On the Mechanism of Inhibition of Fatty Acid Oxidation by 4-Pentenoic Acid in Rat Liver Mitochondria*

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SUMMARY

The effects of 4-pentenoic acid on substrate oxidations and on the distribution of coenzyme A and its derivatives were studied in isolated rat liver mitochondria. CoA-linked substrate, long and short chain fatty acid, pyruvate, and \( \alpha \)-ketoglutarate oxidations were inhibited 60 to 80% by 0.1 mM 4-pentenoic acid. The failure of 4-pentenoic acid to inhibit fatty acid oxidation in uncoupled mitochondria indicated that it was necessary for 4-pentenoic acid to be activated to its CoA ester to become inhibitory. The addition of 4-pentenoic acid caused CoA and acetyl-CoA levels to decrease while acid-soluble and acid-insoluble acyl-CoA levels increased. The observed changes were interpreted as indicating that free CoA was being incorporated into a product of 4-pentenoic acid metabolism, and inhibition of fatty acid oxidation was caused by CoA depletion. These conclusions were supported by the reversal of 4-pentenoic acid inhibition of palmitylcarnitine oxidation in ultrasonically disrupted mitochondria by the addition of CoA, while oxidation of palmityl-CoA was not inhibited in this preparation.

Hypoglycemia induced by 4-pentenoic acid has been attributed to decreased gluconeogenesis secondary to depletion of tissue levels of coenzyme A and carnitine (1-4). In pigeon liver homogenates, 4-pentenoic acid inhibition of palmitate oxidation was reversed by exogenous coenzyme A and (-)-carnitine, and this effect was interpreted as indicating a depletion primarily of extramitochondrial coenzyme A (1). Subsequently, Corredor, Brendel, and Bressler (3) observed an inhibition of pyruvate oxidation, and suggested that this might be caused by a depletion of intramitochondrial CoA and possibly also by an inhibitory action of acrylyl-CoA on pyruvate dehydrogenase.

Other workers using isolated rat liver mitochondria, observed inhibition of pyruvate and \( \alpha \)-ketoglutarate oxidation by 4-pentenoic acid and a partial uncoupling of oxidative phosphorylation (5, 6). They also reported that long chain fatty acid oxidation was strongly inhibited and suggested that decreased gluconeogenesis may be caused by decreased availability of NADH and ATP caused by the diminished rate of \( \beta \) oxidation and by partial uncoupling of oxidative phosphorylation (6, 7).

In this paper, data are presented which show that inhibitions of pyruvate and fatty acid oxidation by 4-pentenoic acid in isolated rat liver mitochondria are mainly caused by a depletion of CoA, although a more specific inhibition of \( \beta \) oxidation by accumulation of short chain acyl-CoA compounds may serve to enhance the primary inhibition because of CoA lack. No evidence for an uncoupling effect of 4-pentenoic acid on oxidative phosphorylation has been obtained.

METHODS AND MATERIALS

Rat liver mitochondria were isolated in a medium containing 225 mM mannitol, 75 mM sucrose, and 50 mM EDTA, essentially by the method of Hogeboom, Schneider, and Palade (8). Mitochondria were washed twice with EDTA-free medium and suspended to give a final concentration of 25 to 35 mg per ml. Protein was determined by the Biuret method (9). Mitochondria were disrupted ultrasonically by the procedure described by Kielley and Bronk (10). The sonically disrupted preparation containing both the soluble and particulate fractions was used after removal of intact mitochondria by centrifugation.

Rates of oxygen consumption were measured polarographically with a Clark oxygen electrode. Mitochondrial flavin (436 nm excitation, 570 nm peak emission) and pyridine nucleotide (366 nm excitation, 460 nm peak emission) fluorescence changes were monitored simultaneously with a dual fluorometer equipped with a rotating disk as a time-sharing device (11). The contents of CoA and acyl-CoA intermediates were determined enzymatically in neutralized perchloric acid extracts (12).

4-Pentenoyl-CoA and \( \beta \)-hydroxypropionyl-CoA were synthesized by the mixed anhydride method (13), and acrylyl-CoA was synthesized from acrylyl imidazole (14, 15). (\( \alpha \))-Palmitylcarnitine was synthesized from (-)-carnitine and palmitoyl chloride (16). (-)-Carnitine was generously provided by the Otsuka Pharmaceutical Factory, Osaka, Japan. 4-Pentenoic acid was purchased from K and K Laboratories, Plainview, New York.

RESULTS

Effects of 4-Pentenoic Acid on Oxygen Consumption of Isolate Mitochondria—The oxidations of fatty acids, pyruvate plus malate, and \( \alpha \)-ketoglutarate were profoundly inhibited in rat liver mitochondria by 0.1 mM 4-pentenoic acid (Fig. 1). The oxidation
rates were determined over a 3- to 5-min interval after a 1-min preliminary incubation of mitochondria with ADP and 4-pentenoic acid. Pyruvate-malate oxidation was about as sensitive as octanoate or palmitoylcarnitine oxidation to inhibition by 4-pentenoic acid, but α-ketoglutarate oxidation was somewhat less affected. Inhibition of pyruvate oxidation was not dependent on the presence of malate, and likewise the presence of malate did not affect the inhibition of other substrate oxidations by 4-pentenoic acid. A 50% inhibition of the State 3 rate of oxygen uptake in the presence of palmitoylcarnitine, palmitate, octanoate, pentanenol, or pyruvate was obtained with 20 to 30 μM 4-pentenoic acid, while α-ketoglutarate oxidation required 60 μM for 50% inhibition. 4-Pentenoic acid itself increased the oxygen consumption of the mitochondrial suspensions during its metabolism by β-oxidation to acetyl-CoA and α-acetylcarnitine (17). The degree of inhibition produced by a given concentration of 4-pentenoic acid tended to increase with all the substrates with increasing times of preliminary incubation with 4-pentenoic acid prior to addition of substrate. The oxidations of non-CoA-linked substrates, namely succinate, and malate-cysteine sulfenic acid, were not significantly affected by 4-pentenoic acid up to concentrations of 1 mM. Furthermore, respiratory control and P:O ratios were unaffected by 4-pentenoic acid with succinate as substrate (Table I).

Since the inhibitory action of 4-pentenoic acid appeared to be linked to CoA, the effects of this inhibitor were investigated in energy-depleted mitochondria, which prevented activation of fatty acids to the CoA derivatives. In Fig. 2 the effects of 4-pentenoic acid on palmitoylcarnitine oxidation are compared in coupled (Fig. 2A) and uncoupled mitochondria (Fig. 2B). Addition of 4-pentenoic acid to mitochondria initially incubated with ADP caused a slight stimulation of respiration followed by inhibition. Palmitoylcarnitine caused no further stimulation of respiration, showing that its oxidation was fully inhibited. Uncoupler failed to release this inhibition. On the other hand, palmitoylcarnitine was oxidized by uncoupled mitochondria (Fig. 2B) at almost the same rate in both the presence and absence of 4-pentenoic acid. It may be concluded, therefore, that the formation of 4-pentenoyl-CoA precedes the inhibition of the oxidation of CoA-linked substrates.

**Effects of 4-Pentenoic Acid on Flavin and Pyridine Nucleotide Oxidation-Reduction State**—A lack of effect of 4-pentenoic acid (up to 0.5 mM) on oxidative phosphorylation with succinate as substrate was confirmed by observing the pyridine nucleotide fluorescence changes of mitochondria during the State 3 to State 4 transition. Also, no reduction of NAD was obtained when 4-pentenoic acid was added to uncoupled substrate-depleted mitochondria incubated in the presence of antimycin A to inhibit electron transfer between cytochromes b and c. Fig. 3 shows data from experiments in which respiratory carriers were first oxidized by additions of ADP to mitochondria incubated in the absence of substrate. Electron transport was inhibited by addition of rutamycin to inhibit energy transfer at the phosphorylation sites, thereby causing a greater proportion of the pyridine nucleotide oxidation-Reduction State.

**TABLE I**

**Effect of 4-pentenoic acid on P:O and respiratory control ratios**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P:O ratio</th>
<th>Respiratory control ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.85 ± 0.04</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>4-Pentenoic acid (1 mM)</td>
<td>1.81 ± 0.04</td>
<td>5.1 ± 0.5</td>
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</table>

**Fig. 2.** Effect of 4-pentenoic acid on palmitoylcarnitine (PC) oxidation in coupled and uncoupled washed rat liver mitochondria (RLM). Reaction conditions were as described in Fig. 1. With coupled mitochondria (A), 4-pentenoic acid prevents any increase in oxygen consumption upon the addition of palmitoylcarnitine (center trace). The upper trace shows the effect of 4-pentenoic acid without added substrate; the lower trace shows the control rate. With uncoupled mitochondria (B), preliminary incubation for 6 min with 40 μM pentachlorophenol, the oxidation of palmitoylcarnitine is only slightly inhibited by 4-pentenoic acid (upper trace). The lower trace shows the control rate. Rates of oxygen consumption expressed in nanomoles per min per mg of protein are given by the numbers shown in the figure.
Recent studies of the nature of the flavoprotein carriers in rat liver mitochondria (18) have established the existence of two main pools; one composed of NADH dehydrogenase and lipoic dehydrogenase which is in near equilibrium with the NAD pool at a potential of about \(-300\) mv (low potential pool), and the other on the oxygen side of the rotenone site of inhibition, composed of respiratory chain flavin, succinic dehydrogenase, and the flavoproteins of fatty acid oxidation, which is in near equilibrium with ubiquinone and cytochrome \(b\) at a potential about \(0\) mv (high potential pool). Oxidation-reduction changes of lipoic dehydrogenase contribute predominantly to the over-all flavoprotein fluorescence changes, although flavoproteins of the high potential pool can contribute 20 to 30\% to the maximum flavin fluorescence decrease observed in the fully reduced state. Addition of 4-pentenoic acid after ADP and rutamycin (Fig. 3, lower panel) resulted in an increase of pyridine nucleotide fluorescence and decrease of flavin fluorescence, denoting a reduction of both carriers. These changes are caused by \(\beta\) oxidation of 4-pentenoic acid. Although the flavin-linked enoyl-CoA dehydrogenase transfers electrons directly to the high potential flavin pool, the large flavin fluorescence response indicates a partial reduction also of the low potential flavins through their equilibrium with the NAD system. This response was similar to that observed after octanoate addition (17). Subsequent addition of pyruvate had no effect, but succinate maximally reduced the high potential flavin pool directly and the pyridine nucleotide and low potential flavin pools by reversed electron transfer (10). Pyruvate added alone after rutamycin and ADP caused almost complete reduction of the fluorescent flavin and pyridine nucleotide pools (Fig. 3, upper panel). 4-Pentenoic acid caused a partial reoxidation of flavins and pyridine nucleotides which had been reduced by pyruvate (Fig. 3, center panel) to about the same degree of reduction caused by the 4-pentenoic acid alone (Fig. 3, lower panel). These results indicate that pyruvate oxidation is sensitive to 4-pentenoic acid inhibition regardless of the order of addition.

Fig. 4 shows a comparison of the pyridine nucleotide oxidation-reduction changes observed upon addition of various fatty acids to mitochondria incubated with ADP and pyruvate in the absence of rutamycin. Under these conditions, pyruvate produced a partial reduction of the NAD(P) pool equivalent to the State 3 level (cf. Fig. 3). Subsequent addition of octanoate caused a further small reduction change, while pentanoate caused a small oxidation. However, 4-pentenoic acid produced a complete reoxidation of pyridine nucleotides. Pyruvate oxidation is inhibited by each of the fatty acids (20), and the different effects observed on the pyridine nucleotide oxidation-reduction state probably reflect differences in the end products of \(\beta\) oxidation of the various fatty acids. Thus, in the absence of malate, octanoate is metabolized completely to ketone bodies, while the end products of \(\beta\) oxidation of octanoic acid and 4-pentenoic acid are propionyl-CoA plus acetyl-CoA (21), and acrylyl-CoA plus acetyl-CoA, respectively. Pyruvate dehydrogenase is known to be inhibited by high ratios of acetyl-CoA:CoA, and propionyl-CoA is also inhibitory (22). If short chain acyl-CoA derivatives from \(\beta\) oxidation of the fatty acids accumulate because of their slow further metabolism, an inhibition of pyruvate oxidation could be caused both by CoA depletion and directly by the accumulated poorly metabolized short chain acyl-CoA.

Effects of 4-Pentenoic Acid on Content of CoA Derivatives in Rat Liver Mitochondria—In order to assess the relative importance
The reaction media contained 8 to 10 mg of mitochondrial protein in 2.4 ml of buffer containing 100 mM KCl, 50 mM sucrose, 20 mM Tris-chloride, 5 mM phosphate-Tris, and 5 mM ADP, pH 7.2. Pentachlorophenol (80 μM) was used as an uncoupler. When present, L-(-)-carnitine was added at a concentration of 1 mM. 4-Pentenoic acid (0.2 mM) was added after 1 min, and the reaction was stopped after a further 2-min incubation by addition of perchloric acid. The values represent the means of several determinations.

The CoA derivatives were then assayed as described under Methods. The CoA content of the CoA derivatives was then assayed as described under "Methods and Materials." The results show that the CoA content had fallen to almost 0.1 nmol per mg of protein. Similar effects were observed when NTP was added along with 4-pentenoic acid instead of ADP before octanoate addition. The results, therefore, indicate that the primary cause of 4-pentenoic acid inhibition is by a CoA depletion effect.

The changes in the levels of CoA intermediates in rat liver mitochondria induced by octanoate, when added before and after 4-pentenoic acid, are shown in Fig. 5. Octanoate addition (Fig. 5A), caused a fall of CoA and acid-insoluble acyl-CoA, and a rise of acetyl-CoA and acid-soluble acyl-CoA. These changes correspond to a rapid activation of the fatty acid to octanoyl-CoA and its relatively slow metabolism by the State 4 respiration. Addition of ADP caused a sharp fall in the content of acid-soluble CoA derivatives, an additional decrease of insoluble acyl-CoA, and a further rise of acetyl-CoA, in accordance with the more rapid metabolism of octanoate to ketone bodies. 4-Pentenoic acid then produced a large rise of acid-soluble acyl-CoA, and decreases in the levels of CoA and acetyl-CoA. The rise of acid-soluble acyl-CoA indicates activation of 4-pentenoic acid to its CoA derivative, but presumably octanoate competed successfully with 4-pentenoate for activation and β oxidation since the CoA level remained relatively high and mitochondrial respiration was little inhibited. On the other hand, after prior addition of ADP plus 4-pentenoic acid, which resulted in a rise of acid-soluble acyl-CoA and a fall of other CoA components (Fig. 5B), octanoate produced relatively small effects, and its oxidation was strongly inhibited. It may be noted that under these conditions the CoA content had fallen to almost 0.1 nmol per mg of protein.

Fig. 6 shows the effects of palmitoylcarnitine followed by ADP and 4-pentenoic acid on CoA intermediates of liver mitochondria. With palmitoylcarnitine as substrate, unlike octanoate, the State 3 rate of respiration was inhibited 65% by 0.02 mM 4-pentenoic acid, when the inhibitor was added last. Palmitoylcarnitine addition caused a complete acylation of the available CoA, mainly to the long chain acid-insoluble form. Subsequent addition of ADP caused a partial release of CoA and an increase of acetyl-CoA, while the major effect of 4-pentenoic acid addition was an increase of acid-soluble acyl-CoA at the expense of acid-insoluble acyl-CoA. These results show that palmitoylcarnitine was more effective than octanoate in sequestering the mitochondrial CoA, and that inhibition of palmitoylcarnitine oxidation occurred without an appreciable change of the CoA content of the mitochondria.

This finding was confirmed in experiments in which palmitoylcarnitine was added 1 min after incubation of mitochondria with 4-pentenoic acid and ADP (Table III). Thus, compared with the controls, 4-pentenoic acid produced only a small further de-
crease of CoA from the low level of 0.27 n mole per mg of protein observed during State 3 oxidation of palmitoylcarnitine. With pyruvate as substrate (Table III), the steady state level of CoA was higher than that observed during palmitoylcarnitine oxidation, and was severely depleted in the presence of 4-pentenoic acid. Acetyl-CoA levels were also decreased by 4-pentenoic acid, while the levels of both acid-soluble and acid-insoluble acyl-CoA derivatives increased. Likewise, addition of palmitate to mitochondria incubated in the presence of 4-pentenoic acid caused an inhibition of respiration, a fall of CoA and acetyl-CoA and rise of acid-soluble and - insoluble acyl-CoA, relative to values obtained in the absence of the inhibitor (Table III). The increase of acid-soluble acyl-CoA which was invariably observed after 4-pentenoic acid addition represents 4-pentenoyl-CoA, acrylyl-CoA, and possibly other CoA intermediates of 4-pentenoic acid metabolism. However, attempts to separate this fraction into its individual acyl-CoA components were not successful. In the light of the failure of 4-pentenoic acid to decrease appreciably CoA levels below control values with palmitoylcarnitine as substrate, the question arises whether CoA depletion represents the sole mechanism of inhibition. If so, then the small amount of residual CoA must be unavailable to the β oxidation enzyme system. Alternatively, the high levels of long chain acyl-CoA derivatives observed in the presence of 4-pentenoic acid suggests the possibility that β oxidation may be inhibited directly by a short chain acyl-CoA derivative of 4-pentenoic acid metabolism.

**Effects of 4-Pentenoic Acid on β Oxidation in Ultrasonically Disrupted Mitochondria**—Because the intact mitochondrial membrane is relatively impermeable to CoA, an ultrasonically disrupted preparation was used to determine whether 4-pentenoic acid inhibition could be reversed by the direct addition of CoA. The soluble fraction containing the fatty acid oxidation enzymes and the particulate fraction containing the electron transport chain were used without separation so that substrate oxidation could be followed polarographically. The preparation was completely uncoupled and was able to oxidize palmitoylcarnitine at 20 to 25% of the rate of intact mitochondria (Fig. 7, left trace). 4-Pentenoic acid was not activated in this uncoupled system and had no inhibitory effect on palmitoylcarnitine oxidation. The addition of ATP after 4-pentenoic acid resulted in an increased rate of oxygen uptake (Fig. 7, right trace). Palmitoylcarnitine caused no increase in oxygen uptake, but CoA addition stimulated respiration to nearly control rates of palmitoylcarnitine oxidation. Palmitoyl-CoA oxidation was not inhibited by the prior addition of 4-pentenoic acid and ATP in the sonically disrupted mitochondrial preparation.

The addition of carnitine (0.1 mM) and acetyl carnitine transferred to the sonically disrupted mitochondria, in order to convert the CoA derivatives of 4-pentenoic acid to carnitine derivative and release CoA, partially relieved the inhibition of palmitoylcarnitine oxidation observed in the presence of ATP. Preliminary incubation with carnitine and acetyl carnitine transferase protected from the inhibition to the same extent that the later addition reversed it. Added CoA relieved the remaining inhibition completely in both cases. These results indicated that although carnitine and acetyl carnitine transferase were able to increase the turnover of CoA somewhat, they were not able to prevent fully the CoA depletion effect.

4-Pentenoyl-CoA or acetyl-CoA added to sonically disrupted mitochondrial suspensions inhibited the oxidation of palmitoylcarnitine up to 50 to 60% (17) but this was not an appreciably greater inhibition than that caused by similar concentrations of palmitylcarnitine as substrate. The reaction mixture, sampling and assay were as described for Fig. 5A. Additions were made to give the following final concentrations: palmitoylcarnitine, 100 μM; ADP, 3 mM; and 4-pentenoic acid, 0.2 mM.

**Table III**

<table>
<thead>
<tr>
<th>Additions to buffer and ADP</th>
<th>Oxygen uptake</th>
<th>CoA</th>
<th>Acetyl-CoA</th>
<th>Other acid-soluble acyl-CoA</th>
<th>Acid-insoluble acyl-CoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitoylcarnitine, 40 μM</td>
<td>55.9 ± 0.6</td>
<td>0.27 ± 0.01</td>
<td>0.61 ± 0.02</td>
<td>0.79 ± 0.09</td>
<td>1.18 ± 0.07</td>
</tr>
<tr>
<td>4-Pentenoic acid, 0.5 mM, and palmitoylcarnitine, 40 μM</td>
<td>11.7 ± 0.6</td>
<td>0.18 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>1.16 ± 0.03</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>Pyruvate, 2 mM</td>
<td>35</td>
<td>0.65</td>
<td>0.35</td>
<td>1.01</td>
<td>0.61</td>
</tr>
<tr>
<td>4-Pentenoic acid, 0.5 mM, and pyruvate, 2 mM</td>
<td>9</td>
<td>0.21</td>
<td>0.16</td>
<td>1.58</td>
<td>1.23</td>
</tr>
<tr>
<td>Palmitate, 40 μM</td>
<td>36</td>
<td>0.93</td>
<td>0.54</td>
<td>1.17</td>
<td>0.90</td>
</tr>
<tr>
<td>4-Pentenoic acid, 0.2 mM, and palmitate, 40 μM</td>
<td>5</td>
<td>0.24</td>
<td>0.10</td>
<td>1.54</td>
<td>1.45</td>
</tr>
</tbody>
</table>

**Fig. 6.** Effect of 4-pentenoic acid added after palmitoylcarnitine on CoA derivatives in liver mitochondria. The reaction mixture, sampling and assay were as described for Fig. 5A. Additions were made to give the following final concentrations: palmitoylcarnitine, 100 μM; ADP, 3 mM; and 4-pentenoic acid, 0.2 mM.

The reaction mixture contained 8 to 10 mg of mitochondrial protein in 2.4 ml of buffer containing 100 mM KCl, 50 mM sucrose, 20 mM Tris-chloride, and 5 mM Tris-phosphate, 5 mM ADP, pH 7.2. 4-Pentenoic acid was added after a 2-min equilibration period. Substrates were added 1 min later or after a 3 min equilibration period for the controls. The reactions were stopped 1 min after the addition of substrate by addition of perchloric acid. Values shown with palmitoylcarnitine as substrate are means ± S.E.M. of four determinations.
Respiratory rates in nanomoles per minute per mg of protein are given by the numbers shown in the figure. Respiratory rates in nanomoles per minute per mg of protein are given by the numbers shown in the figure.

4-Pentenoic acid oxidation, although (−)-carnitine was partly effective in reversing the inhibition in sonically disrupted mitochondria. These results indicate that β oxidation of 4-pentenoyl-CoA is favored over the transferase reaction in intact mitochondria. This preference may be the result of a greater accessibility of the substrate to the β oxidation enzymes than to acylcarnitine transferase since the preference is dependent upon the structural integrity of the mitochondria.

Inhibition of Fatty Acid Oxidation—A difference in the sensitivity to 4-pentenoic acid inhibition between long chain and short chain fatty acid oxidation has been reported (6). For a CoA depletion mechanism of action to be valid, oxidation of short chain fatty acids and other CoA-linked substrates must be affected also. Brendel et al. (4) found that a 15-min preliminary incubation of pigeon liver homogenate with the inhibitor resulted in inhibition of both long and short chain fatty acid oxidation, but only long chain fatty acid oxidation was inhibited without a preliminary incubation period. In our studies with isolated mitochondria, only 1 or 2 min of preliminary incubation was necessary for maximal inhibition of all CoA-linked substrate oxidation. However, oxidation of octanate added after 4-pentenoic acid (Fig. 5) was not inhibited, although oxidation of palmitate or palmitoylcarnitine was still inhibited by this order of addition. These results are compatible with a CoA depletion mechanism of β oxidation inhibition, since 4-pentenoic acid itself must be activated to manifest its inhibitory action. The same acyl-CoA ligase which activates octanoyl-CoA probably activates 4-pentenoic acid (24) and octanoyl-CoA, and then is able to compete successfully for both the enzyme and cofactor if added to mitochondria at the same time or before 4-pentenoic acid. When 4-pentenoic acid is added to mitochondria before octanoyl-CoA, it rapidly traps most of the CoA, and activation of the octanoyl-CoA added subsequently depends on the rate of metabolism of 4-pentenoic-CoA. Palmitate is activated by a different acyl-CoA ligase (25) and this becomes inhibited by limited cofactor availability. Similarly, palmitoylcarnitine does not compete directly with 4-pentenoic acid for the same enzyme, and its transsesterification via palmitoylcarnitine transferase also becomes inhibited by lack of CoA.

In homogenates or whole liver perfusions, it is more difficult to indicate that all CoA-linked substrate oxidation can be inhibited because cytosolic CoA and carnitine are also acylated. Then the transport of long chain fatty acid appears to be primarily affected. However, with long preliminary incubation periods as above (4), or with high concentrations of 4-pentenoic acid (26), it is possible to inhibit mitochondrial fatty acid activation also. Furthermore, the reversal of inhibition of palmitoylcarnitine oxidation with CoA in sonically disrupted mitochondria (Fig. 7) shows that palmitoylcarnitine transferase was not inhibited.

Changes in CoA Derivatives—It was reported in an earlier communication that levels of acid-insoluble acyl-CoA compounds remained high in mitochondria with 4-pentenoic acid inhibition (17). This substrate availability was interpreted to indicate that a specific inhibition of some β oxidation step which occurs after activation was caused by a 4-pentenoic acid derivative. The presence of measurable free CoA in inhibited mitochondria in concentrations comparable to that in mitochondria-oxidizing systems as in the uninhibited systems with palmitoylcarnitine as substrate. However, it may be that the low concentration of free CoA which is measured in 4-pentenoic acid-treated mitochondria is actually not available as such to the fatty acid oxidation system because of compartmentation or some protein-cofactor complex formation which is disrupted upon treatment with perchloric acid. The acid-insoluble acyl-CoA may also consist of nonsubstrate compounds in part, since they have not been rigorously characterized.

In summary, the data presented here are compatible with a CoA depletion mechanism of action of β oxidation inhibition by 4-pentenoic acid. The inhibitions of α-ketoglutarate and pyruvate oxidations can be explained by CoA depletion and by the accumulation of high concentrations of acyl-CoA-like short chain acyl-CoA derivatives. However, the possibility that accumulation of relatively high concentrations of acyl-CoA, an α,β-unsturated acyl-CoA which can react with —SH groups (27, 28), may have specific inhibitory actions, especially over a long period of time, cannot be ruled out.
Acknowledgment—We wish to thank Mrs. Ilga Wohlrab for her skilled assistance with the CoA assays.

REFERENCES

On the Mechanism of Inhibition of Fatty Acid Oxidation by 4-Pentenoic Acid in Rat Liver Mitochondria
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