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The Role of the Adenine Nucleotide Translocator in Oxidative Phosphorylation. A Theoretical Investigation on the Basis of a Comprehensive Rate Law of the Translocator

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Abstract

A minimum model of adenine nucleotide exchange through the inner membrane of mitochondria is presented. The model is based on a sequential mechanism, which presumes ternary complexes formed by binding of metabolites from both sides of the membrane. The model explains the asymmetric kinetics of ADP–ATP exchange as a consequence of its electrogenic character. In energized mitochondria, a part of the membrane potential suppresses the binding of extramitochondrial ATP in competition with ADP. The remaining part of the potential difference inhibits the back exchange of internal ADP for external ATP. The assumption of particular energy-dependent conformational states of the translocator is not necessary. The model is not only compatible with the kinetic properties reported in the literature about the adenine nucleotide exchange, but it also correctly describes the response of mitochondrial respiration to the extramitochondrial ATP/ADP ratio under different conditions. The model computations reveal that the translocation step requires some loss of free energy as driving force. The size of the driving force depends on the flux rate as well as on the extra- and intramitochondrial ATP/ADP quotients. By both quotients the translocator controls the export of ATP formed by oxidative phosphorylation in mitochondria.

Key Words: Mitochondria; adenine nucleotide translocator; kinetics; metabolic control; oxidative phosphorylation.

Introduction

Although the role of the adenine nucleotide translocator in cellular energy metabolism is interpreted differently (Stubbs *et al.*, 1978; Erecinska *et al.*, 1978; van der Meer *et al.*, 1978), we can demonstrate its importance for the distribution of intramitochondrially generated ATP between the intra- and

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extramitochondrial compartments (Kunz *et al.*, 1979, 1981; Küster *et al.*, 1981). The aim of the present paper is to analyze how the translocator is adapted for this function due to its kinetic properties. For this purpose a rate law of the translocator was developed in order to describe its action in phosphorylating mitochondria.

A comprehensive kinetic study on the uptake of ADP and ATP by mitochondria was published by Souverijn *et al.* (1973). It will be shown that their results can be rationalized on the basis of the thermodynamics of adenine nucleotide distribution (Klingenberg and Rottenberg, 1977) by the sequential mechanisms recently proposed by Duyckaerts *et al.* (1980). The final rate law derived in this way differs from that used before in a model of phosphorylating mitochondria (Bohnensack, 1981). Since the earlier rate law was probably incorrect, the model computations were repeated. The obtained results demonstrate that the rate law presented here suitably describes the function of the translocator in phosphorylating mitochondria.

Results

Construction of the Translocator Model

The general structure of the proposed model is derived from the recent observations of Duyckaerts *et al.* (1980). According to these authors, in the ADP_e–ADP_i exchange two forms of the translocator are kinetically important, the free translocator and a ternary complex of the translocator loaded with ADP from both sides of the membrane. If this is extended to the case where ADP and ATP are present on both sides of the membrane, three additional ternary complexes containing ATP from the outer, the inner, or both sides instead of ADP must be expected. The total amount C_0 of the translocator in a given population of mitochondria is then

$$C_0 = [C] + [DeCDi] + [DeCTi] + [TeCDi] + [TeCTi] \qquad (1)$$

where C stands for the free translocator and DeCDi, etc. stand for the different ternary complexes.

For further derivation of the rate equations a rapid equilibrium mechanism (Cleland, 1963) is assumed. The ternary complexes are considered to be in equilibrium with the nucleotides on both sides of the membrane, the corresponding dissociation constants are denoted as K_{DeDi}, etc. The rates of the different exchange reactions are determined by the first-order constants k_{DeDi}, etc. of isomerization of the ternary complexes. In the isomerization process both nucleotide ligands exchange their binding sites. The resulting

rate equations for the four types of exchange are

$$v_{DeDi} = \frac{k_{DeDi} \cdot C_o \cdot [ADP]_e \cdot [ADP]_i}{K_{DeDi} \cdot N} \tag{2}$$

$$v_{DeTi} = \frac{k_{DeTi} \cdot C_o \cdot [ADP]_e \cdot [ATP]_i}{K_{DeTi} \cdot N} \tag{3}$$

$$v_{TeDi} = \frac{k_{TeDi} \cdot C_o \cdot [ATP]_e \cdot [ADP]_i}{K_{TeDi} \cdot N} \tag{4}$$

$$v_{TeTi} = \frac{k_{TeTi} \cdot C_o \cdot [ATP]_e \cdot [ATP]_i}{K_{TeTi} \cdot N} \tag{5}$$

$$N = 1 + \frac{[ADP]_e \cdot [ADP]_i}{K_{DeDi}} + \frac{[ADP]_e \cdot [ATP]_i}{K_{DeTi}} + \frac{[ATP]_e \cdot [ADP]_i}{K_{TeDi}} + \frac{[ATP]_e \cdot [ATP]_i}{K_{TeTi}} \tag{6}$$

The values of the constants in Eqs. (2)–(5) can be estimated from the kinetic date reported for the uptake of external ADP or ATP in exchange for intramitochondrial adenine nucleotides (Pfaff *et al.*, 1969; Klingenberg, 1970; Souverijn *et al.*, 1973; Vignais *et al.*, 1975; Duyckaerts *et al.*, 1980). The relations presented in Table I are obtained as follows. In the absence of external and internal ATP, Eq. (2) directly corresponds to the rate equation found by Duyckaerts *et al.* (1980). The maximum velocity of the ADP_e–ADP_i

Table I. Relations between Rate Constants and Dissociation Constants of the Translocator Model[a]

Relation	Derived from	Reference
$K_{DeTi} = K_{DeDi}$[b]	K_m^D independent of ATP_i/ADP_i	Souverijn *et al.* (1973)
$K_{TeTi} = K_{DeDi} \cdot 10^{-f\Delta\psi/Z}$[c]	$K_m^T \approx K_m^D$	Souverijn *et al.* (1973)
$K_{TeDi} = K_{TeTi}$	analogy with $K_{DeTi} = K_{DeDi}$	cf. text
$k_{DeTi} = k_{DeDi}$	V_D independent of ATP_i/ADP_i	Souverijn *et al.* (1973)
$k_{TETi} = k_{DeDi}$	$V_D = V_T$	Souverijn *et al.* (1973)
$k_{TeDi} = k_{DeDi} \cdot 10^{(1-f)\Delta\psi/Z}$	K_{eq}	Klingenberg and Rottenberg (1977)

[a] The equations were derived by comparison of the rate equations [Eqs. (2)–(6)] with the kinetics of uptake of ADP_e [Eq. (8)] and of ATP_e [Eq. (11)] and the equilibrium distribution of adenine nucleotides [Eq. (14)]. For details see the text.

[b] $K_{DeDi} \approx 0.03\ mM^{-2}$ (Duyckaerts *et al.*, 1980).

[c] $f \approx 0.5$ (assuming $\Delta\psi = -200$ mV).

exchange is

$$V = k_{\mathrm{DeDi}} \cdot \mathrm{C_o} \tag{7}$$

The dissociation constant was determined to be $K_{\mathrm{DeDi}} \approx 0.03$ mM^2 (Duyckaerts *et al.*, 1980).

With respect to the external adenine nucleotides the uptake kinetics are described by Michaelis–Menten equations (Pfaff *et al.*, 1969; Klingenberg, 1970; Souverijn *et al.*, 1973; Vignais *et al.*, 1973; Vignais *et al.*, 1975; Duyckaerts *et al.*, 1980). It follows from Eqs. (2)–(6) that the maximum velocities and Michaelis constants should depend on the internal adenine nucleotides. For the uptake of $\mathrm{ADP_e}$ in exchange for $\mathrm{ADP_i} + \mathrm{ATP_i}$, we have

$$v_{\mathrm{D}} = v_{\mathrm{DeDi}} + v_{\mathrm{DeTi}} \tag{8}$$

For the maximum velocity we have

$$V_{\mathrm{D}} = V\left(\frac{[\mathrm{ADP}]_i}{K_{\mathrm{DeDi}}} + \frac{k_{\mathrm{DeTi}}}{k_{\mathrm{DeDi}}} \cdot \frac{[\mathrm{ATP}]_i}{K_{\mathrm{DeTi}}}\right) \Bigg/ \left(\frac{[\mathrm{ADP}]_i}{K_{\mathrm{DeDi}}} + \frac{[\mathrm{ATP}]_i}{K_{\mathrm{DeTi}}}\right) \tag{9}$$

and for the Michaelis constant we have

$$K_m{}^{\mathrm{D}} = 1 \Bigg/ \left(\frac{[\mathrm{ADP}]_i}{\mathrm{K_{DeDi}}} + \frac{[\mathrm{ATP}]_i}{\mathrm{K_{DeTi}}}\right) \tag{10}$$

The corresponding equations for the uptake of $\mathrm{ATP_e}$ in exchange for $\mathrm{ADP_i} + \mathrm{ATP_i}$ are

$$v_{\mathrm{T}} = v_{\mathrm{TeDi}} + v_{\mathrm{TeTi}} \tag{11}$$

$$V_{\mathrm{T}} = V\left(\frac{k_{\mathrm{TeTi}}}{k_{\mathrm{DeDi}}} \cdot \frac{[\mathrm{ATP}]_i}{K_{\mathrm{TeTi}}} + \frac{k_{\mathrm{TeDi}}}{k_{\mathrm{DeDi}}} \cdot \frac{[\mathrm{ADP}]_i}{K_{\mathrm{TeDi}}}\right) \Bigg/ \left(\frac{[\mathrm{ATP}]_i}{K_{\mathrm{TeTi}}} + \frac{[\mathrm{ADP}]_i}{K_{\mathrm{TeDi}}}\right) \tag{12}$$

$$K_m{}^{\mathrm{T}} = 1 \Bigg/ \left(\frac{[\mathrm{ATP}]_i}{K_{\mathrm{TeTi}}} + \frac{[\mathrm{ADP})_i}{K_{\mathrm{TeDi}}}\right) \tag{13}$$

According to Souverijn *et al.* (1973) the kinetic constants of ADP uptake (V_{D}, $K_m{}^{\mathrm{D}}$) do not depend on the internal ATP/ADP ratio; therefore both the isomerization constant k_{DeTi} and the dissociation constant K_{DeTi} of the ternary complex DeCTi cannot be very different from those of the complex DeCDe [cf. Eqs. (9) and (10)]. The same authors reported that the apparent maximum velocity of the $\mathrm{ATP_e}$–$\mathrm{ATP_i}$ exchange agrees with that of $\mathrm{ADP_e}$ uptake, and so the isomerization constant k_{TeTi} also must agree with k_{DeDi} and k_{DeTi}. But the apparent Michaelis constant for $\mathrm{ATP_e}$ was found to correspond to that of $\mathrm{ADP_e}$ only in uncoupled mitochondria, and a much higher value was determined with energized mitochondria (Souverijn *et al.*, 1973). This

dependence on the energy state can be attributed to the membrane potential for the following reasons. The exchange ADP_e–ATP_i is electrogenic (La Noue *et al.*, 1978; Villiers *et al.*, 1979; Krämer and Klingenberg, 1980a) and the equilibrium distribution of the adenine nucleotides depends on the membrane potential (Klingenberg and Rottenberg, 1977) as expected from the Nernst equation

$$K_{eq} = \frac{[ATP]_e}{[ADP]_e} \cdot \frac{[ADP]_i}{[ATP]_i} = 10^{-\Delta\psi/Z} \tag{14}$$

($Z = 2.3RT/F$). Since in equilibrium the net flux

$$v = v_{DeTi} - v_{TeDi} \tag{15}$$

is zero, it follows from Eqs. (3) and (4) that

$$\frac{K_{TeDi}}{K_{DeTi}} \cdot \frac{k_{DeTi}}{k_{TeDi}} = 10^{-\Delta\psi/Z} \tag{16}$$

In Eq. (16) the constants K_{DeTi} and k_{DeTi} are probably independent of the membrane potential, as indicated by the absence of an effect of the energy state (Souverijn *et al.*, 1973) on the ADP_e uptake. Then only K_{TeDi} and k_{TeDi} are functions of the membrane potential. The dependence of K_{TeDi} on the membrane potential can be estimated from the experimentally found effect of energization on K_{TeTi}. If the inner binding site of the translocator does not discriminate between ADP and ATP ($K_{DeDi} \approx K_{DeTi}$, see above), K_{TeDi} and K_{TeTi} also must be identical. So

$$K_{TeDi} = K_{TeTi} = K_{DeDi} \cdot 10^{-f\Delta\psi/Z} \tag{17}$$

The empirical factor f expresses the fraction of the membrane potential producing the energy-dependent shift of the apparent Michaelis constant for ATP. Its value is available from the measured quantities K_m^D and K_m^T in energized mitochondria. Assuming $\Delta\psi = -200$ mV, the data of Souverijn *et al.* (1973) result in $f \approx 0.5$.

Equation (16) predicts that the remaining portion of the membrane potential $(1 - f)$ causes an energy-dependent inhibition of the ATP_e–ADP_i exchange:

$$k_{TeDi} = k_{DeTi} \cdot 10^{(1-f)\Delta\psi/Z} \tag{18}$$

This is in agreement with the observation of Souverijn *et al.* (1973) that exogeneous ATP is rapidly exchanged for ATP_i whereas the exchange for ADP_i is much slower as long as mitochondria are energized.

With these relations, the rate law of the ADP_e–ATP_i net exchange [Eq. (15)] finally assumes the form

$$v = \frac{V([ADP]_e \cdot [ATP]_i - [ATP]_e \cdot [ADP]_i \cdot 10^{\Delta\psi/Z})}{K_{DeDe} + ([ADP]_e + [ATP]_e \cdot 10^{f\Delta\psi/Z})([ADP]_i + [ATP]_i)} \quad (19)$$

Since in general the concentration of the adenine nucleotides on both sides of the membrane is high enough, the constant K_{DeDe} can be neglected in the numerator. The rate equation may then be approximated as

$$v \approx \frac{V\left(1 - \frac{[ATP]_e}{[ADP]_e} \cdot \frac{[ADP]_i}{[ATP]_i} \cdot 10^{\Delta\psi/Z}\right)}{\left(1 + \frac{[ATP]_e}{[ADP]_e} \cdot 10^{f\Delta\psi/Z}\right)\left(1 + \frac{[ADP]_i}{[ATP]_i}\right)} \quad (20)$$

This equation demonstrates that under this condition the net rate is a function of three quantities, the external and the internal ATP/ADP ratios and the membrane potential, and of two parameters, the maximum rate and the fraction of the membrane potential which suppresses the binding of external ATP.

Description of the Kinetics of Adenine Nucleotide Exchange

The model describes the kinetics of uptake of external adenine nucleotides as illustrated in Figs. 1–3. The concentration dependence is of the Michaelis–Menten type, and the Michaelis constant for the external adenine nucleotide falls with increasing concentration of the internal adenine nucleotides. This is shown in Fig. 1 for the uptake of ADP_e. At a fixed total concentration of internal ADP plus ATP, the rate of ADP uptake depends neither on the internal pattern of adenine nucleotides nor on the membrane potential. ADP_e is exchanged for ADP_i and ATP_i in relation to their internal concentrations (cf. Fig. 1).

The uptake of ADP_e is competitively inhibited by ATP_e (Fig. 2). The inhibition is strongly influenced by the membrane potential, which weakens the inhibitory effect. In the same way the membrane potential increases the Michaelis constant for ATP_e in the uptake of external ATP (Fig. 3). In contrast to the uptake of ADP_e, the uptake of ATP_e depends on the internal adenine nucleotide pattern. The apparent maximum velocity is decreased in proportion to the percentage of internal ADP.

The consequences of these properties for the physiologically important net exchange ADP_e–ATP_i are shown in Fig. 4. There, the net rate is plotted versus the extramitochondrial ATP/ADP ratio for different values of the internal ratio as well as of the membrane potential. The internal ratio determines the apparent maximum rate, which occurs at low external ATP/ADP ratio. This rate is independent of the membrane potential since in this case only the reactions ADP_e–ATP_i and ADP_e–ADP_i take place. The

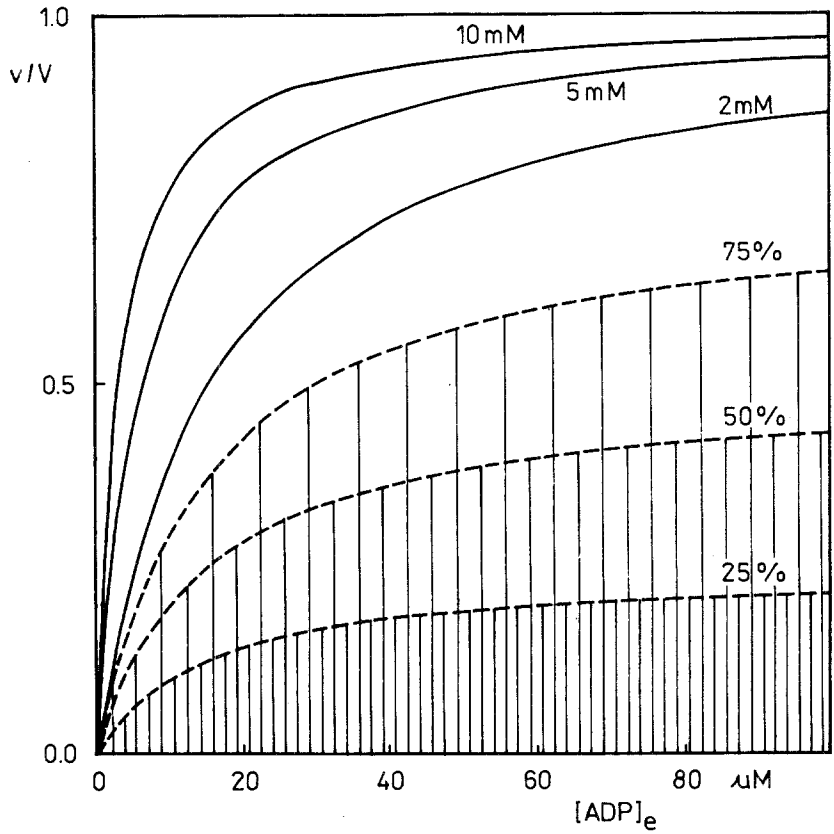

Fig. 1. Simulated concentration dependence of ADP_e uptake. Solid curves indicate rate of ADP_e uptake [Eq. (8)] for different $ADP_i + ATP_i$ sums; the curves are independent of the percentage ADP_i; dashed curves and shaded regions indicate rate of ADP_e–ADP_i exchange [Eq. (2)] at 2 mM $ADP_i + ATP_i$ for different portions of ADP_i (in percent). The rates are given in relation to the maximum velocity [Eq. (7)]. The parameter values are as in Table I. $[ATP]_e = 0$; the curves are independent of $\Delta\psi$.

membrane potential modifies the dependence of the net rate on the external ATP/ADP ratio because it suppresses the inhibitory effect of external ATP on the uptake of ADP_e (cf. Fig. 2).

Simulation of Adenine Nucleotide Translocation in Phosphorylating Mitochondria

In phosphorylating mitochondria neither the internal ATP/ADP ratio nor the membrane potential are constant if oxidative phosphorylation is stimulated by extramitochondrial ADP. For the study of this situation the simplified rate equation of net exchange [Eq. (20)] was incorporated in a

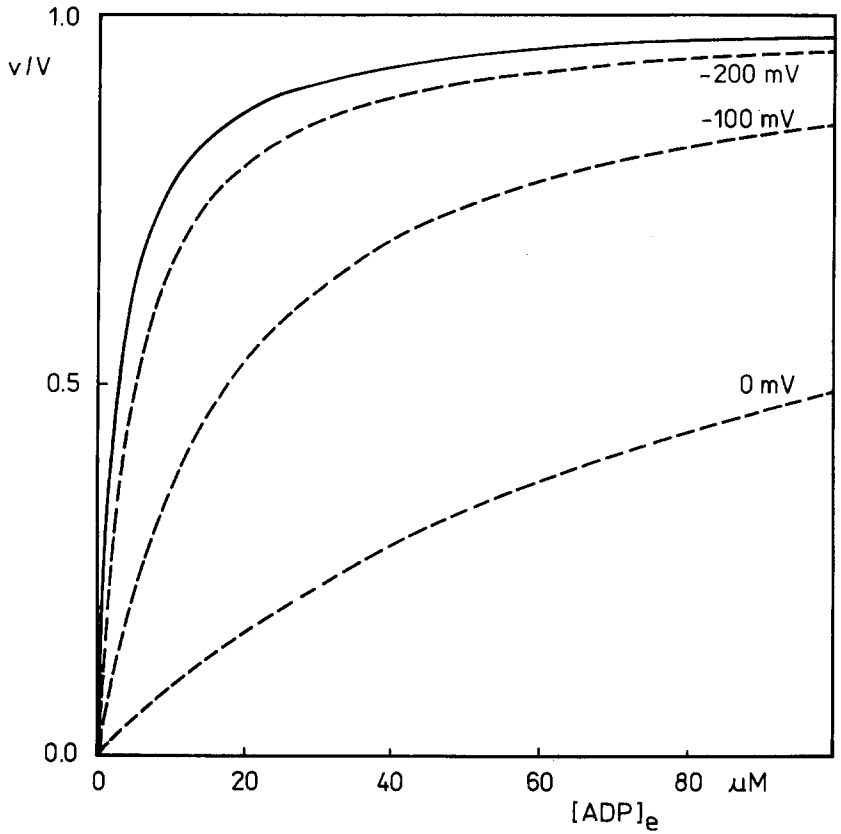

Fig. 2. Simulated inhibition of ADP_e uptake by ATP_e. Solid curve indicates rate of ADP_e uptake in the absence of ATP_e (independent of $\Delta\psi$, cf. legend to Fig. 1); dashed curves indicate the same in the presence of 100 µM ATP_e at different $\Delta\psi$ (values beside the curves). 10 mM ADP_i + ATP_i; other conditions as in Fig. 1.

complex mathematical model of phosphorylating mitochondria (Bohnensack, 1981). Figure 5 demonstrates that this model correctly describes the response of respiration to the extramitochondrial ATP/ADP ratio as a function of inorganic phosphate and in partly uncoupled mitochondria. If the concentration of phosphate is decreased, the apparent maximum velocity falls whereas the control region of the ATP/ADP quotient is only slightly affected. Completely similar results were obtained in experiments (Böhme *et al.*, 1978; Davis and Davis-van-Thienen, 1978; Kunz *et al.*, 1981). Comparison with partially uncoupled mitochondria reveals that at identical rates of respiration the extramitochondrial ATP/ADP ratio is always higher than with tightly coupled mitochondria. High rates of respiration in the presence of high extramitochondrial ATP/ADP ratios were at first described for liver mito-

chondria producing ADP within mitochondria by citrulline synthesis (Letko and Küster, 1979). Meanwhile the same can be demonstrated with partly uncoupled mitochondria (Küster *et al.*, 1981).

Both shifts of the control characteristics, produced by diminished phosphate concentration or increased intramitochondrial energy turnover, are consequences of the energy utilization in the translocation of adenine nucleotides. This is illustrated in Fig. 6, where the translocation rate is plotted versus the loss of free energy in the translocation step for the three cases considered in Fig. 5. This free energy difference is the driving force of the translocation. At high phosphate concentration and low intramitochondrial energy utilization the relation between the flux and the driving force is S-shaped due to the inhibitory effect of external ATP. Since an increasing

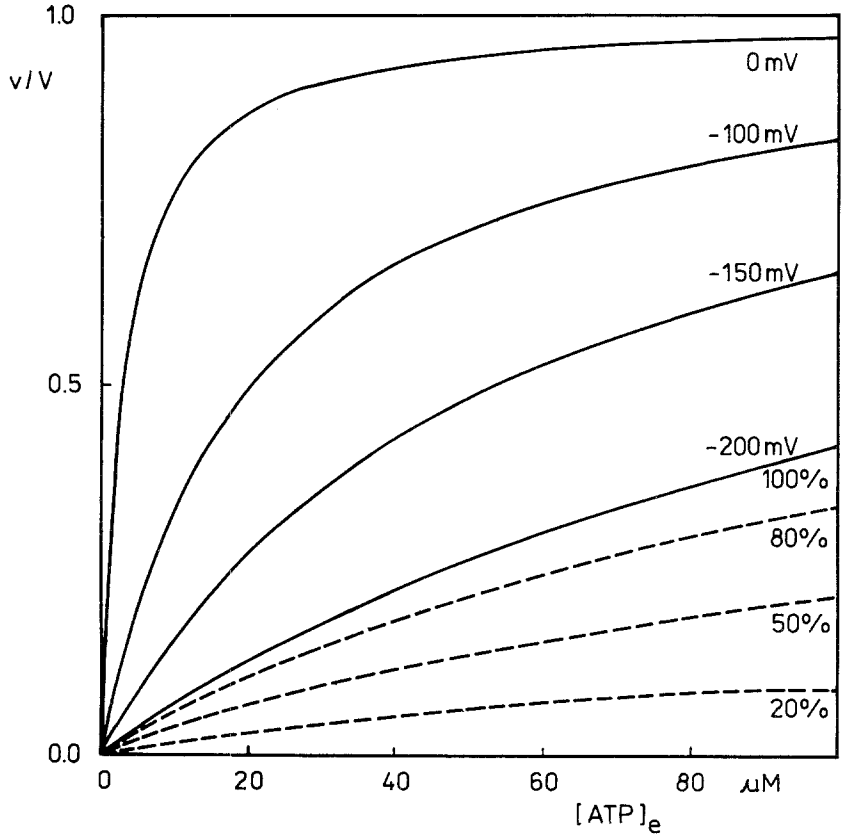

Fig. 3. Simulated concentration dependence of ATP_e uptake. Solid curves indicate rate of ATP_e uptake for different values of the membrane potential at 10 mM ATP_i in the absence of ADP_i; dashed curves indicate the same at $\Delta\psi = -200$ mV and 10 mM ATP_i + ADP_i for different percentages of $ATP_i \cdot [ADP]_e = 0$. Other conditions as in Fig. 1.

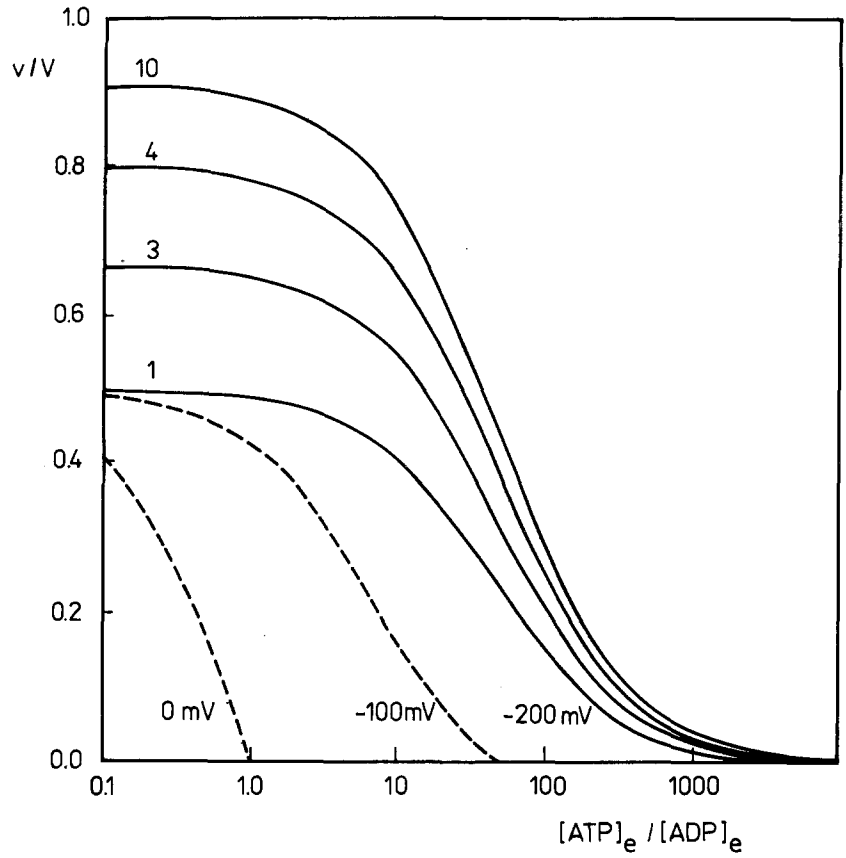

Fig. 4. Simulated rates of ADP_e–ATP_i net exchange. The curves were computed from Eq. (19) with 10 mM ADP_i + ATP_i. Solid curves are for $\Delta\psi = -200$ mV, $[ATP]_i/[ADP]_i$ as given beside the curves; dashed curves are for $\Delta\psi$ as given beside the curves, $[ATP]_i/[ADP]_i = 1$. Other conditions as in Fig. 1.

flux requires a fall in the ATP_e/ADP_e ratio (cf. Fig. 5), this inhibition becomes weaker and weaker. If the concentration of phosphate is decreased or the intramitochondrial energy utilization is increased, comparable fluxes demand lower ATP_e/ADP_e ratios (cf. the shaded areas in Fig. 5). Then the inhibition by ATP_e is not so strong and lower driving forces produce the same fluxes. The lowered energy loss in the translocation step is the reason for the shift of the control characteristics to the right produced by processes such as intramitochondrial ATP turnover or partial uncoupling (Fig. 5). It also explains why the respiration is not controlled by the mass action quotient $[ATP]_e/[ADP]_e \cdot [P_i]_e$). Furthermore, Fig. 6 shows that the apparent maximum velocity of the adenine nucleotide translocation falls with decreas-

ing concentration of phosphate or increasing intramitochondrial energy utilization. In both cases it is caused by the intramitochondrial ATP/ADP ratio, which limits the maximum rate of ADP_e–ATP_i net exchange (cf. Fig. 4). The internal adenine nucleotide ratio is diminished under these conditions, as demonstrated by experiments (Kunz *et al.*, 1979; Letko and Küster, 1979; Kunz *et al.*, 1981) and also reflected by the model (Bohnensack, 1981).

In the translocator model presented here the asymmetric kinetic properties of the adenine nucleotide exchange are attributed to the influence of the

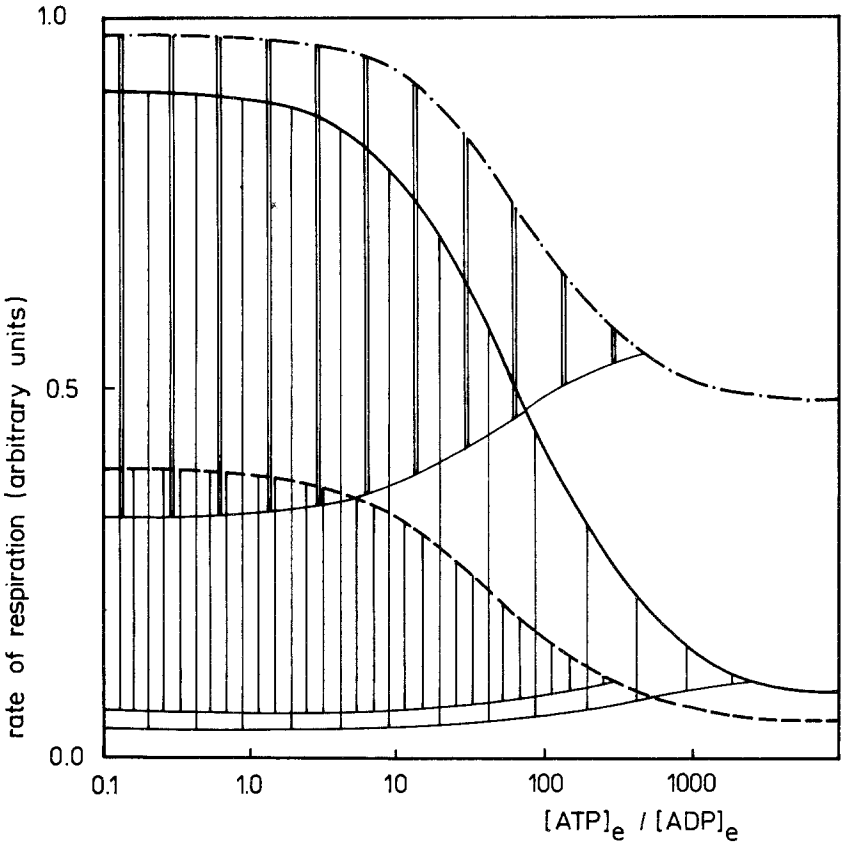

Fig. 5. Simulated effects of inorganic phosphate and of partial uncoupling on the respiration control by the extramitochondrial adenine nucleotides. The curves were computed from a complex model of phosphorylating mitochondria including Eq. (20) for the description of adenine nucleotide translocation. The model, its parameter values, and the procedure of simulation are described elsewhere (Bohnensack, 1981). The parameters of Eq. (20) are $V = 15$ (arbitrary units), $f = 0.5$. Solid curve indicates respiration in the presence of 10 mM P_i; dashed curve, the same at 1 mM P_i; dot-dashed curve, at 10 mM P_i, but tenfold increased proton conductivity of the membrane. The shaded regions show the portion of respiration energy that is utilized for ATP formation.

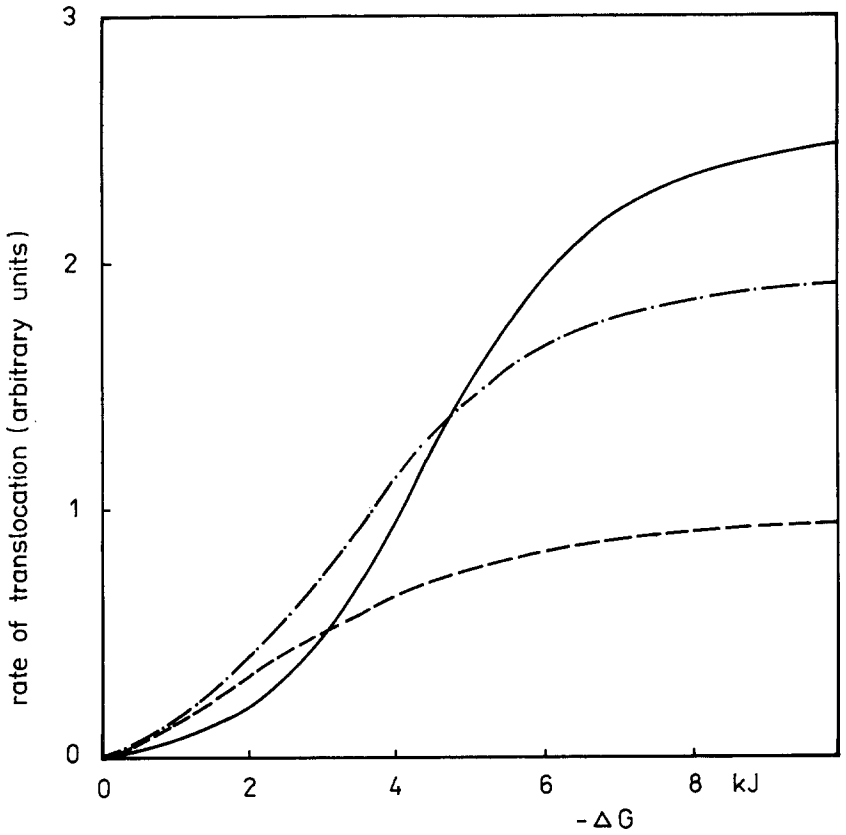

Fig. 6. Net flux through the translocator as a function of the driving force ΔG under the conditions in Fig. 5. The driving force of adenine nucleotide translocation is the loss of free energy in this process, $\Delta G = F\Delta\psi + RT \ln ([ATP]_e/[ADP]_e) \cdot ([ADP]_i/[ATP]_i)$.

membrane potential on the binding of external ATP and on the rate of ATP_e–ADP_i exchange. Both effects are caused by different portions of the total potential difference as expressed by the factor f (cf. Table I). The importance of the size of f for the control of the translocator by the external adenine nucleotides is demonstrated in Fig. 7. If the total potential difference acts on the binding of ATP ($f = 1$), the inhibitory effect of ATP would be much weaker and the sensitivity to the ATP_e/ADP_e ratio would be shifted to very high values. In the other extreme, if the membrane potential has no influence on the binding of ATP ($f = 0$), the translocator is inhibited also at very low ATP/ADP quotients. It seems that the value $f \approx 0.5$ realized in mitochondria guarantees an optimum response of oxidative phosphorylation to the external adenine nucleotide pattern.

Discussion

The translocator model described here depends on the observations of Souverijn *et al.* (1973) in at least two important points. The first is the demonstrated inability of the translocator to exchange external ATP for internal ADP at high rates in energized mitochondria. Such limitation was not found for the uptake of ADP. This behavior cannot be explained on the basis of a gated-pore mechanism (Vignais, 1976; Klingenberg, 1979) which implies that the inward and outward movements are separate processes contributing to the net exchange in a statistical manner (Klingenberg, 1980).

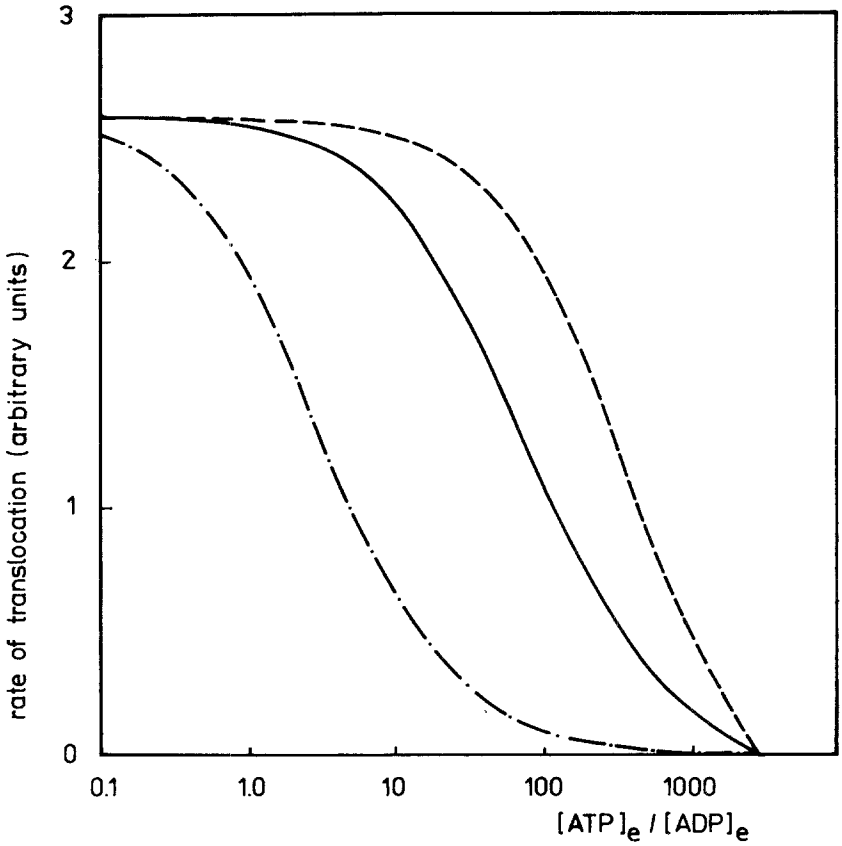

Fig. 7. Simulated rates of adenine nucleotide exchange in phosphorylating mitochondria for different dependences of the translocator kinetics on the membrane potential. Solid curve, conditions as in Fig. 5 ($f = 0.5$); dashed curve, the total membrane potential suppresses the binding of ATP_e ($f = 1.0$); dot-dashed curve, no effect of the membrane potential on the binding of ATP_e ($f = 0.0$).

Consequently the translocator transports ADP or ATP to the outer side independently of the species which are brought in previously. In the sequential mechanism recently proposed by Duyckaerts *et al.* (1980) the inward and outward movements are coupled events so that, on this basis, the experimental observations can be explained without further additional assumptions.

The second point, in which the model depends on the results of Souverijn *et al.* (1973), is the observed influence of the energy state of mitochondria on the affinity to external ATP. A similar behavior was described by others for submitochondrial particles (Villiers *et al.,* 1979). Klingenberg (1980) reported that he was unable to detect drastic changes in this respect. In his interpretation the energization mainly influences the flux rates of ATP through the membrane. But the same author (Klingenberg, 1972) has demonstrated that the latter mechanism ("distribution model") cannot be distinguished from the former ("binding model") if the translocations of ADP and ATP are considered under saturating conditions. This assumption was also used here in order to simplify the rate law for the simulation of phosphorylating mitochondria. Therefore it may be expected that the results presented in Figs. 5–7 are independent of the differences in the basic mechanism.

As shown before, the energy-dependent change in the affinity to ATP can be attributed to the influence of the membrane potential. A possible mechanism is illustrated in Fig. 8. It assumes that a part of the total potential difference is located between the bulk phase and the outer binding site. If the binding of ADP is electroneutral, the binding of the more negatively charged ATP is then necessarily suppressed by the membrane potential. The remaining part of the membrane potential influences the isomerization. From the observations of Souverijn *et al.* (1973), it follows that the three exchange processes ADP_e–ADP_i, ADP_e–ATP_i, and ATP_e–ATP_i have the same maximum velocity independent of the membrane potential. Therefore, only the activation energy for the isomerization of the ATP_e–ADP_i complex must be increased by the membrane potential, but not that of the back reaction ADP_e–ATP_i (cf. Fig. 8).

The rate law of the translocator developed here presents only a minimum variant. Probably, the most serious simplification concerns the complexing of ATP and ADP by magnesium ions. Due mainly to this, the model was not based on data obtained from the reconstituted system (Krämer and Klingenberg, 1980a, 1980b). In such "pure" systems the effect of magnesium ions is not present, in contrast to the conditions in intact mitochondria. Therefore the reference data were taken from the same object for which the model was developed. In addition to this, some reported minor effects of the mitochondrial energy state on the kinetic constants of ADP uptake (Vignais *et al.,* 1973, 1975) were not taken into account.

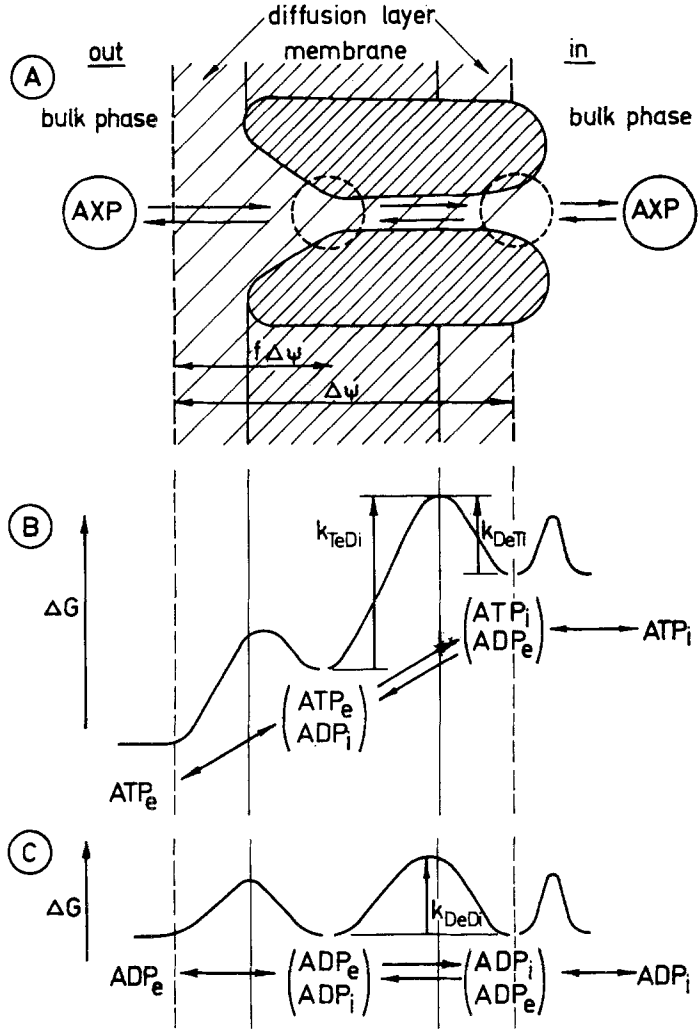

Fig. 8. A possible structural model of the translocator. (A) Arrangement of the translocator in the membrane. The potential difference between the bulk phase and the binding size is $f\Delta\psi$ on the outer side and zero on the inner side; AXP = ATP or ADP. (B, C) Energy profiles of binding of ATP or ADP and of isomerization of the ternary complexes.

In the original model of phosphorylating mitochondria (Bohnensack, 1981) the translocator was represented by a rate law derived from a gated-pore mechanism. That equation contained an additional factor $10^{(1-f)\Delta\psi/Z}$ in the numerator term $[ADP]_i/[ATP]_i$. It shows a remarkable dependence of the kinetic constants of ADP uptake, even in the absence of external ATP, on both the energy state and the internal ATP/ADP ratio. Such effects were not reported by other investigators. The most important consequence resulting from the changed rate law of the translocator for the simulation of oxidative phosphorylation concerns the required maximum rate of the translocator. Its value must be increased four times (cf. the legend of Fig. 5). The reason lies in the probably incorrect assumption that the membrane potential supports the binding of ATP in competition with ADP at the inner side. Then the unproductive ADP_e/ADP_i exchange would be strongly depressed by the membrane potential, which also contradicts the results of Krämer and Klingenberg (1980a).

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References

Böhme, G., Hartung, K. J., and Kunz, W. (1978). In *Bioenergetics at Mitochondrial and Cellular Levels* (Wojtczak, L., Lenartowicz, E., and Zborowski, J., eds.), Nencki Institute of Experimental Biology, Warsaw, pp. 79–102.

Bohnensack, R. (1981). *Biochim. Biophys. Acta* **634,** 203–218.

Cleland, W. W. (1963). *Biochim. Biophys. Acta* **67,** 104–137.

Davis, E. J., and Davis-van-Thienen, W. F. A. (1978). *Biochem. Biophys. Res. Commun.* **83,** 1260–1266.

Duyckaerts, C., Sluse-Goffart, C. M., Fux, J.-P., Sluse, F. E., and Liebecq, C. (1980). *Eur. J. Biochem.* **106,** 1–6.

Erecinska, M., Kula, T., and Wilson, D. F. (1978). *FEBS Lett.* **87,** 139–144.

Klingenberg, M. (1970). In *Essays in Biochemistry* (Campbell, S. N., and Dickens, F., eds.), Vol. 6, Academic Press, New York, pp. 120–159.

Klingenberg, M. (1972). In *Mitochondria/Biomembranes* (Van den Berg, S. G., Borst, F., Van Deenen, L. L. M., Riemersma, J. C., Slater, E. C., and Tager, J. M., eds.), North-Holland, Amsterdam, pp. 147–162.

Klingenberg, M. (1979). *Trends Biochem. Sci.* **4,** 249–252.

Klingenberg, M. (1980). *J. Membr. Biol.* **56,** 97–105.

Klingenberg, M., and Rottenberg, H. (1977). *Eur. J. Biochem.* **73,** 126–130.

Krämer, R., and Klingenberg, M. (1980a). *Biochemistry* **19,** 556–560.

Krämer, R., and Klingenberg, M. (1980b). First European Bioenergetics Conference, Short Reports, Patron Editore, Bologna, pp. 271–272.

Kunz, W., Bohnensack, R., Böhme, G., Küster, U., Letko, G., and Schönfeld, P. (1981). *Arch. Biochem. Biophys.*, **209,** 219–229.

Kunz, W., Bohnensack, R., Küster, U., Letko, G., and Schönfeld, P. (1979). In *Functions and Molecular Aspects of Biomembranes* (Quagliariello, E., Palmieri, F., Papa, S., and Klingenberg, M., eds.) Elsevier/North-Holland, Amsterdam, pp. 313–316.

Küster, U., Letko, G., Kunz, W., Duszynski, J., Bogucka, K., and Wojtczak, L. (1981). *Biochim. Biophys. Acta,* **636,** 32–38.

La Noue, K., Mizani, S. M., and Klingenberg, M. (1978). *J. Biol. Chem.* **253,** 191–198.

Letko, G., and Küster, U. (1979). *Acta Biol. Med. Germ.* **38,** 1379–1385.

Pfaff, E., Heldt, H. W., and Klingenberg, M. (1969). *Eur. J. Biochem.* **10,** 484–493.

Souverijn, J. H. M., Huisman, L. A., Rosing, J., and Kemp, A., Jr. (1973). *Biochim. Biophys. Acta* **305,** 185–198.

Stubbs, M., Vignais, P. V., and Krebs, H. A. (1978). *Biochem. J.* **172,** 333–342.

Van der Meer, R., Akerboom, T. P. M., Groen, A. K., and Tager, J. M. (1978). *Eur. J. Biochem.* **84,** 421–428.

Vignais, P. V. (1976). *Biochim. Biophys. Acta* **456,** 1–38.

Vignais, P. V., Vignais, P. M., and Doussiére, J. (1975). *Biochim. Biophys. Acta* **376,** 219–230.

Vignais, P. V., Vignais, P. M., Lauquin, G., and Morel, F. (1973). *Biochemie* **55,** 763–778.

Villiers, C., Michejda, J. W., Block, M., Lauquin, G. J. M., and Vignais, P. V. (1979). *Biochim. Biophys. Acta* **546,** 157–170.