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Abstract

The consequences of the chemiosmotic theory for the oxidative phosphorylation in mitochondria are analyzed both from a thermodynamic and a kinetic point of view. It is shown that the model implies, that as long as NADH and O_2 is available, the system tries to establish an equilibrium situation, where there are substantial proton concentration and electrical potential differences across the mitochondrial inner membrane, and where the value of $[ATP]/\{[ADP][HPO_4]\}$ in the cytoplasm is removed far from its equilibrium value in a homogeneous system. The exact nature of the equilibrium state depends strongly on the stoichiometries of the proton translocation processes associated with the NADH oxidation and the ATP synthesis. It also depends on the effective electrical capacitance of the mitochondrion.

From the description of the kinetic behaviour it is shown that a steady state situation can arise, where NADH and O_2 is supplied in the mitochondrion and ATP is converted to ADP in the cytoplasm. The metabolic regulation of the oxidative phosphorylation is simple to understand within the model, and it also gives an explanation of the high efficiency of the process.

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THERMODYNAMICS AND CONTROL OF THE OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA. AN INVESTIGATION OF THE CONSEQUENCES OF THE CHEMIOSMOTIC THEORY

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INTRODUCTION

According to the chemiosmotic theory a difference in the electrostatic potential, Φ, and in the proton activity, $10^{-\Delta pH}$, is created over the inner mitochondrial membrane in a respiring system [1]. The free energy stored in this process is supplied mainly from the oxidation of NADH via the electron transport chain, while it is utilized in a number of free energy consuming reactions occuring across the membrane; most notably in the synthesis of ATP from ADP and inorganic phosphate. The total reaction leading from the oxidation of NADH to NAD with oxygen to the conversion of ADP to ATP involves a number of reaction steps, which are affected by the potential Φ and by ΔpH. To describe even the basic functions of the oxidative phosphorylation one has to consider a series of coupled processes. Although the equilibrium constants for many of the reactions are known the (non-linear) couplings between the processes make a quantitative thermodynamic description of the system rather involved. Thus in most studies of the oxidative phosphorylation from a thermodynamic point of view, and within the chemiosmotic model, one has treated only a part of the system, and for example related the conditions for the formation of ATP to ΔpH and Φ [1 - 8].

One of the many intriguing consequences of the chemiosmotic theory is, that through the existance of a dividing membrane it is possible to create a situation, where the reaction between NADH and O_2 to NAD is at, or near, equilibrium with sizeable concentrations of both reactants and product in spite of the fact that in a homogeneous medium the reaction is strongly irreversible. The whole series of reactions of the oxidative phosphorylation has thus the possibility of occuring close to equilibrium in a reversible manner [1,5,9-12]. In this case the control of the processes is related to the thermodynamics and seems to be qualitatively different from the control of, for example, the glycolysis, which is achieved by affecting the kinetic parameters.

For a model of the oxidative phosphorylation based on the chemiosmotic theory we analyze in the present paper the thermodynamic relations quantitatively, and we also present a qualitative description of the dynamic behaviour. The model includes the oxidation of NADH, the synthesis of ATP, additional transport processes, homogeneous equilibrium reactions, and the dependence of the electrostatic potential on the number of translocated charges, so that it is possible to describe a steady state situation, where NADH and O_2 is supplied in the mitochondrial matrix and ATP is converted to ADP in the cytoplasm. From known equilibrium constants and estimated values for parameters, such as total concentrations of the different components, the equilibrium of the system can be calculated. Although the essential correctness of the chemiosmotic theory is now generally accepted the stoichiometric coefficients in several reaction steps are still under debate [13-17]. The value of these coefficients strongly influence the position of the equilibrium, and by comparing calculated ratios [ATP]/ [ADP] for the concentrations in the cytoplasm with experimental values some combinations of coefficients can be ruled out, if the reactions are assumed to occur close to equilibrium. Using a kinetic model it is illustrated how the system responds to a perturbation such as a conversion ATP → ADP in the cytoplasm, showing that the chemiosmotic model also partly accounts for how the control of the ATP synthesis is performed. For a system working at steady state the results from the kinetic model can be used to calculate the free energy losses and thus the efficiency of the ATP synthesis.

In most previous quantitative models of the oxidative phosphorylation [5,7,8,18-20] the description of the system is based on an irreversible thermodynamic formalism using linear approximation. This approach has the advantage of being less model dependent, and one can readily account for proton leakage and other non-specific processes. In our classical thermodynamic approach with equilibrium equations we can, by neglecting leak processes, calculate a resting state, which is independent of the values of the kinetic parameters and only depends on thermodynamic ones. By explicitly separating the thermodynamics from the kinetics the model can be treated without using linearizations, and all the relevant variables in the model can be calculated such as Φ, ΔpH and [ATP], [ADP] and $[HPO_4]$. A novel feature is that the relation between potential and translocated charges is treated explicitly. The closed set of equations obtained also makes it possible to discuss the regulation in quantitative terms. In the kinetic model there is no restriction to a linear regime so that it is possible to describe the kinetic behaviour over regions that are so large that linearizations are no longer valid.

There are thus several advantages of using an equilibrium thermodynamics - conventional kinetics formalism rather than one based on irreversible thermodynamics. However, taken to their logical conclusions, the two approaches are equivalent.

2. Reactions involving a transport across the mitochondrial inner membrane

The key reactions in the oxidative phosphorylation are, according to the chemiosmotic theory, those that involve transport of one or more components across the inner mitochondrial membrane. In a formal description of these reactions it is essential to specify the location, relative to the membrane, of reactants and products for components that cannot penetrate the membrane in a passive process. We have chosen to model the processes, that lead from NADH in the mitochondrion and ADP in the cytoplasm to NAD in the mitochondrion and ATP in the cytoplasm, with four reactions that occur across the membrane [8] as summarized in Fig. 1.

For the oxidation of NADH the total process

$$NADH + \frac{1}{2} O_2 + (m+1)\ H_i^+ \rightleftarrows NAD + H_2O + m\ H_o^+ \qquad (1a)$$

is considered. Here and in the following the subscripts i and o denote species inside or outside the mitochnodrion, respectively. The coefficient m denotes the number of protons that are transported across the membrane in reaction (1a) and according to Mitchell [1,16] m = 6, while in recent studies the possibility m = 8 has been suggested [13-15]. Other coefficients have also been proposed [17]. Since the reaction (1a) involves the transport of m charges across the membrane, the equilibrium in the reaction depends on the difference in electrostatic potential Φ across the membrane, and there is a contribution $me\Phi$ to the free energy, where the potential outside is positive relative to the inside, and e is the unit charge. The equilibrium in the reaction (1a) is then described by

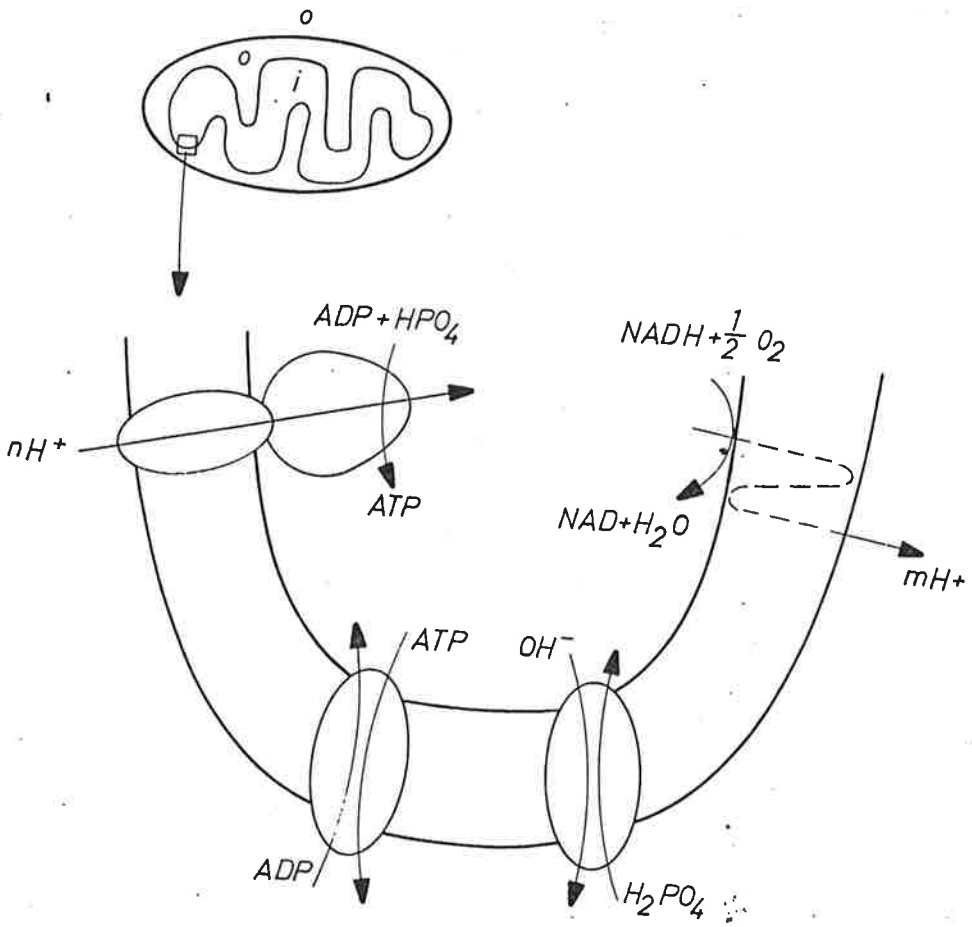


Figure 1. A schematic representation of the four reactions that involve a transport process across the inner mitochondrial membrane.

$$\frac{[NAD][H^+]_o^m}{[NADH]p_{O_2}^{\frac{1}{2}}\,[H^+]_i^{m+1}} = K_1(\Phi) = K_{10} \cdot \exp(-me\Phi/KT) \qquad (1b)$$

where $K_{10} \simeq 5.10^{45}$ is the equilibrium constant in a homogeneous system, which can be calculated from known standard redox potentials [21]. Due to the exponential term in eq. (1b) the effective equilibrium constant K_1 is orders of magnitude smaller than K_{10} in a functioning mitochondrion where Φ is of order 100-200 mV. Furthermore ΔpH can be of the order of 2, and there exists conditions, where the quotient $[NAD]/\{[NADH]p_{O_2}^{1/2}\}$ has physically reasonable values, eg 100, at equilibrium in spite of the large value for K_{10}.

The reaction leading to the formation of ATP inside the mitochondrion is coupled to a proton translocation process and can be written

$$ADP_i^{3-} + HPO_{4i}^{2-} + nH_o^+ \rightleftarrows ATP_i^{4-} + H_2O + (n-1)H_i^+ \qquad (2a)$$

Also for this reaction the stoichiometry is unclear, and values for n ranging from one to three has been suggested [1,13]. In the same way as for reaction (1a) the equilibrium of reaction (2a) is affected by Φ and

$$\frac{[ATP]\,[H^+]_i^{n-1}}{[ADP]_i\,[HPO_4]_i\,[H^+]_o^n} = K_2(\Phi) = K_{20} \cdot \exp(ne\Phi/kT) \qquad (2b)$$

Alberty [22] has investigated equilibria involving ATP and ADP in homogeneous solutions. Both ATP and ADP bind Ca^{2+} and Mg^{2+} with similar strength, and the influence of these ions largely cancel. At pMg = 2 and pH = 7 Alberty gives $\Delta G_2^{o'} = 36$ kJ/mol leading to $K_{20} = 5\ M^{-2}$.

The exchange between ATP and ADP across the membrane

$$ATP_i^{4-} + ADP_o^{3-} \rightleftarrows ATP_o^{4-} + ADP_i^{3-} \qquad (3a)$$

involves the transport of one unit charge and

$$\frac{[ATP]_o\,[ADP]_i}{[ATP]_i\,[ADP]_o} = K_3(\Phi) = K_{30}\exp(e\Phi/kT) \qquad (3b)$$

where $K_{30} = 1$, since the standard states of reactants and products are identical. Similarily, when inorganic phosphate is transported together with a proton (or exchanged with OH^-)

$$H_2PO_{4o}^- + H_o^+ \rightleftarrows H_2PO_{4i}^- + H_i^+ \qquad (4a)$$

the equilibrium constants is unity, and

$$\frac{[H^+]_i\,[H_2PO_4]_i}{[H^+]_o\,[H_2PO_4]_o} = 1 \qquad (4b)$$

since the process does not involve a charge translocation.

If in addition to reactions (1a-4a) oxygen is supplied, and NAD is converted to NADH as in the citric acid cycle, and if ATP is converted to ADP in the cytoplasm in different energy consuming metabolic processes, a steady state situation can be created where m/(n+1) ATP molecules are synthetized per NADH. Thus for m = 6, n = 1 the so called P/O ratio is 3 while for m = 8, n = 2 it is 2.67.

One can note that such a steady state situation can only be created, if there is a proper balance between translocated protons and translocated charges. For example, if in the present scheme the transported phosphate species in reaction (4a) were HPO_4^{2-}, then negative charge would accumulate inside the mitochondrion.

3. Relations valid for the bulk systems

The eqs. (1b-4b) describing the equilibria across the membrane are not sufficient to determine the equilibrium state. Additional relations are obtained by considering the titration equilibria of inorganic phosphate, the constancy of the total amount of different components and the relation between transported charge and Φ.

Inorganic phosphate occur under physiological conditions either as a monovalent or divalent anion with a pK_a of about 6.8 for the titration between the two forms. The equilibrium is considered both inside and outside the mitochondrion and

$$\frac{[HPO_4^{2-}]_{i,o}\,[H^+]_{i,o}}{[H_2PO_4^-]_{i,o}} = K_p \qquad (5,6)$$

where $K_p = 1.6 \cdot 10^{-7}$

Since ATP and ADP are transported in an exchange process, reaction (3a), their total concentration both inside and outside the mitochondrion remain constant and

$$[ATP]_{i,o} + [ADP]_{i,o} = A^{tot}_{i,o} \qquad (7,8)$$

Inorganic phosphate is transported across the membrane so it is only the total amount of phosphate, P^{tot}, in the system that remains constant. The relation between concentrations and amounts of phosphate depends on the effective volume ratio $V = V_o/V_i$, between the cytoplasm and the mitochondrial matrix and

$$[ATP]_i + V[ATP]_o + [HPO_4]_i + V[HPO_4]_o + [H_2PO_4]_i + V[H_2PO_4]_o = (1+V)P^{tot} \qquad (9)$$

For a given reasonable value of $[NAD]/\{[NADH]pO_2^{\frac{1}{2}}\}$ equilibrium in the reaction (1a) can be obtained either through a large value of ΔpH or of Φ. What is actually obtained, depends on the relation between

the effective electrical capacitance of the mitochondrial membrane and the buffer capacity in the mitochondrial matrix. To obtain a relation between the potential Φ and the effective capacitance C in a particular state of the system it is necessary to count the net number of charges that have been translocated. This is performed by referring to a reference equilibrium state where Φ and ΔpH are zero, which can be formally obtained by lowering pO_2. Denoting this reference state with subscript R the total charge of the mitochondrial matrix is

$$Q^{net} = -e V_i N_A \{B(pH_i - pH_{iR}) + [ATP]_i - [ATP]_{iR} + \quad (10a)$$

$$+ 2([HPO_4]_i - [HPO_4]_{iR}) + [H_2PO_4]_i - [H_2PO_4]_{iR}\}$$

where N_A is Avogadros number and the buffer capacity B has been assumed independent of pH. The potential is related to the net number of charges through the capacitance and

$$\Phi = - Q^{net}/C \quad (10b)$$

The eqs. (10a) and (10b) contain two parameters, the buffer capacity B and the electrical capacitance C, whose values are difficult to estimate. The main buffering capacity of the system is due to the inorganic phosphate, the effect of which is included explicitly. The term $B(pH_i - pH_{iR})$ accounts for the titration of compounds present in the mitochondrial matrix. However, for reasonable values of B this term gives only minor contributions to Q^{net} in eq. (10a). The choice of C is more critical and C is strictly not independent of the potential Φ. One major contribution to the capacitance comes from the transport of ions, such as Ca^{2+}, that have not been included explicitly. It is only in the limit of small Φ that these transport processes give a capacitance that is independent of Φ. Due to these uncertainties we have in the calculations investigated the nature of the solution for a number of choices of C.

The relations (1b-4b, 5-9, 10b) constitute the ten equations we explicitly include in the model. The last six of these relations were not explicitly considered by Hill [8] so that in the present work more dependent variables are included giving a more detailed description within the model. Most notably is the dependence of the potential on the number of translocated charges explicitly included, i.e. the chemiosmotic hypothesis is explicitly treated. This gives the possibility of calculating the quotient $[ATP]_o/\{[ADP]_o[HPO_4]_o\}$ obtaining a description of the regulation process. To obtain a fully determined system a number of parameters, that can be estimated from experimental data with reasonable accuracy, has to be specified. The values chosen for these parameters are summarized in Table I.

In the eqs. (1b-4b,5,6) representing equilibria activity coefficients have constantly been neglected. For components in the cytoplasm this is not a serious approximation since one has an essentially aqueous system. Less is known about the conditions in the mitochondrial matrix, and it is conceivable that there are substantial deviations from ideal aqueous solution behaviour. For this reason the concentrations used for species inside the mitochondrion are to a certain extent formal in character and really only representing the activity. These formal concentrations cannot be used strictly in the eqs. (7,9,10a), a complication that is neglected in the calculations.

4. The equilibrium state

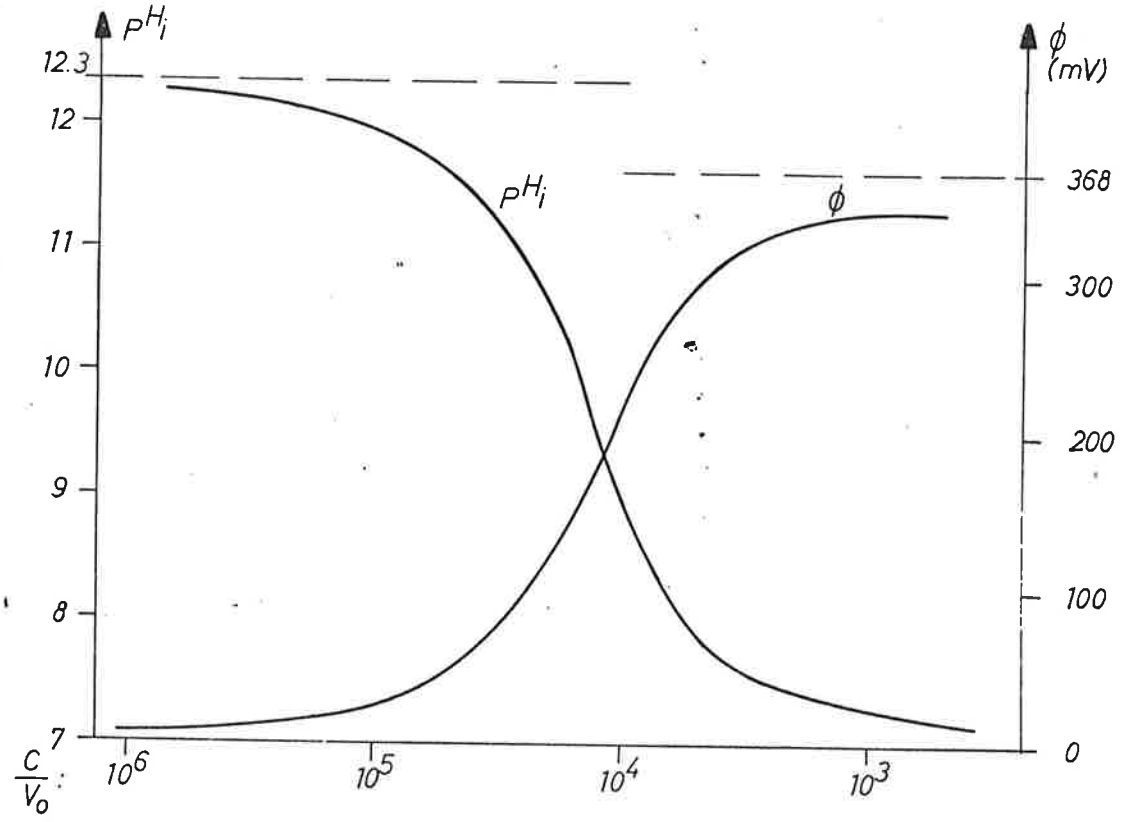
In a system, where the four reactions indicated in Fig. 1 are the only processes that involves a transport across the membrane, the equilibrium state is very different from that where the reactions (1a) and (2a) occur in a homogeneous system. In the model presented in the previous sections there are ten equations and ten unknown viz. pH_i, Φ, $[ATP]_{i,o}$, $[ADP]_{i,o}$, $[H_2PO_4]_{i,o}$ and $[HPO_4]_{i,o}$, so that the equilibrium state is determined. Several of the equations have a strong non-linear character, and a simple analytical solution can not be obtained.

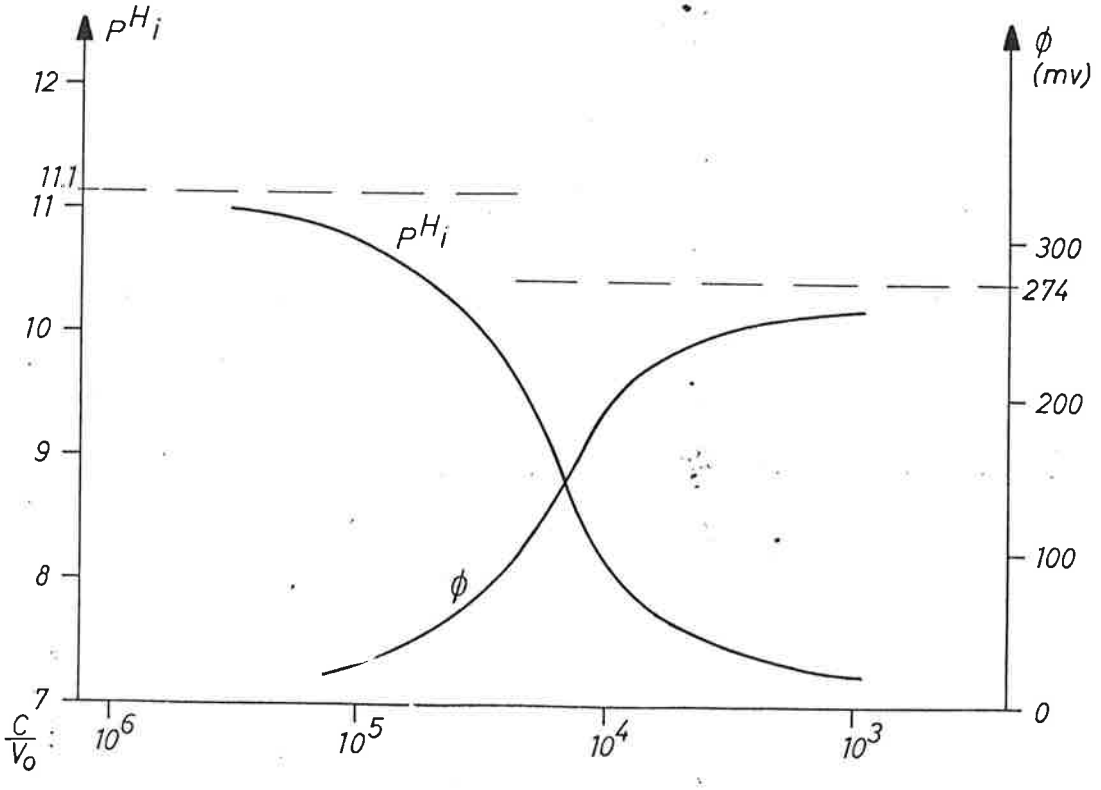
To simplify the equations six of the variables are eliminated analytically, and the remaining set of equations are solved using numerical procedures. For this purpose the C05NAE-routine of the NAG-library [23] is used. The algoritm is based on a combination of Newtons method and the method of steepest-descent.

For the values of the parameters in Table I the solutions to eqs. (1b-4b, 5-9, 10b) have been determined for a range of capacitance/volume ratios C/V_o. The values of stoichiometric coefficients m and n have also been varied. As the capacitance is increased the potential Φ decreases and this drop in Φ is compensated by an increase in pH_i to maintain equilibrium in reaction (1a). This behaviour is illustrated in Fig. 2 where pH_i and are plotted versus C/V_o for the two cases (m=6, n=1) and (m=8; n=2). Figure 2 only shows the variation in two of the variables but the whole solutions changes with C/V_o. To illustrate the general character of the solutions the values of all ten variables for a number of choices of C/V_o and (m,n) are shown in Table II.

For the combinations m=6, n=1, $C/V_o=10^4$ and m=8, n=2 $C/V_o = 1.7 \cdot 10^4$ the calculated ratios $[ATP]_o/[ADP]_o$ are of the same magnitude as those found experimentally [24,25], and it seems that the desired balance between the oxidation of NADH and the synthesis of ATP can be obtained in these cases. For the combination m=6, n=2 too much free energy is stored in Φ and ΔpH at equilibrium, so there is a virtually complete conversion of ADP to ATP. Realistic values for the $[ATP]_o/[ADP]_o$ ratio are obtained only far from equilibrium. For the combination (m=8, n=1) on the other hand enough free energy is not available for the ATP synthesis.

Figure 2. The internal pH (left hand scale) and the potential difference ϕ (right hand scale) at equilibrium as a function of the capacitance - outer volume ratio C/V_o. a) m=6, n=1
b) m=8, n=2. The ratio V_o/V_i between the outer and inner volumes is 10. The remaining parameters are as in Table I.





The two main conclusions that can be made on the basis of these calculations are:

i) The chemiosmotic theory is in quantitative terms consistent with the idea that the so called resting state of the mitochondrion represents an equilibrium situation under the specific constraints imposed by the mitochondrial membrane. This would account for the reversability in the different reaction steps in the oxidative phosphorylation

ii) If the oxidative phosphorylation occurs close to equilibrium only a few combinations of the coefficients m and n are consistent with the experimentally observed $[ATP]_o/[ADP]_o$ ratio and the experimentally determined equilibrium constants of reactions I and II. Possible combinations are m=6, n=1 and m=8, n=2, while m=6, n=2 and m=8, n=1 give unrealistic solutions.

The choice of the values for the parameters in Table I is to a certain extent arbitrary and the assumed values can perhaps be wrong by an order of magnitude. However, this does not affect the conclusions above since the effects of changing the coefficients m and n are clearly larger. Consider for example a change in $[NAD]/\{[NADH]p_{O_2}^{\frac{1}{2}}\}$ by a factor of ten. From eq. (1b) follows that this at most leads to a change by a factor $10^{1/7}=1.2$ in $[H^+]_i$ changing the equilibria in the reactions (2a) and (4a) only slightly.

5. Kinetic model

To obtain further insight into the mechanism behind the respiratory control and into the free energy losses in the synthesis of ATP a model of the dynamic behaviour is advantageous. In the total oxidative phosphorylation process there is a long series of elementary reaction steps, and the dynamics of these steps have only to a limited extent been characterized through experimental studies. In contrast to the equilibrium analysis the description of the kinetics thus has to be of a rather qualitative nature. Some experimental studies indicate that the proton pump and the phosphate transport are markedly faster than the synthesis and the transport of ATP [26], and they are here for simplicity treated as instantaneous in the timescale considered. The synthesis and transport of ATP is therefore discussed in some detail. The case 1 of table II is considered for the explicit dependence of Φ on the dynamics.

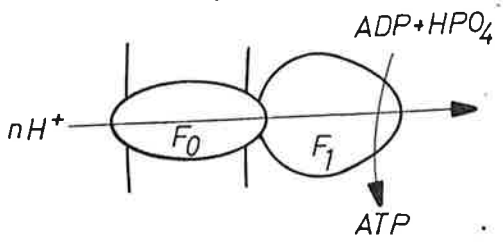


Figure 3. Schematic representation of the F_0-F_1 complex.

The ATP-synthesis may be represented by the following reaction scheme [27] (cf. figure 3)

$$H_o^+ + F_1 \underset{k_{s2}}{\overset{k_{s1}}{\rightleftarrows}} F_1^* \qquad (2c)$$

$$F_1^* + HPO_{4_i} + ADP_i \overset{K_s}{\rightleftarrows} F_1 + ATP_i \qquad (2d)$$

where F_1^* is an activated form of the F_1-complex.

The cross-membrane translocation of H^+ and activation of F_1, (2c), is regarded as the slow process and the only one depending on ϕ. The second step (2d), takes place in the F_1-complex mainly located inside the mitochondrion. Let v_s denote the rate of ATP-synthesis and hence also the H^+-transport rate. Then one probable description will be

$$v_s = k_{s1}\, e^{e\phi/kT} \left[H^+\right]_o \left[F_1\right] - k_{s2} \left[F_1^*\right]$$

$$\frac{F_1 \cdot ATP_i}{F_1^* \cdot \left[HPO_4\right]_i \cdot \left[ADP\right]_i} = K_s$$

$$\left[F_1\right] + \left[F_1^*\right] = F_{tot}$$

Elimination of $\left[F_1\right]$ and $\left[F_1^*\right]$ and comparison with the equilibrium equation (2b) gives

$$v_s = \frac{k_{s1} \cdot k_s \cdot F_{tot} \cdot e^{e\phi/kT} \cdot \left[H^+\right]_o \cdot \left[ADP\right]_i \cdot \left[HPO_4\right]_i - k_{s2} \cdot F_{tot} \cdot \left[ATP\right]_i}{\left[ATP\right]_i + \left[ADP\right]_i \cdot \left[HPO_4\right]_i \cdot K_s}$$

and

$$\frac{k_{s1}}{k_{s2}} \cdot K_s = K_{2o}$$

The ATP-transport

$$ATP_i + ADP_o \underset{k_{t2}}{\overset{k_{t1}}{\rightleftarrows}} ADP_i + ATP_o$$

with the rate $v_t = \frac{d\left[ATP\right]_o}{dt}$ is regarded as symmetrical in ϕ i.e. the factor $e\phi/kT$ in the equilibrium equation is divided equally between the two directions in

$$v_t = k_{t1}\, e^{e\phi/2kT} \cdot \left[ATP\right]_i \cdot \left[ADP\right]_o - k_{t2} \cdot e^{-e\phi/2kT} \cdot \left[ADP\right]_i \cdot \left[ATP\right]_o$$

A comparison with equation (3b) shows that $k_{t1} = k_{t2}$.

In the full kinetic model the equations (1b, 4b, 5-9, 10b) are considered satsified at all times, while the eqs. (2b) and (3b) are replaced by

$$\frac{d}{dt}[ATP]_i = v_s - V \cdot v_t \qquad (2e)$$

$$\frac{d}{dt}[ATP]_o = v_t \qquad (3c)$$

These equations were solved by using an interactive program package SIMNON [28]. The integration routine is based on Hammings predictor-corrector method. The results of the simulations are presented as a phase-plane i.e. in this case $[ATP]_o = f([ATP]_i)$ where both variables are parameterized by time. The constants k_{s1}, k_{s2}, K_s, k_{t1}, k_{t2} and F_{tot} are experimentally unknown, but in the actual example (fig 4) they have been assigned values to reproduce the property that the ATP-synthesis is the slowest reaction ($v_s << v_t$) for small ϕ, while it becomes faster than the ATP-transport when ϕ is increased ($v_t << v_s$). This can actually be achieved for many combinations of parameters, since v_s depends on ϕ as $e^{e\phi/kT}$ and v_t as $e^{e\phi/2kT}$.

In figure 4 the following observations can be made: First, that independent of the startpoint the system develops to the equilibrium. Second that fast and slow modes can be distinguished. If time is eliminated from eqs. (2e) and (3c) to get $\frac{d[ATP]_i}{d[ATP]_o} = \frac{v_s}{v_t} - V$, the trajectory from, for example, the startpoint A to equilibrium E may be interpreted as follows; there is first a fast mode with $\frac{d[ATP]_i}{d[ATP]_o}$ almost equal to $-V$ and $v_t >> v_s$ during which nearly all the stored ATP is transported to the cytoplasm. After that there is a slow quasi-steady-state mode with $\frac{d[ATP]_i}{d[ATP]_o}$ almost $\frac{v_s}{v_t}$ i.e. $v_s >> v_t$ and the ATP-transport is limiting the rate of the system. This fits with the relations between v_s and v_t discussed above.

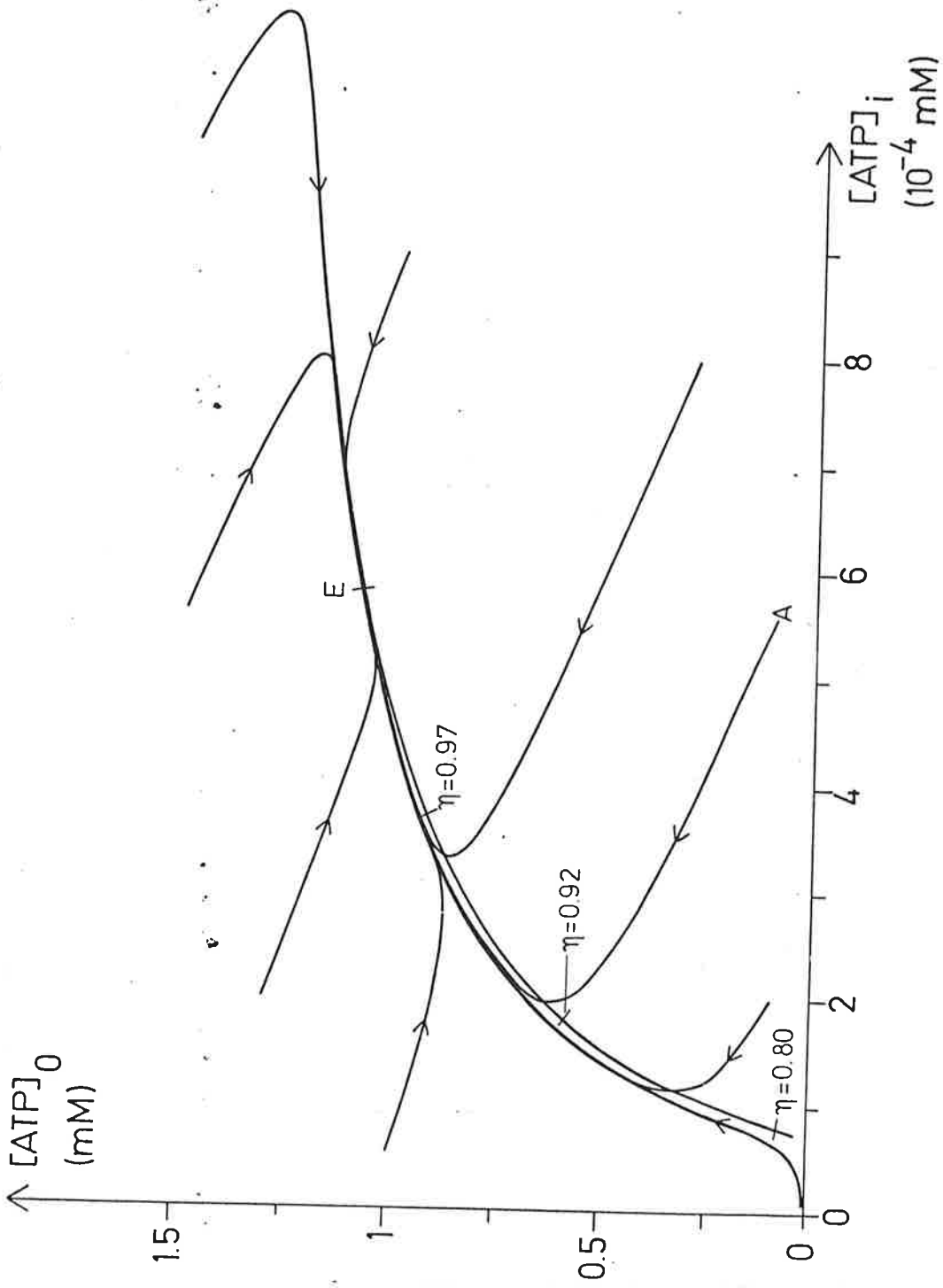


Figure 4. Illustration of the kinetic behaviour of $[ATP]_i$ and $[ATP]_o$ in the system (phase plane). Irrespective of the intitial conditions the values of $[ATP]_o$ and $[ATP]_i$ approach the equilibrium values in E as the arrows indicate. The locus of the steady state is also shown and the efficiency η (see section 7) is calculated at three points.

6. Control mechanisms

In the previous sections is was shown that in the presence of O_2, NADH and adenosine nucleotides the enzyme systems in the mitochondrial membrane establish an equilibrium state with a given ratio $[ATP]_o/[ADP]_o$. In this state there is an equilibrium between the oxidation of NADH and the ATP synthesis representing the resting state (state 4) of a mitochondrion. The chemiosmotic model thus gives a quantitative explanation of how the control of the seemingly irreversible oxidation process is achieved.

Under normal in vivo conditions the various free energy consuming reactions in the cytoplasm lead to a continuous conversion of ATP to ADP which is compensated by a synthesis of ATP in the mitochondrion. This respiring state (state 3) can be simulated by adding a constant term LOAD to eq. (3c) representing the effects on $[ATP]_o$ of the metabolic processes in the cytoplasm

$$\frac{d[ATP]_o}{dt} = v_t - LOAD \qquad (3d)$$

As described by the equations (7-9) this depletion of ATP in the cytoplasm implies a conversion to ADP and HPO_4.

A situation is established where there is a continuous consumption of NADH and O_2. The stoichiometry for the P/O ratio in steady state is constant $m/(n+1)$, while the steady values for the different concentrations depend on the rate constants and the magnitude of the load. This is exemplified in fig. 4, where the locus of the steady state is shown as a function of the load. As expected the steady state locus follows close to the quasi steady state trajectories of the non-stationary conditions discussed in the previous section. It is clear that the [ATP]/[ADP] ratios inside and outside both decrease with increasing load as experimentally found by Brawand et al. [25]. As long as the load is not too large and O_2 and NADH is supplied, the system has the property of maintaining a $[ATP]_o/[ADP]_o$ ratio close to the resting state value also in a steady state situation. This shows that the mitochondrion might function as a regulator of

the [ATP]/[ADP] or [ATP]/{[ADP][HPO_4]} [29] ratio in the cytoplasm.

According to the model presented the control of the oxidative phosphorylation shows an interesting difference from the control of many other metabolic processes. It is commonly found [30], as for example in the glycolysis, that control is achieved by modulating the activity of an enzyme catalysing an irreversible step of a particular reaction sequence. Then the effective rate constant, k_{eff}, of this particular reaction step is dependent on the concentration(s) of one or several effectors E_j so that $k_{eff}(E_j)$. The steady state condition is determined by the function $k_{eff}(E_j)$. In the mitochondrial case the reactions occur across a membrane separating two volumes, and there are no irreversible steps involved in the reaction sequence in the model.

In a real system there will be a certain leakage of protons across the membrane. In the presence of uncouplers, that induce leakage, the oxidation of NADH does not lead to an establishment of a substantial ΔpH and Φ, and the reaction is strongly irreversible with large losses. In the intact native mitochondrion the leakage is much smaller, and it is our assumption that the leak processes do not play an essential role. In the presence of a small leak the steady state with no load should be very close to the equilibrium state calculated in section 4. The control mechanism explained above still applies in such a case. As will be discussed in the next section, being close to equilibrium has the advantage that the efficiency of the total process can be very high.

7. Efficiency

The oxidative phsophorylation is the most fundamental cell process that supports energy in the form of ATP, and it is thus interesting to study the efficiency in the active state (state 3) of the mitochondrion [6].

Based on the results from the kinetic model it is possible to calculate the efficiency, using the expression for the entropyproduction in a chemical reaction. For this purpose the connections with the environment are viewed as in figure 5.

The efficiency is defined as $\eta = \frac{\omega}{\omega_{rev}}$ where ω denotes the free energy used in the cytoplasm per consumed O_2. ω_{rev} relates to the loss free situation at equilibrium (state 4 of mitochondria) where the reactions are reversible. An equivalent way of writing η is $\frac{\omega}{\omega+T\Delta\sigma}$ where $\Delta\sigma$ is the production of entropy in the irreversibel steps namely eqs. (2e) and (3d).

To calculate the useful free energy and the losses, the standard concept of affinity, A, is used. For a reaction in a homogeneous solution A is related to the concentrations c_i through

$$A = RT \ln \{ \prod_i \left(\frac{c_i^{h,eq}}{c_i} \right)^{\nu_i} \}$$

If the reaction occurs across a potential difference the work to transport charges has to be included.

$$A = RT \ln \{ \prod_i \left(\frac{c_i^{eq}}{c_i} \right)^{\nu_i} \} + n F \{ \phi - \phi_{eq} \}$$

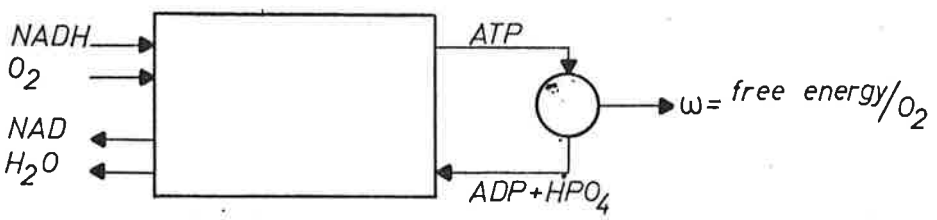


Figure 5. Representation of the mitochondrion as a system that supplies ATP, provided O_2 and NADH is available. The ATP is converted to ADP and inorganic phosphate in an free energy yielding process.

Here $c_i^{h,eq}$ denotes equilibrium concentrations in homogenous solution and $c_i^{eq.}$ relates to the equilibrium for mitochondria. ν_i are the stoichiometric coefficients, n is the number of charges transported and F is Faradays constant.

The useful free energy ω originates from conversion of ATP to ADP in the cytoplasm. Regard the cytoplasm as a homogenous solution. For this reaction the affinity is:

$$A_u = RT \ln \{\frac{1}{K_{20}} \cdot \frac{[ATP]_o}{[H^+]_o \cdot [ADP]_o \cdot [HPO_4]_o}\}$$

The losses appear in the two reactions considered slow namely the synthesis and the transport of ATP. The reference state for these reactions are the resting state 4 of the mitochondrion. The corresponding affinities are thus

$$A_s = RT \ln \{(\frac{[ATP]_i}{[ADP]_i [HPO_4]_i})_{eq.} \cdot (\frac{[ADP]_i \cdot [HPO_4]_i}{[ATP]_i})\} + F(\phi - \phi_{eq})$$

for the synthesis and

$$A_t = RT \ln \{(\frac{[ATP]_o \cdot [ADP]_i}{[ADP]_o \cdot [ATP]_i})_{eq.} \cdot (\frac{[ADP]_o \cdot [ATP]_i}{[ATP]_o \cdot [ADP]_i})\} + F \cdot (\phi - \phi_{eq})$$

for the transport. The efficiency in a steady-state is then

$$\eta = \frac{\text{useful free energy}}{\text{useful free energy + free energy losses}} = \frac{A_u}{A_u + A_s + A_t}$$

where A_s and A_t vanishes as LOAD do, so that $\eta = 1$ in equilibrium. The results of the calculations for different values of LOAD are plotted on the steady-state locus in figure 4. From this it is seen that the efficiency is fairly high as long as the load does not cause a considerable decrease in $\frac{[ATP]_o}{[ADP]_o}$.

From the external point of view the losses are due to the fact that in the steady state the affinity A_u for the hydrolysis of ATP is lower than in the equilibrium state i.e. less free energy can be gained from each synthesized ATP. The affinity A_u or equivalently $[ATP]_o/\{[ADP]_o[HPO_4]_o\}$ is proposed to be a control variable for many extramitochondrial processes [6,29]. It follows from the η-values in Figure 4 that the mitochondrion functions as a regulator for this affinity, which further emphasizes the close connection between control and efficiency.

8. Conclusion

The consequences of the chemiosmotic model have been derived using classical thermodynamics and conventional kinetics to describe a specific set of reactions across the mitochondrial inner membrane. The most restrictive assumptions are, that leak processes can be neglected, and that the system generally operates close to equlibrium. From the calculations a number of observations are made:

i) For certain values of the proton stoichiometries (m,n) in the reactions across the membrane the resulting equilibrium is such that both reactants and products are present in sizeable concentrations in spite of the fact that the oxidation of NADH by O_2 is strongly irreversible in a homogeneous solution. Of the stoichiometries tested the combinations $m = 8\ n = 2$ and $m = 6\ n = 1$ (see reactions 1a and 2a) give the most reasonable values for Φ and ΔpH and other variables (cf. Table II).

ii) The relation between ΔpH and Φ is mainly governed by the effective capacitance of the mitochondrion. One of the main contributions to this capacitance comes from ions that can pass the membrane. For example the effect of valinomycine is to allow potassium to pass, increasing the capacitance.

iii) If NAD is converted to NADH in the mitochondrion in, for example, the citric acid cycle, and if ATP is converted to ADP in the cytoplast through metabolic processes, a steady state will be established, where the ATP is resynthesized converting NADH to NAD under consumption of oxygen. In such a steady state, and only then, is there a stoichiometric relation between the ATP synthesis and the NADH oxidation. There is furthermore the requirement that the reactions across the mitochondrial membrane form a closed cycle in H^+ so that the number of charges translocated equals the number of hydrogens. There is thus a condition on the relation between reactions 3 and 4. This requirement does not seem to have been noticed previously [31].

iv) The chemiosmotic theory does give an explanation of how the free energy stored in NADH is converted into free energy stored in ATP. If accepted, the still unanswered questions are very similar to the ones approached for any enzymatic reaction. What is the precise

stoichiometry of the catalyzed reaction, and how is the specificity realized in the molecular system ?

v) The difference between the resting state (state 4) and the respiratory state (state 3) is a matter of degree. The smaller the flow through the system is, i.e. the smaller the oxygen consumption is, the closer the system is to the resting state (cf. Fig. 4). How far the system is from the equilibrium value is determined by the relation between the rate of the ATP consumption in the cytoplasm and the rate of the enzymatic processes in the membrane. The losses are larger the further the steady state is removed from equilibrium, since ATP is produced at a lower value of the quotient $[ATP]_o/\{[ADP]_o\ [HPO_4]_o\}$.

vi) Without further assumptions the chemiosmotic theory explains how the mitochondrion can function as a regulator of the cytoplasmic free energy reservoir $[ATP]_o/\{[ADP]_o\ [HPO_4]_o\}$ as long as O_2 and NADH is supplied in the mitochondrion. If [ATP] is consumed the system automatically responds by resynthesizing it through the reactions (1-4) [6] (cf. Fig. 4).

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Table I. Values of the different parameters.

K_{10}	$5 \cdot 10^{45}\ M^{-1}(atm)^{-\frac{1}{2}}$
$\frac{[NAD]}{[NADH] \cdot p_{O_2}^{\frac{1}{2}}}$	$40\ (atm)^{-\frac{1}{2}}$
pH_o	7
K_{20}	$5\ M^{-2}$
K_p	$1.6 \cdot 10^{-7} M$
$A_{i,o}^{tot}$	1.55 mM
p^{tot}	12.5 mM
B	1 mM
T	300 K
V	1-100