

# OXIDATIVE PHOSPHORYLATION: THERMODYNAMIC CRITERIA FOR THE CHEMICAL AND CHEMIOSMOTIC HYPOTHESES\*

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*Abstract.*—Oxidative phosphorylation is analyzed by means of nonequilibrium thermodynamics. It is shown that a mitochondrial system may be characterized in terms of the externally fixed affinities (negative free energies) for oxidation and phosphorylation  $A_o^{ez}$  and  $A_p^{ez}$ , without knowledge of internal activities. If the electrochemical potential difference of  $H^+$  is also fixed, a decision can be made between the chemiosmotic and chemical hypotheses. The chemiosmotic hypothesis is shown to be a limiting case of the chemical hypothesis. In general the  $P/O$  ratio cannot be expected to be constant, but will vary with  $A_p^{ez}/A_o^{ez}$ ; for certain ranges the variation may be marked.

*Introduction.*—Experimental and theoretical difficulties have prevented a decision between the chemical and chemiosmotic models of oxidative phosphorylation.<sup>1-6</sup> Much of this difficulty results from the analysis of energetics in terms of equilibrium thermodynamics. As Lehninger, Carafoli, and Rossi have pointed out, "It is misleading. . . to think of the [chemiosmotic] hypothesis in terms of equilibrium thermodynamics. . . . The processes implicit in the chemiosmotic hypothesis are more properly analyzed by nonequilibrium thermodynamic principles."<sup>7</sup> Equilibrium considerations, as well as the assumption of stoichiometry, have often led to emphasis on the magnitudes of forces (e.g., "phosphate potential," pH gradients) in the evaluation of different models. On the other hand, much experimental work centers on the determination of flows alone. The pertinent consideration is not the magnitudes of either forces or flows, but rather the rates of input and output of energy.<sup>8, 9</sup> To quote Lehninger *et al.* again, "The potential work that can be performed by a pH gradient in a nonequilibrium system is a function of the product of the flow and the gradient."<sup>7</sup>

We present here a nonequilibrium thermodynamic analysis and criteria which do not depend on the isolation of an intermediate or the measurement of a component of  $H^+$  flow used for phosphorylation. Although it is necessary to determine the electrochemical potential difference across the mitochondrial membrane  $\Delta\tilde{\mu}_H$ , which we believe to be measurable,<sup>10, 11</sup> the other parameters are evaluated externally.

*Nonequilibrium Thermodynamics of Oxidative Phosphorylation.*—The starting point for a thermodynamic formulation is the specification of the steady-state dissipation function  $\Phi$ , which gives the rate of dissipation of free energy and thus defines the pertinent flows and forces. For the models considered here, under isothermal conditions, it is reasonable to write

$$\Phi = J_P A_P + J_H \Delta\tilde{\mu}_H + J_O A_O. \quad (1)$$

Here the  $J$ 's are generalized flows, and the subscripts  $P$ ,  $H$ , and  $O$  refer, respectively, to phosphorylation, net  $H^+$  flow, and substrate oxidation. The  $A$ 's are the affinities of the chemical reactions,<sup>12, 13</sup> ordinarily the negative free-energy changes per mole. Despite the complexity of the system, equation (1) is adequate; we consider only steady states in which flows such as those of  $Ca^{++}$ ,  $Mg^{++}$ , and  $H_2O$  will have come to a halt.<sup>14</sup>

The above dissipation function is ambiguous as to whether the affinities are to be measured internally or externally. Furthermore, since the flows may be non-conservative, i.e., a substance may be metabolized within the composite membrane, the rate at which substances enter at one surface may not equal the rate at which they leave at the other. Clearly, it would be of great convenience to evaluate the affinities and the reaction rates externally, since internal activities are difficult to determine. Fortunately, this is found to be appropriate (see Appendix I), provided that  $J_H$  is taken as the rate of decrease of  $H^+$  content inside the mitochondrion, and  $\Delta\tilde{\mu}_H$  is defined as  $\tilde{\mu}_H^{in} - \tilde{\mu}_H^{ex}$ . (In the absence of a reaction involving  $H^+$  in the interior of the mitochondrion,  $J_H$  is simply the net outward flux across the inner surface of the membrane.)

Having interpreted our dissipation function in this way, we can now write phenomenological equations:

$$J_P = L_{PA}A_P^{ex} + L_{PH}\Delta\tilde{\mu}_H + L_{PO}A_O^{ex}, \quad (2)$$

$$J_H = L_{PH}A_P^{ex} + L_H\Delta\tilde{\mu}_H + L_{OH}A_O^{ex}, \quad (3)$$

$$J_O = L_{PO}A_P^{ex} + L_{OH}\Delta\tilde{\mu}_H + L_OA_O^{ex}. \quad (4)$$

This general formalism should be applicable near equilibrium whichever hypothesis is correct. For simplicity we have made the usual assumptions of Onsager symmetry, i.e.,

$$L_{ij} = L_{ji}, \quad (5)$$

and linearity.<sup>13, 15</sup> However, the criteria presented are not affected by these assumptions.

As shown in Appendix I, we could have expressed the dissipation function in terms of external flows and internal affinities. This would be impracticable for two reasons. Firstly, there are obvious difficulties in evaluating internal affinities.<sup>16</sup> Secondly, it would be necessary to consider many flows and forces, even when internal concentrations are constant.

*Chemical Hypothesis* (Fig. 1).—In this view substrate oxidation produces a high-energy intermediate, which can drive either phosphorylation or outward  $H^+$  transport (and possibly cation transport). Thus  $J_O$  is positively coupled to both  $J_P$  and  $J_H$ . Since outward  $H^+$  transport may also result from the hydrolysis of ATP,  $J_P$  and  $J_H$  are negatively coupled. It is clear that each flow may be influenced by every force; in phenomenological terms, all coefficients of equations (2)–(4) are nonzero in the most general case. Partial uncoupling of oxidation and phosphorylation could result from breakdown of the high-energy intermediate or by other side reactions or leaks.<sup>8</sup>

*Chemiosmotic Hypothesis* (Fig. 2).—Site I is formally equivalent to Mitchell's

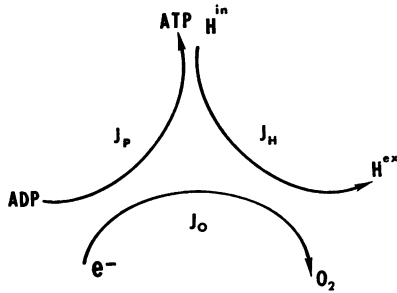


FIG. 1.—Chemical hypothesis.

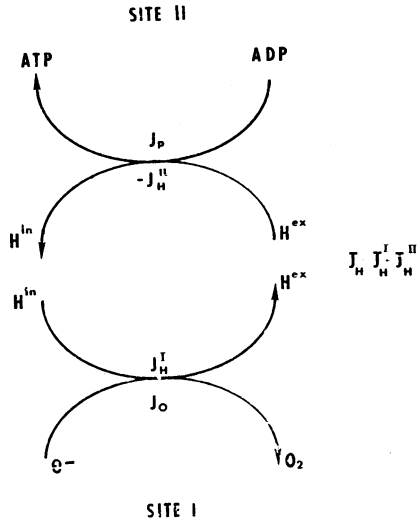


FIG. 2.—Chemiosmotic hypothesis.

“proton-translocating oxido-reduction chain,” site II to his “proton-translocating ATPase system.”<sup>3, 4</sup>  $J_P$  and  $J_O$  are coupled only through circulation of  $H^+$ , rather than through a high-energy intermediate.

Equations (2)–(4) are again applicable. However, in this case we can characterize the over-all coefficients in terms of those of the sites.

Site I:

$$J_H^I = L_H^I \Delta\tilde{\mu}_H + L_{OH}^I A_{O^{ez}} \tag{6}$$

$$J_O = L_{OH}^I \Delta\tilde{\mu}_H + L_O^I A_{O^{ez}} \tag{7}$$

Site II:

$$J_P = L_P^{II} A_P^{ez} + L_{PH}^{II} \Delta\tilde{\mu}_H \tag{8}$$

$$J_H^{II} = L_{PH}^{II} A_P^{ez} + L_H^{II} \Delta\tilde{\mu}_H \tag{9}$$

Thus, at site I oxidation results in the transport of  $H^+$  against  $\Delta\tilde{\mu}_H$ , while at site II the spontaneous flow of  $H^+$  results in phosphorylation.

The net flow of  $H^+$  is given by

$$J_H = J_H^I + J_H^{II}, \tag{10}$$

so that adding equations (6) and (9) gives

$$J_H = L_{PH}^{II} A_P^{ez} + (L_H^I + L_H^{II}) \Delta\tilde{\mu}_H + L_{OH}^I A_{O^{ez}} \tag{11}$$

Equations (8), (11), and (7) correspond, respectively, to equations (2), (3), and (4). The straight coefficients  $L_O$  and  $L_P$  and the cross coefficients  $L_{OH}$  and  $L_{PH}$  are simply those of the appropriate elemental sites, while  $L_H$  is the sum  $L_H^I + L_H^{II}$ . The important observation is that

$$L_{PO} = 0 \text{ (chemiosmotic hypothesis),} \tag{12}$$

i.e., if  $\Delta\tilde{\mu}_H$  were maintained constant, phosphorylation and oxidation would be independent. In particular, if  $\Delta\tilde{\mu}_H = 0$  uncoupling is complete, and  $J_P = 0$  if  $A_P^{ez} = 0$ , irrespective of  $J_O$ , while  $J_O = 0$  if  $A_O^{ez} = 0$ , irrespective of  $J_P$ .<sup>17</sup>

*Degree of Coupling and Effectiveness of Energy Utilization.*—A normalized dimensionless parameter, useful in evaluating the effectiveness of energy utilization, is the “degree of coupling.”<sup>18, 19</sup> This is most conveniently defined in terms of resistance coefficients:

$$q_{ij} = -\frac{R_{ij}}{\sqrt{R_i R_j}}; \quad (13)$$

$q_{ij}$  measures the extent to which the  $i$ th flow “drags” the  $j$ th flow when the  $j$ th force and other flows are zero. For any pair of flows

$$-1 \leq q \leq 1. \quad (14)$$

For complete coupling (stoichiometry),  $q = \pm 1$ ; for complete uncoupling,  $q = 0$ . Although the efficiency of energy conversion depends on both  $q$  and the forces, the maximum efficiency is uniquely determined by  $q$ . Furthermore,  $q$  influences the efficacy with which energy can be utilized to establish a force, e.g., a concentration gradient or a reaction affinity.<sup>13, 18</sup>

For oxidative phosphorylation we are concerned with  $q_{PH}$ ,  $q_{OH}$ , and  $q_{PO}$ . These are readily derived from the inverse phenomenological equations:

$$A_P^{ez} = R_P J_P + R_{PH} J_H + R_{PO} J_O, \quad (15)$$

$$\Delta\tilde{\mu}_H = R_{PH} J_P + R_H J_H + R_{OH} J_O, \quad (16)$$

$$A_O^{ez} = R_{PO} J_P + R_{OH} J_H + R_O J_O. \quad (17)$$

If the chemiosmotic hypothesis holds, a special relationship applies, viz.:<sup>20</sup>

$$q_{PO} = -q_{PH} q_{OH}. \quad (18)$$

(Since  $J_P$  and  $J_H$  are negatively coupled,  $q_{PH}$  is negative.) Whenever such a relationship holds, a 3-flow system is equivalent to two 2-flow systems sharing a circulating flow. Thus, equation (18) is both a necessary and a sufficient condition for the chemiosmotic hypothesis.

*Discussion.*—The above treatment should be applicable to the steady-state analysis of oxidative phosphorylation in a variety of experimental circumstances, irrespective of the detailed mechanisms. (A similar treatment may be applicable to photosynthetic phosphorylation.) It is simplest to consider situations in which the dissipation function is reduced to two terms, so that each flow depends on only two forces. It is then useful to consider the degree of coupling which determines the effectiveness of energy utilization under the specific conditions considered.<sup>19, 21</sup>

If  $A_P^{ez}$  and  $A_O^{ez}$  are fixed at any desired values,  $J_H$  will become zero in the stationary state. For this case  $q = q_{PO}$  (Appendix II). As is shown in Figure 3,  $J_P/J_O$  is in general a function of the ratio of the forces; true stoichiometry exists only for the limiting case  $q = 1$ . The region of primary interest is the left upper quadrant, where oxidation “drives” phosphorylation. In this region, even for

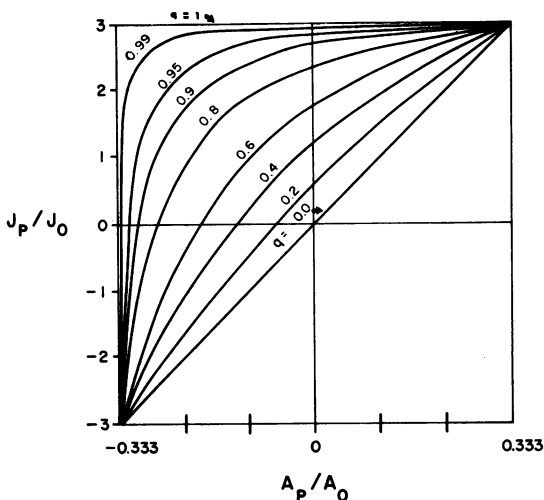


FIG. 3.—Dependence of the  $P/O$  ratio ( $J_P/J_O$ ) on the ratio of reaction affinities ( $A_P^{ez}/A_O^{ez}$ ). In the left upper quadrant oxidation “drives” phosphorylation (for complete coupling the  $P/O$  ratio has been taken to be 3).

(a)  $J_H = 0$ :  $q = q_{PO}$ ; (b)  $\Delta\tilde{\mu}_H = 0$ :  $q = (q_{PO} + q_{PH}q_{OH})/\sqrt{(1 - q_{PH}^2)(1 - q_{OH}^2)}$ .

high degrees of coupling, stoichiometry is approached only in a limited range. Outside this range marked deviations can occur.

Figure 3 also applies to the case where  $\Delta\tilde{\mu}_H$  is maintained at zero. In this case  $q = (q_{PO} + q_{PH}q_{OH})/\sqrt{(1 - q_{PH}^2)(1 - q_{OH}^2)}$  (Appendix II). Introducing equation (18) shows that for the chemiosmotic hypothesis, setting  $\Delta\tilde{\mu}_H = 0$  uncouples oxidation from phosphorylation, so that in the range where the phenomenological equations are linear  $J_P/J_O$  is directly proportional to  $A_P^{ez}/A_O^{ez}$ . With nonzero but constant  $\Delta\tilde{\mu}_H/A_O^{ez}$ , proportionality will be lost, but linearity retained. To the extent that the relation between  $J_P/J_O$  and  $A_P^{ez}/A_O^{ez}$  becomes nonlinear (i.e.,  $q$  differs from zero) the system deviates from the chemiosmotic theory, which is therefore seen to be a limiting case. If the system is not chemiosmotic (i.e.,  $L_{PO} \neq 0$ ), oxidation would drive phosphorylation as shown in the left upper quadrant, even when  $\Delta\tilde{\mu}_H = 0$ . If all three cross-coefficients were nonzero, a combination of mechanisms would be operative.<sup>8</sup>

Obviously there are substantial experimental difficulties in these applications of the phenomenological equations. However, it should be possible to vary and measure the affinities  $A_O^{ez}$  and  $A_P^{ez}$  over a considerable range. Furthermore, it seems that both the pH difference and the electrical potential difference may be evaluated and regulated in functioning mitochondria.<sup>10, 11</sup> Although it may be difficult to fix  $\Delta\tilde{\mu}_H$  exactly at zero, it seems possible to make it small.<sup>11</sup>

Many workers have emphasized the significance of stoichiometric ratios in the thermodynamic analysis of oxidative phosphorylation (see, for example, refs. 2, 4–6). Although specific mechanisms may suggest stoichiometric ratios, they are not required by thermodynamic considerations. An instance of the use of stoichiometry is the attempt to calculate the magnitude of  $\Delta\tilde{\mu}_H$  necessary for

chemiosmotic oxidative phosphorylation. This calculation requires knowledge of  $J_H^{II}$ , the rate of hydrogen transport at the phosphorylation site, under the conditions of the experiment. It should not be assumed that  $J_H^{II}$  is a constant and integral multiple of  $J_P$ .<sup>8</sup>

*Appendix I.*—We consider the mitochondrial oxidation of a substrate to a product. The steady-state rate of dissipation of free energy isothermally is

$$\Phi = -\sum_j (\dot{n}_j^{in} \tilde{\mu}_j^{in} + \dot{n}_j^{ex} \tilde{\mu}_j^{ex}), \quad (\text{A1})$$

in which  $\tilde{\mu}_j$  and  $n_j$  represent electrochemical potential and number of moles of species  $j$  ( $\dot{n}_j = dn_j/dt$ ). The superscripts *in* and *ex* refer, respectively, to the interior of the mitochondria and the external medium. The species  $j$  include substrate, product,  $O_2$ ,  $CO_2$ ,  $H_2O$ , ATP, ADP,  $P_i$ ,  $H^+$ , and possibly additional components such as  $K^+$ ,  $Ca^{++}$ , and  $Mg^{++}$ . Designating the  $r$ th metabolic reaction by  $J_r$  and the stoichiometric coefficients by  $\nu_{jr}$ ,

$$\sum_r \nu_{jr} J_r = \sum_r (\dot{n}_{jr}^{in} + \dot{n}_{jr}^{ex}) = (\dot{n}_j^{in} + \dot{n}_j^{ex}). \quad (\text{A2})$$

It is immaterial whether the reactions take place entirely within the mitochondrial membrane or within the interior of the mitochondrion as well. Substituting equation (A2) in (A1),

$$\Phi = -\sum_j (\dot{n}_j^{in} \Delta\tilde{\mu}_j + \sum_r \nu_{jr} J_r \tilde{\mu}_j^{ex}). \quad (\text{A3})$$

The affinity  $A_r$  is defined as

$$A_r = -\sum_j \nu_{jr} \tilde{\mu}_j. \quad (\text{A4})$$

Combining equations (A3) and (A4),

$$\Phi = -\sum_j \dot{n}_j^{in} \Delta\tilde{\mu}_j + \sum_r J_r A_r^{ex}. \quad (\text{A5})$$

When the interior of the mitochondrion is in a stationary state for all components,  $\dot{n}_j^{in} = 0$  for all  $j$ , and consequently for our example

$$\Phi = \sum_r J_r A_r^{ex} = J_P A_P^{ex} + J_O A_O^{ex}. \quad (\text{A6})$$

Thus in this stationary state it suffices to measure changes in the external solution only, although in general  $J_P$  and  $J_O$  are given by the sum of the rates of change on both sides of the membrane. Equation (A6) is an example of the general principle that if any forces are not controlled, the system will reach a stationary state in which their conjugate flows are zero.<sup>15</sup> The flows  $J_P$  and  $J_O$  do not vanish since  $A_P^{ex}$  and  $A_O^{ex}$  are fixed experimentally.

We are also interested in the stationary state in which experimental control of  $\Delta\tilde{\mu}_H$  prevents the relaxation of  $-\dot{n}_H^{in} = J_H$  to zero; in this case equation (A5) gives

$$\Phi = J_P A_P^{ex} + J_H \Delta\tilde{\mu}_H + J_O A_O^{ex}. \quad (\text{A7})$$

In the approach to the above stationary states, equation (A5) applies; i.e.,

each flow is a function of several forces. However, in the stationary state of equation (A7), each flow is a function of only three forces. If  $\Delta\tilde{\mu}_H$  is not directly controlled,  $J_H$  becomes zero, giving the stationary state of equation (A6), in which each flow is a function of only two forces. Although neither flow is now an explicit function of  $\Delta\tilde{\mu}_H$ , the existence of the  $H^+$  transport mechanism influences the values of the phenomenological coefficients.<sup>22</sup>

As indicated earlier, the application of equation (1) requires a decision as to whether flows and affinities should be evaluated internally or externally. Equation (A6) indicates that the reaction flows and affinities should be evaluated externally. Since the whole mitochondrion is here considered in a stationary state, this might seem self-evident. However, the situation is complicated if there is nonconservative flow, e.g., that of  $H^+$ , in that it is not intuitively evident whether the flow should be taken as  $-\dot{n}_H^{in}$  or  $-\dot{n}_H^{ex}$ . This question is resolved by equation (A5): if the affinities are defined externally,  $H^+$  flow must be evaluated internally. Symmetry considerations show that we could have expressed the dissipation function in terms of external flows ( $-\dot{n}_j^{ex}$ ) and internal affinities. However, this formulation does not lead to simple dissipation functions, since the external flows need not be zero when the mitochondrion is in a stationary state.

*Appendix II.*—We consider two cases corresponding to equation (A6).

(a)  $J_H = 0$ . Equations (15) and (17) then become

$$A_P^{ex} = R_P J_P + R_{PO} J_O, \quad (A8)$$

$$A_O^{ex} = R_{PO} J_P + R_O J_O. \quad (A9)$$

Consequently, the effective degree of coupling is given by

$$q = -R_{PO}/\sqrt{R_P R_O} = q_{PO} \quad (J_H = 0). \quad (A10)$$

(b)  $\Delta\tilde{\mu}_H = 0$ . Equations (15), (16), and (17) then give

$$A_P^{ex} = R_P (1 - q_{PH}^2) J_P - \sqrt{R_P R_O} (q_{PO} + q_{PH} q_{OH}) J_O, \quad (A11)$$

$$A_O^{ex} = -\sqrt{R_P R_O} (q_{PO} + q_{PH} q_{OH}) J_P + R_O (1 - q_{OH}^2) J_O. \quad (A12)$$

Consequently,

$$q = \frac{q_{PO} + q_{PH} q_{OH}}{\sqrt{(1 - q_{PH}^2)(1 - q_{OH}^2)}} \quad (\Delta\tilde{\mu}_H = 0). \quad (A13)$$

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‡ USPHS Career Development Awardee, 5-K3-HE-24, 481-03.

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<sup>3</sup> Mitchell, P., *Nature*, **191**, 144 (1961).

<sup>4</sup> Mitchell, P., *Biol. Rev.*, **41**, 445 (1966).

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<sup>7</sup> Lehninger, A. L., E. Carafoli, and C. S. Rossi, in *Advances in Enzymology*, ed. F. F. Nord (New York: Interscience, 1967), p. 259.

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- <sup>10</sup> Rottenberg, H., Ph.D. thesis, Harvard University (1968).
- <sup>11</sup> Rottenberg, H., in preparation.
- <sup>12</sup> Prigogine, I., *Introduction to Thermodynamics of Irreversible Processes* (New York: Interscience, John Wiley & Sons, 1955).
- <sup>13</sup> Essig, A., and S. R. Caplan, *Biophys. J.*, **8**, 1434 (1968).
- <sup>14</sup> It is clear that electroneutrality necessitates that a net flow of  $H^+$  be accompanied by an equivalent flow of anions and/or cations. If these flows occur through leak pathways, they are not coupled to the metabolic processes and so need not be included in this dissipation function, i.e., each flow of equation (1) is a function of only three forces. If some compensating flow is coupled to metabolism, its contribution may be completely eliminated from the dissipation function by making its conjugate force zero. It seems that the important compensatory ion is potassium. We believe that there is good evidence that potassium flow is not coupled to metabolism.<sup>11</sup> However, even if it were, the means may exist for making  $\Delta\bar{\mu}_K$  extremely small (e.g., exposure to valinomycin).<sup>10, 11</sup>
- A related consideration is the possibility of  $H^+/K^+$  exchange. This would tend to keep  $\Delta pH$  small, but we do not feel that the evidence cited for such exchange diffusion is compelling. If in fact it exists, equation (1) will continue to apply, provided that  $J_K \Delta\bar{\mu}_K$  is zero.
- <sup>15</sup> Katchalsky, A., and P. F. Curran, *Nonequilibrium Thermodynamics in Biophysics* (Cambridge, Mass.: Harvard University Press, 1965).
- <sup>16</sup> Note that, except at equilibrium, internal and external reaction affinities will be unequal. Consider an enclosed region into which a reactant  $\alpha$  diffuses and forms a product  $\beta$  which diffuses outward. Then  $\mu_{\alpha}^{e2} > \mu_{\alpha}^{i2}$  and  $\mu_{\beta}^{i2} > \mu_{\beta}^{e2}$ . Adding these,  $\mu_{\alpha}^{e2} - \mu_{\beta}^{e2} > \mu_{\alpha}^{i2} - \mu_{\beta}^{i2}$ , i.e.,  $A^{e2} > A^{i2}$ . A similar consideration applies to oxidative phosphorylation.
- <sup>17</sup> For simplicity the argument is developed with linear equations. However, since there is no direct interaction between oxidation and phosphorylation, higher-order terms such as  $L_{POO}A^{e2}A^{o2}$  are also zero. Although linearity is therefore not necessary, for sufficiently small forces it would be expected.
- <sup>18</sup> Kedem, O., and S. R. Caplan, *Trans. Faraday Soc.*, **61**, 1897 (1965).
- <sup>19</sup> Caplan, S. R., *J. Theoret. Biol.*, **10**, 209 (1966); **11**, 346 (1966).
- <sup>20</sup>  $L_{ij} = R'_{ij}/D$ , where  $R'_{ij}$  is the cofactor of  $R_{ij}$  and  $D$  the determinant of the resistance matrix. Since  $L_{PO} = 0$  for the chemiosmotic hypothesis,  $R'_{PO} = R_{PH}R_{OH} - R_{PO}R_H = 0$ . Equation (18) follows.
- <sup>21</sup> Rottenberg, H., S. R. Caplan, and A. Essig, in *Membrane Metabolism and Ion Transport* ed. E. E. Bittar (London: John Wiley & Sons), in press.
- <sup>22</sup> Katchalsky, A., and R. Spangler, *Quart. Rev. Biophys.*, **1**, 127 (1968).