Biochem. J. (1981) **196**, 803–809
Printed in Great Britain

2-Mercaptoacetate administration depresses the β-oxidation pathway through an inhibition of long-chain acyl-CoA dehydrogenase activity

Françoise BAUCHÉ, Dominique SABOURAULT, Yves GIUDICELLI, Joseph NORDMANN and Roger NORDMANN
Laboratoire de Biochimie de la Faculté de Médecine de Paris-Ouest et Groupe de Recherches (U.72) de l'I.N.S.E.R.M., 45 rue des Saints-Pères, 75270 Paris Cédex 06, France

(*Received 31 December 1980/Accepted 6 March 1981*)

To elucidate the mechanisms through which 2-mercaptoacetate administration inhibits fatty acid oxidation in the liver, the respiration rates induced by different substrates were studied polarographically in rat hepatic mitochondria isolated 3h after 2-mercaptoacetate administration. Palmitoyl-L-carnitine oxidation was almost completely inhibited in either the absence or presence of malonate. Octanoate oxidation was also inhibited, and the intramitochondrial acyl-CoA content was markedly increased. The oxidation rate of pyruvate and 2-oxoglutarate on the one hand and of 3-hydroxybutyrate, succinate and glutamate on the other was either normal or only slightly decreased. In the presence of 2,4-dinitrophenol, the extent of the inhibition of palmitoyl-L-carnitine oxidation was unchanged. All these results are consistent with the hypothesis that the 2-mercaptoacetate inhibition of fatty acid oxidation is due to an inhibition of the β-oxidation pathway itself. Finally, the mitochondrial defect responsible for this inhibition was shown to be an inhibition of palmitoyl-CoA dehydrogenase activity (EC 1.3.99.3).

In previous studies (Sabourault *et al.*, 1976), we have reported that 2-mercaptoacetate is, like 2-mercaptoethanol (Nordmann & Nordmann, 1971*a*,*b*), a potent fatty-liver-inducing drug in the rat. In fact, we found that as early as 3h after the intraperitoneal administration of this compound (40μmol/100g body wt.), the hepatic triacylglycerol content was increased 3-fold, and 24h after this administration it reached more than 14 times the control value. Experiments performed to elucidate the mechanisms of this effect have shown that 2-mercaptoacetate administration is followed by an early increase in peripheral lipolysis and by a marked inhibition of both ketogenesis and fatty acid oxidation in the liver (Sabourault *et al.*, 1979).

The purpose of the present study was to elucidate the mechanism through which 2-mercaptoacetate administration inhibits fatty acid oxidation in the liver. This was done by studying the oxidation rate of different substrates in rat hepatic mitochondria isolated 3h after the administration of 2-mercaptoacetate. The results presented here provide evidence that 2-mercaptoacetate administration directly inhibits the β-oxidation pathway by interfering with the palmitoyl-CoA dehydrogenase activity (EC 1.3.99.3).

Materials and methods

Mitochondrial isolation

Wistar female rats (200±20g) were starved overnight before the experiments. 2-Mercaptoacetate (40μmol/100g body wt.) was administered intraperitoneally as previously described (Sabourault *et al.*, 1976); control rats received an equal volume of 0.9% NaCl. Animals were killed by decapitation 3h later. Liver mitochondria were prepared by the method of Beattie (1968). The fluffy layer was discarded, and the mitochondrial pellet was washed three times by suspension and centrifugation in 0.25M-sucrose at 4°C and was finally suspended in the same medium at 4°C at a protein concentration of 40–60mg/ml. Protein was determined as described by Lowry *et al.* (1951).

Respiration

The rate of mitochondrial respiration was measured with a Gilson oxygraph at 25°C by using a Clark oxygen electrode. The reaction medium (pH7.4) usually consisted of 58mM-KCl, 25mM-NaCl, 6mM-$MgCl_2$, 13mM-K_2HPO_4, 3mM-KH_2PO_4, 12mM-NaF and mitochondria equivalent to 2.5–3mg of protein (final volume 1.6ml). When

0306-3283/81/060803-07$01.50/1

pyruvate, 2-oxoglutarate and octanoate were the respiratory substrates, NaF was omitted from this medium. Final concentrations of added respiratory substrates were: sodium DL-3-hydroxybutyrate, 14mM; sodium succinate, 6mM; sodium pyruvate, 10mM; sodium 2-oxoglutarate, 10mM; sodium L-glutamate, 5mM; sodium L-malate, 5mM; palmitoyl-L-carnitine, 25 μM; sodium octanoate, 50 μM. In some experiments, sodium malonate (5mM), rotenone (1.5 μM), oligomycin (10 μg) and 2,4-dinitrophenol (50 μM) were also added to the incubation medium. Respiration was initiated by the addition of the substrates (state 4), after which 150 μM-ADP was added to induce an immediate increase in the rate of oxygen utilization (state 3 as defined by Chance & Williams, 1955). This state is maintained until the added ADP is phosphorylated. At this time, the rate of oxygen consumption decreases (state 4'). State-3 and state-4 and -4' activities were determined by calculating the nmol of oxygen consumed/min and relating this to mg of mitochondrial protein added. The respiratory control ratio is determined by dividing state-3 activity by that of state 4'. The ADP/O ratio is calculated by dividing the nmol of ADP phosphorylated by ng-atoms of O consumed during each interval at state 3.

Measurement of palmitoyl-CoA dehydrogenase activity

The oxidation rate of palmitoyl-L-carnitine was also measured by using the spectrophotometric assay of Osmundsen & Bremer (1977), which selectively measures the flux through the acyl-CoA dehydrogenases of β-oxidation. In this method, reduction of ferricyanide, the final acceptor of reducing equivalents, is followed by recording the decrease in A_{420} with time, with 475nm as the reference wavelength. The assay medium consisted of 130mM-KCl, 10mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], 0.1mM-EDTA, 1mM-KCN, 0.5mM-$K_3Fe(CN)_6$, 1mM-ADP, 1mM-P_i and 4.5mg of defatted bovine serum albumin, pH7.2. After addition of mitochondria (up to 2mg of protein), 3 μg of rotenone was added, followed by addition of palmitoyl-L-carnitine (20 μM) and 10mM-oxaloacetate. The final assay volume was 3ml.

Measurement of mitochondrial long-chain acyl-CoA

For this 2vol. of 1.8M-$HClO_4$ were added to 1vol. of mitochondrial suspension and the samples were subsequently treated as described by Garland (1974) to isolate the fraction containing long-chain acyl-CoA (C_{10} and above). CoA in this fraction was determined after alkaline hydrolysis with phosphotransacetylase as described by Stadtman *et al.* (1951).

Materials

2-Mercaptoacetate was obtained from E. Merck, Darmstadt, Germany, and was of analytical grade. Palmitoyl-L-carnitine, rotenone (grade II), 2,4-dinitrophenol (grade II), oligomycin, Hepes and bovine serum albumin (essentially fatty acid-poor; fraction V) were purchased from Sigma Chemical Co., St Louis, MO, U.S.A. Oxaloacetic acid was from Boehringer, Mannheim, Germany. All other reagents were of analytical grade or of highest purity available and were from Sigma or Merck.

Results

Data in Table 1 show that, in the absence of any added substrate, the oxygen consumed by mitochondria from 2-mercaptoacetate-treated animals was markedly increased (+109%), whereas, as expected from previous studies from this laboratory (Sabourault *et al.*, 1979), respiration with palmitoyl-L-carnitine was almost completely inhibited (−86% in state 3 and −30% in state 4). As a consequence of this inhibition, the respiratory control ratio was markedly depressed (less than unity) and the ADP/O ratio could not be calculated. These data suggest that these effects could result from an inhibition of one or more of the following metabolic processes: (1) palmitoyl-L-carnitine transport into the mitochondria, (2) β-oxidation, (3) citrate cycle, (4) respiratory chain, (5) coupling of oxidation to phosphorylation and (6) adenine nucleotide translocase activity.

The possibility that 2-mercaptoacetate administration could inhibit the transport of palmitoyl-L-carnitine into the mitochondria was investigated by following the oxidation rate of octanoate, a substrate which is believed not to require carnitine acyltransferase to enter into the mitochondria (Fritz, 1959), and also by measuring the intramitochondrial amount of long-chain acyl-CoA ($\geqslant C_{10}$). As shown in Table 1, the rate of octanoate oxidation was also markedly decreased after 2-mercaptoacetate administration. Conversely, long-chain acyl-CoA concentration was drastically increased in the mitochondria of the treated animals (2.16 ± 0.16nmol/mg of protein, compared with 0.46 ± 0.09nmol/mg in the control) (means $\pm$ S.E.M. for four determinations). These results thus provide the evidence that the inhibition of fatty acid oxidation found in the liver after 2-mercaptoacetate administration is not related to a disturbance of the transport of acyl-CoA into the mitochondria.

As stated above, inhibition of the citrate cycle could be also a mechanism through which 2-mercaptoacetate blocks fatty acid oxidation. To test this hypothesis, the rate of palmitoyl-L-carnitine oxidation was studied in the presence of malonate

Table 1. *Rates of fatty acid oxidation and oxidative phosphorylation in rat liver mitochondria, after the administration of 2-mercaptoacetate (40 μmol/100 g body wt. intraperitoneally)*

Experiments were performed as described in the text. Each value is the mean ± S.E.M. for the number of determinations in parentheses, each determination being performed on mitochondria isolated from two pooled livers removed 3 h after the administration of either saline (control) or 2-mercaptoacetate (treated). Statistically significant differences were determined by using Student's *t* test. Abbreviation and symbol: N.D., not determined; †, cannot be calculated.

Substrates	Animals	O_2 consumption (nmol/min per mg of protein) State 4	O_2 consumption (nmol/min per mg of protein) State 3	Respiratory control ratio	ADP/O	O_2 consumption (nmol/min per mg of protein) in the presence of 2,4-dinitrophenol (0.050 mM)
None	Control	4.2 ± 0.4 (8)	—	—	—	—
	Treated	8.8 ± 1.0 (7)	—	—	—	—
		$P < 0.001$	—	—	—	—
Palmitoyl-L-carnitine (0.025 mM)	Control	11.2 ± 1.4 (5)	40.8 ± 1.9 (5)	3.3 ± 0.3	2.25 ± 0.08	32.2 ± 1.1 (5)
	Treated	7.8 ± 1.0 (10)	5.6 ± 1.7 (10)	<1	†	6.1 ± 0.5 (5)
		$0.001 < P < 0.01$	$P < 0.001$	$P < 0.001$		$P < 0.001$
Palmitoyl-L-carnitine (0.025 mM) + sodium malonate (5 mM)	Control	13.3 ± 1.5 (5)	38.2 ± 2.5 (5)	3.0 ± 0.2	2.05 ± 0.10	26.6 ± 0.8 (3)
	Treated	5.4 ± 1.3 (5)	4.5 ± 1.2 (5)	<1	†	3.6 ± 0.6 (3)
		$P < 0.001$	$P < 0.001$	$P < 0.001$		$P < 0.001$
Sodium octanoate (0.050 mM)	Control	7.3 ± 0.6 (5)	21.0 ± 2.0 (5)	2.4 ± 0.3	1.70 ± 0.10	N.D.
	Treated	6.2 ± 0.4 (5)	6.2 ± 0.4 (5)	1.0 ± 0.0	†	N.D.
		$0.01 < P < 0.02$	$P < 0.001$	$P < 0.001$		

(5 mM). Under these conditions (Table 1), 2-mercaptoacetate administration still decreased the oxidation of palmitoyl-L-carnitine in both state 3 (90% inhibition) and state 4 (66% inhibition). From these data, it can be concluded that the effects of 2-mercaptoacetate administration on fatty acid oxidation observed in the absence of malonate are not linked to citrate-cycle inhibition.

To consider the possibility that 2-mercaptoacetate may alter the activity of adenine nucleotide translocase or the respiratory-chain coupling, the rate of palmitoyl-L-carnitine oxidation was studied in mitochondria incubated with 2,4-dinitrophenol (50 μM) in place of ADP. As shown in Table 1, the inhibition of palmitoyl-L-carnitine oxidation was of the same order of magnitude (81% inhibition) whether mitochondria from treated animals were incubated in the presence of 2,4-dinitrophenol or ADP (state 3). These data thus indicate that, if the occurrence of an inhibition of either adenine nucleotide translocase or of coupling cannot be completely excluded (see below), these mechanisms do not play a major role in the inhibitory effect of 2-mercaptoacetate on palmitoyl-L-carnitine oxidation. It seems therefore likely that an alteration of either the respiratory-chain activity or the β-oxidation pathway is involved in the mechanism of action of 2-mercaptoacetate on fatty acid oxidation.

To test the first of these two hypotheses, we have studied the influence of 2-mercaptoacetate administration on the respiration of hepatic mitochondria oxidizing nicotinamide-nucleotide-linked (3-hydroxybutyrate, glutamate and malate) and flavin-linked (succinate) substrates. In the presence of these different substrates, state-4 activities were significantly increased, and this effect was particularly marked in the presence of succinate (+176%). After addition of ADP (state 3), however, the oxidation rate of all the substrates studied was decreased: −37% with 3-hydroxybutyrate, −56% with glutamate and −26% with succinate. As a consequence of these alterations, calculation of the respiratory control ratio for each of these substrates yielded very low values in the treated group. Data in Table 2 also show that when 2,4-dinitrophenol was added in place of ADP, the oxidation rates of these substrates were similar in mitochondria from treated and control animals. This provides evidence that 2-mercaptoacetate administration has no effect on the activity of the respiratory chain and suggests therefore that an inhibition of β-oxidation is the probable mechanism which is involved in the inhibitory effect of 2-mercaptoacetate on fatty acid oxidation in the liver.

Several mechanisms can be proposed to explain the modifications described in Table 2. Among them, an uncoupling action of 2-mercaptoacetate is a possible mechanism, since, beside the fact that it would explain the opposite alterations of state-4 and -3 activities, it would also explain the similar respiration rates which are found when mitochondria from control and treated rats are incu-

Table 2. *Effects of 2-mercaptoacetate administration on the oxidation of nicotinamide-nucleotide-linked and flavin-linked substrates in rat liver mitochondria*

Experimental conditions and statistical analysis are as described in Table 1.

Substrates	Animals	O_2 consumption (nmol/min per mg of protein) State 4	O_2 consumption (nmol/min per mg of protein) State 3	Respiratory control ratio	ADP/O	O_2 consumption (nmol/min per mg of protein) in the presence of 2,4-dinitrophenol (0.050 mM)
Sodium DL-3-hydroxybutyrate (14 mM)	Control	7.7 ± 0.9 (6)	29.9 ± 2.6 (6)	4.0 ± 0.1	2.60 ± 0.07	16.3 ± 2.0 (7)
	Treated	13.0 ± 1.5 (8)	19.0 ± 3.7 (8)	1.7 ± 0.1	2.40 ± 0.10	17.0 ± 1.9 (5)
		$P < 0.001$	$P < 0.001$	$P < 0.001$	$0.001 < P < 0.01$	$P > 0.02$
Sodium L-glutamate (5 mM) + sodium L-malate (5 mM)	Control	13.4 ± 0.4 (5)	68.5 ± 2.2 (5)	5.2 ± 0.6	2.60 ± 0.20	45.9 ± 2.4 (5)
	Treated	19.2 ± 3.2 (4)	29.8 ± 4.1 (4)	2.2 ± 0.3	2.45 ± 0.05	47.0 ± 2.0 (5)
		$0.001 < P < 0.01$	$P < 0.001$	$P < 0.001$	$P > 0.02$	$P > 0.02$
Sodium succinate (6 mM)	Control	13.9 ± 2.6 (5)	63.7 ± 2.2 (5)	5.4 ± 0.4	1.70 ± 0.20	62.7 ± 7.3 (7)
	Treated	38.4 ± 5.0 (5)	47.2 ± 5.3 (5)	1.5 ± 0.2	1.60 ± 0.10	59.2 ± 5.3 (8)
		$P < 0.001$	$0.001 < P < 0.01$	$P < 0.001$	$P > 0.02$	$P > 0.02$

Table 3. *Effects of 2-mercaptoacetate administration on the oxidation of some CoA-dependent substrates in rat liver mitochondria*

Experimental conditions and statistical analysis are as described in Table 1.

Substrates	Animals	O_2 consumption (nmol/min per mg of protein) State 4	O_2 consumption (nmol/min per mg of protein) State 3	Respiratory control ratio	ADP/O	O_2 consumption (nmol/min per mg of protein) in the presence of 2,4-dinitrophenol (0.050 mM)
Sodium pyruvate (10 mM)	Control	5.7 ± 1.0 (4)	16.3 ± 2.4 (4)	2.9 ± 0.4	2.20 ± 0.10	10.5 ± 2.0 (4)
	Treated	10.5 ± 0.9 (5)	17.6 ± 2.7 (5)	1.6 ± 0.1	1.95 ± 0.10	14.9 ± 1.2 (5)
		$P < 0.001$	$P > 0.02$	$P < 0.001$	$0.01 < P < 0.02$	$0.001 < P < 0.01$
Sodium 2-oxoglutarate (10 mM)	Control	6.4 ± 1.0 (5)	19.4 ± 1.2 (5)	2.3 ± 0.2	2.10 ± 0.04	16.4 ± 2.5 (5)
	Treated	9.9 ± 0.8 (5)	19.1 ± 1.7 (5)	1.9 ± 0.1	2.05 ± 0.10	16.9 ± 2.7 (5)
		$P < 0.001$	$P > 0.02$	$0.001 < P < 0.01$	$P > 0.02$	$P > 0.02$

bated with the uncoupler 2,4-dinitrophenol. In addition, an uncoupling action of 2-mercaptoacetate, which would also explain the increased respiration rate in state 1 (Table 1), seems all the more likely, as other thiol compounds have been shown to behave as slight uncouplers of oxidative phosphorylation (Cederbaum & Rubin, 1976). Increased respiration rates in both state 1 (without substrate) and 4 (with 3-hydroxybutyrate, glutamate or succinate) could also result from an increased ATPase (EC 3.6.1.3) activity in the mitochondria of the treated animals. However, increased state-4 respiration with succinate as the substrate was not reversed by oligomycin (results not shown), suggesting thus (Lardy *et al.*, 1958) that ATPase activity was probably not modified by the administration of 2-mercaptoacetate. A decreased respiration rate in state 3 could be due to a partial inhibition of adenine nucleotide translocase activity: in fact several authors have reported an inhibition of this enzyme by enhanced acyl-CoA concentration (Lerner *et al.*, 1972; Gordon, 1973), a situation that was found to apply to the mitochondria of 2-mercaptoacetate-treated rats (see above).

Regardless of the mechanism involved in the alterations of the mitochondrial functions depicted in Table 2, it is clear that these alterations are not sufficient to explain the almost complete inhibitory effect of 2-mercaptoacetate administration on fatty acid oxidation. As stated above, this action appears likely to be related to an inhibition of one or more of the β-oxidation steps. A possible cause of β-oxidation inhibition could be a decreased availability of the mitochondrial CoA pool after 2-mercaptoacetate administration. To test this hypothesis, we have studied the rates of pyruvate and 2-oxoglutarate oxidation, which are dependent on free CoA availability. As shown in Table 3, state-4 and -3 activities were found respectively increased and normal in mitochondria from the treated

Table 4. *Effects of 2-mercaptoacetate administration on the rates of palmitoyl-L-carnitine-dependent reduction of ferricyanide in rat liver mitochondria*

Palmitoyl-CoA dehydrogenase activity was determined as described in the text. Experimental conditions and statistical analysis are as described in Table 1.

Substrates	Animals	Rates of ferricyanide reduction (nmol/min per mg of protein)
Palmitoyl-L-carnitine (0.020mM)	Control	75.8 ± 1.7 (4)
	Treated	19.4 ± 5.9 (4)
		$P < 0.001$
Palmitoyl-L-carnitine (0.020mM) + rotenone (0.0025mM)	Control	12.5 ± 1.9 (4)
	Treated	3.2 ± 2.2 (4)
		$P < 0.001$
Palmitoyl-L-carnitine (0.020mM) + rotenone (0.0025mM) + oxaloacetate (10mM)	Control	34.8 ± 3.9 (4)
	Treated	9.8 ± 2.1 (4)
		$P < 0.001$

animals. It can thus be concluded that the 2-mercaptoacetate-induced inhibition of β-oxidation is not related to decreased availability of mitochondrial free CoA.

To investigate further the mechanism through which 2-mercaptoacetate administration inhibits β-oxidation, the activity of acyl-CoA dehydrogenase was studied by the method proposed by Osmundsen & Bremer (1977). According to this method, in the absence of rotenone, ferricyanide is reduced by the electrons originating from both flavin-linked acyl-CoA dehydrogenase and nicotinamide-nucleotide-linked 3-hydroxyacyl-CoA dehydrogenase. In the presence of rotenone, or of rotenone plus oxaloacetate, ferricyanide is reduced by electrons originating solely from acyl-CoA dehydrogenase activity, allowing thus the measurement of this enzyme activity. As shown in Table 4, the rate of palmitoyl-L-carnitine dehydrogenation was greatly depressed (−75%) in the mitochondria from treated animals under the three different experimental conditions used. Finally, comparison of these data with the inhibition rate of palmitoyl-L-carnitine oxidation in state 3 (Table 1) shows essentially identical depression. These findings led us to conclude that the action of 2-mercaptoacetate on β-oxidation is completely accounted for by an inhibition of palmitoyl-CoA dehydrogenase activity.

Discussion

In previous reports from this laboratory (Nordmann & Nordmann, 1971*a*,*b*) it was shown that 2-mercaptoethanol administration to the rat is followed by the induction of a fatty liver, which could be prevented by pyrazole (Sabourault *et al.*, 1977), a potent inhibitor of alcohol dehydrogenase (EC 1.1.1.1) (Goldberg & Rydberg, 1969). As 2-mercaptoethanol is a substrate of alcohol dehydrogenase (Lambe & Williams, 1965), these experiments suggested that this fatty liver was due to a metabolite of 2-mercaptoethanol rather than to 2-mercaptoethanol itself. Evidence in favour of this hypothesis was provided by the finding that the administration of 2-mercaptoacetate to the rat also induced a fatty liver (Sabourault *et al.*, 1976, 1977), which was due, at least in part, to both an increased peripheral lipolysis (Sabourault *et al.*, 1977) and an inhibition of fatty acid oxidation in the liver (Sabouralt *et al.*, 1979).

This study was undertaken to elucidate the mechanism through which 2-mercaptoacetate inhibits the rate of fatty acid oxidation in the liver. The results presented here clearly demonstrate that this inhibition does not result from altered transport of acyl-L-carnitine esters into the mitochondria, altered citrate-cycle activity, altered respiratory-chain activity, altered nucleotide translocase activity or altered coupling of oxidation to phosphorylation, but is the consequence of depression of β-oxidation through inhibition of the reaction catalysed by acyl-CoA dehydrogenase.

Mitochondrial dehydrogenation of acyl-CoA is catalysed by three enzymes with different chain-length specificity, all containing FAD as the prosthetic group (Wakil & Barnes, 1971). The electron acceptor for the hydrogenases is another flavoenzyme, electron-transfer flavoprotein, which also requires FAD (Beinert, 1963). The present data, showing an increase in the mitochondrial content of long-chain fatty acyl-CoA as well as the inhibition of palmitoyl-CoA dehydrogenation, suggest that palmitoyl-CoA dehydrogenase and/or the associated electron-transfer flavoprotein are inhibited after 2-mercaptoacetate administration. However, in the absence of a reliable quantitative test for the electron-transfer flavoprotein, it is not possible to establish which of these factors is inhibited.

During the last 10 years, attention has been

focused on the mechanisms through which different compounds interfere with β-oxidation. One of the most studied inhibitors is pent-4-enoic acid, which is believed to act through a metabolite of pent-4-enoyl-CoA, which inhibits 3-oxo acyl-CoA thiolase (EC 2.3.1.9) (Holland & Sherratt, 1973; Holland *et al.*, 1973; Sherratt & Osmundsen, 1976). The same mechanism has also been shown to apply for arsenite, another potent inhibitor of β-oxidation (Rein *et al.*, 1979). Some metabolites of hypoglycin (methylenecyclopropylacetate and methylenecyclopropylpyruvate) have also been reported to inhibit β-oxidation through their conversion into methylenecyclopropylacetyl-CoA, an inhibitor of butyryl-CoA dehydrogenase (EC 1.3.99.2) but not of palmitoyl-CoA dehydrogenase and octanoyl-CoA dehydrogenase (Sherratt & Osmundsen, 1976). Other inhibitors, such as decanoyl-(+)-carnitine and 2-bromopalmitoyl-CoA, have been shown to inhibit selectively the activity of carnitine palmitoyltransferase (EC 2.3.1.21) (Fritz & Marquis, 1965), whereas fluoride ions, which inhibit pyrophosphatase (EC 3.6.1.1), indirectly prevent fatty acid oxidation by an inhibitory effect of pyrophosphate on acyl-CoA synthetase (EC 6.2.1.3) (Battenburg & van den Bergh, 1973). The mechanisms of action of all these inhibitors appear thus to be markedly different from that involved in the inhibition of fatty acid oxidation by 2-mercaptoacetate administration.

Another inhibitor of β-oxidation is erucic acid. The observation that feeding rapeseed oil causes cardiac lipidosis (Rocquelin *et al.*, 1971) has led to the discovery that erucate inhibits palmitate oxidation (Christophersen & Bremer, 1972). From subsequent experiments, it seems that erucic acid acts at the same site as 2-mercaptoacetate. In fact, Christophersen & Christiansen (1975) and Osmundsen & Sherratt (1978) have provided evidence that the carnitine ester of this acid probably inhibits palmitoyl-CoA dehydrogenase competitively. More recently, riboflavin deficiency has also been reported to induce an inhibition of fatty acid oxidation, an effect which results from an inhibition of acyl-CoA dehydrogenase activities (Hoppel *et al.*, 1979). Beside this vitamin deficiency, it appears therefore that the only two compounds whose administration inhibits β-oxidation through a decrease in palmitoyl-CoA dehydrogenase activity are 2-mercaptoacetate and erucate.

Since all the data reported in the present study were obtained after the administration of 2-mercaptoacetate *in vivo*, it seems important to determine whether 2-mercaptoacetate itself is able or not to induce directly the same effects on isolated mitochondria. It is in fact not unusual to observe different influences of metabolically active agents on mitochondria, when animals are treated *in vivo* and when the isolated organelles are exposed to the compounds *in vitro*. This has been shown especially for ethanol (Higgins & Friend, 1972), hydrazine (Higgins & Banks, 1971) and ethionine (Higgins *et al.*, 1978). From preliminary experiments (F. Bauché, D. Sabourault, Y. Giudicelli, J. Nordmann & R. Nordmann, unpublished work), it seems, however, that the addition of 2-mercaptoacetate to isolated mitochondria results also in an inhibition of the oxidation of palmitoyl-L-carnitine.

Another important question is to determine whether the carboxy or the thiol group is responsible for the effects reported in this study. Recent investigations (Vahlkamp *et al.*, 1979; Wilms *et al.*, 1980) have shown that alkylthiols such as methanethiol and ethanethiol alter the mitochondrial electron transfer through a selective inhibition of cytochrome *c* oxidase (EC 1.9.3.1). In those studies, attempts to reproduce the same inhibition with 2-mercaptoacetate and other charged mercaptans were unsuccessful, demonstrating the necessity for mercaptans to be uncharged to inhibit cytochrome *c* oxidase (Wilms *et al.*, 1980). Our results confirm *in vivo* these observations *in vitro* by showing the lack of effect of 2-mercaptoacetate administration on the respiratory-chain activity. Although the effects of methanethiol and ethanethiol on β-oxidation were not studied, it seems reasonable to assume that the effects of 2-mercaptoacetate administration on β-oxidation are structurally linked to both the thiol and carboxy groups. This seems all the more likely as, in most if not all cases, the active metabolites of the carboxylic inhibitors of β-oxidation are their CoA and/or their L-carnitine esters (Osmundsen & Sherratt, 1978). Finally, the 2-mercaptoacetate inhibition of β-oxidation appears to be specific for this compound since, as shown previously (Sabourault *et al.*, 1976), administration of the next higher analogue of 2-mercaptoacetate, 2-mercaptopropionate, fails to induce any steatogenous effect in the liver.

To conclude, this study has shown that 2-mercaptoacetate administration induces an almost complete inhibition of β-oxidation in the liver. Compared with other β-oxidation inhibitors, the mechanism of this inhibition appears all the more original, as it involves a direct inhibition of fatty acyl-CoA dehydrogenase activity. However, further experiments performed *in vitro* are necessary to determine which of 2-mercaptoacetate, its CoA or its L-carnitine ester is responsible for this action.

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale, the Ecole Pratique des Hautes Etudes (3ème Section) and the Université René Descartes.

References

Battenburg, J. J. & van den Bergh, S. G. (1973) *Biochim. Biophys. Acta* **316**, 136–142

Beattie, D. S. (1968) *Biochem. Biophys. Res. Commun.* **31**, 901–907

Beinert, H. (1963) *Enzymes, 2nd Ed.* **7**, 467–476

Cederbaum, A. I. & Rubin, E. (1976) *Biochem. Pharmacol.* **25**, 963–973

Chance, B. & Williams, G. R. (1955) *J. Biol. Chem.* **217**, 409–427

Christophersen, B. O. & Bremer, J. (1972) *Biochim. Biophys. Acta* **280**, 506–514

Christophersen, B. O. & Christiansen, R. Z. (1975) *Biochim. Biophys. Acta* **388**, 402–412

Fritz, I. B. (1959) *Am. J. Physiol.* **197**, 297–304

Fritz, I. B. & Marquis, N. R. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **54**, 1226–1233

Garland, P. B. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 4, pp. 2015–2016, Academic Press, New York

Goldberg, L. & Rydberg, U. (1969) *Biochem. Pharmacol.* **18**, 1749–1762

Gordon, E. R. (1973) *J. Biol. Chem.* **248**, 8271–8280

Higgins, E. S. & Banks, W. L. (1971) *Biochem. Pharmacol.* **20**, 1313–1324

Higgins, E. S. & Friend, W. H. (1972) *Proc. Soc. Exp. Biol. Med.* **141**, 944–947

Higgins, E. S., Friend, W. H. & Rogers, K. S. (1978) *Experientia* **35**, 578–579

Holland, P. C. & Sherratt, H. S. A. (1973) *Biochem. J.* **136**, 157–171

Holland, P. C., Senior, A. E. & Sherratt, H. S. A. (1973) *Biochem. J.* **136**, 173–184

Hoppel, C., DiMarco, J. P. & Tandler, B. (1979) *J. Biol. Chem.* **254**, 4164–4170

Lambe, R. F. & Williams, D. C. (1965) *Biochem. J.* **97**, 475–478

Lardy, H. A., Johnson, D. & MacMurray, W. C. (1958) *Arch. Biochem. Biophys.* **78**, 587–597

Lerner, E., Shug, A. L., Elson, C. & Shrago, E. (1972) *J. Biol. Chem.* **247**, 1513–1519

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Nordmann, R. & Nordmann, J. (1971*a*) *Rev. Eur. Etud. Clin. Biol.* **16**, 564–566

Nordmann, R. & Nordmann, J. (1971*b*) *Biochimie* **53**, 705–708

Osmundsen, H. & Bremer, J. (1977) *Biochem. J.* **164**, 621–633

Osmundsen, H. & Sherratt, H. S. A. (1978) *Biochem. Soc. Trans.* **6**, 84–88

Rein, K. A., Borrebaek, B. & Bremer, J. (1979) *Biochim. Biophys. Acta* **574**, 487–494

Rocquelin, J. L., Sergiel, J. P., Warkin, B. & Leclerc, J. (1971) *J. Am. Oil Chem. Soc.* **48**, 728–732

Sabourault, D., Giudicelli, Y., Nordmann, R. & Nordmann, J. (1976) *Biochim. Biophys. Acta* **431**, 241–248

Sabourault, D., Giudicelli, Y., Nordmann, J. & Nordmann, R. (1977) *Lipids* **12**, 641–644

Sabourault, D., Bauché, F., Giudicelli, Y., Nordmann, J. & Nordmann, R. (1979) *FEBS Lett.* **108**, 465–468

Sherratt, H. S. A. & Osmundsen, H. (1976) *Biochem. Pharmacol.* **25**, 743–750

Stadtman, E. R., Novelli, G. D. & Lipman, F. (1951) *J. Biol. Chem.* **191**, 365–376

Vahlkamp, T., Meijer, A. J., Wilms, J. & Chamuleau, R. A. F. (1979) *Clin. Sci.* **56**, 147–156

Wakil, S. J. & Barnes, E. M., Jr. (1971) *Compr. Biochem.* **18S**, 57–104

Wilms, J., Lub, J. & Wever, R. (1980) *Biochim. Biophys. Acta* **589**, 324–335