hline \text{net reaction:} & \mathrm{A(e)} \rightleftharpoons \mathrm{A()} + \mathrm{e^-} \end{array}$$

This will theoretically give the correct result in all cases. However, a check of the equilibrium relationship:

$$\mathrm{A()\cdot M(e)/A(e)\cdot M()} = K$$

or

$$\mathrm{M()/M(e)} = (1/K)\cdot(\mathrm{A()/A(e)})$$

reveals that when M is at its midpoint, A will be far from its midpoint unless K is close to 1, implying that the midpoint potentials are equal. If A is far from its midpoint, then the ratio A()/A(e) is either much less than, or much greater than 1; which implies that either A() or A(e) is

very small. The measurement of these small quantities is difficult and open to a number of experimental artifacts. This means that, in practice, the mediator must be close to the cytochrome in midpoint potential for accurate results (12).

C. Summary of Experimental Observations

1. Phosphate Potential Induced Shift

Figure 4 shows the experimentally observed redox behavior of cytochromes $\underline{b}_t$ and $\underline{a}_3$. Notice that increasing the so-called "phosphate potential", (ATP)/(ADP)·(Pi), of the system results in a shift in the observed midpoint potential V^o, and that the direction of this chift can be positive or negative depending on the cytochrome in question. The magnitude of the shift varies as $\pm$ $\ln P^{\mp}$ with a slope of from 40 to 60 mV/phosphate potential unit.

The direction of the shifts has not received the attention which it deserves. The midpoint potential is related to the standard free energy by the equation:

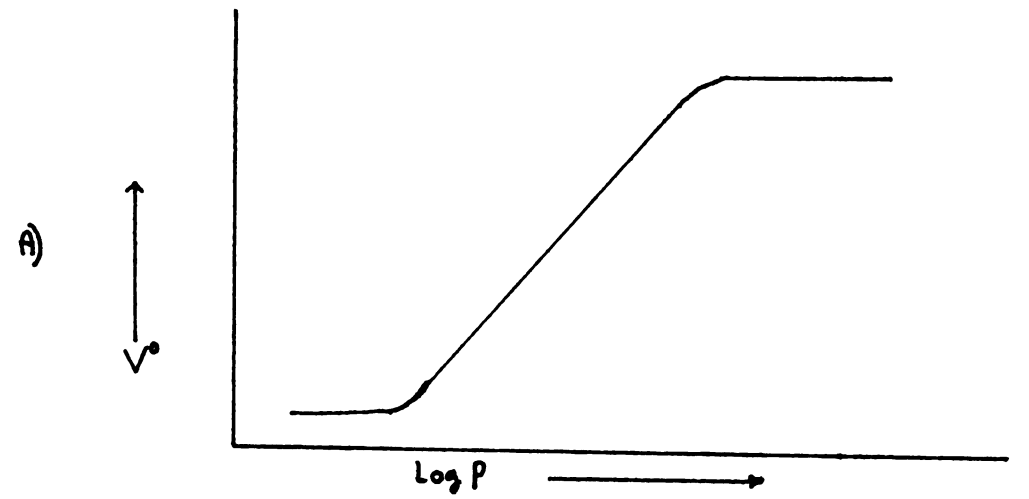
$$\text{II.C.1} \quad \Delta G^o = -nFV^o$$

Thus, increasing the midpoint potential of a redox system is equivalent to decreasing the standard free energy of the system. At the midpoint, the standard free energy is the free energy. Naively applying this to cytochrome $\underline{b}_t$ implies that adding ATP dissipates free energy from cytochrome $\underline{b}_t$.

Since this is unacceptable, we are forced to conclude

FIGURE 4

Relationship between the midpoint potentials of cytochromes $\underline{b}_t$ and $\underline{a}_3$, and the phosphate potential. a) cytochrome $\underline{b}_t$. b) cytochrome $\underline{a}_3$. (After 75).



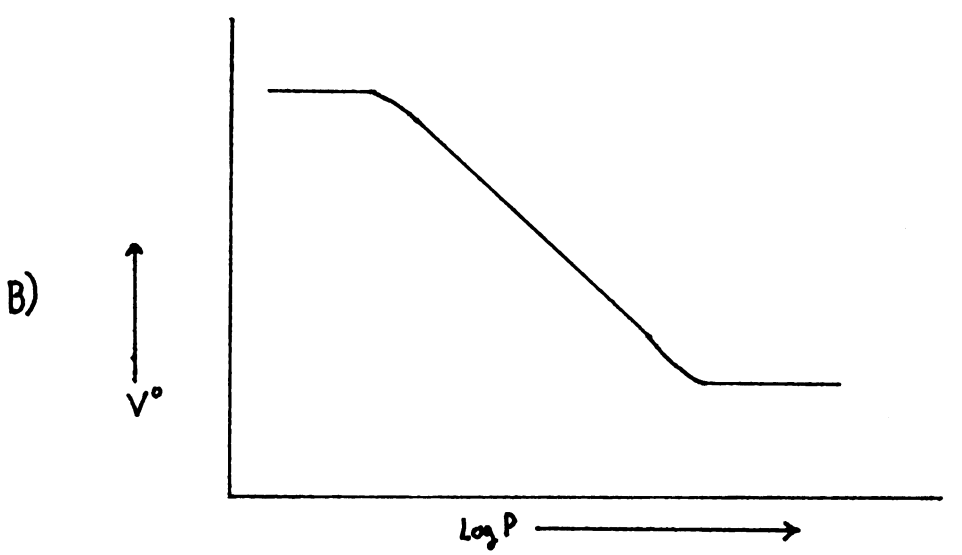


Figure 4

that the midpoint potentials as they are measured are not simple functions of the "true" standard free energies of the cytochromes. While none of the theories put forth to explain the shifts state this point explicitly, it is recognized implicitly. One group of theories suggest that there is a second form of the cytochrome present, with a different midpoint potential. This second form is indistinguishable spectroscopically from the usual form, but measureable electrically. In this theory, some average of the two froms is measured electrically, with ATP causing the ratio of the two forms to vary (16, 54, 55, 57, 79). Another theory suggests that one does not measure cytochrome $\underline{b}_t$ or $\underline{a}_3$ at all electrically, but only cytochrome $\underline{c}$ (32, 67). Adding ATP to the system will then result in reversed electron transport according to the equations:

II.C.2 $\underline{b}_t^{red} + \underline{c}^{ox} + ADP + Pi = \underline{b}_t^{ox} + \underline{c}^{red} + ATP$

and

II.C.3 $\underline{c}^{red} + \underline{a}_3^{ox} + ADP + Pi = \underline{c}^{ox} + \underline{a}_3^{red} + ATP$

respectively. The midpoint potential calculated by mistakenly applying the Nernst equation to the measurements is off by essentially $\pm$ ℓn P, the positive sign applying to cytochrome $\underline{b}_t$ and the negative sign to cytochrome $\underline{a}_3$.

In summary, we will mention what we feel to be the strongest points made by either side. The two cytochrome theory has argued that if reverse electron transport is the cause of the shifts, then all electron carriers be-

tween cytochromes $\underline{b}_t$ and $\underline{c}$ on the one hand, and between cytochromes $\underline{a}_3$ and $\underline{c}$ on the other hand, should show the same shift (35). In return, the reverse electron transfer advocates argue that it is an amazing coincidence that the signs of the shifts should be exactly that predicted by their theory (32).

2. Uncoupler Effects

Bohme and Cramer (5) have reported that uncouplers lower the midpoint potential of plant mitochondria cytochrome $\underline{b}_6$. The data of Wilson and Dutton (76) for cytochrome $\underline{b}_t$ also show this effect, although they prefer to interpret it differently. The data of the $\underline{a}$ cytochromes are ambiguous (49, 71, 77).

Bohme and Cramer (5) explain their uncoupler effect as resulting from a change in the local dielectric constant, this change being induced by the binding of the uncoupler. This leaves the phosphate potential shifts unexplained. Similarly, the phosphate potential theories make no effort to explain uncoupler shifts, instead regarding an uncoupler as a drug which sets the phosphate potential equal to zero. In short, no theory fully explains all the data. Using the pair model, we shall now attempt to explain both effects.

III. OVERVIEW OF THE ANSWERS TO THE PROBLEM

If all chemical species are in equilibrium with one another we may describe the system by classical thermodynamics. For a perfect gas mixture, the chemical potential of a species i is given by (14):

$$\text{III.1} \quad \mu_i = \mu_i^\circ + RT \log (i).$$

where: μ_i = chemical potential of species i

μ_i° = standard chemical potential of species i, a constant depending only of how the standard state is defined.

R = gas constant

T = Absolute temperature

(i) = concentration of species i (we will often drop the bracket notation when it is clear that concentrations are meant.)

If this perfect gas mixture undergoes a chemical reaction, then the chemical potentials are related by

$$\text{III.2} \qquad \sum \nu_i \mu_i = 0$$

where the ν_i are defined as the coefficients in the reaction formula

$$\nu_1 \mu_1 + \nu_2 \mu_2 + \cdots = \nu_j \mu_j + \cdots + \nu_n \mu_n$$

being positive if they appear on the left and negative if on the right. If we substitute III.1 into III.2 we find

$$\sum \nu_i \mu_i = \sum \nu_i \left(\mu_i^\circ + RT \ln [i] \right)$$

$$\text{III.3} \qquad \sum \nu_i \mu_i = \sum \nu_i \mu_i^\circ + RT \ln \prod [i]^{\nu_i} = 0$$

Which can be put into the form

III.4 $\Delta G = \Delta G^{\circ} + RT \ln \prod [i]^{\nu_i}$

If the reaction is at equilibrium, $\prod [i]^{\nu_i}$ is equal to the equilibriumconstant K. Substituting into III.4 we find

III.5 $\Delta G^{\circ} = -RT \log K$

The difference between gas phase reactions and solution reactions is that the chemical species no longer obey the Ideal gas law and we must replace concentrations by activities in order to remain theoretically correct. However, as the argument of Appendix I shows, our purposes will be satisfied if we make the approximation of using concentrations instead of activities.

In a redox reaction the free energy released is equal to the work done in transferring the electrons. Thus

III.6 $\Delta G = -nFV$

where n is the number of electrons transferred per mole, V is the electrical potential, and F is Faraday's constant. In reaction steps of the electron transfer chain, n equals one. Finally, we substitute III.6 into III.4 and find

$$\Delta G = -nFV$$
$$= \Delta G^{\circ} + RT \ln \prod [i]^{\nu_i}$$

III.7 $V = V^{\circ} - (RT/nF) \ln \prod [i]^{\nu_i}$

where $V^{\circ} = -\Delta G^{\circ}/nF$.

To keep the signs consistent we have to agree on how we shall write the reaction equation. Without going into a

long discussion about sign conventions, we simply state that the usual convention leaves III.7 looking like

$$\text{III.8} \quad V = V^{o} + \frac{RT}{F} \ln \frac{[\text{oxidized species}]}{[\text{reduced species}]}$$

for an electron transfer reaction written as

$$\text{III.9} \quad A(e) = A() + e^{-}$$

If the reaction is reversed, V^{o} becomes negative.

Thus far, our argument has been perfectly general. Any explanation of a shift must therefore arise from III.7, and that leaves only three classes of possibilities:

1) V^{o} changes
2) $\frac{[\text{oxidized species}]}{[\text{reduced species}]}$ changes
3) there is something overlooked in the system.

It is more convenient to discuss these classes in reverse order, beginning with class 3).

A. PH Effects

One point which our discussion has ignored thus far is the influence of pH in the redox measurements. We have been able to do this because the experiments we wish to explain are done in buffered media. However, as Figure 5 shows, the midpoint potential of cytochrome b is strongly dependent on the pH. It also shows that the high-potential form b_t is unaffected by pH. Fortunately, these effects can be explained in a way applicable to all classes

FIGURE 5
pH dependence of the midpoint potential of cytochrome $\underline{b}_t$.

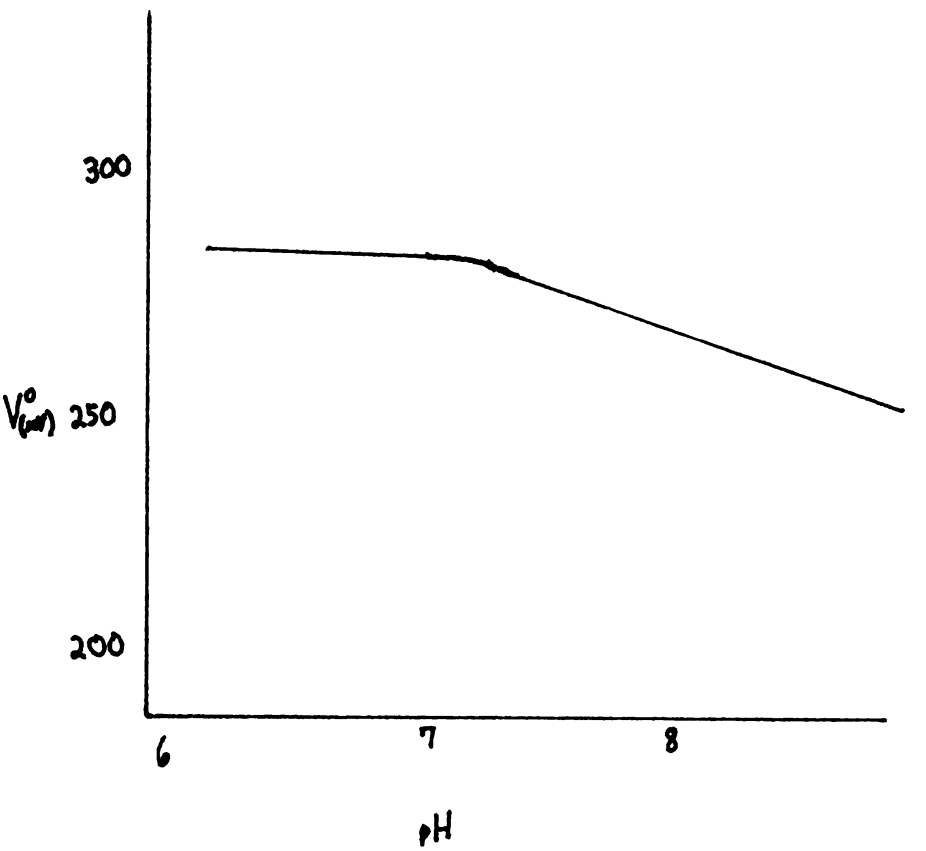


Figure 5

of theories (20).

This explanation is that for many biomolecules, including the cytochromes, the pK of the reduced form is larger than that of the oxidized form. This means that at physiological pH's the oxidized form will be unprotonated, while the reduced form will be protonated. Then one can write the reaction:

$$\text{III.A.1} \quad \underline{b}(e)\text{-H} = \underline{b}() + H^+$$

from which one can write a Nernst expression:

$$\text{III.A.2} \quad V = V^o + (RT/F)\ln\left\{[\underline{b}()]\ [H^+]/[\underline{b}(e)\text{-H}]\right\}$$
$$= V^o + (RT/F)\ln\ [H^+] + (RT/F)\ln\left\{[\underline{b}()]/[\underline{b}(e)\text{-H}]\right\}$$

Allowing one to define the midpoint potential as:

$$\text{III.A.3} \quad V^{o'} = V^o - (RT/F)pH$$

This does not explain the lack of pH dependence of the high-potential form. Proponents of the class 1 & 2 theories say this indicates that there is a second form of the cytochrome, with a different pK. The class 3 advocates, however, reply that it merely shows how the cytochrome interacts with the proton gradient of the chemiosmotic theory. As was the case with the exchange reactions, the data cannot distinguish between the two leading alternatives.

B. Class 3 theories - Something is missing

1. Chemiosmotic explanations

We can now move on to specific models. Since the

most natural subdivision is between the class 3 theories and the class 1 & 2 theories, we will first discuss the class 3 theories. We begin by specifically considering one of our tacit assumptions. This assumption is that we may ignore the possible presence of an electric field. Since redox potentials are actually measurements of electronic chemical potentials, in electrical units, the presence of an electrical field does affect the redox potential. If there is an electric field present, something causes it to change, and the observer is not aware of this, the change in the electric field would appear to the observer to be a change in the midpoint potential.

Fortunately, the possible sources of electric fields in membranes are few. Indeed, the only biological source of an electric field is the transmembrane potential. To be responsible for the redox shifts, it would have to vary with the phosphate potential. A priori, there is no reason why it should. This is, however, Mitchell's original interpretation of the effect (42).

We may refer to Mitchell's model as the chemiosmotic interpretation. It relates the midpoint potential to the transmembrane potential by

$$\text{III.B.1} \qquad V^{o} = V_{0}^{o} + \chi\psi$$

where ψ is the transmembrane potential and χ is a fudge factor describing how far the cytochrome is inside the membrane (and hence how much of it is affected by either side's

potential) Uncoupling, in this version of the chemiosmotic theory, destroys the membrane potential; ATP, by energizing the system, increases the potential. Depending on the sign of χ, and on how ψ depends on P, one can have the midpoint potential increase or decrease.

While this was a reasonable argument when it was proposed, knowledge of the relationships between the electron carriers and the inner membrane has improved since then, and the theory is no longer viable. Thus, while one could speculate in 1970 that cytochrome oxidase was embedded in the membrane, we now know that it extends into the aqueous phase. Yet cytochromes a and a_3 show a shift while cytochrome c does not. Moreover, there is no evidence of any differences in membrane relationships between cytochromes b_t and b_k, yet one shows a shift and the other does not. Finally, this theory suggests that most electron carriers should show a shift, while the opposite is the case. In brief, the chemiosmotic explanation is not correct, and we must look elsewhere for a complete explanation.

2. Reversed electron transport theory

Let us now be very specific to see what, besides the membrane potential, could be missing from our equations. We have a mediator M, and only M, reacting at the electrode. We also have cytochrome b reacting with the mediator. The equations are

$$\text{III.B.2} \quad M(e) = M() + e^-$$

$$\text{III.B.3}\quad \underline{b}(e) + M() = M(e) + \underline{b}()$$

with

$$\text{III.B.4}\quad K = [M(e)]\,[\underline{b}()] \,/\, [M()]\,[\underline{b}(e)]$$

The redox potential measured at the electrode is

$$\text{III.B.5}\quad V_m = V_m^o + (RT/F)\ln [M()]/[M(e)]$$

Now III.B.3 is the sum of the two half-reactions

$$\text{III.B.6}\quad \underline{b}(e) = \underline{b}() + e^-$$

$$\text{III.B.7}\quad M() + e^- = M(e)$$

Note that III.B.7 is the reverse of III.B.2. Thus ΔG_3, the free energy of III.B.3, is just

$$\text{III.B.8}\quad \Delta G_3 = \Delta G_6 + \Delta G_7 = \Delta G_6 - \Delta G_2$$

From III.A.5:

$$\text{III.B.9}\quad \Delta G_3^o = -RT \ln K$$

From III.B.4:

$$[M()]/[M(e)] = (1/K)([\underline{b}()]/[\underline{b}(e)])$$

Thus:

$$V_m = V_m^o + (RT/F) \ln (1/K)([\underline{b}()]/[\underline{b}(e)])$$

$$\text{III.B.10}\quad V_m = V_m^o - (RT/F)\ln K + (RT/F) \ln [\underline{b}()]/[\underline{b}(e)]$$

But, from III.A.7 and III.A.8

$$\text{III.B.11}\quad V_m^o = -\Delta G_2^o/F \;;\; V_b^o = -\Delta G_6^o/F$$

Plugging III.B.11, III.B.8, and III.B.9 into III.B.10:

$$V_m^o = -\Delta G_2^o/F - \Delta G_3^o/F + (RT/F) \ln [\underline{b}()]/[\underline{b}(e)]$$

$$= -\Delta G_2^o/F - \Delta G_6^o/F + \Delta G_2^o/F + (RT/F) \ln [\underline{b}()]/[\underline{b}(e)]$$

$$\text{III.B.12}\quad = V_b^o + (RT/F) \ln [\underline{b}()]/[\underline{b}(e)]$$

Consequently, what we measure is exactly what we desire.

The only other possible complication is to add additional reactants to the system. We know that cytochrome c reacts with b:

III.B.13 $\underline{b}() + \underline{c}(e) = \underline{b}(e) + \underline{c}()$

Repeating the above calculation on the system III.B.2, III.B.3, and III.B.13 yields

III.B.14 $V_m = V_c^o + (RT/F)\ln[\underline{c}()]/[\underline{c}(e)]$

The final complication is to include ATP synthesis, replacing III.B.13 with

III.B.15 $\underline{b}(e) + \underline{c}() + Pi + ADP = \underline{b}() + \underline{c}(e) + ATP$

where we have ignored the water. Again, III.B.12 can be derived. However, III.B.14 must now be modified. Rather than derive the new equation, we will change III.B.3 and derive the corresponding result for b. This makes our system

III.B.2 $M(e) = M() + e^-$

III.B.16 $M() + \underline{c}(e) = M(e) + \underline{c}()$

III.B.15 $\underline{b}(e) + \underline{c}() + Pi + ADP = \underline{b}() + \underline{c}(e) + ATP$

The same argument as before readily yields

III.B.14 $V_m = V_c^o + (RT/F)\ln[\underline{c}()]/[\underline{c}(e)]$

But now, when we try to express V_m in terms of b the complication arises. For, instead of eq. III.B.8 we have

III.B.17 $\Delta G_{15} = \Delta G_6 - \Delta G_c + \Delta G_p$

where G_p is the free energy of ATP formation.

Once again, we substitute into eq. III.B.14:

$$V_m = V_c^o + (RT/F)\ln\frac{[\underline{b}()]\,[ATP]}{K[\underline{b}(e)]\,[Pi]\,[ADP]}$$

$$V_m = V_c^o + (RT/F) \ln \frac{[ATP]}{[Pi][ADP]}$$

$$+ (RT/F) \ln [\underline{b}()]/[\underline{b}(e)] - (RT/F) \ln K$$

$$= V_c^o + \frac{1}{F} (\Delta G_c^o - \Delta G_c^o + \Delta G_p^o) + (RT/F) \ln [\underline{b}()]/[\underline{b}(e)]$$

$$+ (RT/F) \ln \frac{ATP}{[Pi][ADP]}$$

$$\text{III.B.18} = V_b^o + (RT/F) \ln [\underline{b}()]/[\underline{b}(e)] + \frac{1}{F} (\Delta G_p^o + RT \ln \frac{ATP}{[Pi][ADP]})$$

which clearly depends upon the logarithm of the phosphate potential.

This is the explanation of the redox shifts put forward by Slater (51) and also championed by Wikstrom (68). It has been presented here in some detail to indicate exactly how the phosphate potential dependence enters in. The phosphate potential dependent redox shift is explained as an artifact caused by the mediator not reacting directly with the cytochrome measured spectroscopically. The uncoupler effect is then explained by postulating that it sets the effective phosphate potential to zero.

Before discussing this theory further we must illustrate another possible effect, that of the short circuit. Suppose we add eq. III.B.3 to the system of III.B.2, III.B.15 and III.B.16. Then the electron need not go through the energy transducing machinery to get from <u>b</u> to <u>c</u>; it can travel via the mediator. If we add up the equations we discover that the net reaction is

$$\text{III.B.19} \quad Pi + ADP = ATP$$

or ATP hydrolysis.

This result should not be surprising. The electron transfer system and the ATP synthesizing system balance each other out energetically. If something is done to remove all restraints on one, the other should be expected run down also. As we see, this is what in fact happens.

Experimentally, this means that some electron carrier must not be reacting with the mediator. There are several ways to accomplish this. The most direct is to use a mediator which reacts so slowly with one of the cytochromes that the reaction never reaches equilibrium. Another possibility is to allow the mediator-cytochrome reaction to reach equilibrium, but arrange it so that the cytochrome does not equilibrate with other cytochromes. This last suggestion will make more sense after we have examined some of the more complicated reaction mechanisms.

We can now return to discussing the reversed-electron transport theory. The overriding experimental fact in the discussion is that ATP hydrolysis does not occur. Consequently, it is an experimental fact that some step in the set of all possible reactions does not occur. If it could be demonstrated that the missing step is eq. III.B.3 the problem would be solved and Slater would be correct.

Unfortunately, negative statements are difficult to prove. Lambowitz and Wikstrom (32) and Dutton and Storey (19) have demonstrated that short-circuiting occurs in mung-bean mitochondria. Wikstrom has also shown that

massive quantities of mediator can short-circuit animal mitochondria (69). This has been taken as proof that the reverse electron transport theory is correct. However, all this proves is the uncontested statement that short-circuited mitochondria catalyze ATP hydrolysis.

Chance's group has collected much evidence that the mediators are reacting with electron carriers in equilibrium with the cytochromes, if not with the desired cytochromes themselves (18, 20, 80). This would mean that the desired cytochrome is indeed measured electrically, and would contradict the reverse electron transport theory.

The most telling argument against the theory is the observation that only cytochrome $\underline{b}_t$ shows the shift in complex III, and only cytochromes $\underline{a}$ and $\underline{a}_3$ in complex IV. If, as the reverse electron transport theory requires, the shift is the result of the mediator only reacting with cytochrome $\underline{c}$, then everything between $\underline{b}_t$ and $\underline{c}$, and between $\underline{c}$ and $\underline{a}$ should show a similar shift. This is not the case. Moreover, the size of the shift of cytochrome $\underline{a}$ is less than half that of $\underline{a}_3$, and in the opposite direction. This is also difficult to reconcile with reversed electron transport (35).

C. Introduction to Class 1 and 2 Theories

The members of the other two classes of explanation have in common the feature that there is some change in

the cytochrome being measured, resulting in a real mid-point potential change. To understand this, suppose that there exists two forms of cytochrome $\underline{b}$, which are spectroscopically indistinguishable:

III.C.1 $\underline{b}_1(e) = \underline{b}_1() + e^- \; ; \; \underline{b}_2(e) = \underline{b}_2(\;) + e^-$

For simplicity, let

III.C.2 $[\underline{b}_1()]/[\underline{b}_1(e)] = B_1 \; ; \; [\underline{b}_2()]/[\underline{b}_2(e)] = B_2$

If the ratios can be measured, they can be adjusted experimentally, and we can take $B_1 = B_2 = B$. Then, given that a redox system contains some form of $\underline{b}$, the measured potential will be either

III.C.3 $V_1 = V_1^o + (RT/F) \ln B$

or

III.C.4 $V_2 = V_2^o + (RT/F) \ln B$

D. Class 2 Theories: $[ox]/[red]$ Changes

Suppose that in the system under discussion the mediator only reacts with $\underline{b}_1$, while the spectroscope measures both $\underline{b}_1$ and $\underline{b}_2$. This presumption is made in all class 2 theories. Then the true potential is

III.D.1 $V_c = V_1^o + RT/F \ln [\underline{b}_1()]/[\underline{b}_1(e)]$

but one calculates

III.D.2 $$V_c = V_1^o + RT/F \ln \frac{[\underline{b}_1() + \underline{b}_2()]}{[\underline{b}_1(e) + \underline{b}_2(e)]}$$

Suppose

III.D.3 $\underline{b}_2() = K_o(x)\, \underline{b}_1()$

and

$$\text{III.D.4} \quad \underline{b}_2(e) = K_e(x)\ \underline{b}_2(e)$$

Then

$$\text{III.D.5} \quad V_c = V_1^o + (RT/F) \ln \frac{[\underline{b}_1()](1 + K_o(x))}{[\underline{b}_1(e)](1 + K_e(x))}$$

$$= V_1^o + (RT/F) \ln \frac{(1 + K_o(x))}{(1 + K_e(x))}$$

$$+ (RT/F) \ln [\underline{b}_1()]/[\underline{b}_1(e)]$$

Depending on what the parameter, x, is, the midpoint potential will appear to vary. X is usually taken to be the phosphate potential.

Before proceeding we must discuss the assumption of spectral indistinguishability. The situation can be best illustrated with the a cytochromes. If we monitor 605 nm, a wavelength characteristic of the a cytochromes, and perform a redox titration, we observe the sigmoidal curve characteristic of two redox components. From this, we can calculate the midpoint potentials of these components. If we repeat the experiment in the presence of a high phosphate potential we obtain two different values. However, only about one-half of the 605 absorbance is involved in these changes (71, 75).

There are three possible interpretations of these observations:

1) Cytochromes a and a_3 both contribute to the 605 nm band, but have different midpoint potentials.

2) Either cytochrome a or a_3, but not both, contri-

bute to the 605 band, and they have the same midpoint potential.

3) Some combination of the above.

Explanation number 2 is somewhat unusual, and requires some additional explanation. It was originally put forth by Nicholls, (44) and has recently been adopted by Wikstrom and by Chance (71). Its advantage is that it allows cytochromes *a* and a_3 to have separate, well defined absorbance peaks, and thus keeps them spectrally distinguishable. Its problem is that as thus far stated there is no way to produce the sigmoidal curve, since *a* and a_3 are given the same midpoint potential. The problem is solved by assuming that both *a* and a_3 can exist in either of two different forms, with each form having a different midpoint potential. Moreover, the value of these midpoint potentials depends upon the phosphate potential. Furthermore, it is postulated that which state *a* is in depends upon the state of a_3, and vice versa.

Since this seems *ad hoc*, one may wonder why explanation 1) does not win by default. The answer is that 1) is not completely free of problems. The chief problem is that the extinction coefficients of cytochrome *a* appear to depend upon what, if anything, is bound to a_3. These extinction shifts largely disappear if the data are interpreted according to explanation 2).

Since both explanations lead to undesirable consequences,

the choice becomes one of a choice of evils. To us, explanation 1) is the lesser evil, since its basic premises seem more reasonable. The only advantage of explanation 2) is that smaller shifts in extinction coefficients are required, but some shifts are needed even in this case.

1. Marbach and Vignais

We may now return to our discussion of Class 2 models. The model of Marbach and Vignais (39) is poorly developed, but appears to belong in Class 2. The novel feature of this model is the postulated existence of a long "arm" on the F1 complex which can rest in either of two stable positions. One position has the end of the armeffectively complexed to cytochrome b, while the other has the end of the arm in the middle of the ATP synthetase. Denoting the position of this arm-end as I, and the ATP synthetase as F, we can describe the steps as follows:

III.D.7 $F + QH_2 + 2\underline{b}()\text{-}I = 2\underline{b}(e) + Q + F\text{-}(IH)_2$

$2\underline{b}(e) + ADP + Pi + F\text{-}(IH)_2 = 2\underline{b}(e)\text{-}I + ATP + H_2O + F$

$2\underline{b}(e)\text{-}I + 2\underline{c}() = 2\underline{b}()\text{-}I + 2\underline{c}(e)$

In the scheme of III.D.1 - III.D.5, $\underline{b}_1$ corresponds to b, and $\underline{b}_2$ to b-I. While one could go on and develop the quantitative details, Marbach and Vignais are content to stop here, and relate their model to experimental evidence purely on a qualitative basis.

The primary proponents of Class 2 theories are Wilson

FIGURE 6
Fundamental scheme of the Class 2 theories.

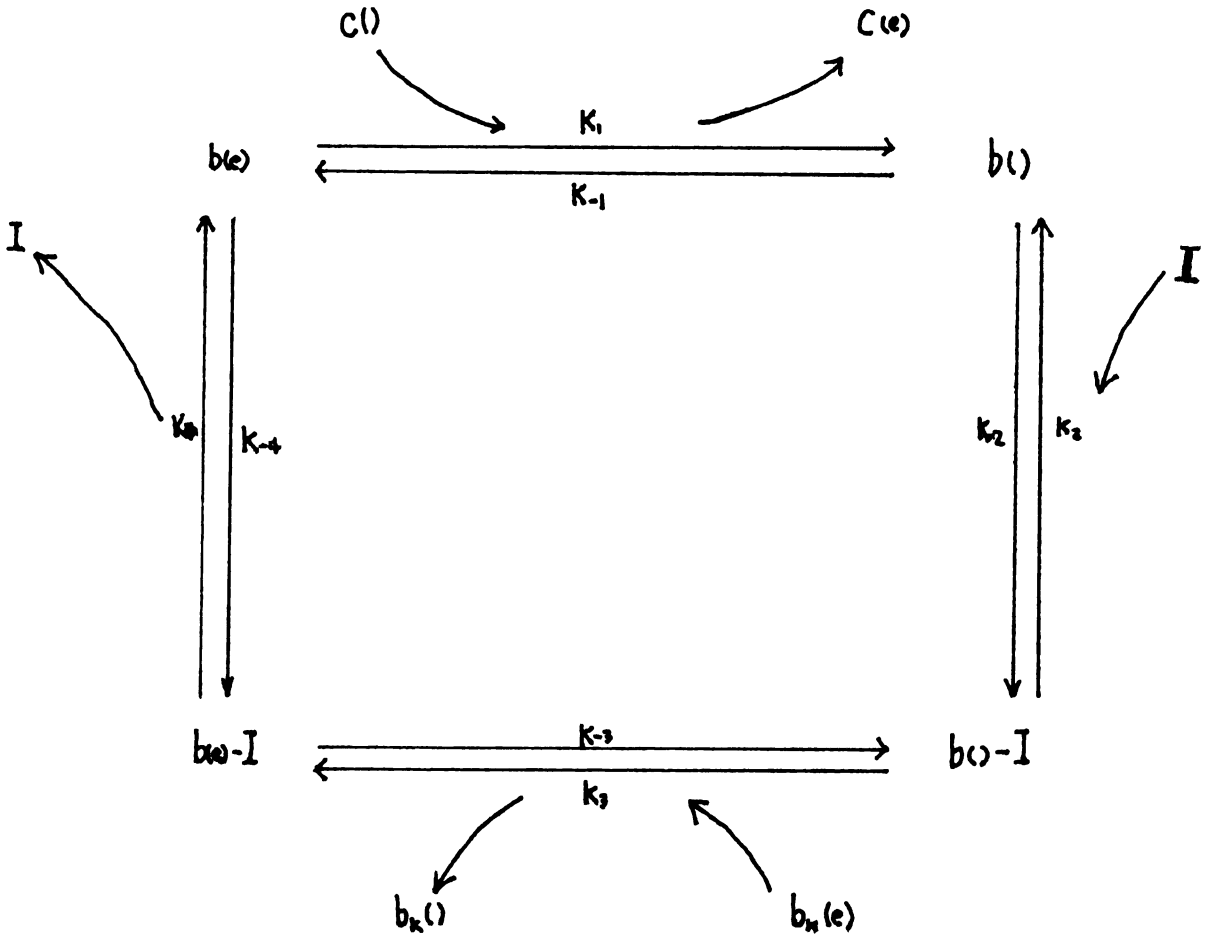


Figure 6

and co-workers, such as DeVault. The simplest way to see their interrelationships is to consider Figure 6. If in Figure 6 the mediator reacts only with $\underline{b}()$ and $\underline{b}(e)$ we can use the same analysis as before and find:

$$\text{III.D.8} \quad V_b = V_b^o + (RT/F)\ln K_2/K_4 + (RT/F)\ln \frac{[\underline{b}()]}{[\underline{b}(e)]}$$

The trouble with Figure 6 is that the scheme is too cyclic; the net reaction is 0 = 0, and there is nothing produced which can transfer energy. Wilson and DeVault have proposed ways to overcome these problems.

2. DeVault

While the exact diagram has varied somewhat from paper to paper (16, 20), DeVault's latest scheme is much like Figure 6 except that he has it transforming I into I (20) (Figure 7).

As DeVault notes, there are other places one can insert the I's into the square; consideration of these additional models, however, reveals no new features. The gist of all of them is to produce I , which can react to form ATP:

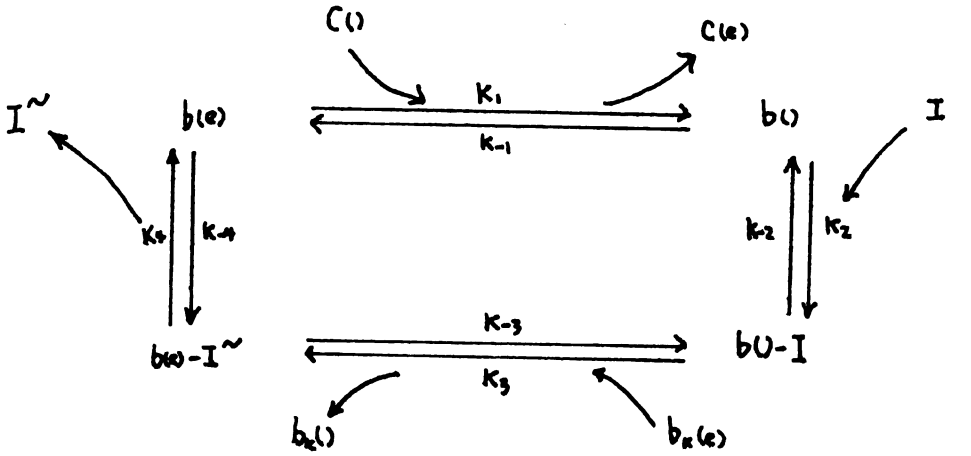
$$\text{III.D.9} \quad ADP + Pi + I\sim = ATP + I$$

This model is, then, part of the chemical theory. Thus, the considerations discussed earlier apply. The only new feature introduced is the matter of short-circuiting, or, whether the $\underline{b}()/\underline{b}(e)$ couple operate at the same redox potential as the $\underline{b}()$-I/$\underline{b}(e)$-I couple.

FIGURE 7

DeVault's mechanism of energy coupling.
A) Basic scheme converting I into I
B) Scheme with a reaction "cut" to prevent energy dissipation.

A)


C()
C(e)
K1
K-1
I~
b(e)
b()
I
K+
K-+
K-2
K2
K-3
b(e)-I~
b()-I
K3
b(e)
b(e)

B)

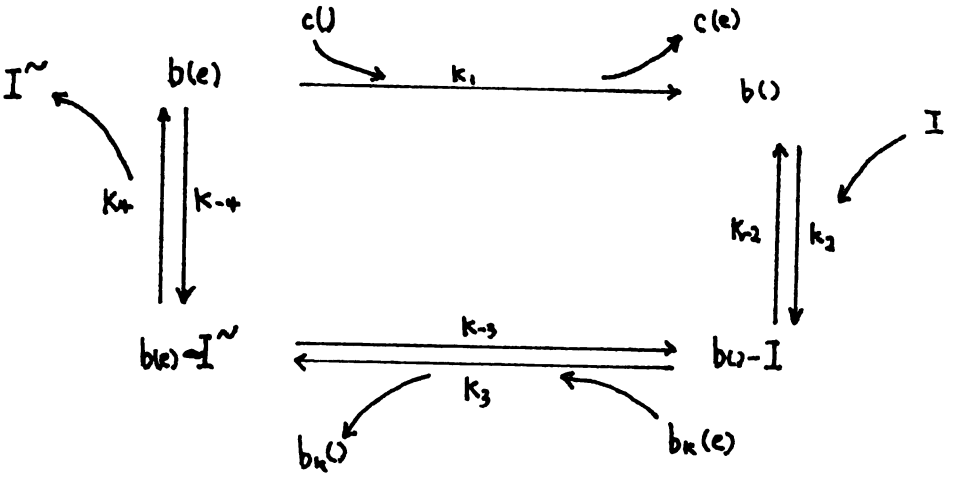

c()
c(e)
K1
I~
b(e)
b()
I
K+
K-+
K-2
K2
K-3
b(e)-I~
b()-I
K3
b(e)
b(e)

Figure 7

First of all, if the four reactions of Figure 7 are all in equilibrium, then $\underline{b}$ and $\underline{b}$-I will in fact be at the same redox potential. The proof of this is somewhat laborious, but straightforward:

III.D.10a $\quad \underline{b}(e) + \underline{c}() \underset{K_{-1}}{\overset{K_1}{=}} \underline{b}() + \underline{c}(e)$

III.D.10b $\quad \underline{b}() + I \underset{K_{-2}}{\overset{K_2}{=}} \underline{b}()\text{-}I$

III.D.10c $\quad \underline{b}()\text{-}I + \underline{b}_k(e) \underset{K_{-3}}{\overset{K_3}{=}} \underline{b}(e)\text{-}I^{\sim} + \underline{b}_k()$

III.D.10d $\quad \underline{b}(e)\text{-}I^{\sim} \underset{K_{-4}}{\overset{K_4}{=}} I^{\sim} + \underline{b}(e)$

III.D.10e $\quad$ net: $\quad I^{\sim} \underset{K_-}{\overset{K}{=}} I$

We wish to measure

III.D.11 $\quad V_{b\text{-}I} = V^{o}_{b\text{-}I} + (RT/F)\ln[\underline{b}()\text{-}I]/[\underline{b}(e)\text{-}I]$

but

$$|\underline{b}()\text{-}I| = K_2[\underline{b}()][I] \quad \text{and} \quad [\underline{b}(e)\text{-}I] = [\underline{b}(e)][I^{\sim}]K_4^{-1}$$

$$V_{b\text{-}I} = V^{o}_{b\text{-}I} + (RT/F)\ln (K_2[I][\underline{b}()])/(K_4^{-1}[I^{\sim}]\underline{b}(e))$$

III.D.12 $$= V^{o}_{b\text{-}I} + (RT/F)(\ln K_2 + \ln K_4 + \ln [I]/[I^{\sim}] + \ln [\underline{b}()]/[\underline{b}(e)])$$

From III.D.10e:

III.D.13 $\quad [I]/[I^{\sim}] = K_1K_2K_3K_4$

Substituting III.D.13 into III.D.12:

$$V_{b\text{-}I} = V^{o}_{b\text{-}I} + (RT/F)\left(\ln K_2 + \ln K_4 - \ln K_1K_2K_3K_4 + \ln \frac{\underline{b}()}{\underline{b}(e)}\right)$$

III.D.14 $\quad V_{b\text{-}I} = V^{o}_{b\text{-}I} - (RT/F)(\ln K_1 + \ln K_3 - \ln [\underline{b}()]/\underline{b}(e))$

But

$$(RT/F)\ln K_1 = -V_b^o \quad \text{and} \quad (RT/F)\ln K_3 = V_{b-I}^o \ ;$$

thus:

$$\text{III.D.15} \quad V_{b-I} = V_{b-I}^o + V_b^o - V_{b-I}^o + (RT/F)\ln[\underline{b}()]/[\underline{b}(e)]$$
$$= V_b$$

Since DeVault explicitly says he wishes to keep the two potentials distinct, he only allows the mediator to react with one species. In addition, although he does not seem to realize it, the preceeding analysis shows that one of the reactions must be "cut" so that equilibrium is not attained. The most natural choice is reaction III.D.10a, resulting in Figure 7B. Then the observed redox potential will be given by either:

$$V_{obs} = V_b^o + (RT/F)\ln\frac{[\underline{b}()] + [\underline{b}()\text{-}I]}{[\underline{b}(e)] + [\underline{b}(e)\text{-}I]}$$
$$= V_b^o + (RT/F)\ln\frac{[\underline{b}()] + K_2[I][\underline{b}()]}{[\underline{b}(e)] + K_4^{-1}[I\sim][\underline{b}(e)]}$$

$$\text{III.D.16} \quad V_{obs} = V_b^o + (RT/F)\ln\frac{1 + K_2[I]}{1 + K_4^{-1}[I\sim]} + (RT/F)\ln[\underline{b}()]/[\underline{b}(e)]$$

or:

$$V_{obs} = V_{b-I}^o + (RT/F)\ln\frac{[\underline{b}()] + [\underline{b}()\text{-}I]}{[\underline{b}(e)] + [\underline{b}(e)\text{-}I]}$$

$$\text{III.D.17} \quad = V_{b-I}^o + (RT/F)\ln\frac{1 + K_2^{-1}[I]}{1 + K_4K_p[I\sim]} + (RT/F)\ln\frac{[\underline{b}()\text{-}I]}{[\underline{b}(e)\text{-}I]}$$

depending upon which species the mediator reacts with. Because of III.D.9, I and I depend upon P, and hence redox potentials will depend upon P. As before, if we try to froce the reaction into a simple Nernst equation, the observed midpoint potentials will appear to depend on P. If we also assume that the mediator only reacts with $\underline{b}_k$ or $\underline{c}$, we can make the shift positive or negative.

3. Wilson

Wilson, on the other hand, modifies this scheme so that it becomes possible to bring both species to the same redox potential. (79) His scheme is Figure 8. In this scheme L is some hypothetical electrically active ligand. It is seen that in the part of the scheme corresponding to Figures 6 and 7, one step has been explicitly cut. It thus becomes possible to have all of these steps at the same redox potential without short-circuiting the system. However, these steps are on a side chain of the electron transfer chain, which consists of reactions 1, 2, 5, and 6. The mediator cannot react with all of the latter steps without short-circuiting the system. Wilson makes the same assumption we did in eq. III.D.1, that only one species is measured electrically. His redox expression, then, is a special case of III.D.5:

$$\text{III.D.18}\quad V = V^{o} + (RT/F)\cdot\left(\ln \frac{K_p K_2 [I]P + 1}{K_p K_4 [I]P + 1} + \ln \frac{[b()-L()-I]}{[b(e)-L()-I]}\right)$$

FIGURE 8
Wilson's scheme of energy coupling. The portion in dotted lines corresponds to the basic scheme of Fig. 5.

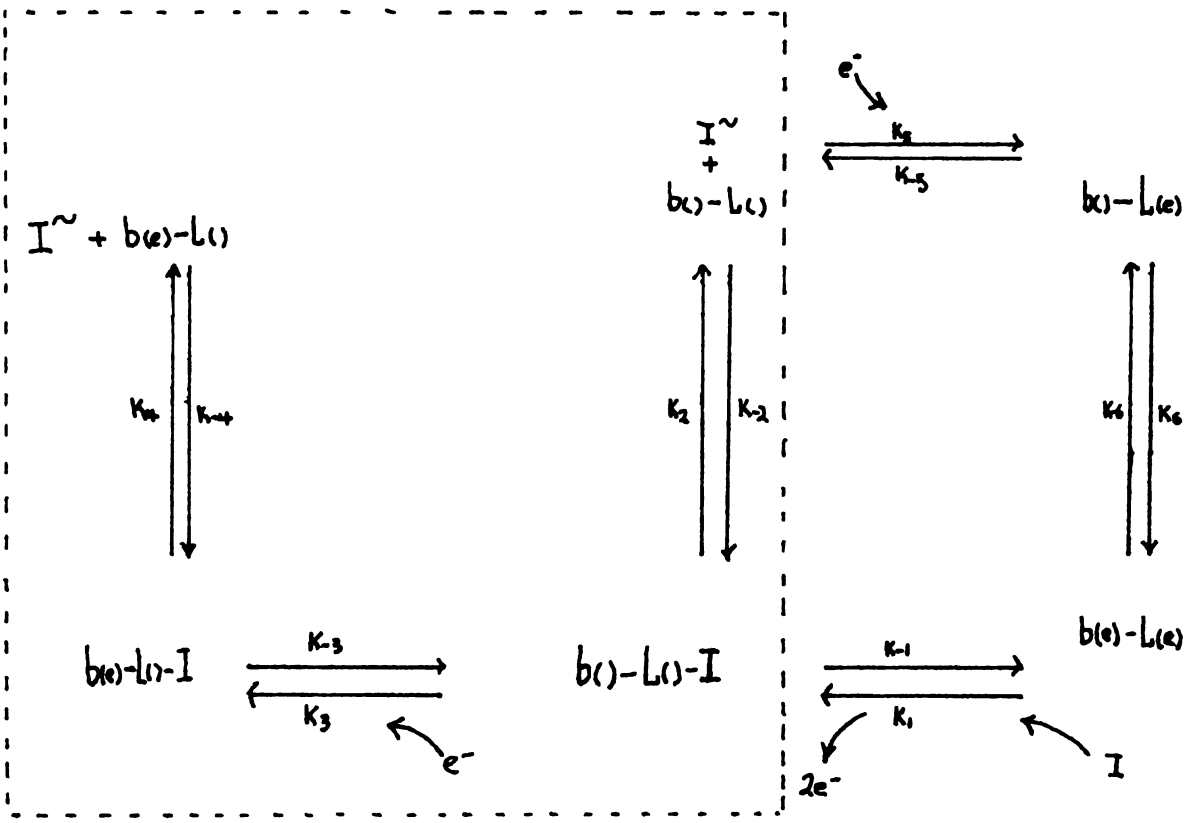


Figure 8

IV. THE PAIR MODEL

Before discussing the details of the pair model, we must first say a word about methodology. The usual method of calculating redox potentials involves writing down the chemical reactions of the system and applying the Nernst equation. This method has limited usefulness for our problem, as the system is not readily specified by ordinary chemical reactions. Instead, we shall first write out the grand partition function of the system. From this, the chemical potential of an electron in the system can be calculated, and the redox potential then calculated from the chemical potential.

Besides the electron e^- our system contains an alternative negative charge a^-, and besides the positive charge p^+ we have an alternative positive charge u^+. This gives us four possible pairs in the system, e^--p^+, e^--u^+, a^--p^+, a^--u^+; with energies E_{ep}, E_{eu}, E_{ap}, E_{au}. We assume that the system is in thermodynamic equilibrium and that the pairs do not interact with one another. Denoting the number of cytochromes by C, and the number of e^--p^+, e^--u^+, a^--p^+, a^--u^+ pairs by N_{ep}, N_{eu}, N_{ap}, N_{au}, we can write the grand partition function Z_g as (27):

$$\text{IV.1} \quad Z_g = \left[1 + z_e z_p \exp(-\beta E_{ep}) + z_e z_u \exp(-\beta E_{eu}) + z_a z_p \exp(-\beta E_{ap}) + z_a z_u \exp(-\beta E_{au}) \right]^C$$

where the z's are the fugacities of the charges, with $z_i = \exp(\beta \mu_i)$ and μ_i the electrochemical potential. Since the expectation value for the number os electrons is (27)

$$\langle e \rangle = Z_e \frac{\partial}{\partial Z_e} \ln Z_g$$

it follows that

IV.2
$$\langle e \rangle = \frac{C Z_e \left[Z_p \exp(-\beta E_{ep}) + Z_u \exp(-\beta E_{eu}) \right]}{1 + Z_e \left[Z_p \exp(-\beta E_{ep}) + Z_u \exp(-\beta E_{eu}) \right] + Z_a \left[Z_p \exp(-\beta E_{ap}) + Z_u \exp(-\beta E_{au}) \right]}$$

This can be easily solved for z_e:

IV.3
$$z_e = \frac{1 + Z_a \left[Z_p \exp(-\beta E_{ap}) + Z_u \exp(-\beta E_{au}) \right]}{Z_p \exp(-\beta E_{ep}) + Z_u \exp(-\beta E_{eu})} \cdot \frac{\langle e \rangle}{C - \langle e \rangle}$$

Since $z_e = \exp(\beta \mu_e)$ and $\mu_e = -FV$, where V is the half-cell redox potential, we can immediately write for the redox potential

$$V = -\frac{RT}{F} \ln \frac{1 + Z_a \left[Z_p \exp(-\beta E_{ap}) + Z_u \exp(-\beta E_{au}) \right]}{Z_p \exp(-\beta E_{ep}) + Z_u \exp(-\beta E_{eu})} + \frac{RT}{F} \ln \frac{C - \langle e \rangle}{\langle e \rangle}$$

and for the midpoint potential:

IV.4
$$V^\circ = -\frac{RT}{F} \ln \frac{1 + Z_a \left[Z_p \exp(-\beta E_{ap}) + Z_u \exp(-\beta E_{au}) \right]}{Z_p \exp(-\beta E_{ep}) + Z_u \exp(-\beta E_{eu})}$$

There are three special cases to consider.

Case 1: Only the $e^- - p^+$ pair is present.

Then $z_a = z_u = 0$ and the midpoint potential for this special case reduces to

IV.5 $V_1^\circ = (1/F)(\mu_p - E_{ep})$

In the original formulation of the pair theory, the positive charge p^+ is an ionophore containing Mg^{++} and

either Pi^- or ADP^-. Therefore μ_p is the chemical potential of the ionophoric complex. We can obtain a more useful expression for the midpoint potential if we assume the ionophoric complex dissociation reaction is in equilibrium:

$$p \cdot I = p + I$$

where I stands for ionophore and care should be taken not to confuse p, the general positive charge, with the phosphate potential P.

We then have the relationship:

$$\langle p \rangle \langle I \rangle / \langle p\text{-}I \rangle = K$$

$$\langle p\text{-}I \rangle = K^{-1} \langle p \rangle I = K^{-1}(I_t \langle p \rangle - \langle p\text{-}I \rangle \langle p \rangle)$$

where I_t = total number of ionophores present.

$$\langle p\text{-}I \rangle \simeq K^{-1} I_t \langle p \rangle$$

if I_t <p-Ionophore> . This is true whenever the system is not saturated with p. Then we can write the desired result:

$$\langle p\text{-}I \rangle = K' \langle p \rangle$$

where $K' = K^{-1} I_t$.

Now

$$\mu_p = \mu_p^o + RT \ln \langle pI \rangle$$

The midpoint potential may then be expressed as

$$V_I^o = F^{-1}(\mu_p^o + RT \ln \langle p\text{-}I \rangle - E_{ep})$$

$$= F^{-1}(\mu_p^o - E_{ep} + RT \ln K' \langle p \rangle)$$

IV.6 $$= F^{-1}(\text{Const.} + RT \ln \langle p \rangle)$$

We see that the midpoint potential is proportional to the concentration of the electron's partner, implying that

variations in the concentration of the partner cause the midpoint potential to vary. This is a real shift in the midpoint potential, and not an apparent shift due to measurement of the wrong cytochrome, or the creation of a new species not measureable spectroscopically.

Case 2: Only e^--p^+ and e^--u^+ pairs are present.

In this case $z_a = 0$ and the midpoint potential reduces to

$$V_2^o = + (RT/F) \ln (z_p \exp(-\beta E_{ep}) + z_u \exp(-\beta E_{eu}))$$

or

$$\text{IV.7} \quad V_2^o = V_1^o + (RT/F) \ln \; 1 + (z_u/z_p)\exp(-\beta(E_{eu}-E_{ep}))$$

This means that adding a second type of positive charge causes the midpoint potential to shift positive relative to V_1^o. Since this also means that there are two types of reduced cytochromes present, those with e^--p^+ pairs and those with e^--u^+ pairs, we can express this by saying that alternative pairing at the reduced cytochrome increases the midpoint potential. Discussion of the physical significance of this will be deferred until a later section.

Case 3: Only e^--p^+ and a^--p^+ pairs present.

This is similar to Case 2, except that we now have two types of oxidized cytochrome and $z_u = 0$. The midpoint potential is

$$\text{IV.8} \quad V_3^o = V_1^o - (RT/F) \ln(1 + z_a \exp(-\beta E_{ap}))$$

and it can be seen to have shifted negatively relative to V_1^o. Thus alternative pairing at the oxidized cytochrome

decrease the midpoint potential. Again, the discussion of this case will be deferred.

A. The Complete Model and Calculations.

To turn these simple systems into a system capable of producing ATP we need a way to insert the ATP precursors into the pairs, and a mechanism for forming ATP from these precursors. Let us ignore the latter complication for the moment, and concentrate on the former, for it was this consideration which led to the first formulation of the pair model.

We start with two of our simple systems, one containing e^--p_1^+ pairs, and the other e^--p_2^+ pairs. We arrange these systems so that each transfers its pair between two cytochromes, denoted by X and Y, and then transfers the positive charge in its pair to a common site. If we interpret p_1^+, the positive charge in the first system, as being a Pi containing positively charged ionophore; p_2^+ as an ADP containing positively charged ionophore; and the common site as the F1 complex, we have a master system capable of transducing electronic energy into ATP.

These considerations readily explain the fact that two electrons are required to make one ATP molecule at a phosphorylation site. There are two possible ways to arrange a two-electron transfer in the electron transfer chain. One way is to use a single element, and have this element

transfer two electrons, one at a time:

$$\underline{b}_t \rightarrow \underline{b}_k \rightarrow \underline{c} \qquad \text{(Scheme A)}$$

The other way is to use two elements in parallel, and have each transfer one electron:

$$\begin{matrix} \underline{b}_t \rightarrow \underline{b}_k \rightarrow \underline{c} \\ \underline{b}_t \rightarrow \underline{b}_k \rightarrow \underline{c} \end{matrix} \qquad \text{(Scheme B)}$$

Experimentally, it has not been possible to distinguish between the two, although the first scheme is commonly assumed (33). For our purposes, however, the second is more useful, for two reasons. First, scheme (A) can be regarded as a special case of scheme (B), so that only one calculation is needed. Second, in the original pair model, where p_1 and p_2 are different charges, it is easier to imagine two cytochromes, each specialized for one charge, than one cytochrome which can transport either.

Figure 9 diagrams the main features of the model. p_1^- and p_2^- are the ordinary, deprotonated, p_1 and p_2, while p_1^+ and p_2^+ are p_1^- and p_2^- complexed with an Mg^{++} containing ionophore. We treat the ionophore as a site in the system for p_1^- and p_2^-; thus we ignore its chemistry except as an influence on the energy of p_1^+ and p_2^+. Again, we assume we have C of each cytochrome. We can then write for the grand partition function:

$$\text{IV.A.1} \quad Z_g = \left[Z_X(1) \cdot Z_X(2) \cdot Z_Y(1) \cdot Z_Y(2) \right]^C$$

Where

FIGURE 9

Steps in the original pair model process for coupling oxidative phosphorylation to the electron transfer chain. The initial state of the system, (A), contains no pairs. The first step is the entry of a $P_1^- - H^+$ and a $P_2^- - H^+$ pair, (B), each of which associate with a X cytochrome. The next step is the entry of two $e^- - H^+$ pairs (D), which also associate with a X cytochrome. This step may preceed step (B) as in (C). After both pairs are in place, P_1^- and P_2^- enter a Mg^{++} containing ionophore, to form complexes we denote by P_1^+ and P_2^+. These change pairs with the electrons to form $e^- - P_1^+$ and $e^- - P_2^+$ pairs, with the protons returning to the solution (E). Next, the pairs are transferred to the Y cytochromes (F). Next, the P's are transported to the F1 complex, where they are combined. The electron then pairs to a proton as in (G). If the P_1-P_2 complex remains after the electrons have left the Y cytochromes, we have the situation in (H), whereas, if the electrons remain after P_1-P_2 is removed, we have (I).

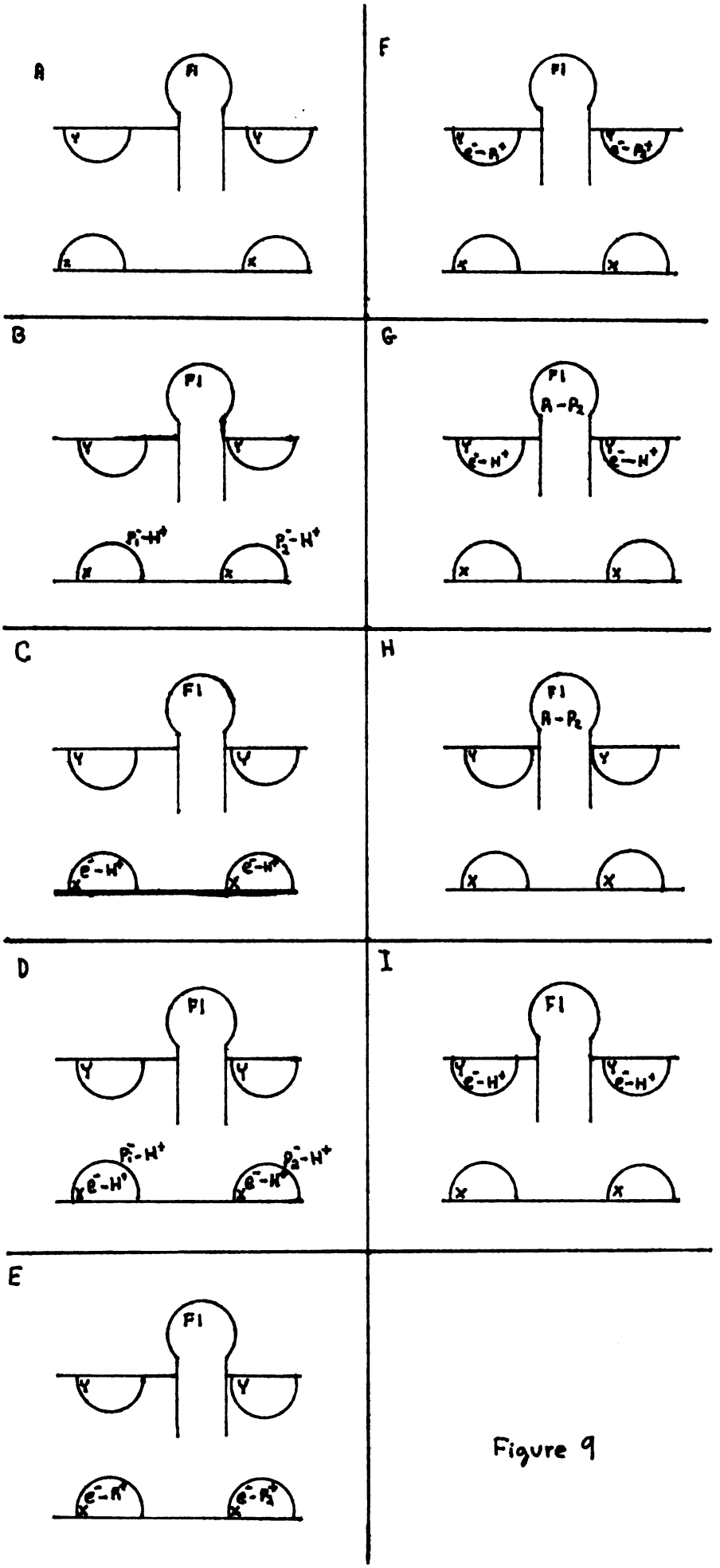


Figure 9

with the expression 'pi' to be replaced by either p1 or p2, depending upon the argument of Z_X or Z_Y. The various terms can be understood by studying Figure 9.

We have assumed that X is independent of Y. This is only an approximation for cytochrome $\underline{a} + \underline{a}_3$ (71, 35), but this assumption leads to interesting results in its own right.

Once again, we can compute the expectation value for electrons on each cytochrome:

IV.A.2

$$\langle e_X(i)\rangle = \frac{Cz_e\left[z_H \exp(-\beta E_{X_{e,H}}) + z_{Pi} z_H^2 \exp\left(-\beta E_{X_{e,Pi,H}}\right) + z_{Pi} \exp\left(-\beta E_{X_{e,Pi,H}}\right)\right]}{Z_{X(i)}}$$

IV.A.3

$$\langle e_{Y(i)}\rangle = \frac{Cz_e\left[z_{P_i} \exp(-\beta E_{Y_{e,Pi}}) + z_H \exp(-\beta E_{Y_{e,H}}) + z_H z_{P_1} z_{P_2} \exp(-\beta E_{Y_{e,P_1,P_2}})\right]}{Z_{Y(i)}}$$

From these, we can express the redox potential in terms of either $\langle e_X(i)\rangle$ or $\langle e_Y(i)\rangle$:

IV.A.4a

$$V = -\frac{RT}{F} \ln \frac{1 + z_{Pi} z_H \exp\left(-\beta E_{X_{e,Pi,H}}\right)}{z_H \exp(-\beta E_{X_{e,H}}) + z_{Pi} z_H^2 \left(-\beta E_{X_{e,Pi,H}}\right) + z_{Pi} \exp\left(-\beta E_{X_{e,Pi}}\right)} \frac{\langle e_X(i)\rangle}{C - \langle e_X(i)\rangle} \equiv V_X(i)$$

IV.A.4b

$$= -\frac{RT}{F} \ln \frac{1 + z_{P_1} z_{P_2} \exp\left(-\beta E_{Y_{P_1,P_2}}\right)}{z_H \exp\left(-\beta E_{Y_{e,H}}\right) + z_{P_1} z_{P_2} z_H \exp\left(-\beta E_{Y_{P_1,P_2}}\right) + z_{Pi} \exp\left(-\beta E_{Y_{e,Pi}}\right)} \frac{\langle e_Y(i)\rangle}{C - \langle e_Y(i)\rangle} \equiv V_Y(i)$$

where the $V_X(i)$ and $V_Y(i)$ are all equal to the actual redox potential V, but are expressed in terms of different $\langle e \rangle$.

At this point, let us insert the observation that the redox potential will depend upon z_H, and hence upon the pH. We do so because the next step in the argument is to hold the pH constant, and consequently pH dependence will not be apparent in the final expression. Before taking this step, however, we wish to describe in greater detail why explicit pH dependence expressions are not useful.

As mentioned, the redox potential can be expressed in terms of the fugacity of a proton paired to an electron. This proton is attached by weak forces, rather than covalent bonds. However, covalently bound protons also can affect the redox potential if the pH is shifted (20). Consequently, there are two effects present, and either will mask the other. For this reason explicit expressions will not be derived.

To appreciate the implications of these results, let us interpret p_1 as Pi, p_2 as ADP, and p_1p_2 as ATP; and let us express the fugacities in terms of solution concentrations as we did in equation IV.5. We shall simplify the expressions by holding the pH, and hence z_H, constant, and by expressing the coefficients as X_{11}, X_{12}, etc., instead of writing out the constants explicitly. We may now write the midpoint potentials as:

IV.A.5a $$V_X^o(1) = -(RT/F)\ \ln((1+X_{11}\langle Pi\rangle)/(X_{12}+X_{13}\langle Pi\rangle))$$

IV.A.5b $$V_X^o(2) = -(RT/F)\ \ln\left(\frac{1+X_{21}\langle ADP\rangle}{X_{22}+X_{23}\langle ADP\rangle}\right)$$

IV.A.5c $$V_Y^o(1) = -(RT/F)\ \ln\left(\frac{1 + Y_{11}\langle ATP\rangle}{Y_{12}+Y_{13}\langle Pi\rangle + Y_{14}\langle ATP\rangle}\right)$$

IV.A.5d $$V_Y^o(2) = -(RT/F)\ \ln\left(\frac{1 + Y_{21}\langle ATP\rangle}{Y_{22}+Y_{23}\langle ADP\rangle + Y_{24}\langle ATP\rangle}\right)$$

Since $\langle Pi\rangle \neq \langle ADP\rangle$, the midpoint potentials of X_1 and X_2, and of Y_1 and Y_2, will not, in general, be equal. This implies the apparent existence of four cytochromes instead of two. In addition, the midpoint potentials are dependent on $\langle Pi\rangle$, $\langle ADP\rangle$, and $\langle ATP\rangle$ and not on the phosphate potential, contrary to experimental observations. Consequently, this analysis is incomplete.

The reason that $V_X^o(1) \neq V_X^o(2)$ and $V_Y^o(1) \neq V_Y^o(2)$ is that we have tacitly assumed the 1 and 2 cytochromes to be distinguishable. Physically, this is an unrealistic assumption. A better assumption is that cytochromes X_1 and X_2 on the one hand, and Y_1 and Y_2 on the other, are physically indistinguishable. In that case we can only discuss $\langle e_X\rangle$ and $\langle e_Y\rangle$:

$$\langle e_X\rangle \equiv \langle e_X(1)\rangle + \langle e_X(2)\rangle$$

$$\langle e_Y\rangle \equiv \langle e_Y(1)\rangle + \langle e_Y(2)\rangle$$

If we attempt to solve for z_e we find

IV.A.6
$$\langle e_X \rangle = \sum_{i=1}^{2} \frac{C z_e \left[z_H \exp(-\beta E_{X e H}) + Z_{P_i} Z_H^2 \exp(-\beta E_{Z e P_i H}) + z_{P_i} \exp(-\beta E_{X e P_i}) \right]}{Z_{X(i)}}$$

By setting

IV.A.7
$$\begin{cases} Z_H \exp(-\beta E_{X e H}) = Q_1 \\ \exp(-\beta E_{X e P_1}) + Z_H^2 \exp(-\beta E_{X e_1 H}) = Q_2 \\ \frac{1}{Z_{P_1}} + Z_H \exp(-\beta E_{X P_1 H}) = Q_3 \\ \exp(-\beta E_{X e_2}) + Z_H^2 \exp(-\beta E_{X e P_2 H}) = R_2 \\ \frac{1}{Z_{P_2}} + Z_H \exp(-\beta E_{X e P_2 H}) = R_3 \end{cases}$$

we can simplify to

$$\langle e_X \rangle = \frac{C Z_e [Q_1 + Q_2 Z_{P_1}]}{Q_3 Z_{P_1} + Z_e [Q_1 + Q_2 Z_{P_1}]} + \frac{C Z_e [Q_1 + R_2 Z_{P_2}]}{Z_{P_2} R_3 + Z_e [Q_1 + R_2 Z_{P_2}]}$$

leading to

IV.A.8
$$0 = Z_e^2 (2C - \langle e_X \rangle)(Q_1 + R_2 Z_{P_2})(Q_1 + Q_2 Z_{P_1}) + Z_e (C - \langle e_X \rangle)\left[R_3 Z_{P_2} (Q_1 + Q_2 Z_{P_1}) + Q_3 Z_{P_1} (Q_1 + R_2 Z_{P_2}) \right] - \langle e_X \rangle Q_3 R_3 Z_{P_1} Z_{P_2}$$

If the measurements are made near the midpoint, $C \simeq \langle e_X \rangle$ and the middle term on the right is approximately zero. Then

IV.A.9
$$Z_e = \frac{Q_3 R_3 Z_{P_1} Z_{P_2}}{(Q_1 + R_2 Z_{P_2})(Q_1 + Q_2 Z_{P_1})} \frac{\langle e_X \rangle}{2C - \langle e_X \rangle}$$

Again expressing the fugacities as concentrations, and simplifying the coefficients, we find:

IV.A.10
$$V_x^\circ = -\frac{RT}{2F} \ln \frac{X_1 \langle P_1 P_2 \rangle}{X_2 + X_3 \langle P_1 \rangle + X_4 \langle P_2 \rangle + X_5 \langle P_1 P_2 \rangle}$$

Dividing numerator and denominator by $\langle p_1 \rangle \langle p_2 \rangle$, and setting p_1=Pi, p_2=ADP, and $\langle p_1 p_2 \rangle / \langle p_1 \rangle \langle p_2 \rangle = P$ results in

IV.A.11

$$V_X^o = -\frac{RT}{2F} \ln\left\{ \frac{X_1 P}{\frac{X_2}{\langle Pi\rangle\langle ADP\rangle} + \frac{X_3}{\langle ADP\rangle} + \frac{X_4}{\langle Pi\rangle} + X_5 P} \right\}$$

A similar argument for the Y cytochromes produces:

IV.A.12

$$V_Y^o = -\frac{RT}{2F} \ln \frac{\frac{1}{\langle Pi\rangle\langle ADP\rangle} + Y_1 P + Y_2 \langle Pi\rangle\langle ADP\rangle P}{Y_3 + \frac{Y_4}{\langle Pi\rangle} + \frac{Y_5}{\langle ADP\rangle} + Y_6 P + \frac{Y_7}{\langle Pi\rangle\langle ADP\rangle} + \frac{Y_8\langle Pi\rangle}{P^2} + Y_9\langle ADP\rangle P + Y_{10}\langle ATP\rangle}$$

The various undefined coefficients are easy to obtain and are omitted for brevity.

We now have midpoint potential expressions which depend upon the phosphate potential. Unfortunately, they depend upon other parameters as well. This is not necessarily fatal to the original pair theory, as one may assume that the contribution of these terms is small. However, this lends to unrealistic physical assumptions. As an example, to eliminate most of the single concentration terms one has to assume that the cytochromes work in perfect synchrony, that is, that Pi and ADP enter the system simultaneously, and then progress through the subsequent steps simultaneously.

Besides the difficulties with the phosphate potential measurements, the original pair theory has some other weaknesses; we will briefly discuss two of them. The first weakness concerns the reaction mechanism. The second weakness concerns the stoichiometry of the nucleotide carriers.

A complete energy coupling theory must contain a mole-

cular reaction mechanism for the phosphorylation reaction. At the current state of the art, it only seems possible to postulate general schemes; the molecular mechanisms can only be speculated. This is particularly evident in the two principal energy coupling theories, the chemical and the chemiosmotic.

In the chemical theory, energy is coupled through chemical reactions with a high energy intermediate. The specific mechanism cannot be stated without knowledge of this intermediate, and the weakness of the theory is that the intermediate has not been isolated. In the chemiosmotic theory electronic energy is first converted into concentration and potential gradients, and then these gradients power phosphorylation. At present, one can only speculate as to how gradients energize the phosphorylation reaction.

The original pair theory fits into this pattern. The model explains very well how energy may be transferred to ADP and Pi; it does not explain how the energized ADP and Pi are combined into ATP. Blondin and Green (3) have suggested that there is a specific ionophore which catalyzes the reaction; this must be considered speculation until some evidence for the existence of this ionophore is obtained. Until a detailed reaction mechanism is exhibited, the pair model cannot be said to have been demonstrated.

While the mechanism problem is not unique to the pair model, the phosphate carrier problem is. A quick examination of the mechanism of Figure 2 reveals that both Pi and ADP transport should be stoichiometrically coupled to electron transport. Unfortunately, there are data which are not easily reconciled with this requirement. For example, there is evidence that the ADP/ATP exchange is only partially electrogenic (64). Also, the drug atractyloside blocks ADP transport without uncoupling the phosphorylation of internal ADP (33, 64).

To these two difficulties we may now add the problem of the phosphate potential dependence of the midpoint potentials. These problems hav in common their origin in the coupling of the phosphate carriers to the electron transport chain. Therefore, we shall now investigate a modification of the pair model, which removes these objections, while retaining the pair model characteristics.

B. New Pair Model and Calculations

Our starting point is the suggestion by Williams (73) that the driving force in oxidative phosphorylation is a high local concentration of protons in the immediate vicinity of the phosphorylation site, with the electron transfer chain supplying the energy needed to maintain these "protons in the membrane". Since the acid catalyzed dehydration of Pi and ADP into ATP is a well-established che-

mical reaction (72), the problem of the reaction mechanism is taken care of nicely. The pair theory can provide a method to supply the energy necessary to deliver these protons to the F1 complex by modifying our model and taking p_1^+ and p_2^+ of Figure 9 to be "high energy protons" H_h^+. These protons are formally distinguished by the subscript h to indicate that they cannot be exchanged with a "low energy" solution proton H^+ without the energy being dissipated.

Since Pi and ADP no longer pair with the electron we have eliminated the problem of the phosphate carriers being stoichiometrically coupled to the electron transfer chain. Technically, we have a new problem, that of energizing these carriers. We know of no evidence that the carriers by themselves directly affect the redox potentials; therefore we shall ignore this complication in this paper.

We shall now explore the problem of the phosphate potential dependence by deriving the new expressions for the midpoint potentials. Since the positive charges are now exclusively protons, we have to consider a third cytochrome, Z, as in Figure 10. The problem of whether these cytochromes are arranged in scheme (A) or (B) of Section 4 no longer exists, as we no longer have two different positive charges p_1^+ and p_2^+. This means that if scheme (B) is in fact correct, and there are, for example, two "Y's"

FIGURE 10

Diagram of the modified pair theory. The diagram is constructed to emphasize the flow of the state, rather than the states themselves. A high energy proton, H_h^+, paired to an electron at X, is transferred to Y. There, the high energy proton is replaced with a low energy proton H^+. The high energy proton, now at the F1 complex, pairs with an anion a^- and is used to catalyze the formation of ATP. The H^+-e^- pair is transferred to Z, where H^+ is free to exchange with solution protons.

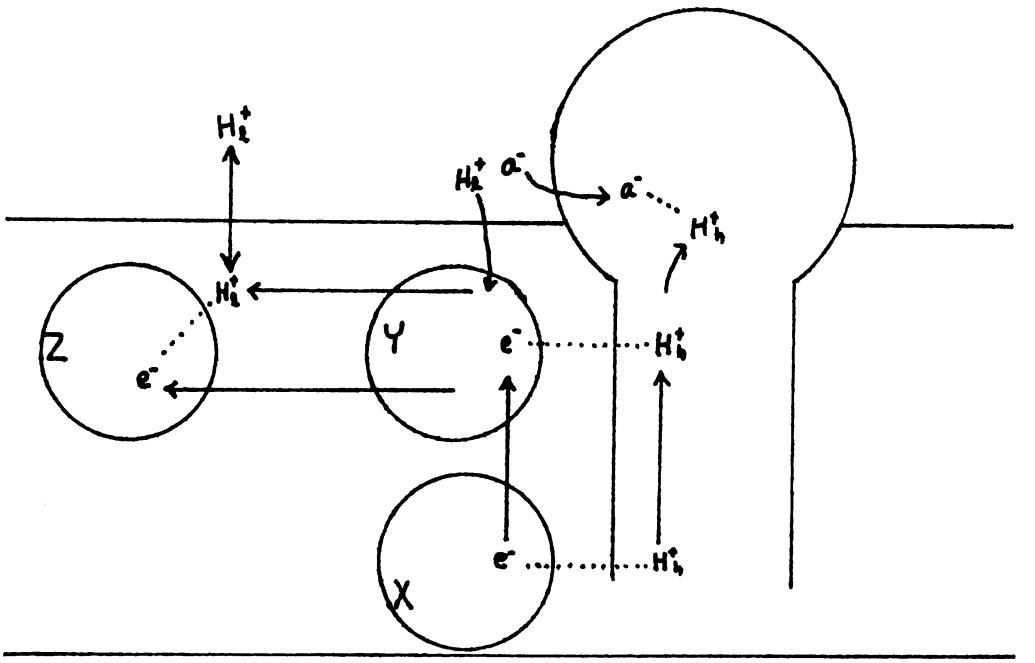


Figure 10

present in an electron transfer chain, that the same possible states exist for both "Y's", and in computing the grand partition function the only difference between a partition function based on scheme (A) and one based on scheme (B) would be in the interpretation of the cytochrome concentration factor. Since this interpretation is simpler in scheme (A), that scheme will be assumed.

The grand partition function now becomes:

IV.B.1 $$Z_g = (1+Z_e Z_h \exp(-\beta E_{x_{eh}}))^C (1+Z_e Z_h \exp(-\beta E_{y_{eh}}) + Z_e Z_h \exp(-\beta E_{y_{eh}}) + Z_e Z_l \exp(-\beta E_{y_{el}}))^C \cdot (1+Z_e Z_l \exp(-\beta E_{z_{el}}))^C$$

neglecting the possibility of two pairs being associated with Y for sake of simplicity. The previous arguments lead to the following expressions for the midpoint potentials:

IV.B.2a $$V_x^\circ = -\frac{RT}{F} \ln\left(1/Z_h \exp(-\beta E_{x_{eh}})\right)$$

IV.B.2b $$V_y^\circ = -\frac{RT}{F} \ln \frac{1 + Z_e Z_h \exp(-\beta E_{y_{eh}})}{Z_h \exp(-\beta E_{y_{eh}}) + Z_l \exp(-\beta E_{y_{el}})}$$

IV.B.2c $$V_z^\circ = -\frac{RT}{F} \ln\left(1/Z_l \exp(-\beta E_{z_{el}})\right)$$

At the F1 complex the following reaction occurs:

IV.B.3 $$k\, H_h^+ + Pi + ADP = ATP + kH^+$$

with the exact value of k depending upon the exact stoichiometry of the reaction. While many believe k to be 2 (33), Williams (72) believes 1 to be the correct value. For either case we may write

$$(z_{ATP}/z_{ADP} z_{Pi})(z_l/z_h)^k = K_p$$

or

IV.B.4 $z_h^k = z_\ell^k z_p / K_p$

with

IV.B.5 $z_p = z_{ATP}/z_{ADP} z_{Pi}$

Z_p is proportional to the phosphate potential P.

Substituting:

IV.B.6a $V_x^o = -\frac{RT}{F} \ln \frac{1}{A_1 z_p^{\nu_k}}$

IV.B.6b $V_y^o = -\frac{RT}{F} \ln \left\{ (1 + B_1 z_p^{\nu_k}) / (B_2 + B_3 z_p^{\nu_k}) \right\}$

IV.B.6c $V_z^o = -\frac{RT}{F} \ln C_1^{-1}$

with

IV.B.7a $A_1 = z_1 \exp(-\beta E_{x_{ch}}) / K_p^{\nu_k}$

IV.B.7b $B_1 = z_a z_1 \exp(-\beta E_{y_{ch}}) / K_p^{\nu_k}$

IV.B.7c $B_2 = z_1 \exp(-\beta E_{y_{cl}})$

IV.B.7d $B_3 = z_1 \exp(-\beta E_{y_{ch}}) / K_p^{\nu_k}$

IV.B.7e $C_1 = z_1 \exp(-\beta E_{z_{cl}})$

C. New Pair Model - Discussion

1. Phosphate Potential

In this model we can be more specific concerning the direction of the shifts. V_Z^o, not being associated with any H_h^+ containing pair, does not depend upon the phosphate potential. V_X^o varies from $-\infty$ to $+\infty$ as z_p varies from 0

to ∞, while V_Y^o varies from $-(RT/F)\ \ln\ (1/B_2)$ to $-(RT/F)$ $\ln\ (B_1/B_3)$. In the last case, the negative algebraic component of the shift is B_1, which arises from an alternative pairing for the oxidized cytochrome, as in case 3 of the simple model. There is a positive component to the shift, B_2, due to an alternative pairing of the reduced cytochrome, as in case 2 of the simple model. These same effects are present in the original pair model, but are more difficult to see.

To calculate the direction in which V_Y^o varies with the phosphate potential, let us first assume that $B_1/B_3 > 1/B_2$. From eq. IV.B.7 this inequality may be written:

$$\text{IV.C.1} \qquad \frac{Z_a Z_h \exp(-\beta E_{Y_{ah}})}{Z_h \exp(-\beta E_{Y_{eh}})} > \frac{1}{Z_l \exp(-\beta E_{Y_{el}})}$$

Thus far, all of the quantities involved in the inequality may be properly taken as constant. We now multiply both sides by z_e^{-1}:

$$\text{IV.C.2} \qquad \frac{Z_a Z_h \exp(-\beta E_{Y_{ah}}) Z_e Z_l \exp(-\beta E_{Y_{el}})}{Z_e Z_h \exp(-\beta E_{Y_{eh}})} > 1$$

z_e of course, depends upon a number of parameters, including the phosphate potential.

The left side of eq. IV.C.2 may be rewritten as

$$\frac{Z_a Z_h \exp(-\beta E_{Y_{ah}}) Z_e Z_l \exp(-\beta E_{Y_{el}})}{Z_e Z_h \exp(-\beta E_{Y_{eh}})} = \frac{\langle Y_{ah}\rangle \langle Y_{el}\rangle}{\langle Y_{eh}\rangle \langle Y_o\rangle}$$

as may be seen by calculating the expectation values from the partition function and forming their product. This product is analogous to the equilibrium constant for a chemical reaction, and may be interpreted the same way. The denominator contains the states of the system prior to energy transfer to the F1 complex, and the numerator those states present after energy transfer. Thus, the numerator must be larger than the denominator if forward electron transfer is to be the favored direction for the reaction. It is logical to assume the system to be so constructed that forward electron transfer is, in fact, the favored reaction, consequently, the numerator is larger than the denominator, eq. IV.C.2 is correct as written, and our initial assumption about the inequality was correct:

$$B_1/B_3 > 1/B_2$$

$$(RT/F) \ln B_1/B_3 > (RT/F) \ln 1/B_2$$

IV.C.3 $$V_Y^{o(p=\infty)} = -\frac{RT}{F} \ln \frac{B_1}{B_3} \quad < \quad -\frac{RT}{F} \ln \frac{1}{B_2} = V_Y^{o}(p=0)$$

Consequently, V_X^o increases with z_p, while V_Y^o decreases.

"X" represents a schematic cytochrome lying before a "phosphorylation site", "Y" the cytochrome associated with the site, and "Z" the cytochrome after the site. The modified pair theory predicts that the midpoint potentials should shift in opposite directions depending upon whether the cytochrome lies directly before the site, or

is associated with it. This shift results in X becoming a more effective electron acceptor and Y becoming a less effective electron acceptor upon increasing the phosphate potential, which will express itself by inhibiting forward electron transport at high phosphate potentials. This can explain respiratory control. Moreover, the cytochromes known to have phosphate potential dependent midpoint potentials, $\underline{b}_t$, $\underline{a}$, and $\underline{a}_3$, appear to be located where the direction of the their shifts would require: $\underline{b}_t$ before the site in complex III, $\underline{a}$ before the site in complex IV, and $\underline{a}_3$ at the site in complex IV (80).

A new problem, however, has arisen. The model predicts that for each phosphorylation site there should be two cytochromes with varying midpoint potentials, one increasing with the phosphate potential and the other decreasing. In complex III only $\underline{b}_t$ has been shown to have a phosphate potential dependent midpoint potential. Cytochrome $\underline{b}_k$ appears to be the electron acceptor from $\underline{b}_t$, and would thus correspond to the "Y" of our model. Its midpoint potential is unaffected by changes in the phosphate potential. There are two possible explanations for this discrepancy in the model. The first one is that there may be an interaction between the two cytochromes just as there is between $\underline{a}$ and $\underline{a}_3$. If this interaction was not accounted for, a shift in $\underline{b}_k$ could be ascribed to $\underline{b}_t$. The second possibility is that there is an as yet unidentified

electron carrier between $\underline{b}_t$ and $\underline{b}_k$ which accepts the electrons instead of $\underline{b}_k$.

We mentioned that the slope of the $\underline{b}_t$ midpoint potential versus log P plot was 60 mV/unit. The pair model predicts that the slope should be RT/kF. This implies that the value of k in eq. IV.B.4 should be 1. Since two protons are transferred per pair of electrons, this means one of the protons is dissipated, probably by equilibrating with the solution before it can be used to dehydrate Pi and ADP.

In complex IV, $\underline{a}_3$ corresponds to Y, while $\underline{a}$ corresponds to X. Most researchers agree that the midpoint potential of $\underline{a}$ depends upon the phosphate potential; what is debatable is the sign of the shift. Some investigators measure a positive shift, as the pair model predicts (75); others interpret the spectroscopy differently (71) and conclude that there is negative shift. The problem lies in the nature of the interaction between $\underline{a}$ and $\underline{a}_3$, and must at present be regarded as undecided (contrast 71 with 45 and 49).

The slope for cytochrome $\underline{a}_3$ requires a more involved analysis. The slope is predicted to be

$$\frac{\partial V_4^o}{\partial \ln Z_p} = \frac{RT}{F}(\beta_1\beta_2 - \beta_3) Z_p^{\nu_K} \left[k(1+\beta_1 Z_p^{\nu_K})(\beta_2 + \beta_3 Z_p^{\nu_K}) \right]$$

$$< \frac{RT}{F}(\beta_1\beta_2 - \beta_3) Z_p^{\nu_K} / (k\beta_1\beta_2 Z_p^{\nu_K})$$

Table 1

Midpoint potential changes in cytochromes with increasing phosphate potential

Cytochrome	Role	Predicted	Observed	k
b_t	X	+	+	1
b_k	Y	-	0	
a	X	+	±?	
a_3	Y	-	-	1

$$\text{IV.C.4} \quad = \frac{RT}{kF}\left(1 - \frac{B_3}{B_1 B_2}\right)$$

However, eq. IV.C.3 showed that B_3/B_1B_2 is less than one. Consequently, the right side of eq. IV.C.4 will be less than RT/kF, making a value of 40mV/unit reasonable for this expression, which corresponds to the "Y" cytochrome. This cytochrome corresponds to cytochrome a_3, which is the one with a slope of 40 mV/unit. These results are summarized in Table I.

2. Uncoupler Effects

We have mentioned that uncouplers affect the midpoint potential. To explain this, we must first describe how uncoupling occurs in the pair model. Since the energy is stored in a pair, the only way to dissipate that energy is to modify the pair. Thus, in the pair model, uncouplers are postulated to act by inducing the formation of a new pairing of the electron, a pair containing less energy than the normal pair. This new pair, while functionally part of the electron transfer chain, is unable to provide energy for oxidative phosphorylation. Thus uncoupling is really the substitution of one coupled process for another (4).

We will not specify anything about the nature of this new pair, except to list some possibilities. The electron could be paired with the uncoupler itself, most likely with the uncoupler functioning as an ionophore complexed to a cation (4). Or the uncoupler could modify the ener-

getics so that the electron now pairs to a "low energy" proton. Finally, the electron could pair to some other positive charge injected by the uncoupler (5).

The net result of these new pairs being formed is that we are left with a situation much like case 2 of the simple model, with uncoupler pairs and regular pairs competing. If in addition the anionic form of the uncoupler is also involved in a binding of the cytochrome, we have case 3 of the simple model. Since case 2 produces a positive shift, and case 3 a negative shift, an uncoupler can conceivably shift the midpoint potential in either direction.

Because this effect has not been studied in detail experimentally, we can only speak qualitatively about it, and hence we will not develop any formalism beyond that provided by the simple model. However, an examination of this model shows that the magnitude of the shift should depend upon the uncoupler fugacity, and therefore, upon the uncoupler concentration. This has, in fact, been observed experimentally (45).

D. Comparison with Other Models

Without going into great detail, we shall now discuss how the pair model relates to other explanations of the redox potential shifts. The most convenient way to organize these theories is to first consider the Nernst equation:

IV.D.1 $V = V^{o} + (RT/F) \ln (\langle ox \rangle / \langle red \rangle)$

If V shifts, there are clearly four possible explanations:

1) V^{o} has shifted

2) $\langle ox \rangle / \langle red \rangle$ has shifted

3) There is a missing term on the right which has shifted

4) Some combination of the above

We shall ignore possibility (4), as it adds nothing new, and look at (1) - (3).

The reversed-electron transfer theory belongs to class (3). As mentioned in the introduction, a redox system containing a redox couple in equilibrium with ATP synthesis and a mediator reacts with, but for the other cytochromes between the phosphorylation site and the mediator the correct relationship is:

IV.D.2 $$V = V^{o} + (RT/F) \ln(\langle ox \rangle / \langle red \rangle) \pm (V_{p}^{o} + (RT/F) \ln P)$$

where the + or - sign depends upon which side of the phosphorylation site the mediator is located.

This explanation is in contrast to that offered by the pair model, where it is assumed that z_e is known or, in experimental terms, that the electrode measures the "correct" potential. Therefore, the calculated shifts occur regardless which cytochromes the mediator react with. The effects produced by driving electron transport backwards are incorporated in the pair model. Changing the

phosphate potential will tend to drive the reaction forwards or backwards. This driving is the source for the shifts in the midpoint potentials through the pairings. The pairs do come to equilibrium but not the electrons by themselves.

This position disposes of the vexing question regarding the site of action of the mediator. Thus far there is no answer which is satisfactory to all experimentalists; the only point on which there is agreement being that the mediator cannot react with every cytochrome in the electron transfer chain without short-circuiting the entire system. We have to be careful by what we mean by "reaction" here. If the mediator is capable of transferring electrons between different cytochromes short-circuiting indeed will take place. The transfer, however, will be effective only if it goes from pair states to pair states and moving only electrons will not accomplish this. Unless the possibility of pairing for the transferred electron and its abandoned partner are simultaneously offered, there will be no short-circuit even though the mediator "reacts" with every cytochrome.

One objection against the reverse electron theory is that if the mediators react with a specific cytochrome, then all cytochromes on the same side of that cytochrome should show identical P dependent shifts in midpoint potential. This objection does not apply to the pair model, because

if the electron is paired to a low energy proton, i.e. one which can equilibrate with the solution without dissipating free energy, its midpoint potential is independent of the phosphate potential. We have chosen the corresponding cytochrome Z to be after the transducing cytochromes, but it could have been placed in front as well.

The other common explanations fall under class (2) in the above scheme. With them there exists a second form of the cytochrome, which greatly increases in concentration upon energization of the mitochondrion. Since the two forms are spectroscopically indistinguishable, this means that the logarithmetic term in eq. IV.D.1 should actually be written

IV.D.3 $(RT/F)\ \ln\ (\langle ox\rangle_1 + \langle ox\rangle_2/\langle red\rangle_1 + \langle red\rangle_2)$

However, the two forms are in equilibrium with one another, and IV.D.3 can be solved in terms of the phosphate potential and whichever cytochrome reacts with the mediator, giving an expression similar to eq. IV.B.6b.

Formally, this explanation is close to the pair model, as the pair model assumes alternative pairings. However, there is a physical distinction, in that these alternative pairs do not result in a second chemically distinct form of cytochrome. This is because the pairs involve weak bonds, and chemical distinctions require chemical bonds. Thus one should expect that the absorption spectra should not reveal these alternative pairs, while one must wonder

why the alternative cytochromes have not been seen.

Indeed, the pair model falls under class (1) of our scheme. This seems paradoxical, since V^o is a constant for simple systems. Actually, V^o is constant for a given specification of a cytochrome, but we have shown that the pair theory requires that the number and energy states of all pairs be known to fully specify a cytochrome. Modifying the cytochrome by introducing new pairs changes its mid-point potential. We may consider the pair model to unite the features of both the two cytochrome and reverse electron transport theories into a unified, more general model.

V. FURTHER SPECULATIONS AND SUGGESTIONS FOR EXPERIMENTALISTS

By this time it should be apparent that the data are capable of a variety of interpretations. Some of the ambiguities can only be resolved by further experimentation; others could not be discussed earlier because a complete analysis needed the formalism of the pair model. We shall now take a look at some of these ambiguities.

A. "Real vs. "Apparent" Redox Shifts

Not surprisingly, the most important experimental question has not yet been resolved. This is the question of whether the shifts occur through reversed electron transport, with the mediator reacting only with cytochrome c instead of a or b; or whether the shifts are real, with the mediator reacting with the desired cytochrome. We have previously discussed how the known experimental data can be interpreted in terms of either theory. This leaves the decision between the two interpretations to be made on the basis of probability and consistency with other theories.

To understand why we are so pessimistic, let us consider the two most direct approaches to the problem. First,

let us try to demonstrate experimentally that cytochrome b reacts with a mediator. To do this, we must experimentally extract cytochrome b from the mitochondria. Thus far, it has not been possible to extract b in an active form, so this experiment is impossible at the present atate of the art. However, even if this were possible, it would not be conclusive, since the extracted b would be independent of the membrane, and the membrane undoubtably contributes to the necessity of using mediators. Consequently, no experiments done on membrane free cytochromes can be conclusive.

These considerations lead to the second approach: to remove all cytochromes but b from the mitochondrion. Again, however, only cytochrome c is readily extractable from mitochondria. Consequently, if the same shifts occur in the extracted mitochondrion nothing is proved, since one can argue that the mediator is still not reacting with b but with some component other than c. Conversely, if the chifts now disappear, it does not necessarily mean that the mediator only reacts with c, since one can argue that the salt solution used for extraction denatured the membrane so that the b-mediator reaction can no longer occur.

Therefore, as we have suggested earlier, the decision between real and apparent redox shifts must be made on the basis of probabilities. On this basis, we feel that the arguments put forth for real redox shifts are stronger

than those for apparent shifts. In particular, the observation that not all electron carriers show a shift, while the reversed electron transfer theory suggests that they should (35), is a particularly strong argument, stronger than the arguments of Slater and Wikstrom.

However, on the basis of consistency with energy coupling theories the reversed electron transport theory becomes strong indeed. The reason is that this theory is independent of the mechanism of energy coupling, meaning in particular that it is consistent with the chemiosmotic theory. The alternative theories we have divided into class 2 and class 1 theories. The class 2 theories all involve the cytochrome in the energy transduction process, meaning that they are variations of the chemical theory. The class 1 theory is the pair model. This means that the chemiosmotic theory is consistent only with the reversed electron transport explanation for the redox shifts. Therefore, if one believes the discussion of the redox shifts, one is led to doubt the chemiosmotic hypothesis.

B. Semi-reversed Electron Transport Theory

Wikstrom's latest theory is that a real shift is occuring with the a cytochromes, but that the b cytochromes display reversed electron transport. The reason for this is that Wikstrom now believes that phosphorylation in complex IV takes place between cytochrome a_3 and

molecular oxygen, rather than between a and a_3. This means that the redox reaction between cytochrome c and a_3 is no longer considered to be in equilibrium with the phosphorylation reaction, and there is nothing left to reverse. Wikstrom now explains the redox shifts as being due to conformational changes in the cytochromes, the changes' function being respiratory control (70).

We have earlier discussed how Wikstrom and Chance have reinterpreted the spectroscopic data. What needs to be added here is an acknowledgment that the pair model worked out in Section IV is inadequate insofaras the cytochrome oxidase data are concerned, since it does not incorporate interaction effects. The next step in the development of the pair theory is to remove this restriction and see if the behavior of cytochrome oxidase can be satisfactorily modeled. Without actually performing the calculation, we will sketch out how this model may be constructed.

We have written the partition function for a two cytochrome system containing but one class of pair as

$$Z_\phi = \sum_{j=0}^{c} \sum_{i=0}^{c} \frac{c!}{(c-i)!\,i!} z^i e^{-\beta E_i} \frac{c!}{(c-j)!\,j!} z^j e^{-\beta E_j}$$

with Ei=iEx where Ex is the energy of one pair on cytochrome X, and similarly for Y. Since the terms containing i are separate from those containing j, we may sum the partition function using the binomial theorem. However, this separability is the result of our assumption regarding

the lack of interactions. A more general expression is to write

$$E_X = E_X + E_{XY}$$

where E_X = as before, and E_{XY} is the energy change due to having a pair on Y. One can replace these in the partition function, and calculate a new function , which includes interaction effects.

The difficult part is to write the correct form for E_{XY}. It will have to depend upon the spin state of Y, as well as on any ligands which may be present. Once constructed, however, the free energy of X follows immediately, from which one may predict the spectra of X. And by repeating our calculations one may compute the redox potential of X.

C. Phosphate Potential Dependence

We have presented two pair models, differing observationally in the redox potential dependence of the phosphate potential. We argued that the modified pair theory was the more plausible partly because its redox potentials depended strictly upon the phosphate potential and not on Pi, ADP, or ATP by themselves, and that this was what the data are. However, our suspicious minds keep thinking that the reason no data have appeared showing dependence on individual nucleotides is because no experimentalist believed that such could be the case, and thus all such

data have been surpressed. This is a point which can easily be proven by experimentation.

D. Uncoupler Effects

Equally untouched experimentally are the effects of uncouplers upon the redox potential. Thus far uncouplers have been used in experiments srtictly as a control; to demonstrate that a particular effect is due to redox energy. While uncoupler effects have been reported, there has not been any place for them in the energy coupling theories. The pair model does have a place for uncoupler effects however, and they no longer can be ignored.

We explained that the pair model views uncouplers as providing an alternative partner for the electron. This new pair should show all of the effects the usual pair does, in particular, its redox energy should depend upon the uncoupler concentration. More experiments along these lines are in order.

Fianlly, our new model removes one of the objections which uncoupler experiments have raised against the pair theory, that of maximum uncoupler to phosphorylation site ratios of less than one. In the original formulation (3), where the uncoupler molecule itself had to pair with the electron, these ratios were difficult to reconcile. In the new model, where either high or low energy protons pair with the electron, all the uncoupler need do is amplify

the natural source of low energy protons. There are many ways in which this can be accomplished.

E. Conclusions

The new pair model, related to the proton in the membrane hypothesis of Williams, has been shown to be generally consistent with the experimental data concerning the phosphate potential and uncoupler effects upon the midpoint potentials of cytochromes. The model's strongest point is that it makes midpoint potential shifts a natural feature of energy transduction. As with all theories proposed to date, some ad hoc postulates are necessary, suggesting that more information is needed before a completely definitive theory can be proposed. Such a theory would have to consider the interaction of several electrons with each other.

APPENDIX

APPENDIX I

ON ACTIVITY COEFFICIENTS

I. Problem:

Given a reaction system $A() + B(e) \rightleftharpoons A(e) + B()$

We can calculate the half-cell redox potential by

$$V_A = V_A^\circ + \frac{RT}{nF} \ln \frac{a_{A()}}{a_{A(e)}}$$

where a = the activity of the species in question.

Now upon the addition of ½mM of Uncoupler U, or ATP, we observe that for the same concentrations of A() and A(e) we now measure V_A° . Writing the activities as the product of an activity coefficient γ and a concentration, this effect can be described as

$$V_A' = V_A^\circ + \frac{RT}{nF} \ln \frac{\gamma_{A()}[A()]}{\gamma_{A(e)}[A(e)]} + X$$

II. Explanation:

Now this can be explained in two ways:

A) The added chemical causes $\gamma_{A()}$ to shift to $\gamma_{A()}+\delta$, resulting in

$$V_A' = V_A^\circ + \frac{RT}{nF} \ln \frac{\gamma_{A()}[A()]}{\gamma_{A(e)}[A(e)]} + X$$

$$\text{WITH } X = \frac{RT}{nF} \ln \frac{\delta[A()]}{\gamma_{A(e)}[A(e)]}$$

B) V_A° shifts. This implies a change of some sort in at least one of the species, say A(). Thus A() becomes A'().

However, $\gamma_{A(\cdot)} \approx \gamma_{A'(\cdot)}$ and $[A(\cdot)] = [A'(\cdot)]$ so the sole observable effect is in V_A^o.

$$V'_A = V^{o\prime}_A + \frac{RT}{nF} \ln \frac{\gamma_{A(\cdot)}[A(\cdot)]}{\gamma_{A(\omega)}[A(\omega)]}$$

III. Reasons for Preferring B) over A):

In the absence of independent information about , there is no way of confirming one explanation and denying the other. Any decision to use one scheme over the other is to an extent arbitrary. However, there are some reasons for preferring B) to A).

1) Perfect solutions (Solutions obeying the same assumptions as ideal gasses, eg., no interactions) can be described by the equation

$$\mu_i = \mu^o_i + RT \ln [i]$$

This is an exact equation for these assumptions. Activity coefficients do not occur. In this kind of redox reaction any change in V_A has to involve V_A^o because under these assumptions there is nothing to change in the logarithmic term.

Now, the point is that the pair model we have developed contains the assumption of no interactions. Yet, this model does predict a shift in V_A, with the shift appearing in the V_A^o term. It seems unnatural to try to force this shift into a change in activity coefficients.

2) The change will have to be in the wrong direction.

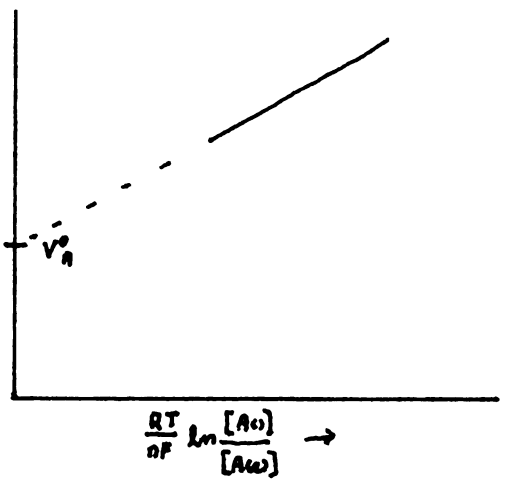
FIGURE 11

Method of computation of midpoint potentials.

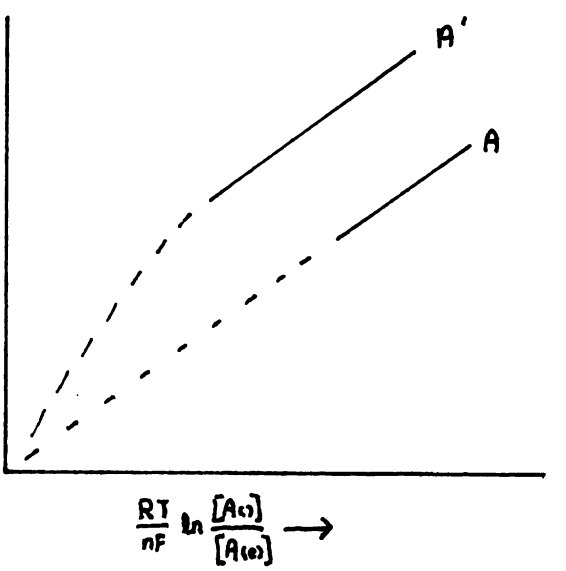
a) Extrapolation to equal concentrations.

b) Extrapolation to equal concentrations and zero concentrations for A'() and A().

A)



B)



To see this, we need to understand how midpoint potentials are actually computed. A collection of V_A^o vs. $\ln \frac{[A()]}{[A(e)]}$ values are measured, and V_A plotted against $\frac{RT}{nF} \ln \frac{[A_{ox}]}{[A_{red}]}$, as in Figure 11a. The value of V where $\frac{[A()]}{[A(e)]} = 1$ is approximately V_A^o, and would be exactly V_A^o if $\gamma_{ox}/\gamma_{red} = 1$.

Now the exact way to calculate V_A^o is to repeat the calculation for different concentrations of $[A()] = [A(e)]$, and extrapolate to zero concentration where the activity coefficient is defined to be unity. This has not been done for our system. However, one can easily imagine how the results would have to look in order for the actual midpoint potentials to be identical under the two conditions. This has been done in Figure 11b, where it is seen that adding ATP or Uncoupler causes the extrapolated curve to shift markedly. This implies that the magnitude should be dependent upon the value of $[A()] = [A(e)]$, which has not been observed.

APPENDIX II

Table 2

Table of Experiments with Redox Shifts

System	V^o ATP	Uncoupled	Mediator	Uncoupler	Additional Components of System	Ref.
Spinach Chloroplast b_6	5	-140	1,2 naphthoquinone 1,4 naphthoquinone 2,5 dihydroxybenzequinone 2-hydroxy-1, 4-naphthoquinone	FCCP NH_4Cl	unstated	5
Rat liver cyt. a	230	245	midpoint potentials were estimated from transmembrane potentials	Choline chloride	.135 KCl or .135 Choline Cl .25M Sucrose 3mM glycylglycine	26
	205	245		FCCP		

System	V^o ATP	Uncoupled	Mediator	Uncoupler	Additional Components of System	Ref.
Rat liver b_t	245	-30	TMPD PMS	5-Cl,3-(p-phenyl) 2',4',5'-trichloro-salicylanilide	.22M mannitol .05M Sucrose .015M K morphlolinopropane sulfonate 5uM rotenone	76
Beef heart b_k	118	154	DAD PES	FCCP	.25M Sucrose	2
" " b_t	270	-28	duroquinone		.05M tris .001M EDTA	
" " c	lower	higher	pyocyanine		5uM rotenone	
Pigeon heart submito. b_k	30	30	DAD PMS	pentachlorophenol	.023M mannitol	37
" b_t	230	-30	PES DOQ		.007M Sucrose	
" c	235	235	pyocyanine 2 hydroxy-1-4 naphthoquinone			

System	V^o ATP	Uncoupled	Mediator	Uncoupler	Additional Components	Ref.
Pigeon heart *a*	260	215	PMS DAD	FCCP	.2M mannitol .04M Sucrose 600uM EDTA	75
" " a_3	155	385				
Pigeon heart a_3	260	220	as ref.59	as ref.59	as ref.59	36
Pigeon heart a_3	155	375				
Iron sulfur 1 complex I	-405	-305	PES duroquinone pyocyanine 2OH-naphthoquinone phenosafranine benzyl viologen methylviologen	FCCP	unstated	46
Cyt.*c* Beef Heart	280	235	DAD PMS	None; '-' charged phospholipids added	.225M mannitol .075M Sucrose .020M MOPS buffer no ATP	62
Cyt.*c* Horse Heart	285	240				
Cyt.*c* Baker's Yeast	255	235				
Cyt.*c* *Candida Mycoderna*	275	240				

APPENDIX III

LIST OF SYMBOLS

Symbol	Meaning	Page Introduced
A(),A(e)	Oxidized or reduced electron carrier	11
ADP	Adenosine Diphosphate	1
ATP	Adenosine Triphosphate	1
e^-	unpaired electron	10
E_i	Free energy of particle 'i'	43
F	Faraday's Constant	21
F1	ATP-ase Complex	2
G_i	Free energy change in species 'i'	8
I	Intermediate in chemical theory	11
K_i	Equilibrium constant of reaction number 'i'	20
L	Ligand in Wilson's theory	42
M	Mediator	29
N_i	Number of particles of type 'i' present in ensemble	43
P	Phosphate Potential	8
Pi	Inorganic Phosphate	1
R	Gas Constant	8
T	Absolute Temperature	8
V, V^o	Redox, or midpoint, potential	21
Z_i	Grand partition function of system i	43

Ψ	Transmembrane potential	28
μ_i	Chemical potential of species 'i'	8
ν_i	Stoichiometric coefficient	24
χ	Chemiosmotic correction factor	28
$\sim$	High energy bond symbol	11

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