Journal of Bioenergetics and Biomembranes (1976) 8, 293–311

REVIEW
Effect of Long-Chain Fatty Acids and Acyl-CoA on Mitochondrial Permeability, Transport, and Energy-Coupling Processes

Lech Wojtczak

Department of Cellular Biochemistry,
Nencki Institute of Experimental Biology,
Pasteura 3, 02-093 Warsaw, Poland

Received 30 January 1976

Introduction

Long-chain fatty acids, after being activated to give acyl-CoA thioesters and being transferred into the mitochondrion by means of the carnitine shuttle, are good respiratory substrates whose oxidation is coupled to the phosphorylation of ADP to ATP [1, 2]. They also constitute the main endogenous respiratory substrate in isolated liver mitochondria [3]. On the other hand, however, uncoupling properties of long-chain fatty acids have been observed for more than two decades. More recently, long-chain fatty acids and their CoA thioesters have been found to affect the transport of certain metabolites and ions across the mitochondrial membrane, thus throwing a new light on the mechanism of the interference by fatty acids in mitochondrial functioning and on their possible role in regulation of energy metabolism of the cell.

The purpose of this article is to give an account of the present knowledge on the influence of fatty acids on the permeability of mitochondrial membranes, carrier-mediated transport processes, and energy-coupling reactions in isolated mitochondria. The extrapolation of these effects to in vivo conditions and possible importance for the regulation of cellular metabolism will also be briefly discussed.

©1976 Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission of the publisher.

Inhibition of Carrier-Mediated Metabolite Transport

Translocation of Adenine Nucleotides

Wojtczak et al. [4, 5] described an inhibition by fatty acids of the translocation of adenine nucleotides in liver mitochondria. This was confirmed by Pfaff and Klingenberg (quoted according to Weidemann et al. [6]) in heart mitochondria. According to Wojtczak et al. [5], this inhibition could explain, at least partly, several effects of long-chain fatty acids on mitochondrial energy-coupling reactions, such as lowering of the P/O ratio, inhibition of the ATP–P_i exchange reaction, and inhibition, at low concentrations of fatty acids, of the uncoupler-stimulated mitochondrial ATPase. Subsequently, however, it was demonstrated [7–11] that CoA thioesters of long-chain fatty acids are very potent inhibitors of the translocase, and it was suggested therefore that acyl-CoA thioesters rather than free fatty acids are responsible for the inhibition observed when fatty acids are added to mitochondrial suspensions.

The inhibition of adenine nucleotide translocase by long-chain acyl-CoA is now well established [7–15] and the best documented among various effects of fatty acids and their CoA thioesters on mitochondria. It is of a competitive character [13, 14, 16] (Fig. 1) and strongly depends on the size and structure of the fatty acid moiety.

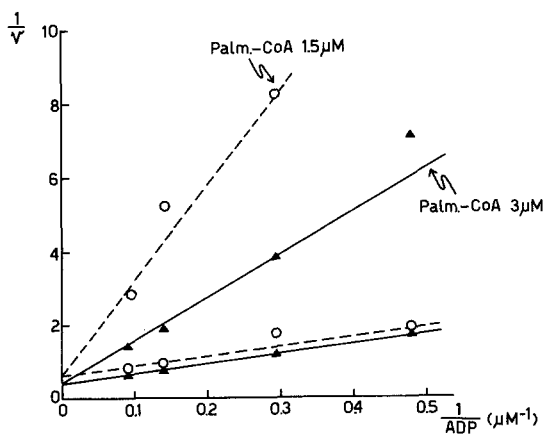

Figure 1. Competitive inhibition by palmitoyl-CoA of ADP translocation in rat-liver mitochondria. Double reciprocal plots. Incubation media were (1) 250 mM sucrose, 1 mM EDTA, 10 mM triethanolamine-HCl, pH 7.4 (empty circles, dashed lines); (2) 110 mM KCl, 1 mM EDTA, 10 mM triethanolamine-HCl, pH 7.4 (full triangles, solid lines). K_m values calculated from this experiment are 5.0 μM and 6.6 μM, K_i values 0.2 μM and 0.9 μM in sucrose and KCl media, respectively. From [16].

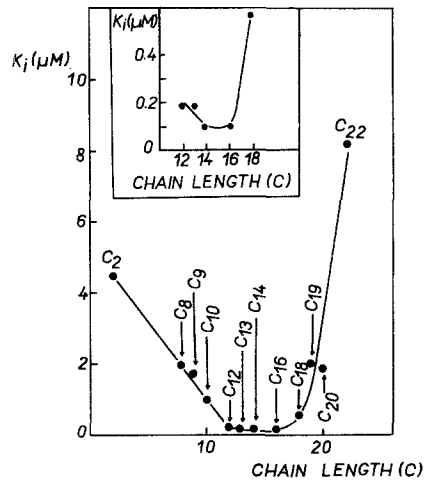

Figure 2. Relation between chain length of acyl-CoA thioesters and the inhibition of adenine nucleotide translocation. From [13].

Among thioesters of saturated fatty acids the strongest inhibition is exerted by myristoyl($C_{14:0}$)-CoA and palmitoyl($C_{16:0}$)-CoA [13, 14] (Fig. 2). Introduction of double bonds into the carbon atom chain of the fatty acid has variable effects on the inhibitory potency of acyl-CoA (or free fatty acid). In case of long-chain fatty acids, above C_{16}, a single double bond of *cis* configuration potentiates the inhibition [13, 17], whereas in medium-chain fatty acids, below C_{14}, unsaturation may decrease their inhibitory property [17]. Since double bonds of *cis* configuration produce a bending of the carbon atom chain and thus shorten the distance between the methyl and the carboxyl ends of the molecule, it may be supposed that this distance, the so-called hydrodynamic length, rather than the number of carbon atoms or the degree of unsaturation, is important for the ability of acyl-CoA to inhibit the translocase. This speculation is supported by the observation that a double bond of *trans* configuration has little effect on the inhibitory potency and that linoleoyl($C_{18:2cis,cis}$)-CoA is less inhibitory than oleoyl($C_{18:1cis}$)-CoA[13]. It seems likely that a certain distance between the methyl and the carboxyl ends of the fatty acid chain is required to assure the optimum "fitness" of the molecule to the membrane-embedded translocase. This length should correspond to that of myristic ($C_{14:0}$), palmitic ($C_{16:0}$), and oleic ($C_{18:1cis}$) acids. The hydrodynamic lenght of linoleic acid ($C_{18:2cis,cis}$) is probably too low and that of elaidic acid ($C_{18:1trans}$) too high.

Mitochondria can indeed bind long-chain acyl-CoA. The ratio of bound to free acyl-CoA remains practically constant over a wide

concentration range and amounts to 2 for oleoyl-CoA and to 1.2–1.4 for palmitoyl-CoA [13]. This ratio increases when Mg^{2+} or other divalent cations are present in the medium [16]. It has also been shown that palmitoyl-CoA inhibits in a competitive way the binding of ADP to mitochondrial membrane fragments [14].

Acyl-CoA thioesters are strongly surface-active agents, and it has been shown [18] that common anionic and nonionic detergents, like

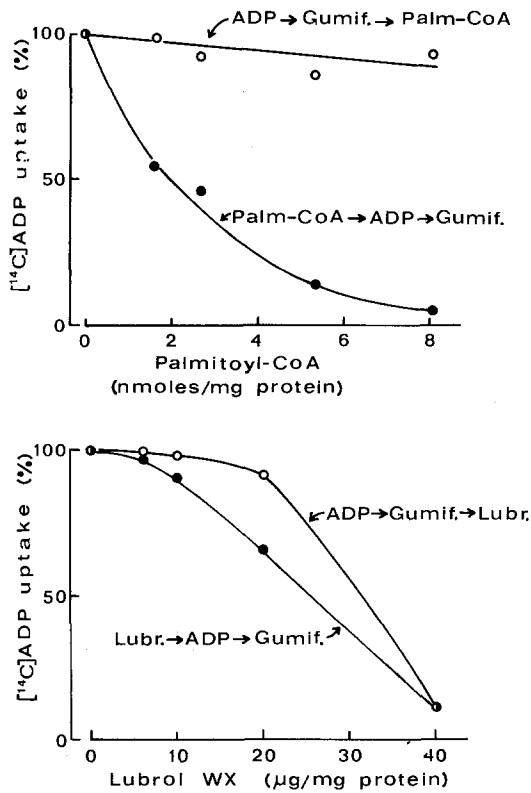

Figure 3. Effect of palmitoyl-CoA and nonionic detergent Lubrol WX on ADP translocation in rat-liver mitochondria. Empty points represent samples to which palmitoyl-CoA or Lubrol were added after the translocation had been terminated by carboxyatractyloside (Gummiferin, abbreviated as Gumif.). These curves show that a passive, carboxyatractyloside-insensitive, outflow of [^{14}C]ADP occurs at Lubrol concentrations above 20 μg/mg mitochondrial protein and that there is practically no such outflow with palmitoyl-CoA up to at least 8 nmole/mg protein. Full points show the effect on the translocase with superimposed effect on the passive outflow. One hundred percent uptake indicates the rate of translocation (as measured by the uptake of [^{14}C]ADP) in the absence of palmitoyl-CoA and Lubrol. From [18], slightly modified.

deoxycholate, dodecylsulphate, and Lubrol WX, inhibit the translocase as well. However, effective concentrations of these detergents are one to two orders of magnitude higher than those of palmitoyl-CoA. Besides, the difference between the concentration producing an inhibition of the translocase and that inducing passive permeability of the membrane to adenine nucleotides (lytic effect) is much smaller in the case of the detergents than in the case of palmitoyl-CoA (Fig. 3). This indicates that the effect of acyl-CoA on the translocase is in a sense more specific and not only due to the surfactant activity. A cationic detergent, cetyltrimethylammonium bromide, is not inhibitory in sublytic concentrations and, on the contrary, it can abolish the inhibitory effect of acyl-CoA [18]. This can be explained by assuming that the negatively charged polar group of acyl-CoA is involved in the inhibition. This conclusion is supported by the observation that the inhibition is weaker in saline than in sucrose media (Fig. 1, compare K_i values in sucrose and KCl solutions).

The inhibition is diminished by Mg^{2+} and other divalent metal cations [16] which are known to form complexes with adenosine phosphates. This again points to the importance of the adenosine phosphate group of the CoA moiety in the mechanism of the inhibition. The structural similarity of this group to ADP is obvious.

The following mechanism of the inhibition of adenine nucleotide translocase may therefore be proposed [18, 19] (Fig. 4). Molecules of long-chain acyl-CoA become attached to the mitochondrial membrane by their hydrocarbon chains of fatty acid residues (Fig. 4C). This increases the local concentration of adenosine phosphate groups of acyl-CoA in the vicinity of the translocase (more properly, of the ADP-specific receptor site of the translocase) and facilitates a competitive binding (Fig. 4D). Cationic detergent, cetyltrimethylammonium, electrically attracts acyl-CoA molecules and removes them from the receptor site of the translocase (Fig. 4F). Inhibition by anionic detergents is smaller because of a poorer structural fitness to the receptor site of the translocase (Fig. 4G).

In experiments carried out so far only externally generated or added long-chain acyl-CoA has been used. Because of the well-known impermeability of the inner mitochondrial membrane to CoA thioesters, the question still remains open whether the translocase can also be affected by intramitochondrial acyl-CoA, i.e., acting on the inner side of the inner membrane.

Although much more attention has recently been paid to the action of acyl-CoA thioesters, it seems likely that free fatty acids have some inhibitory effect on the translocase as well. This is, however, difficult to demonstrate directly because of the formation of small amounts of acyl-CoA by intact mitochondria under the conditions of the assay. The inhibition by free fatty acids is likely because of their similarity to

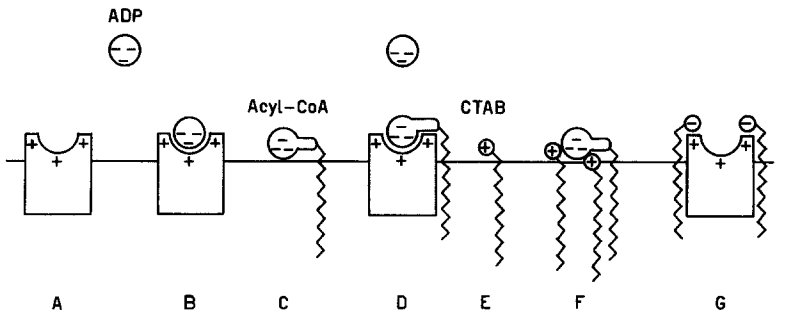

Figure 4. Mechanism of the inhibition by long-chain acyl-CoA and anionic detergents of adenine nucleotide translocase. A—Free receptor site of the translocase; B—receptor site occupied by an ADP molecule; C—acyl-CoA bound to the membrane by the nonpolar hydrocarbon chain of the fatty acid moiety; D—receptor site of the translocase occupied by the adenosine phosphate group of acyl-CoA; the translocase is immobilized and inactivated; E—cationic detergent, cetyltrimethylammonium, bound to the membrane by the nonpolar hydrocarbon chain; F—cetyltrimethylammonium cations attract acyl-CoA molecules and remove them from the receptor site; the translocase becomes reactivated; G—molecules of an anionic detergent, e.g., dodecylsulphate or deoxycholate, bound to the membrane by nonpolar groups and blocking partially the receptor site by means of negatively charged polar groups. From [19].

anionic detergents. Besides, it has been shown [20] that oleate diminishes atractyloside-sensitive binding of ATP to mitochondrial membrane fragments.

Possible Regulatory Role of the Inhibition by Acyl-CoA of Adenine Nucleotide Translocation

Long-chain acyl-CoA thioesters are thus naturally occurring inhibitors of adenine nucleotide translocation. The question is therefore discussed whether or not they play a regulatory role in the energy metabolism of the cell. In isolated mitochondria long-chain fatty acids and acyl-CoA increase the carboxylation of pyruvate [21–23] and propionyl-CoA [24], processes which require ATP and are inhibited by ADP. A regulatory role of acyl-CoA in ketogenesis [25] and in the utilization of fatty acids for oxidation and triglyceride synthesis [7] has also been proposed. In brown adipose tissue, acyl-CoA is believed to control the utilization of the high-energy state of the mitochondrion for either ATP synthesis or heat production [26]. The mechanism by which acyl-CoA interferes in all these processes is probably similar, namely, the inhibition of the ATP–ADP exchange across the mitochondrial membrane leads to an increase of the intramitochondrial and a decrease of the external phosphorylation potential.

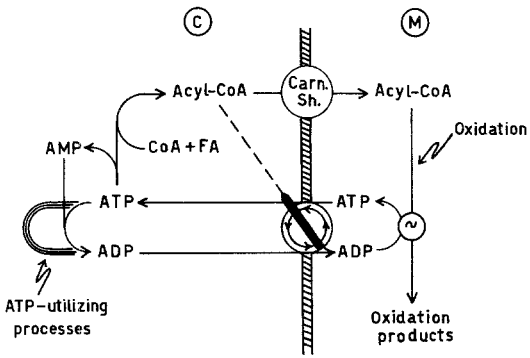

Figure 5. Schematic representation of a hypothetical regulatory role of long-chain acyl-CoA on the transport of adenine nucleotides between mitochondria and the cytoplasm. Formation of acyl-CoA from CoA and long-chain fatty acids (FA) is controlled by the level of available ATP in the cytoplasmic compartment (C) of the cell. Acyl-CoA is subsequently transported by the carnitine shuttle (Carn. Sh.) into the inner mitochondrial compartment (M) where it is oxidized. Any excess of long-chain acyl-CoA on the external side of the inner mitochondrial membrane will result in a partial inhibition of adenine nucleotide translocation and will thus diminish ATP/ADP ratio in the cytoplasm. Presented at the 9th International Congress of Biochemistry [24].

At equilibrium conditions, mitochondria are able to maintain a much higher phosphorylation potential in the surrounding medium than in their internal compartment [27, 28]. This is due to a preference of the translocase for the uptake of external ADP in exchange for intramitochondrial ATP in coupled mitochondria [29]. However, when ATP generated in mitochondria is extensively utilized in the cytoplasmic compartment, this high gradient of phosphorylation potentials may be diminished, since the translocase is assumed [30] to be, at least in liver, the rate-limiting step for oxidative phosphorylation. Therefore, any change in the efficiency of the translocation rate may result in a change of intramitochondrial and, especially, extramitochondrial phosphorylation potentials (Fig. 5).

On the other hand, however, doubts have also been expressed as to the regulatory role of acyl-CoA in the cell. It has been calculated [11] that long-chain acyl-CoA amounts to 0.8 nmole/mg protein in rat liver. Moreover, most of this acyl-CoA is thought to be bound to, or located in, mitochondria [31]. If so and if the sensitivity of the translocase in mitochondria in situ is equal or similar to that in isolated particles [13, 18] one may wonder how the transport of ADP and ATP between mitochondria and cytoplasm is ever possible. This difficulty may be explained, at least partly, by our recent observations [16] that the inhibitory effect of acyl-CoA is modified by the ionic composition of the

medium and especially by the presence of divalent cations. This suggests that under in vivo conditions the inhibition by long-chain acyl-CoA may be much smaller than assessed from in vitro experiments and is subject to a subtle secondary regulation by the ionic composition in the immediate vicinity of the inner mitochondrial membrane. In connection with this, the presence of large amounts of magnesium in the mitochondrial intermembrane compartment or loosely associated with the outer surface of the inner membrane [32] may be of particular importance.

In order conclusively to prove or to disprove the assumption that acyl-CoA plays a regulatory role of this kind in the cell it should be necessary to show whether or not an increase in cellular acyl-CoA produces a decrease of the cytoplasmic and an increase of the mitochondrial phosphorylation potentials and affects, in a positive or negative way, processes depending on ATP concentration in these two cellular compartments. Such an attempt has recently been made using isolated hepatocytes in which a decrease of the cytoplasmic ATP/ADP ratio and of the rate of glucose synthesis and fructose phosphorylation proceeds along with the accumulation of long-chain acylCoA [31, 33]. However, conclusive evidence for an in situ increase of the mitochondrial phosphorylation potential is still missing.

Di- and Tricarboxylate Transport

Carriers for di- and tricarboxylate anions are also inhibited by long-chain acyl-CoA [34]. As in the case of the adenine nucleotide translocase, this inhibition is competitive and depends strongly on the chain length of the fatty acid moiety [13]. However, carboxylate transport is less sensitive to acyl-CoA than is ADP transport (Table I).

The molecular mechanism of the inhibition is probably similar to that of the inhibition of adenine nucleotide translocase, namely, the negatively charged polar group of acyl-CoA interacts with the positively charged receptor site of the carrier while the nonpolar hydrocarbon

TABLE I. K_i values for palmitoyl-CoA of mitochondrial transport systems[a]

Transport system	K_i (μM)
Adenine nucleotide	0.10–0.25 (4)[b]
Citrate	3.2 –4.5 (2)
Malate	7.1 –9.5 (5)
Phosphate	25 (1)

[a] Reproduced from [13].

[b] Number of experiments in parentheses.

chain of the fatty acid moiety attaches the whole molecule to the membrane. It may also, however, be supposed that acyl-CoA inhibits the transport by complexing membrane-bound metal ions which, as postulated [35], are essential for dicarboxylate transport.

Doubts have been expressed [13] whether the inhibition of carboxylate transport by long-chain acyl-CoA may have any physiological importance because of the relatively high K_i value. On the other hand, however, the concentration of some organic anions, e.g., citrate, in liver is about one order of magnitude lower than that of adenine nucleotides. Therefore, under physiological conditions, the inhibition of citrate transport and adenine nucleotide transport could be similar (for further discussion of this point see [36]).

Phosphate Transport

Phosphate carrier is also inhibited by acyl-CoA thioesters [13]. The Ki value is, however, still higher than for di- and tricarboxylate transport (Table I). The inhibition depends on the chain length of the fatty acid moiety [13]. It is, however, interesting to note that this dependence is slightly different for phosphate, carboxylate, and adenine nucleotide carriers. For adenine nucleotides the highest inhibition is obtained with myristoyl($C_{14:0}$)-CoA and palmitoyl($C_{16:0}$)-CoA, while for citrate there is a sharp maximum with myristoyl-CoA only and for phosphate with lauroyl($C_{12:0}$)-CoA [13]. This may reflect a different spatial localization of these carriers in the lipid core of the membrane.

Free fatty acids have also been found to inhibit phosphate transport and this effect is not due to formation of small amounts of acyl-CoA since it cannot be abolished by carnitine [37]. The highest inhibition is obtained with lauric ($C_{12:0}$), myristic ($C_{14:0}$), and oleic ($C_{18:1}$) acids. The effect of acyl-CoA and free fatty acids on the phosphate carrier can best be measured by following their effect on mitochondrial swelling in isotonic ammonium phosphate solution as described by Chappell and Crofts [38]. By this method, a progressive inhibition of the swelling by increasing concentrations of palmitate can easily be observed (Fig. 6). On the other hand, however, it has also been found [37] that palmitate as well as palmitoyl-CoA partially release the inhibition produced by mersalyl, a specific inhibitor of phosphate carrier [39]. It can thus be concluded that acyl-CoA thioesters and long-chain free fatty acids block phosphate carrier in the mitochondrial inner membrane but, on the other hand, they also increase carrier-independent permeability of the membrane to phosphate.

Preliminary experiments using ^{36}Cl have shown (Wojtczak, unpublished) that long-chain fatty acids also increase mitochondrial permeability to chloride anions. Increased permeability to Cl^- has been

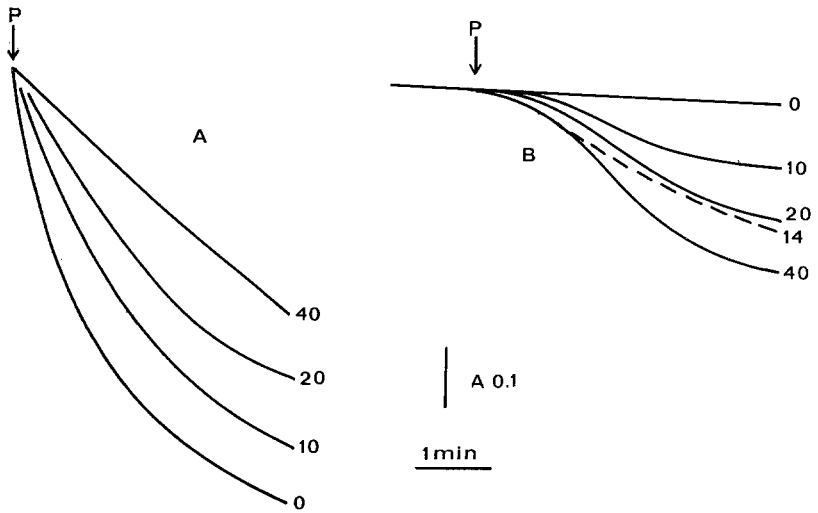

Figure 6. Effect of palmitate and palmitoyl-CoA on swelling of rat liver mitochondria in ammonium phosphate solution. The medium contained 100 mM ammonium phosphate (pH 7.4), 1 mM EDTA, and 5 μM rotenone. Swelling was monitored by measuring absorbance at 546 nm. A—Mitochondria were added to the medium, immediately followed by palmitate, as shown by the arrow (P). B—The medium contained in addition 0.3 mM mersalyl; palmitate (solid lines) or palmitoyl-CoA (dashed line) was added as indicated by the arrow (P). The numbers indicate the amounts of added palmitate or palmitoyl-CoA in nmole/mg mitochondrial protein. From [37].

observed previously [40] in mitochondria isolated from the brown adipose tissue and might be related to a high content of nonesterified long-chain fatty acids in these mitochondria [41, 42].

Inhibition of Nicotinamide Nucleotide Transhydrogenase

It has been found by Rydström et al. [43] that long-chain acyl-CoA thioesters are potent inhibitors of mitochondrial nicotinamide nucleotide transhydrogenase. Both energy-independent and energy-linked transhydrogenation processes are equally inhibited. The inhibition is competitive with respect to NADP(H) but not with respect to NAD(H). It strongly depends on the chain length of the fatty acid moiety, the lowest K_i value of 0.15 μM being found for palmitoyl-CoA.

It is assumed [43] that the inhibition is due to the interaction of the adenosine phosphate group of the CoA moiety with the NADP(H)-specific site of the enzyme. The nonpolar hydrocarbon chain of the fatty acid moiety attaches the molecule to the membrane and thus facilitates

this interaction. The molecular mechanism of the inhibition is therefore probably similar to that of the adenine nucleotide translocase inhibition [18].

It has been speculated [43] that the inhibition of the transhydrogenase by acyl-CoA has a regulatory role in switching between the oxidative breakdown and the reductive synthesis of fatty acids. However, the problem is not clear. Since the inhibition by acyl-CoA has been demonstrated so far only on sonic submitochondrial particles and since the transhydrogenase seems to be located on the internal side of the inner mitochondrial membrane (which corresponds to the external side of the sonic particles), it is logical to assume that this enzymic system can be affected by the intramitochondrial pool of long-chain acyl-CoA only. Therefore, the inhibitory effect may be largely counteracted by a fairly high concentration of intramitochondrial NADP(H). Furthermore, high intramitochondrial content of magnesium [32] may also partly abolish the inhibition.

Changes in the Permeability to Cations

First observations on increased outflow of intramitochondrial potassium as induced by fatty acids were made by Pressman and Lardy [44, 45] and Berger [46]. Only recently, however, the ionophore effect of fatty acids and their CoA thioesters has been directly demonstrated [47]. These compounds do not only increase the outflow of mitochondrial K^+ but also promote an energy-dependent uptake of monovalent cations from the medium. Unlike valinomycin but similar to gramicidin [48–50], fatty acids increase the permeability of the inner mitochondrial membrane to all alkali metal cations, including Li^+ and Na^+. The effect depends on the hydrodynamic chain length, being the highest for laurate ($C_{12:0}$), myristate ($C_{14:0}$), and oleate ($C_{18:1\,cis}$) [47].

Fatty-acid-induced uptake of cations is not that strongly dependent on the presence of penetrating anions, e.g., acetate or phosphate, as is the case with antiobiotic-induced cation uptake [38, 50]. This is probably due to the fact that fatty acids somewhat increase the permeability of the membrane to Cl^- as well (Wojtczak, unpublished). These findings provide a logical explanation for the long-known effect of fatty acids on mitochondrial swelling in saline solutions [51–53]. In fact, the effect of chain length of the fatty acids on swelling [52, 54, 55] and on the permeability to cations [47] (see also chain-length dependence of ATPase stimulation by fatty acids [56], which process is also related to cation permeability, as will be discussed in the following section of this article) is identical.

It is proposed [47] that fatty acids may function either as mobile carriers for monovalent cations or as channel-forming agents. The mobile carrier hypothesis is supported by the observation that fatty acids

promote the extraction of K^+ from water into a butanol-benzene phase [47]. On the other hand, however, acyl-CoA thioesters can act by the channel mechanism only, since it is well known that they do not penetrate across the inner mitochondrial membrane. The ionophore effect of fatty acids is further supported by observations (Wojtala and Wojtczak, unpublished) that they increase the permeability to cations of bimolecular phospholipid membranes. In mitochondrial membranes the increased permeability to cations is abolished by Mg^{2+}. This effect can be interpreted as due to immobilization of fatty acids in the membrane in the form of magnesium salts. It may also explain the increase by chelating agents of mitochondrial permeability to cations [57]. The latter effect might be due to endogenous mitochondrial fatty acids whose ionophore action is normally blocked by magnesium ions bound to the membrane.

Besides fatty acids and ionophore antibiotics a number of factors are known to increase the permeability of the inner mitochondrial membrane to cations, e.g., certain detergents, heavy metals, SH-blocking reagents, and chelating agents (for references see [47]). Among them, however, only fatty acids and acyl-CoA thioesters are normal cellular constituents and, therefore, are good candidates for natural ionophores which can control monovalent cation movements across the mitochondrial membrane in vivo. These movements, i.e., an uptake under high-energy conditions and release in low-energy states, are most likely responsible for the well-known configurational changes of mitochondria as observed both in vitro [58–62] and in vivo (in situ) [59, 62, 63].

Possible consequences of the increased permeability to cations in energy-coupling processes of mitochondria will be discussed in the following section.

Effect on Energy Coupling

ATPase, ATP-P_i Exchange, P/O Ratio, and Energy State of Mitochondria

First reports on the uncoupling effect of long-chain fatty acids came from Pressman and Lardy [44, 45, 56], who found a stimulation of mitochondrial ATPase. This was subsequently confirmed by other investigators [64–67]. On the other hand, at low concentrations fatty acids have been reported to inhibit the uncoupler-stimulated ATPase [5, 66–71]. The inhibitory effect of long-chain fatty acids on the ATP–P_i exchange reaction in mitochondria has also been observed [64–66, 72, 73]. Lowering by fatty acids of the P/O ratio has also been known for a long time [45, 51, 56, 64–66, 74]. However, at low concentrations and under conditions of active respiration (state 3), long-chain fatty acids increase intramitochondrial carboxylation of propionyl-CoA and pyruvate [21–24], processes which are indicative of a high energy state of mitochondria.

These various and often opposite effects can now be reconciled in view of the present knowledge of the mechanisms by which fatty acids affect the inner mitochondrial membrane. The *inhibition* of mitochondrial uncoupler-stimulated ATPase and of the ATP–P_i exchange reaction and lowering of the P/O ratio as measured with extramitochondrial phosphate acceptors can simply be explained by the inhibitory effect of fatty acids [4, 5] and/or acyl-CoA formed therefrom [7–11] on adenine nucleotide translocase. Suggestions have also been proposed [17, 71] that, apart from this, fatty acids can exhibit an oligomycinlike inhibitory effect on mitochondrial ATPase.

The *stimulation*, at higher concentrations of fatty acids, of mitochondrial ATPase [5, 44, 45, 56, 64–70] is of a more complex nature. It probably represents the sum of three different mechanisms: (1) the detergent effect on mitochondrial membranes (first postulated by Pressman and Lardy [56]), (2) energy dissipation due to ion pumping [47], and (3) true uncoupling due to an increased permeability to protons. The first mechanism is supported by the observations that fatty-acid-stimulated ATPase is no longer sensitive to atractyloside [5] and, at very high concentrations of fatty acids, is insensitive to oligomycin [17]. It is, however, absolutely dependent on Mg^{2+} [5, 17, 71], probably because of enhanced leakage of intramitochondrial magnesium [5]. The second mechanism, i.e., the stimulation of the ATPase activity by increased cation movements, is supported by a striking similarity of the dependence of both processes on the chain length and the degree of unsaturation of fatty acids [17, 47, 52, 54–56, 68]. Energy drain produced by cation pumping is further potentiated by an increased permeability of the mitochondrial membrane to Cl^-, as induced by fatty acids. This may also contribute to the stimulation of mitochondrial respiration by fatty acids under state 4 conditions. A similar explanation has been proposed [50] for the stimulation by valinomycin of mitochondrial respiration. The stimulation by fatty acids is, however, never as high as by uncouplers [5], thus confirming the assumption that mitochondria are not fully uncoupled. Fatty acids should therefore be thought of as producing a semi-uncoupled or "loosely coupled" state characterized by lack of respiratory control but preserved ability to synthesize ATP and to run other energy-dependent reactions, e.g., ion pumping. Such a state has been observed in isolated liver mitochondria under certain conditions (e.g., [75]). It is also observed in mitochondria isolated from the brown adipose tissue [42] and is believed to represent the main factor controlling heat generation in this tissue (for review see [76]). It should then be stressed that fatty acids at low concentration, e.g., for oleate below 50 nmole/mg mitochondrial protein, do not uncouple the mitochondrial energy-generating system in the sense that they do not collapse the transmembrane electrochemical potential [77, 78]. This type of uncoupling may appear

at higher fatty acid concentrations only and be the result of increased permeability of the mitochondrial membrane to protons.

Mitochondrial Respiration, Reverse Respiratory Control

The effect of fatty acids on mitochondrial respiration is bimodal. As already described, they increase substrate oxidation in state 4. Contrary to this, in state 3 and in uncoupled mitochondria, they inhibit the oxidation of various substrates [5, 64, 79, 80]. The explanation of the latter effect is not quite clear. It may be partly due to an impaired transport of respiratory substrates into the mitochondrion. But most likely it is mainly related to the increased permeability of the inner membrane to cations. It has been observed [81] that high osmolarity of the medium decreases oxidative processes in mitochondria. The mechanism of this inhibition is probably somehow connected with the condensation and the dehydration of the mitochondrial matrix. Mitochondria of the brown adipose tissue, which are more permeable to cations than liver mitochondria, exhibit a depressed oxidation even when placed in hypotonic solutions of nonpenetrating solutes, e.g., sucrose, because they are unable to maintain their own internal tonicity in such media [82].

Since long-chain fatty acids increase the permeability of mitochondria to cations, it can be expected that they will produce an outflow of intramitochondrial potassium ions when added to mitochondria under low energy states, i.e., in the presence of an uncoupler or ADP. This outflow may lead to a decreased internal osmolarity and, consequently, to dehydration of the matrix. A similar situation may appear when ADP or an uncoupler is added to mitochondria whose permeability to cations has already been incrased by a previous addition or endogenous formation of fatty acids. An inhibition instead of an activation of the respiration may then occur. This has been repeatedly observed under various experimental conditions [80, 83–86] and described as "reverse acceptor control" [83, 84].

In mitochondria from kidney cortex such reverse respiratory control in NaCl medium has been correlated with an increased permeability of these mitochondria to Na^+ [87]. We have shown [88] that nonesterified long-chain fatty acids whose level is elevated in kidney mitochondria may, at least partly, contribute to this increased permeability and therefore be responsible for the unusual behavior of these mitochondria towards phosphate acceptor and uncoupler. When kidney cortex mitochondria are isolated in the presence of serum albumin a normal ("positive") respiratory control is observed, even in NaCl medium. However, addition of small amounts of myristate to such mitochondria results in the appearance of reverse control with respect to ADP or an

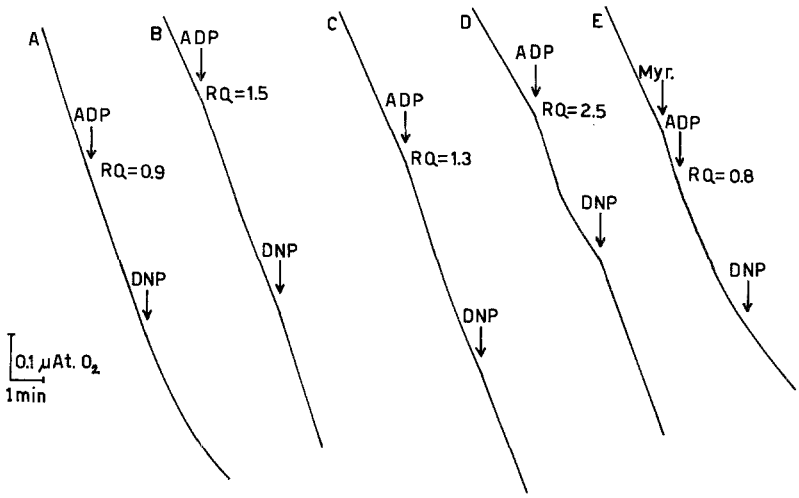

Figure 7. Respiratory control in rat kidney cortex mitochondria. Respiration was measured amperometrically at 25° C in the following medium: 135 mM NaCl, 2.5 mM EDTA, 10 mM triethanolamine-HCl, 5 mM phosphate (pH 7.2), 3.3 mM glutamate, 1.7 mM L-malate and mitochondria 0.8 mg protein/ml. In traces B and D the medium also contained bovine serum albumin 7 mg/ml. In traces C, D, and E mitochondria were isolated in the medium containing bovine serum albumin 3 mg/ml. Additions: ADP, 0.175 mM; 2,4-dinitrophenol (DNP) 0.1 mM; myristate (Myr.), 88 nmoles/mg mitochondrial protein. From [88].

uncoupler (Fig. 7). Reversed respiratory control has occasionally been observed by us in fatty-acid-treated rat liver mitochondria (Zaluska and Wojtczak, unpublished results).

It has once been postulated [89, 90] that fatty acids may interact with high-energy intermediates of oxidative phosphorylation. The compound thus formed might react either with CoA to give acyl-CoA or with H_2O, leading to a discharge of the energy. The latter process might account for the uncoupling effect of fatty acids. This postulation was based on observations [89–91] that long-chain fatty acids could be activated and oxidized or esterified in mitochondria at very high concentrations, resulting in a complete uncoupling. This hypothesis was also compatible with the observation [92] that mitochondria catalyze an exchange of oxygen between water and the carboxylic group of fatty acids. It was, however, subsequently shown by van den Bergh and his coworkers [17, 71, 93, 94] that the activation of fatty acids under uncoupled conditions could entirely be explained by substrate-level phosphorylation.

Conclusions

The following effects of fatty acids and acyl-CoA thioesters on energy metabolism of mitochondria can now be assumed:

(1) Inhibition of adenine nucleotide translocation. This effect may increase the energy state of mitochondria respiring under state 3 conditions and decrease phosphorylation potential in the surrounding medium (the cytoplasm).

(2) Increased permeability to monovalent cations. This may lead to a partial energy dissipation due to a futile recycling of K^+ (or another cation), namely an energy-dependent uptake and a passive outflow.

(3) True uncoupling due to increased permeability to protons. This effect probably occurs at high concentrations of fatty acids only.

(4) Substrate effect. Fatty acids in the form of acyl-CoA are excellent respiratory substrates for mitochondria of most tissues. Their oxidation is coupled to the generation of high energy state of the mitochondrial membrane and, consequently, to ATP synthesis.

References

1. S. Skrede and J. Bremer, *Acta Chem. Scand.*, 19 (1965) 1995–1997.
2. J. Bremer and E.J. Davis, *Biochim. Biophys. Acta*, 275 (1972) 298–301.
3. J. Bryla, Z. Kaniuga, and B. Frackowiak, *Biochim. Biophys. Acta*, 143 (1967) 285–291.
4. L. Wojtczak and H. Załuska, *Biochem. Biophys. Res. Commun.*, 28 (1967) 76–81.
5. L. Wojtczak, K. Bogucka, M.G. Sarzała, and H. Załuska, in *Mitochondria, Structure and Function*, L. Ernster and Z. Drahota, eds., Academic Press, London and New York (1969), pp. 79–92.
6. M.J. Weidemann, H. Erdelt, and M. Klingenberg, *Eur. J. Biochem.*, 16 (1970) 313–335.
7. S.V. Pande and M.C. Blanchaer, *J. Biol. Chem.*, 246 (1971) 402–411.
8. A. Shug, E. Lerner, Ch. Elson, and E. Shrago, *Biochem. Biophys. Res. Commun.*, 43 (1971) 557–563.
9. E. Lerner, A.L. Shug, C. Elson, and E. Shrago, *J. Biol. Chem.*, 247 (1972) 1513–1519.
10. R.A. Harris, B. Farmer, and T. Ozawa, *Arch. Biochem. Biophys.*, 150 (1972) 199–209.
11. W.J. Vaartjes, A. Kemp Jr., and J.H.M. Souverijn, *FEBS Lett.*, 23 (1972) 303–308.
12. A.L. Shug and E. Shrago, *Biochem. Biophys. Res. Commun.*, 53 (1973) 659–665.
13. F. Morel, G. Lauquin, J. Lunardi, J. Duszyński, and P.V. Vignais, *FEBS Lett.*, 39 (1974) 133–138.
14. E. Shrago, A. Shug, C. Elson, T. Spennetta, and C. Crosby, *J. Biol. Chem.*, 249 (1974) 5269–5274.
15. C.H. Ho and S.V. Pande, *Biochim. Biophys. Acta*, 369 (1974) 86–94.
16. J. Duszyński and L. Wojtczak, *FEBS Lett.*, 50 (1975) 74–78.

17. W.J. Vaartjes, *Langketen vetzuren en het mitochondriale energiemetatolisme,* thesis, University of Utrecht (1973).
18. J. Duszyński and L. Wojtczak, *FEBS Lett.*, 40 (1974) 72–76.
19. J. Duszyński, *Transport nukleotydow adeninowych w mitochondriach,* thesis, Nencki Institute of Experimental Biology, Warsaw (1975).
20. L. Wojtczak and H. Zaluska, *Biochim. Biophys. Acta,* 189 (1969) 455–456.
21. J.W. Stucki, F. Brawand, and P. Walter, *Eur. J. Biochem.*, 27 (1972) 181–191.
22. A.B. Wojtczak, E. Lenartowicz, M. A. Rodionova, and J. Duszyński, *FEBS Lett.*, 28 (1972) 253–258.
23. M. Lopes-Cardozo, W.J. Vaartjes, and S.G. van den Bergh, *FEBS Lett.*, 28 (1972) 265–270.
24. L. Wojtczak, J. Duszyński, M. Komosińska, and A.B. Wojtczak, *9th International Congress of Biochemistry, Stockholm* (1973) International Union of Biochemistry Abstract book, 351.
25. W.J. Vaartjes, M. Lopes-Cardozo, and S.G. van den Bergh, *FEBS Lett.*, 26 (1972) 117–122.
26. E.N. Christiansen, Z. Drahota, J. Duszyński, and L. Wojtczak, *Eur. J. Biochem.* 34 (1973) 506–512.
27. H.W. Heldt, M. Klingenberg, and M. Milovancev, *Eur. J. Biochem.*, 30 (1972) 434–440.
28. E.C. Slater, J. Rosing, and A. Mol, *Biochim. Biophys. Acta,* 292 (1973) 534–553.
29. E. Pfaff and M. Klingenberg, *Eur. J. Biochem.*, 6 (1968) 66–79.
30. H.W. Heldt and M. Klingenberg, *Eur. J. Biochem.*, 4 (1968) 1–8.
31. T.P.M. Akerboom and P.F. Zuurendonk, *9th FEBS Meeting, Budapest* (1974), Abstracts, 494.
32. K. Bogucka and L. Wojtczak, *Biochem. Biophys. Res. Commun.*, 44 (1971) 1330–1337.
33. M.J.H. Geelen, A.B. Wojtczak, M. Lopes-Cardozo, and S.G. van den Bergh, *10th FEBS Meeting, Paris* (1975), Société de Chimie Biologique, Paris, Abstracts, No. 1438.
34. M.L. Halperin, B.H. Robinson, and I.B. Fritz, *Proc. Natl. Acad. Sci. U.S.A.*, 69 (1972) 1003–1007.
35. S. Passarella, F. Palmieri, and E. Quagliariello, *FEBS Lett.*, 38 (1973) 91–95.
36. A.J. Meijer and K. van Dam, *Biochim. Biophys. Acta,* 346 (1974) 213–244.
37. H. Zaluska and L. Wojtczak, manuscript in preparation.
38. J.B. Chappell and A.R. Crofts, in *Regulation of Metabolic Processes in Mitochondria,* J.M. Tager, S. Papa, E. Quagliariello, and E.C. Slater, eds. Elsevier, Amsterdam (1966), pp. 293–316.
39. D.D. Tyler, *Biochem. J.*, 107 (1968) 121–123.
40. D.G. Nicholls and O. Lindberg, *Eur. J. Biochem.*, 37 (1973) 523–530.
41. Z. Drahota, E. Honová, and P. Hahn, *Experientia (Basel),* 24 (1968) 431–432.
42. J.I. Pedersen and H.J. Grav, *Eur. J. Biochem.*, 25 (1972) 75–83.
43. J. Rydström, *Eur. J. Biochem.*, 31 (1972) 496–504.
44. B.C. Pressman and H.A. Lardy, *J. Biol. Chem.*, 197 (1952) 547–556.
45. B.C. Pressman and H.A. Lardy, *Biochim. Biophys. Acta,* 18 (1955) 482–487.
46. M. Berger, *Biochim. Biophys. Acta,* 23 (1957) 504–509.
47. L. Wojtczak, *FEBS Lett.*, 44 (1974) 25–30.
48. C. Moore and B.C. Pressman, *Biochem. Biophys. Res. Commun.*, 15 (1964) 562–567.
49. J.B. Chappell and A.R. Crofts, *Biochem. J.*, 95 (1965) 393–402.
50. B.C. Pressman, *Proc. Natl. Acad. Sci. U.S.A.*, 53 (1965) 1076–1083.
51. A.L. Lehninger and L.F. Remmert, *J. Biol. Chem.*, 234 (1959) 2459–2464.
52. Y. Avi-Dor, *Biochim. Biophys. Acta,* 39 (1960) 53–61.

53. L. Wojtczak and A.L. Lehninger, *Biochim. Biophys. Acta,* 51 (1961) 442–456.
54. K. Utsumi, S. Ohara, G. Yamamoto, K. Inaba, H. Urakami, and M. Yamamoto, *Acta Med. Okoyama,* 16 (1962) 317–331.
55. J. Zborowski and L. Wojtczak, *Biochim. Biophys. Acta,* 70 (1963) 596–598.
56. B.C. Pressman d and H.A. Lardy, *Biochim. Biophys. Acta,* 21 (1956) 458–466.
57. C.T. Settlemire, G.R. Hunter, and G.P. Brierley, *Biochim. Biophys. Acta,* 162 (1968) 487–499.
58. L. Packer, E. Corriden, and R.H. Marchant, *Biochim. Biophys. Acta,* 78 (1963) 534–536.
59. E.C. Weinbach, J. Garbus, and H.G. Sheffield, *Expt. Cell Res.,* 46 (1967) 129–143.
60. C.R. Hackenbrock, *J. Cell Biol.,* 37 (1968) 345–369.
61. R.A. Harris, J.T. Penniston, J. Assai, and D.E. Green, *Proc. Natl. Acad. Sci. U.S.A.,* 59 (1968) 830–837.
62. U. Muscatello, V. Guarriera-Bobyleva, and P. Buffa, *J. Ultrastruct. Res.,* 40 (1972) 235–260.
63. C.R. Hackenbrock and T.G. Rehn, *7th International Congress of Electron Microscopy, Grenoble* (1970) Société Française de Microscopie Electronique, Paris, Abstracts, Vol. 3, pp. 143–144.
64. W.C. Hülsmann, W.B. Elliott, and E.C. Slater, *Biochim. Biophys. Acta,* 39 (1960) 267–276.
65. L. Wojtczak and A.B. Wojtczak, *Biochim. Biophys. Acta,* 39 (1960) 277–286.
66. P. Borst, J.A. Loos, E.J. Christ, and E.C. Slater, *Biochim. Biophys. Acta,* 62 (1962) 509–518.
67. C.J. Bos and P. Emmelot, *Biochim. Biophys. Acta,* 64 (1962) 21–29.
68. W. Chefurka and T. Dumas, *Biochemistry,* 5 (1966) 3904–3911.
69. R. Cereijo-Santaló, *Can. J. Biochem.,* 45 (1967) 897–909.
70. M. Erecińska, *4th FEBS Meeting, Oslo* (1967) Universitetsforlaget, Oslo, Abstracts, 94.
71. S.G. van den Bergh, C.P. Modder, J.H.M. Souverijn, and H.C.J.M. Pierrot, in *Mitochondria, Structure and Compartmentation,* L. Ernster and Z. Drahota, eds, Academic Press, London (1969), pp. 137–144.
72. K. Ahmed and P.G. Scholefield, *Nature (London),* 186 (1960) 1046–1047.
73. A.B. Falcone and R.L. Mao, *Biochim. Biophys. Acta,* 105 (1965) 233–245.
74. P.G. Scholefield, *Can. J. Biochem. Physiol.,* 34 (1956) 1227–1232.
75. L.F. Remmert and A.L. Lehninger, *Proc. Natl. Acad. Sci. U.S.A.,* 45 (1959) 1–5.
76. T. Flatmark and J.I. Pedersen, *Biochim. Biophys. Acta,* 416 (1975) 53–103.
77. P. Mitchell, in *Mitochondria, Structure and Compartmentation,* L. Ernster and Z. Drahota, eds, Academic Press, London (1969), pp. 219–232.
78. V.P. Skulachev, A.A. Jasaitis, V.V. Navickaite, and L.S. Yaguzhinsky, in *Mitochondria, Structure and Compartmentation,* L. Ernster and Z. Drahota, eds, Academic Press, London (1969), pp. 275–284.
79. P.G. Scholefield, *Can. J. Biochem. Physiol.,* 34 (1956) 1211–1225.
80. L. Vázquez-Colón, F.D. Ziegler, and W.B. Elliott, *Biochemistry,* 5 (1966) 1134–1139.
81. D.G. Nicholls and O. Lindberg, *FEBS Lett.* 25 (1972) 61–64.
82. D.G. Nicholls, H.J. Grav, and O. Lindberg, *Eur. J. Biochem.,* 31 (1972) 526–533.
83. A.L. Lehninger and C.T. Gregg, *Biochim. Biophys. Acta,* 78 (1963) 12–26.
84. C.T. Gregg and A.L. Lehninger, *Biochim. Biophys. Acta,* 78 (1963) 27–44.
85. F.D. Ziegler, L. Vázquez-Colón, W.B. Elliott, A. Taub, and C. Gans, *Biochemistry,* 4 (1965) 55–560.
86. A.B. Wojtczak, E. Lagwińska, and L. Wojtczak, *Acta Biochim. Polon.,* 15 (1968) 15–29.
87. M.W. Weiner and H.A. Lardy, *J. Biol. Chem.,* 248 (1973) 7682–7687.

88. L. Wojtczak, manuscript in preparation.
89. L. Wojtczak, H. Zaluska, and Z. Drahota, *Biochim. Biophys. Acta*, **98** (1965) 8–18.
90. L. Wojtczak, Z. Drahota, H. Zaluska, and J. Zborowski, in *Regulation of Metabolic Processes in Mitochondria*, J.M. Tager, S. Papa, E. Quagliariello, and E.C. Slater, eds, Elsevier, Amsterdam (1966), pp. 134–142.
91. Z. Drahota and E. Honová, *Acta Biochim. Polon.*, **15** (1968) 227–234.
92. A.B. Falcone and R.L. Mao, *Biochim. Biophys. Acta*, **105** (1965) 246–252.
93. S.G. van den Bergh, *Biochim. Biophys. Acta*, **98** (1965) 442–444.
94. S.G. van den Bergh, in *Regulation of Metabolic Processes in Mitochondria*, J.M. Tager, S. Papa, E. Quagliariello, and E.C. Slater, eds, Elsevier, Amsterdam (1966) pp. 126–133.