Review Letter

Control of mitochondrial respiration

J.M. Tager, R.J.A. Wanders, A.K. Groen, W. Kunz+, R. Bohnensack+, U. Küster+, G. Letko+, G. Böhme+, J. Duszynski* and L. Wojtczak*

Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, P.O. Box 20151, 1000 HD Amsterdam, The Netherlands, +Physiologisch-Chemisches Institut, Medizinische Akademie Magdeburg, Leipzigerstrasse 44, Magdeburg, DDR and **Nencki Institute of Experimental Biology, 3 Pasteur Street, 02-093 Warsaw, Poland*

Received 2 November 1982

The control theory of Kacser and Burns [*in:* Rate Control of Biological Processes (Davies, D.D. ed) pp. 65–104, Cambridge University Press, London, 1973] and Heinrich and Rapoport [Eur. J. Biochem. (1974) 42, 97–105] has been used to quantify the amount of control exerted by different steps on mitochondrial oxidative phosphorylation in rat-liver mitochondria. Inhibitors were used to manipulate the amount of active enzyme. The control strength of the adenine nucleotide translocator was measured by carrying out titrations with carboxyatractyloside. In state 4, the control strength of the translocator was found to be zero. As the rate of respiration was increased by adding hexokinase, the control strength of the translocator increased to a maximum value of ~30% at ~80% of state 3 respiration. In state 3, control of respiration is distributed between a number of steps, including the adenine nucleotide translocator, the dicarboxylate carrier and cytochrome *c* oxidase. The measured values for the distribution of control agree very well with those calculated with the aid of a model for mitochondrial oxidative phosphorylation developed by Bohnensack et al. [Biochim. Biophys. Acta (1982) 680, 271–280].

Oxidative phosphorylation *Control strength* *Adenine nucleotide translocator*
Mitochondrial respiration *Computer simulation of respiration* *Rat-liver mitochondria*

1. INTRODUCTION

The fact that respiration is linked to the synthesis of ATP was first recognised by Engelhardt [1,2] whilst subsequent investigations by Kalckar [3,4], Belitzer and Tzibakowa [5] and Ochoa [6] emphasised the close coupling between oxidation and phosphorylation. In systematic studies on the control of respiration in isolated mitochondria, Lardy and Wellman [7] and Chance and Williams [8] observed a hyperbolic relationship between the rate of respiration and the extramitochondrial ADP concentration; both groups concluded that the extramitochondrial ADP concentration is the primary factor controlling the rate of oxidative phosphorylation in mitochondria.

In a series of important studies Klingenberg and coworkers [9–11] varied not only the ADP concentration but also that of ATP and P_i. They concluded [9–11] that respiration is a function of the extramitochondrial phosphate potential, defined as [ATP]/[ADP][P_i]. More extensive studies using the same experimental approach were carried out by Wilson and coworkers [12–14], who also came to the conclusion that respiration is controlled by [ATP]/[ADP][P_i].

Chance and Williams [8] in their initial studies paid particular attention to the 'resting state' (state 4), in which lack of ADP limits respiration, and to the 'active state' (state 3), in which ADP is present in excess. The resulting concepts and terminology of Chance and Williams [8] have been of particular

Published by Elsevier Biomedical Press
00145793/83/0000—0000/$3.00

importance in understanding the dynamics of oxidative phosphorylation. However, in the intact cell the rate of mitochondrial respiration varies according to the energy requirements of the cell, so that the concepts of resting and active states as defined for isolated mitochondria are of limited applicability.

Several systems have been developed [15–21] in which respiration in isolated mitochondria is poised between state 4 and state 3. However, the results obtained have not lent themselves to unequivocal interpretation. For instance, opinions differ with regard to the question of whether respiration is controlled by the extramitochondrial ATP/ADP ratio [15–18] or by the extramitochondrial phosphate potential [9–14, 22–24]. It has been implicitly assumed that one particular reaction limits respiration, for instance the adenine nucleotide translocator [15,18] or cytochrome *c* oxidase (review [25]). However, little attention has been paid to the possibility that respiration may be controlled by more than one reaction.

Recent investigations in three different laboratories have resulted in the emergence of a common concept for the control of mitochondrial respiration. These studies will be described in this communication and discussed in relation to data in the literature.

2. RELATIONSHIP BETWEEN MITOCHONDRIAL RESPIRATION AND THE EXTRAMITOCHONDRIAL ATP/ADP RATIO

Is the adenine nucleotide translocator displaced from equilibrium?

In isolated mitochondria, different steady-state rates of respiration can be obtained by using F_1-ATPase [15–17], glucose–hexokinase [18,22–24], or creatine–creatine kinase [19–21] as an extramitochondrial ADP-regenerating system. The groups of Davis and Kunz [15–18] concluded that the rate of mitochondrial respiration is primarily controlled by the extramitochondrial ATP/ADP ratio rather than by the extramitochondrial phosphate potential, and that the adenine nucleotide translocator is the rate-limiting step. Indeed, when these groups varied the phosphate concentration in their respective experiments, they observed no direct relationship between the rate of respiration and $[ATP]/[ADP][P_i]$ [17,26,27], which would indicate that the translocator is out of equilibrium.

This conclusion is in contrast with the concept of Wilson's group [12–14,25,28–31]. The main difference between the two concepts is that according to Wilson's group the adenine nucleotide translocator catalyses a near-equilibrium reaction at all rates of respiration (reviews [32,33]), whereas according to the group of Kunz the translocator reaction is out of equilibrium already in the resting state [34], disequilibrium progressively becoming greater with increasing rates of respiration.

Mitochondrial respiration can also be stimulated by an intramitochondrial ATP-utilizing reaction such as citrulline synthesis [27,35–38]. Theoretically, if the adenine nucleotide translocator catalyses a near-equilibrium reaction, the relationship be-

Table 1

Adenine nucleotide patterns in mitochondria during respiration stimulated by citrulline synthesis (A) and by phosphorylation of glucose (B)

Nucleotide	Amount or concentration	
	Intramitochondrial (nmol/mg protein)	External (μM)
(A) Citrulline synthesis		
ATP	10.2 ± 0.9	153 ± 4
ADP	3.1 ± 0.2	2.9 ± 0.7
AMP	0.4 ± 0.1	3.3 ± 0.3
Total	13.6 ± 1.0	159 ± 4
ATP/ADP	3.3 ± 0.5	53 ± 14
(B) Hexokinase-glucose system		
ATP	9.6 ± 0.6	151 ± 3
ADP	3.0 ± 0.4	6.6 ± 1.6
AMP	0.4 ± 0.1	3.7 ± 0.2
Total	13.0 ± 0.7	161 ± 3
ATP/ADP	3.2 ± 0.6	23 ± 6

Mitochondrial respiration was adjusted to approximately the same value (43% and 44.5% of the fully active state in A and B, respectively), either via citrulline synthesis (A) or via glucose 6-phosphate synthesis (B). The extrapolation technique described in [34] was used to differentiate between intramitochondrial and extramitochondrial adenine nucleotides. Values are means ± SD (n = 7). (Data from [39])

tween the rate of oxygen uptake and the extramitochondrial ATP/ADP ratio (at constant phosphate concentration) should be independent of the site of ATP utilization. This was tested by Küster et al. [39], who stimulated mitochondrial respiration to ~45% of the maximal rate, either via extramitochondrial ATP utilization (formation of glucose 6-phosphate), or via intramitochondrial ATP utilization (citrulline synthesis). As shown in table 1, the extramitochondrial ATP/ADP ratio differed markedly under the two conditions, whereas the intramitochondrial ATP/ADP ratio was the same (see also [40]). Since intramitochondrial ATP^{4-} is exchanged for extramitochondrial ADP^{3-}, so that the transport of adenine nucleotides is electrogenic and hence dependent upon the magnitude of the membrane potential [41–46], one explanation for the above finding could be that the value of $\Delta\psi$ across the mitochondrial membrane differs under the two conditions. However, Duszynski and coworkers [47,48] measured $\Delta\psi$ under both conditions and observed no difference, in accordance with the results of Williamson et al. [49]. Thus, it can be concluded that the adenine nucleotide translocator is displaced from equilibrium – at least when respiration is 45% of the maximum rate. However, in these experiments it was not possible to quantify the extent to which the translocator reaction was out of equilibrium, since during citrulline synthesis under the conditions used (Mg^{2+} added) there is still considerable flux through the translocator due to the presence of adhering ATPases in the mitochondrial preparation.

Wanders et al. [40] circumvented this problem by omitting Mg^{2+} from the medium when respiration was stimulated by citrulline synthesis and by making use of the finding that at a particular rate of respiration the intramitochondrial ATP/ADP ratio is the same regardless of whether respiration is stimulated by intramitochondrial or extramitochondrial processes (see also [47]). The same applies to $\Delta\psi$ (see [48,49]); these are two of the three parameters involved in the adenine nucleotide translocator reaction. Thus the ΔG of the reaction could be calculated from the value of the extramitochondrial ATP^{4-}/ADP^{3-} ratio during citrulline synthesis and glucose-6-phosphate formation, respectively. As shown in table 3 (taken

Table 2

Membrane energy state of mitochondria under different conditions

Conditions	Respiration rate (nmol O_2/min·mg)	$\Delta\psi$ (mV)	ΔpH (mV)
State 4	8.9	166	52
Ornithine	25.0	152	51
Limiting hexokinase	24.2	151	53
Excess hexokinase	45.2	145	n.d.

Mitochondria (8.5 mg protein) were suspended in 1.1 ml medium with glutamate as substrate and containing 0.15 mM ADP, 0.1 mM [^{14}C]acetate and 0.15 nM [^{3}H]TPMP. Where indicated, 10 mM ornithine or hexokinase was added. After 3 min at 25°C, samples were layered on silicone oil and centrifuged. Radioactivity was measured in the bottom and supernatant $HClO_4$ extracts. Sucrose and water spaces were measured in parallel runs. (Data from [48])

Table 3

Calculation of the free-energy difference of the adenine nucleotide translocator reaction

Parameter	Condition (i) (synthesis of glucose-6-phosphate)	Condition (ii) (synthesis of citrulline)
J_o (natom/min·mg)	69.0	68.7
$([ATP_{out}]/[ADP_{out}])_{total}$	23.6	77.0
$([ATP^{4-}_{out}]/[ADP^{3-}_{out}])_{free}$	1.7	57.5

$$\Delta G_T = 2.3\,RT \log \frac{1.7}{57.5} = -8.7 \text{ kJ/mol}$$

Mitochondria (1.53 mg protein/ml) were incubated in a medium containing 100 mM KCl, 50 mM Tris–HCl, 1 mM EGTA, 10 mM potassium phosphate, 10 mM succinate, 1 mM malate, 20 mM glucose, 16.6 mM $KHCO_3$, 10 mM $MgCl_2$, 2.0 mM ATP and 2 μg/ml rotenone. Final pH, 7.4. In condition (ii) Mg^{2+} was omitted and 10 mM ornithine plus 5 mM NH_4Cl were added. Under condition (i), sufficient hexokinase was added to give the same rate of respiration as under condition (ii). (Data from [40])

from [40]), the adenine nucleotide reaction is 8.7 kJ/mol out of equilibrium at 40% of maximal respiration.

3. COMPUTER SIMULATIONS OF OXIDATIVE PHOSPHORYLATION

The problem of whether the rate of respiration is controlled by the extramitochondrial ATP/ADP ratio or by the extramitochondrial [ATP]/[ADP][P_i] has also been approached in a theoretical way. Bohnensack and coworkers [50–52] developed a computer model for this purpose in which the following processes were considered: proton translocation by the respiratory chain, the production of ATP by ATPase, the translocation of adenine nucleotides and of phosphate by their respective translocators, and a passive backflow of protons through the mitochondrial membrane. Following the concept of Wilson (review [25]), the first two sites of the respiratory chain were considered to be in near-equilibrium with the intramitochondrial phosphate potential and the cytochrome *c* oxidase reaction was considered to be irreversible (see also [53]). The adenine nucleotide translocator was incorporated as an enzyme with a ping-pong mechanism (see [46,54,55]). The kinetic parameters for the different reactions considered were taken from the literature. Fig.1 shows a comparison between the model [52] and experimental results taken from Wanders et al. [40]. In the experiment and in the model, the rate of respiration was

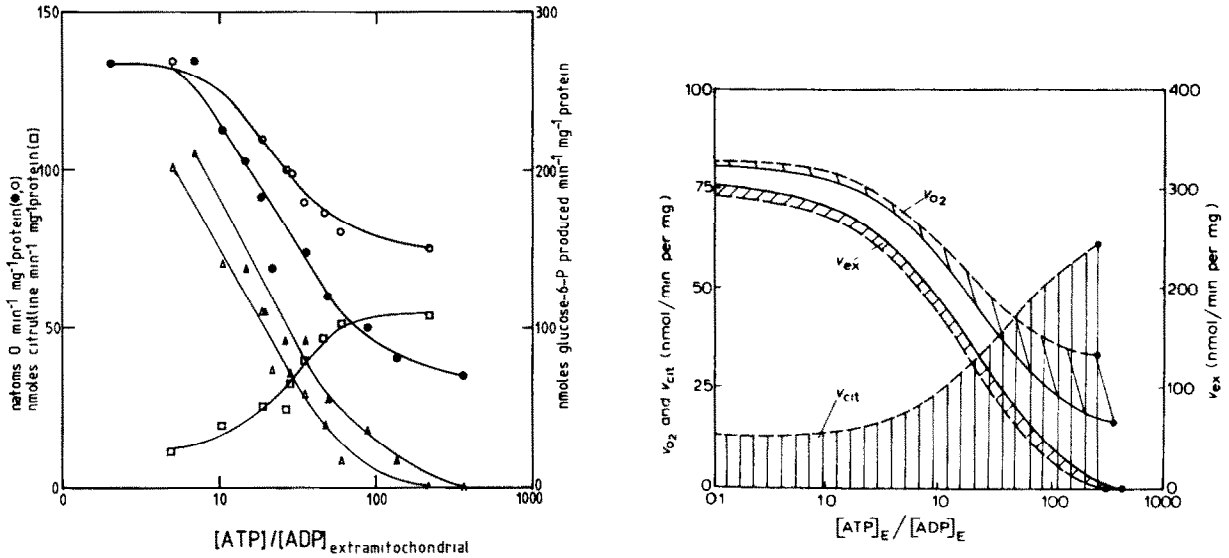

Fig.1. Relationship between rate of O_2 uptake, glucose-6-phosphate and citrulline synthesis and the extramitochondrial ATP/ADP ratio.

(A) Rat-liver mitochondria (1.93 mg protein/ml) were incubated in the reaction mixture described in table 3. Different concentrations of hexokinase either alone (●,▲) or together with 5 mM NH_4Cl and 10 mM ornithine (○,△,□) were added. Intramitochondrial ATP and ADP were measured in neutralised perchloric acid extracts of the mitochondria after separation by centrifugation-filtration through silicone oil. Total ATP and ADP were corrected for intramitochondrial ATP and ADP. ATP, ADP, glucose-6-phosphate (△,▲) and citrulline (□) were measured in the neutralised perchloric acid extracts; see [40].

(B) Simulated competition between citrulline synthesis and net phosphorylation of extramitochondrial ADP and its effect on mitochondrial respiration. The stationary ratio of respiration (v_{O_2}), that of citrulline synthesis (v_{cit} = 0.5 v_i), and that of net phosphorylation of external ADP (v_{ex}) were computed for two conditions and plotted versus the extramitochondrial ATP/ADP ratio, either in the presence of citrulline synthesis (-----) or in its absence (———). The other parameter values are described in [52], from which the experiment is taken.

varied either via the glucose/hexokinase system or via citrulline synthesis.

Both in the experiment and in the computer simulation a sigmoidal relationship was observed between the rate of oxygen uptake (and the rate of glucose-6-phosphate production) and the logarithm of the extramitochondrial ATP/ADP ratio. Introduction of an intramitochondrial ATP-utilizing reaction leads to a shift of the curve towards higher ATP/ADP ratios, this being caused by the disequilibrium of the adenine nucleotide translocator. Even though the major characteristics of the Wilson concept about oxidative phosphorylation have been incorporated into Bohnensack's model, the computer simulation predicts disequilibrium of the adenine nucleotide translocator, which is also what is observed experimentally.

4. DISTRIBUTION OF CONTROL IN OXIDATIVE PHOSPHORYLATION

It has often been suggested [56–60], that a reaction can only be rate-controlling if it operates significantly out of equilibrium. In the previous sections we have indicated that the adenine nucleotide translocator is, indeed, displaced from equilibrium. Does this imply that the translocator controls respiration? This need not necessarily be the case. Kacser and Burns [61] have shown that there is no direct relationship between the free-energy difference of a reaction and the amount of control exerted by that step on pathway flux. Thus, in theory even a near-equilibrium translocator could have significant control on flux (Kacser and Burns [61]).

To quantify the amount of control exerted by an enzyme on pathway flux, Kacser and Burns [61,62] and Heinrich and Rapoport [63,64] introduced a quantitative measure of control referred to either as sensitivity coefficient [61,62] or control strength [63,64], which is defined as the fractional change in pathway flux induced by a fractional change in the amount of the enzyme under consideration. In mathematical terms sensitivity coefficient or control strength is defined as:

$$C_i = \left(\frac{dJ/J}{dE_i/E_i}\right)_{ss} \quad (1)$$

where C_i is the control strength of enzyme E_i, and J is the flux through the pathway in the steady state (ss). Kacser and Burns [61,62] and Heinrich and Rapoport [63,64] have shown that the sum of all control strengths in a pathway is unity, provided that the concentrations of the first substrate and the end product are kept constant. An obvious way to determine control strength is to manipulate the amount of active enzyme by using specific inhibitors. There are numerous reports in the literature of inhibitor titration studies on oxidative phosphorylation [26,65–69]. In these studies it has been presumed that the shape of the inhibitor titration curve gives information about whether a step does or does not control flux. A step is assumed to be rate-controlling if a linear relationship is observed and not rate-controlling if the titration curve is sigmoidal (see [26,66–70]). However, as shown by Akerboom [71] and Groen et al. [72], the shape of an inhibition curve in itself does not give unequivocal information about the amount of control exerted; the amount of inhibitor used should also be taken into account [71–73].

The control theory of Kacser and Burns [61,62] and Heinrich and Rapoport [63,64] provides a suitable theoretical framework to allow quantitative conclusions to be drawn from inhibitor studies (see [72,73]). When the amount of inhibitor added is translated into a certain fractional decrease in enzyme activity, the control strength of a particular reaction can be determined from the inhibition curve. Irreversible inhibitors are most suitable for this purpose [72,73], since the amount of inhibitor added is proportional to the amount of enzyme inactivated. Carboxyatractyloside is an irreversible inhibitor of the adenine nucleotide translocator [54]. In fig.2 a carboxyatractyloside titration is shown of mitochondrial respiration between the resting and the active states. From the initial slope of the inhibitor titration curves the control strength of the adenine nucleotide translocator can be calculated in a simple way (see [72,73]). It is clear that the initial slope increases as respiration increases to about 85% of maximal respiration and then decreases somewhat as respiration increases still further. That is, the control strength of the adenine nucleotide translocator increases and then declines again. Since the maximal value reached for the control strength is about 0.3 (30%), the adenine nucleotide translocator cannot be con-

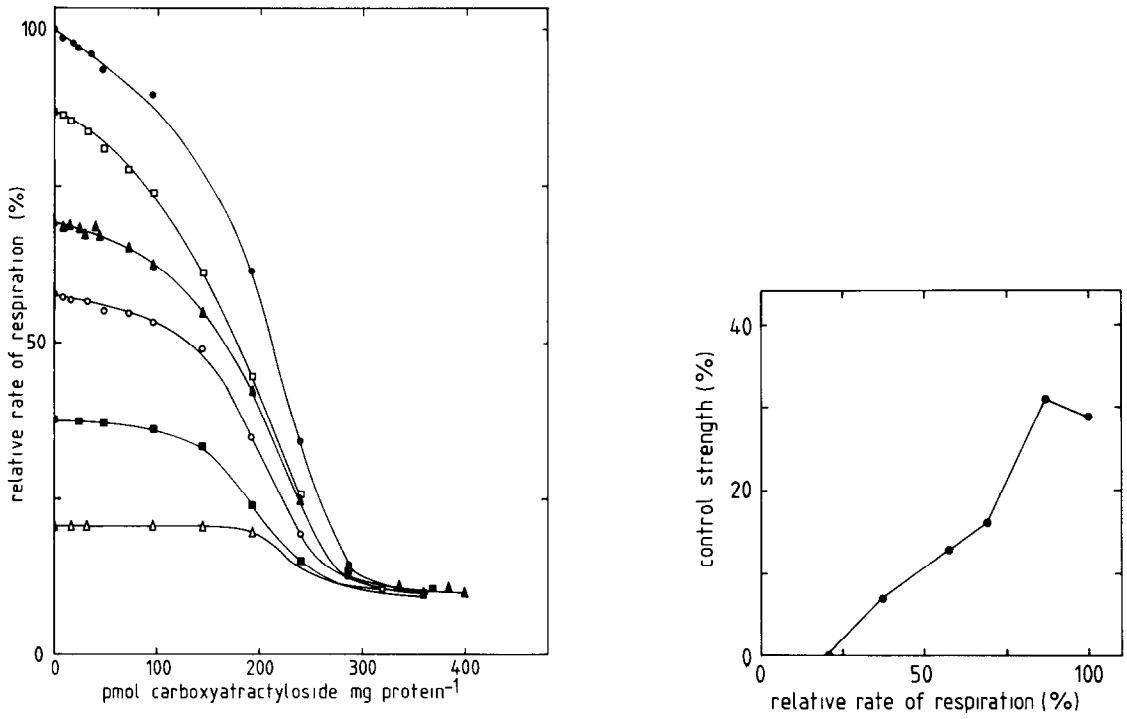

Fig.2. Control strength of the adenine nucleotide translocator at different rates of respiration in rat-liver mitochondria. Mitochondria were incubated in a medium containing 100 mM KCl, 50 mM Tris–HCl, 10 mM potassium phosphate, 1 mM EGTA, 1 mM ATP, 20 mM succinate, 2 mM malate, 20 mM glucose, 2 mM glucose 6-phosphate, 10 mM $MgCl_2$ and 1 μg rotenone/ml. Final pH, 7.4. Different rates of respiration were adjusted via limiting amounts of hexokinase. Carboxyatractyloside was used to inhibit the adenine nucleotide translocator specifically. Each point in the figure represents a separate incubation. The data from fig.2A were used to calculate the control strength of the adenine nucleotide translocator (fig.2B) at the different rates of respiration, as in [72,73]; unpublished results.

sidered as the only rate-controlling step in oxidative phosphorylation (i.e., it is not *the* rate-limiting step).

It should be pointed out that the value of the control strength of the translocator also depends on the ADP-regenerating system used. Qualitatively this can be seen in the experiments of Lemasters and Sowers [68]. They observed that atractyloside inhibited far more effectively at the same rate of respiration, if respiration was varied via phosphate than if glucose/hexokinase was used. An important experiment of Kunz et al. [27], shown in fig.3A, provides an explanation for the low control strength of the translocator in the glucose/hexokinase system. Respiration was adjusted to about 50% of the maximal rate with hexokinase. Upon addition of carboxyatractyloside a biphasic response is observed. Initially, there is a strong inhibition of oxygen uptake, which, however, is subsequently largely overcome due to the fact that also hexokinase has to adjust to a new lower rate. This can only be achieved if the ATP/ADP ratio decreases. On the other hand, lowering of the ATP/ADP ratio stimulates the translocator, which partly compensates the inhibitory action of carboxyatractyloside. The extent to which the ATP/ADP ratio decreases is, of course, a function of the kinetic constants of both hexokinase and the adenine nucleotide translocator for ATP and ADP. Thus, the control strength of the translocator is a function of the dependence of the rate of both reactions on ATP and ADP. This is one of the fundamental concepts of the control theory of Kacser and Burns [61,62]. Already in 1973 Kacser

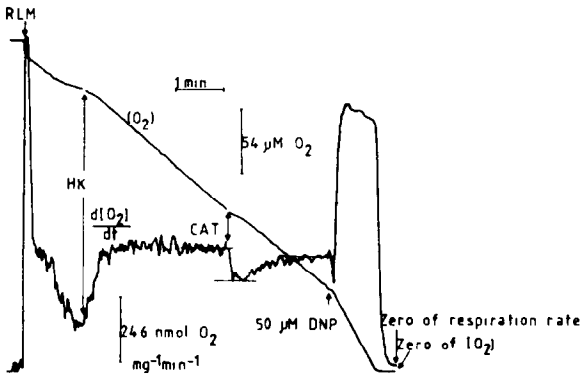

Fig.3. Time course of the effect of a small amount of carboxyatractyloside on the respiration rate in an intermediate state. Incubation of rat-liver mitochondria (RLM) (0.80 mg protein/ml) in standard medium with 9.2 mM succinate, 1 μM rotenone and 4.6 mM ATP. A stationary respiration rate of 61.8 nmol $O_2 \cdot mg^{-1} \cdot min^{-1}$ was adjusted by addition of an appropriate activity of hexokinase (HK). Then 0.116 nmol carboxyatractyloside (CAT)/mg mitochondrial protein was added. The rates of respiration in the different phases of inhibition are marked by lines in the first derivative of the oxygen electrode signal. For comparison, the respiration uncoupled by 2,4-dinitrophenol (DNP) is recorded; data from [27].

Table 4

Distribution of control strength among different steps during State 3 respiration of rat-liver mitochondria

Step	Control strength	
	Measured	Simulated
Adenine nucleotide translocator	0.29 ± 0.05	0.32
Proton leak	0.04 ± 0.01	0.02
Dicarboxylate carrier	0.33 ± 0.04	0.44[a]
Cytochrome *c* oxidase	0.17 ± 0.01	0.17
b–c_1 complex	0.03 ± 0.005	0.01
Hexokinase	0	0
H^+-ATPase	n.d.	0.04
Total	0.86 ± 0.06	1.00

[a] Dicarboxylate carrier plus succinate dehydrogenase

Rat-liver mitochondria were incubated as described in fig.2; excess hexokinase was present. The control strength of the various steps was measured as in [73] (see text). The values are means ± SE of 4 different preparations and are taken from [73]. In the last column the values for the control strength were simulated using the model in [52]

and Burns quantified this qualitative statement by showing that there is an inverse relationship between the control strengths of two adjacent enzymes and their elasticities towards the common intermediate. Elasticity is defined as the fractional change in rate of an enzymic reaction induced by a fractional change in substrate concentration [61,62]. Thus, since in the experimental set-up used hexokinase is insensitive towards ATP and ADP (low elasticity) compared to the translocator, a major part of the control at intermediate states of respiration is located at hexokinase (see [73]).

In column 2 of table 4 (taken from [73]), the values of the control strengths in state 3 respiration are given. The control strength of the different steps was measured by using the following inhibitors: carboxyatractyloside for the adenine nucleotide translocator, azide for cytochrome *c* oxidase, phenylsuccinate for the dicarboxylate translocator and HQNO for the b–c_1 complex. The passive permeability of the mitochondrial inner membrane for protons was varied with uncoupler (see [73]). It is clear that control is distributed over several different steps including cytochrome *c* oxidase and the adenine nucleotide translocator.

Whereas the use of irreversible inhibitors to measure control strength is straightforward, there are several difficulties inherent in the use of competitive or non-competitive inhibitors (discussed in [72]). The computer model offers the advantage that the concentration of enzymes can easily be manipulated. The values of the control strength of different steps in state 3 respiration as calculated with the aid of the model are given in column 3 of table 4 (taken from [52]). The calculated and measured values are very similar, thus reinforcing the conclusion that control of respiration is distributed among several steps.

5. CONCLUDING REMARKS

It is clear from the investigations described above that the adenine nucleotide translocator is displaced from equilibrium even at rather low rates

of respiration. This finding shows that the extramitochondrial phosphate potential is not the primary factor in the control of respiration in isolated mitochondria, in contrast to previous suggestions [22–25]. Furthermore, the results show that the adenine nucleotide translocator contributes significantly to the control of respiration. However, it is not the only rate-controlling step in oxidative phosphorylation, in contrast to earlier suggestions [15–18]. Other steps, including the cytochrome *c* oxidase reaction (cf. [25]), contribute significantly to control of respiration. Moreover, the finding that the adenine nucleotide translocator is not the only rate-controlling step implies that the extramitochondrial ATP/ADP ratio is not the only parameter controlling respiration. Indeed, the extramitochondrial P_i concentration and the supply of hydrogen, which provide the other substrates for oxidative phosphorylation, play a significant role in controlling respiration.

The studies with isolated mitochondria have shown that the distribution of control among different steps is a function of the rate of respiration. It can be anticipated that a similar situation will be encountered in the intact cell. Our present efforts are directed towards elucidation of the factors involved in the control of respiration in vivo.

REFERENCES

[1] Engelhardt, W.A. (1930) Biochem. Z. 227, 16–38.
[2] Engelhardt, W.A. (1932) Biochem. Z. 251, 343–368.
[3] Kalckar, H. (1937) Enzymologia 2, 47–52.
[4] Kalckar, H. (1939) Biochem. J. 33, 631–641.
[5] Belitzer, V.A. and Tzibakowa, E.T. (1939) Biokhimiya 4, 516–535.
[6] Ochoa, S. (1940) Nature 146, 267.
[7] Lardy, H.A. and Wellman, H. (1952) J. Biol. Chem. 195, 215–224.
[8] Chance, B. and Williams, G.R. (1955) J. Biol. Chem. 217, 409–427.
[9] Klingenberg, M. and Schollmeyer, P. (1960) Biochem. Z. 333, 335–350.
[10] Klingenberg, M. (1963) Angew. Chem. 75, 900–907.
[11] Klingenberg, M. (1969) in: The Energy Level and Metabolic Control in Mitochondria (Papa, S. et al. eds) pp. 189–193, Adriatica Editrice, Bari.
[12] Wilson, D.F., Owen, C.S., Mela, L. and Weiner, L. (1973) Biochem. Biophys. Res. Commun. 53, 326–333.
[13] Owen, C.S. and Wilson, D.F. (1974) Arch. Biochem. Biophys. 161, 581–591.
[14] Holian, A., Owen, C.S. and Wilson, D.F. (1977) Arch. Biochem. Biophys. 181, 164–171.
[15] Davis, E.J., Lumeng, L. and Bottoms, D. (1974) FEBS Lett. 39, 9–12.
[16] Davis, E.J. and Lumeng, L. (1975) J. Biol. Chem. 250, 2275–2282.
[17] Davis, E.J. and Davis-Van Thienen, W.I.A. (1978) Biochem. Biophys. Res. Commun. 83, 1260–1266.
[18] Küster, U., Bohnensack, R. and Kunz, W. (1976) Biochim. Biophys. Acta 440, 391–402.
[19] Walter, P. and Stucki, J.W. (1970) Eur. J. Biochem. 12, 508–519.
[20] Stucki, J.W., Brawand, F. and Walter, P. (1972) Eur. J. Biochem. 27, 181–191.
[21] Brawand, F., Folly, G. and Walter, P. (1980) Biochim. Biophys. Acta 590, 285–289.
[22] Van der Meer, R., Westerhoff, H.V. and Van Dam, K. (1980) Biochim. Biophys. Acta 591, 488–493.
[23] Van Dam, K., Westerhoff, H.V., Krab, K., Van der Meer, R. and Arents, J.C. (1980) Biochim. Biophys. Acta 291, 240–250.
[24] Stucki, J.W. (1980) Eur. J. Biochem. 109, 269–283.
[25] Wilson, D.F. (1980) in: Membrane Structure and Function (Bittar, E.E. ed) pp. 153–195, Wiley, New York.
[26] Böhme, G., Hartung, K.J. and Kunz, W. (1978) in: Bioenergetics at Mitochondrial and Cellular Levels (Wojtczak, L. et al. eds) pp. 79–102, Nencki Institute of Experimental Biology, Warsaw.
[27] Kunz, W., Bohnensack, R., Böhme, G., Küster, U., Letko, G. and Schönfeld, P. (1981) Arch. Biochem. Biophys. 209, 219–229.
[28] Erecinska, M., Veech, R.L. and Wilson, D.F. (1974) Arch. Biochem. Biophys. 160, 412–421.
[29] Wilson, D.F., Stubbs, M., Oshino, N. and Erecinska, M. (1974) Biochemistry 13, 5305–5311.
[30] Wilson, D.F., Stubbs, M., Veech, R.L., Erecinska, M. and Krebs, H.A. (1974) Biochem. J. 140, 57–64.
[31] Erecinska, M., Wilson, D.F. and Nisniki, K. (1978) Am. J. Physiol. 234, 273–281.
[32] Stubbs, M. (1979) Pharmac. Ther. 7, 329–349.
[33] Stubbs, M. (1981) in: Short-Term Regulation of Liver Metabolism (Hue, L. and Van de Werve, G. eds) pp. 411–425, Elsevier Biomedical, Amsterdam, New York.
[34] Letko, G., Küster, U., Duszynski, J. and Kunz, W. (1980) Biochim. Biophys. Acta 593, 196–203.
[35] Siekevitz, P. and Potter, V.R. (1953) J. Biol. Chem. 201, 1–13.

[36] Letko, G. and Küster, U. (1979) Acta Biol. Med. Germ. 38, 1379–1385.
[37] Wanders, R.J.A., Van Woerkom, G.M., Nooteboom, R.F., Meijer, A.J. and Tager, J.M. (1981) Eur. J. Biochem. 113, 295–302.
[38] Tager, J.M., Wanders, R.J.A., Groen, A.K., Van der Meer, R., Akerboom, T.P.M. and Meijer, A.J. (1981) Acta Biol. Med. Germ. 40, 895–906.
[39] Küster, U., Letko, G., Kunz, W., Duszynski, J., Bogucka, K. and Wojtczak, L. (1981) Biochim. Biophys. Acta 636, 32–38.
[40] Wanders, R.J.A., Groen, A.K., Meijer, A.J. and Tager, J.M. (1981) FEBS Lett. 132, 201–206.
[41] Pfaff, E. and Klingenberg, M. (1968) Eur. J. Biochem. 6, 66–79.
[42] Pfaff, E., Heldt, H.W. and Klingenberg, M. (1969) Eur. J. Biochem. 10, 484–493.
[43] Klingenberg, M., Heldt, H.W. and Pfaff, E. (1969) in: The Energy Level and Metabolic Control in Mitochondria (Papa, S. et al. eds) pp. 237–253, Adriatica Editrice, Bari.
[44] Klingenberg, M. and Rottenberg, H. (1977) Eur. J. Biochem. 73, 125–130.
[45] LaNoue, K., Mizani, S.M. and Klingenberg, M. (1978) J. Biol. Chem. 253, 191–198.
[46] Klingenberg, M. and Heldt, H.W. (1982) in: Metabolic Compartmentation (Sies, H. ed) pp. 101–122, Academic Press, New York.
[47] Duszynski, J., Küster, U., Letko, G., Bogucka, K., Kunz, W. and Wojtczak, L. (1980) in: First European Bioenergetics Conference, Short Reports, pp. 309–310, Pàtron Editrice, Bologna.
[48] Duszynski, J., Bogucka, K., Letko, G., Küster, U., Kunz, W. and Wojtczak, L. (1981) Biochim. Biophys. Acta 637, 217–223.
[49] Williamson, J.R., Steinman, R., Coll, K. and Rich, T. (1981) J. Biol. Chem. 256, 7287–7297.
[50] Bohnensack, R. and Kunz, W. (1978) Acta Biol. Med. Germ. 37, 97–112.
[51] Bohnensack, R. (1981) Biochim. Biophys. Acta 634, 203–218.
[52] Bohnensack, R., Küster, U. and Letko, G. (1982) Biochim. Biophys. Acta 680, 271–280.
[53] Bienfait, H.F., Jacobs, J.M.C. and Slater, E.C. (1975) Biochim. Biophys. Acta 376, 446–457.
[54] Vignais, P.V. (1976) Biochim. Biophys. Acta 456, 1–38.
[55] Klingenberg, M. (1980) J. Membr. Biol. 56, 97–105.
[56] Krebs, H.A. (1946) Enzymologia 12, 88–100.
[57] Krebs, H.A. (1957) Endeavour 16, 125–132.
[58] Bücher, T. and Rüssmann, W. (1963) Angew. Chem. 19, 882–893.
[59] Rolleston, F.S. (1972) Curr. Top. Cell. Regul. 5, 47–75.
[60] Newsholme, E.A. and Crabtree, B. (1981) in: Short-Term Regulation of Liver Metabolism (Hue, L. and Van de Werve, G. eds) pp. 3–41, Elsevier Biomedical, Amsterdam, New York.
[61] Kacser, H. and Burns, J.A. (1973) in: Rate Control of Biological Processes (Davies, D.D. ed) pp. 65–104, Cambridge University Press, London.
[62] Kacser, H. and Burns, J.A. (1979) Biochem. Soc. Trans. 7, 1149–1160.
[63] Heinrich, R. and Rapoport, T.A. (1974) Eur. J. Biochem. 42, 97–105.
[64] Heinrich, R. and Rapoport, T.A. (1975) BioSystems 7, 130–136.
[65] Wilson, D.F. and Chance, B. (1966) Biochem. Biophys. Res. Commun. 23, 751–756.
[66] Bertina, R.M. (1972) The Interaction of Oligomycin and Aurovertin with the ATPase Complex in Intact Mitochondria, PhD Thesis, University of Amsterdam, Gerja, Waarland.
[67] Kröger, A. (1972) in: Biochemistry and Biophysics of Mitochondrial Membranes (Azzone, G.F. et al. eds) pp. 101–111, Academic Press, New York.
[68] Lemasters, J.J. and Sowers, A.E. (1979) J. Biol. Chem. 254, 1248–1251.
[69] Grunwald, R. and Lemasters, J.J. (1982) in: Short Reports of the Second European Bioenergetics Conference (EBEC), pp. 269–270, LBTM–CNRS, Villeurbanne.
[70] Rognstad, R. (1979) J. Biol. Chem. 254, 1875–1878.
[71] Akerboom, T.P.M. (1979) Compartmentation of Adenine Nucleotides in Rat Hepatocytes, PhD Thesis, University of Amsterdam, Krips Repro, Meppel.
[72] Groen, A.K., Van der Meer, R., Westerhoff, H.V., Wanders, R.J.A., Akerboom, T.P.M. and Tager, J.M. (1982) in: Metabolic Compartmentation (Sies, H. ed) pp. 9–37, Academic Press, New York.
[73] Groen, A.K., Wanders, R.J.A., Westerhoff, H.V., Van der Meer, R. and Tager, J.M. (1982) J. Biol. Chem. 257, 2754–2757.