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INHIBITION OF MITOCHONDRIAL FATTY ACID OXIDATION IN PENTENOIC ACID-INDUCED FATTY LIVER

A POSSIBLE MODEL FOR REYE'S SYNDROME

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Abstract—Rats treated with six to eight doses (80 mg/kg, i.p.) of 4-pentenoic acid, an inhibitor of mitochondrial fatty acid oxidation *in vitro*, during a 48-hr starvation period developed microvesicular fatty infiltration of the liver similar to that observed in Reye's Syndrome. Hepatic triglycerides were elevated an average of 5-fold, although considerable variability was found between individual rats. Fed rats did not develop fatty liver upon similar treatment with pentenoic acid. Liver mitochondria isolated from rats with pentenoic acid-induced fatty liver showed a persistent inhibition of fatty acid oxidation. Rates of oxidation of palmitoylcarnitine and decanoylcarnitine were decreased about 70%, while that of octanoylcarnitine was decreased 50%. Carnitine-independent oxidation of octanoate was also inhibited. Oxidation rates for substrates other than fatty acids, including glutamate, succinate, pyruvate, and α-ketoglutarate, were unaffected. Measurements of flavoprotein reduction in intact mitochondria indicated that neither palmitoylcarnitine nor palmitoyl CoA plus L-carnitine could elicit reduction of acyl-CoA dehydrogenase and electron transferring flavoprotein in mitochondria from rats with pentenoic acid-induced fatty liver. These results support a site of inhibition of mitochondrial β-oxidation at the level of acyl-CoA dehydrogenase for pentenoic acid treatment *in vivo*, and they suggest a role for nutritional or hormonal factors in the metabolic disposition of pentenoic acid *in vivo* and in the development of fatty liver.

The childhood disease Reye's Syndrome, originally described as "encephalopathy and fatty degeneration of the viscera of unknown origin" [1], has been associated with an antecedent viral infection, often influenza B or varicella [2, 3]. Reye's Syndrome is characterized by the onset of severe and protracted vomiting, followed rapidly by progressive deterioration of neurologic function and coma [2, 3]. These patients also exhibit severe hepatic dysfunction, as indicated by increases in serum transaminase activities, prothrombin time, ammonia and free fatty acid levels, and altered serum amino acid patterns [3]. Histologically, the liver exhibits a characteristic microvesicular fatty degeneration [3–5]. At an ultrastructural level, mitochondria in hepatocytes exhibit gross alterations in structure, including swelling and pleomorphism [6, 7]. Although the pathogenesis is not understood, biochemical studies have suggested that mitochondria may be a principal target in Reye's Syndrome [6]. Measurements in liver biopsies have revealed decreased activities for several mitochondrial enzymes [5, 8–10]. Overall, the pathology of the liver in Reye's Syndrome resembles toxic liver injury.

Although a precise animal model of Reye's Syndrome has not been developed, Glasgow and Chase [11] have shown that rats treated chronically with multiple doses of 4-pentenoic acid develop fatty infiltration of hepatocytes similar to that seen in Reye's Syndrome as well as some of the characteristic alterations in serum chemistry. Yoshida [12] has reported that ultrastructural alterations of hepatic mitochondria precede the appearance of fat in the liver during chronic treatment of rats with pentenoic acid. Pentenoic acid is a structural analog of hypoglycin [13]. The latter has been identified as the causative agent for Jamaican Vomiting Sickness, the symptoms of which are similar to those of Reye's Syndrome [14].

In vitro studies with isolated mitochondria have shown that pentenoic acid inhibits fatty acid oxidation after being converted to a CoA derivative [15], although the precise mechanism of inhibition has not been clarified [16]. Pentenoic acid also decreases free CoASH* levels in isolated mitochondria leading to an inhibition of other CoA dependent reactions, such as pyruvate oxidation [17–19]. One previous report concerning *in vivo* effects of pentenoic acid has demonstrated that liver mitochondria isolated from rats 1–5 hr following administration of a single high dose (350 mg/kg) of pentenoic acid exhibit pronounced inhibition of palmitoylcarnitine oxidation and partial inhibition of pyruvate oxidation [20].

The goal of the present study was to characterize alterations of mitochondrial function in relationship to the development of pentenoic acid-induced fatty liver as a possible model for mitochondrial abnormalities which occur in Reye's Syndrome. In this paper we report data showing that the nutritional state of

* Abbreviations: CoASH, coenzyme A (free form); Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; and EGTA, ethylene glycol-bis(β-aminoethyl) *N,N,N',N'*-tetraacetic acid.

the rat plays a role in the development of fatty liver in response to pentenoic acid treatment, and that pentenoic acid-induced fatty liver is associated with persistent inhibition of mitochondrial fatty acid β-oxidation. We also report measurements of flavoprotein reduction in intact mitochondria which suggest that the site of inhibition of β-oxidation resulting from pentenoic acid treatment *in vivo* is acyl-CoA dehydrogenase.

EXPERIMENTAL PROCEDURES

Materials. 4-Pentenoic acid was obtained from ICN Pharmaceuticals, Inc., Plainview, NY. For injection into rats, a 0.5 M solution neutralized to pH 7.4 with NaOH was prepared. Purity of pentenoic acid dosing solutions was greater than 90%, as determined by gas chromatography using a column designed for separation of volatile short chain fatty acids (GP 10% SP 1200/1% H_3PO_4 on 80/100 Chromosorb WAW, Suppelco, Inc., Bellefonte, PA).

Pentenoic acid treatment. Male, Sprague–Dawley rats (100–250 g) were treated with sodium pentenoate solution by intraperitoneal injection. In the "chronic" pentenoic acid treatment protocol as originally described by Glasgow and Chase [11] for the production of fatty liver, rats had been treated with pentenoic acid at a dose of 50 mg/kg every 4 hr for ten doses followed by a single dose of 200 mg/kg, administered 1–2 hr after the tenth dose, and subsequently killed 20–45 min later for study. However, to distinguish persistent effects of pentenoic acid treatment arising from administration of multiple low doses from the effects of a higher acute dose given just prior to killing the rat, we have modified their treatment protocol by eliminating the high acute dose and altering the dosing schedule. In the present study, a dose of 80 mg pentenoic acid/kg body weight was administered three times per day (9:00 a.m., 1:00 p.m. and 5:00 p.m.) for 2 days prior to an experiment on the morning of day 3. Some rats received additional 80 mg/kg doses of pentenoic acid at 9:00 p.m. on day 2 (seventh dose) and at 9:00 a.m. on the day of the experiment (eighth dose). Rats that received an injection on the morning of the experiment were killed 2 hr later. Control rats received a similar volume of 0.15 M NaCl. For rats designated as "starved", food was withdrawn at the time of the first injection of pentenoic acid. Rats designated as "fed" were allowed standard rat chow *ad lib.* throughout the treatment period. Both groups received water *ad lib.* Rats were kept in wire-bottom cages during the treatment period.

Serum chemistry. Blood was collected from neck vessels at the time rats were decapitated. Serum chemistry profiles were determined with a Technicon SMA-12 Autoanalyzer. Serum ammonia was determined enzymatically using glutamate dehydrogenase [21].

Lipid determination. A sample of each liver (1 g) was extracted by the method of Folch *et al.* [22]. Total lipids were determined gravimetrically. Triglyceride content was determined enzymatically by measuring glycerol following hydrolysis in ethanolic KOH [23].

Histology. Frozen sections were prepared and stained with Oil Red 0 according to standard procedures. For electron microscopy, 1 mm^3 pieces of liver were fixed in phosphate-buffered glutaraldehyde (2%), post-fixed in OsO_4 (1%), sectioned, and stained with uranyl acetate and lead citrate according to standard techniques [24].

Preparation of mitochondria. Liver mitochondria were isolated by differential centrifugation of a homogenate prepared in 0.25 M sucrose [25]. In some experiments, a medium containing fatty acid-free bovine serum albumin was used for isolation, as previously described [26, 27].

Respiration. Oxygen uptake was measured polarographically with a Clark O_2 electrode at 25° in a temperature-controlled chamber of 1.6 ml volume. The medium used was 130 mM KCl, 10 mM Na^+/Hepes, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 0.5 mM EGTA, pH 7.5, containing about 2 mg mitochondrial protein/ml. Substrates used were 10 mM glutamate plus 5 mM malate, 10 mM succinate plus 2 μM rotenone, 10 mM pyruvate plus 1 mM malate, or 10 mM α-ketoglutarate. State 3 rates of respiration were determined after addition of 200 nmoles of ADP [28]. Respiratory control ratios and ADP/O ratios were calculated from the polarographic recordings by standard procedures [28, 29]. Oxidation of 20 μM fatty acyl-carnitines or 0.2 mM octanoate was measured similarly in the presence of 5 mM malonate and 0.6 mM ADP [15, 16]. In all cases, at least two determinations were averaged for each respiration experiment.

Flavoprotein reduction. Reduction of mitochondrial flavoproteins was monitored by dual wavelength spectroscopy at 475 nm, with reference at 510 nm [30], using an Aminco DW-2A spectrophotometer.

Protein. Mitochondrial protein concentration was measured by the cyanide biuret method [31] using bovine serum albumin as a standard.

Statistical analysis. Statistical significance of differences was evaluated by Student's *t*-test.

RESULTS

Role of nutritional state in the development of fatty liver. Starvation of the rats was necessary for the development of fatty liver in response to pentenoic acid treatment (Table 1). Fed rats did not develop fatty liver at comparable doses of pentenoic acid. Furthermore, treatment for longer periods, up to 4 days (cumulative dose: 1040 mg/kg), also did not produce fatty liver in fed rats. Administration of six to eight doses (each 80 mg pentenoic acid/kg body wt) over a 48-hr starvation period produced an average 5-fold elevation in hepatic triglyceride content as compared to saline-treated control rats. There was, however, wide variation between individual rats in the degree of fatty liver which developed in response to pentenoic acid treatment. Some treated rats appeared to be unsusceptible, exhibiting control triglyceride levels, while others had triglyceride levels greater than ten times control values. The increase in triglyceride content was also reflected by an increase in hepatic total lipid content (Table 1). The content of non-triglyceride lipids, mainly phospholipids, was unaffected by pentenoic acid treatment. Rats with pentenoic acid-induced fatty liver

Table 1. Effect of nutritional state and dose dependence for production of fatty liver by pentenoic acid treatment*

Total dose of pentenoic acid† (mg/kg)	Schedule‡	Hepatic triglyceride (mg/g liver) Starved rats	Hepatic triglyceride (mg/g liver) Fed rats	Hepatic total lipids (mg/g liver) Starved rats	Hepatic total lipids (mg/g liver) Fed rats
0	Saline	6.4 ± 0.5 (12)	6.8 ± 0.8 (6)	33.4 ± 1.1 (12)	30.8 ± 1.6 (4)
300	6 × 50 mg/kg	11.5 ± 2.4 (6)	4.0 ± 1.0 (3)	40.8 ± 4.6 (6)	31.7 ± 2.6 (3)
480	6 × 80 mg/kg	32.1 ± 6.1 (15)	6.0 ± 1.1 (4)	58.0 ± 7.3 (14)	29.5 ± 4.4 (5)
560	7 × 80 mg/kg	25.0 ± 3.4 (27)	7.2 ± 1.7 (5)	50.0 ± 3.3 (27)	30.4 ± 1.6 (5)
640	8 × 80 mg/kg	30.4 ± 2.5 (11)	8.3 ± 0.9 (3)	58.6 ± 2.5 (11)	25.7 ± 2.8 (3)

* Hepatic triglycerides and total lipids were determined for rats treated with various doses of pentenoic acid administered according to schedules described in Experimental Procedures. Values are expressed as mean ± S.E.M.; numbers in parentheses indicate the number of rats.

† Total dose administered over 48 hr.

‡ Number of injections × dose per injection administered at times indicated in Experimental Procedures.

showed a 30% increase in liver to body weight ratio [treated: 0.039 ± 0.005 (S.D.) (N = 48); control: 0.030 ± 0.005 (N = 12) g liver/g body wt; $P < 0.001$]. A variety of doses and schedules for pentenoic acid administration were tested. At 80 mg/kg, a mortality rate of about 12% was observed. Such rats usually died after only two pentenoic acid injections and did not exhibit fatty liver. Multiple injections of pentenoic acid were necessary for the development of fatty liver. Higher triglyceride levels were elicited by doses of 80 mg/kg administered within the 48-hr starvation period as compared to doses of 50 mg/kg. After six doses, administration of one or two additional 80 mg/kg doses (at 12 or 2 hr, respectively) prior to killing the rat did not further increase hepatic triglyceride content. However, with more doses, corresponding to a larger cumulative dose, the degree of variation in triglyceride levels between individual rats appeared to decrease.

Histologic observations confirmed that the fat deposited in response to pentenoic acid treatment was microvesicular and evenly distributed throughout affected hepatocytes. Frozen sections stained with Oil Red O, a fat stain, revealed a preferential localization of the triglyceride deposits in periportal regions of the liver. Electron microscopic studies also confirmed microvesicular fat deposits in hepatocytes from pentenoic acid-treated starved rats (Fig. 1). Occasional mitochondria exhibited swelling and pleomorphism in rats with fatty liver. However, by contrast with a previous report [12], mitochondrial ultrastructural alterations were not prominent in these animals. In agreement with biochemical data, fed rats treated with pentenoic acid did not exhibit hepatic fat deposits or mitochondrial ultrastructural alterations.

Serum chemistry alterations. Rats with pentenoic acid-induced fatty liver had serum ammonia levels about 60% higher than controls [treated: 238 ± 44 μM (N = 12); control: 145 ± 33 μM (N = 5); $P < 0.01$]. Blood urea nitrogen levels were also increased marginally [treated: 29 ± 4 mg/100 ml (N = 19); control: 22 ± 4 mg/100 ml (N = 6); $P < 0.05$]. Serum levels of calcium, phosphate, glucose, uric acid, cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, lactate dehydrogenase, and gluta-

Table 2. Effect of pentenoic acid treatment on palmitoylcarnitine oxidation*

	State 3 oxidation rate (n atoms O/min/mg protein)
48-hr starved rats	
Control (N = 11)	65.3 ± 4.2
Treated, high triglyceride (N = 8)	21.1 ± 5.3†
Treated, low triglyceride (N = 6)	40.2 ± 4.3†
Fed rats	
Control (N = 5)	57.6 ± 7.2
Treated (N = 8)	40.0 ± 5.4‡

* Palmitoylcarnitine oxidation was measured with liver mitochondria isolated from rats treated chronically with six to eight doses (80 mg/kg) of pentenoic acid and saline-injected control rats. Data for pentenoic acid-treated starved rats are grouped to indicate rats that developed fatty liver ("high triglyceride", 27.2 ± 4.9 mg/g liver) and those that did not ("low triglyceride", 6.8 ± 1.1 mg/g liver). Triglyceride levels for other groups were equivalent to values presented in Table 1. Numbers in parentheses represent the number of preparations. Values are expressed as mean ± S.E.M.

† Significantly different from control value at $P < 0.01$.

‡ Significantly different from control value at $P < 0.05$.

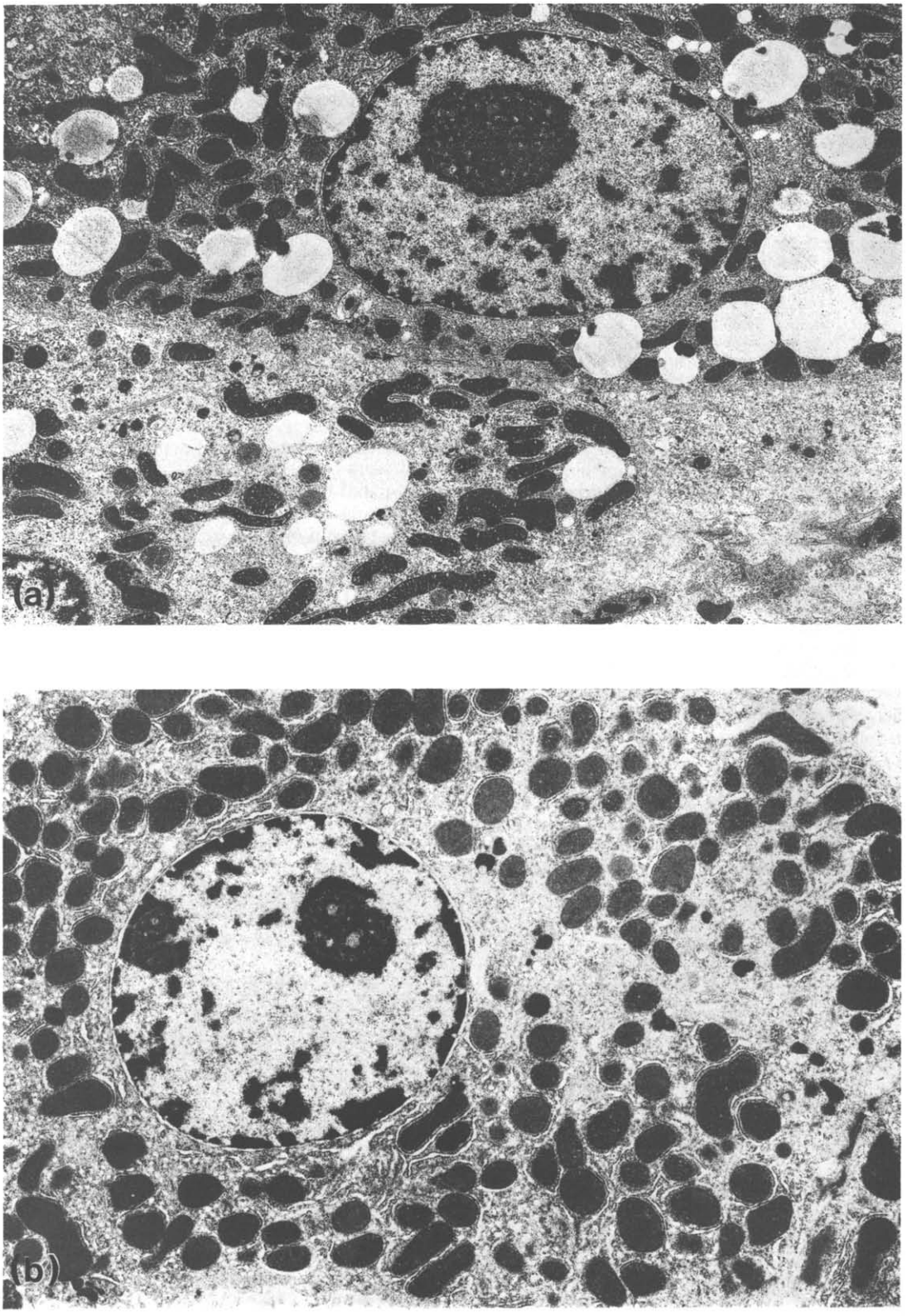

Fig. 1. Hepatocyte ultrastructure in pentenoic acid-induced fatty liver. Representative electron micrographs of hepatocytes from a rat treated with pentenoic acid (total dose 560 mg/kg) during 48 hr starvation (a) and a saline-treated 48-hr starved control rat (b), demonstrating microvesicular fat deposits following pentenoic acid treatment. Final magnification: 7250× for both.

Table 3. Inhibition of oxidation of various chain length fatty acid-type substrates*

Substrate	% Inhibition†	
	High triglyceride	Low triglyceride
L-Palmitoylcarnitine	67.7 ± 7.6	37.7 ± 5.7
DL-Decanoylcarnitine	68.6 ± 5.9	34.7 ± 11.3
DL-Octanoylcarnitine	48.7 ± 8.0	35.7 ± 7.4
Octanoate	58.9 ± 6.3	50.7 ± 13.9

* Inhibition of State 3 oxidation rates for the indicated substrates was determined with mitochondria isolated from pentenoic acid-treated starved rats that developed fatty liver ("high triglyceride") and those that did not ("low triglyceride"), as described in Table 2. Values are expressed as mean ± S.E.M.

† Percent inhibition compared to control.

mic-oxaloacetic acid transaminase were not significantly different from control values. Serum triglyceride content was also not elevated.

Alterations of mitochondrial function associated with pentenoic acid-induced fatty liver. The oxidation of several substrates representing different aspects of mitochondrial function was tested with mitochondria isolated from pentenoic acid-treated rats. The major functional alteration of mitochondria resulting from this treatment was a pronounced inhibition of the oxidation of fatty acid-type substrates, as shown for palmitoylcarnitine in Table 2. However, the extent of inhibition of fatty acyl-carnitine oxidation observed with different mitochondrial preparations from treated rats varied in a systematic manner that correlated with hepatic triglyceride levels and reflected the variability, as noted above, between individual rats in the development of fatty liver. Accordingly, the mitochondrial data for treated starved rats have been grouped to reflect rats that exhibited fatty liver and those that did not. The groups have been designated "high triglyceride" and "low triglyceride" respectively. Triglyceride values for the "low triglyceride" group were within the mean plus two standard deviations of the mean for control rats (i.e. ≤ 10 mg/g liver).

Oxidation of fatty acyl-carnitines was measured by recording oxygen uptake in the presence of ADP (State 3 conditions) and malonate, an inhibitor of the citric acid cycle. Previous studies have shown that under these conditions acyl-carnitines are oxidized to acetoacetate, and that the rate of oxygen uptake reflects maximal flux through fatty acid β-oxidation [15, 16]. With mitochondria from rats displaying fatty liver ("high triglyceride" group), State 3 rates of palmitoylcarnitine oxidation were decreased an average of about 70% compared to control values (Table 2). In some preparations, the rate of oxygen uptake in the presence of palmitoylcarnitine was identical to that observed in the absence of added substrate (endogenous respiration) and, thus, reflected maximal inhibition. Mitochondria isolated from rats with fatty liver consistently exhibited pronounced inhibition of fatty acid oxidation, regardless of whether the fatty liver had been induced by treatment with six, seven or eight doses of pentenoic acid (cumulative dose 480, 560, or 640 mg/kg) in which case the final 80 mg/kg dose had been given 16, 12 or 2 hr prior to preparation. Mitochondria isolated from similarly treated starved rats which did not exhibit fatty liver ("low triglyceride" group) showed less inhibition (35%) of palmitoylcarnitine oxidation. Mitochondria isolated from treated fed rats also exhibited a 30% inhibition of palmitoylcarnitine oxidation compared to fed control rats. Nutritional state alone, however, had little effect on the rate of palmitoylcarnitine oxidation (Table 2).

Oxidation of shorter chain acyl-carnitines was likewise inhibited, as was oxidation of octanoate (Table 3). The latter can undergo carnitine-independent oxidation [32]. The inhibition of acyl-carnitine oxidation could not be reversed by addition of 0.2 mM L-carnitine.

To localize the site of inhibition of fatty acid oxidation more specifically, reduction of flavoproteins involved in the fatty acid oxidation branch of the electron transfer chain was monitored in intact mitochondria by dual wavelength spectroscopy. Previous studies with rat liver mitochondria have shown that the absorbance change characteristic of flavoprotein

Table 4. Comparison of State 3 respiratory rates*

Substrate	Control	High triglyceride (natoms O/min/mg protein)	Low triglyceride
Glutamate/malate	85.6 ± 5.2	87.1 ± 5.6	79.3 ± 7.3
Succinate	126.4 ± 4.7	104.3 ± 9.0	92.4 ± 10.4
Pyruvate/malate	36.2 ± 2.0	39.3 ± 2.3	35.0 ± 2.7
α-Ketoglutarate	45.7 ± 3.8	38.9 ± 6.4	55.9 ± 7.0

* State 3 oxidation rates for the indicated substrates were measured with mitochondria isolated from control and pentenoic acid-treated starved rats that developed fatty liver ("high triglyceride") and those that did not ("low triglyceride"), as described in Table 2. Each value is the mean ± S.E.M. for four to eleven preparations.

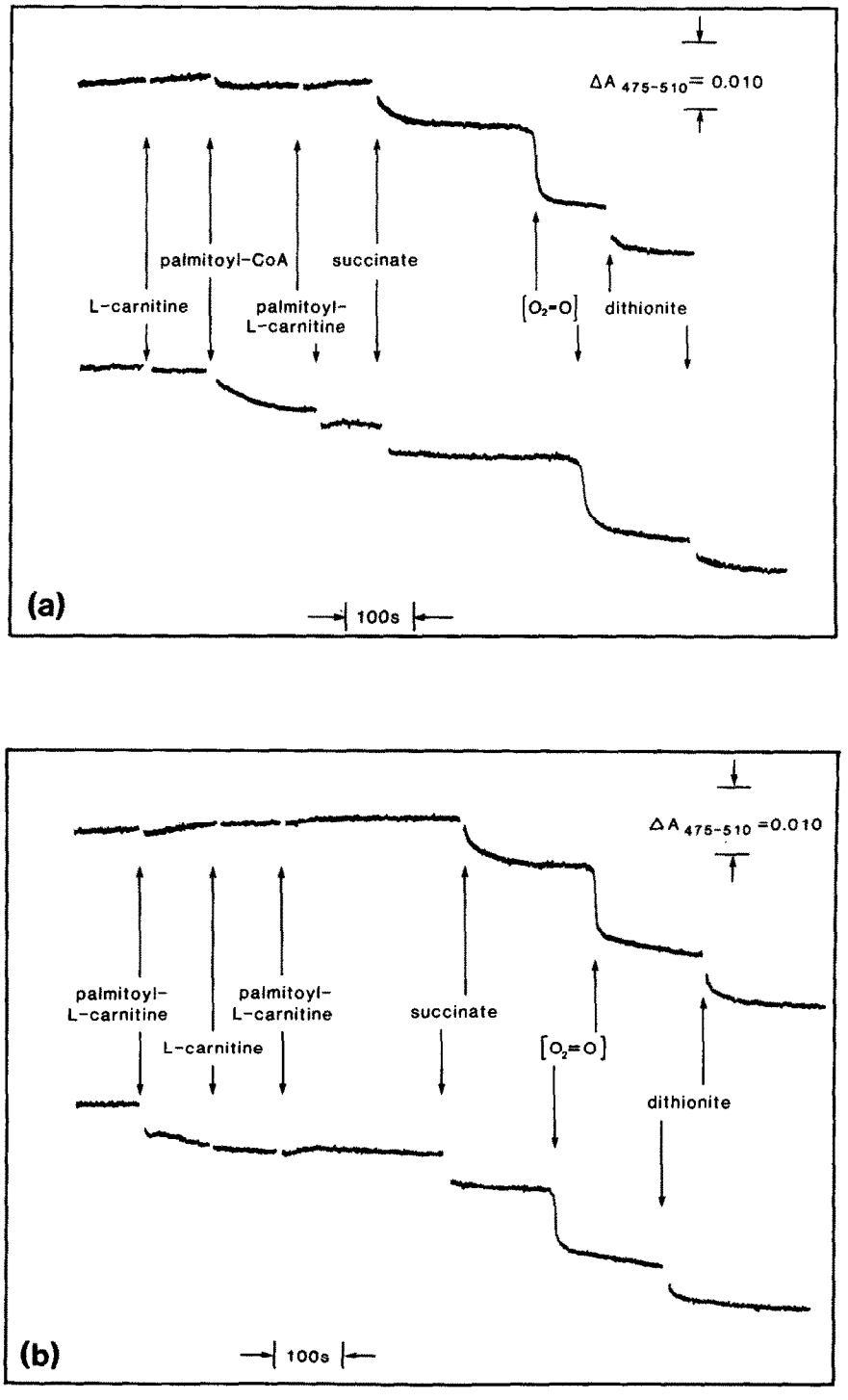

Fig. 2. Reducibility of flavoproteins in mitochondria from rats with pentenoic acid-induced fatty liver. Reduction of flavoproteins in intact mitochondria was monitored by dual wavelength spectroscopy using the wavelength pair 475 minus 510 nm. Mitochondria from a rat with pentenoic acid-induced fatty liver (upper tracings in panels a and b, 2.7 mg protein/ml) or a control rat (lower tracings in panels a and b, 2.1 mg protein/ml) were suspended in a medium containing 130 mM KCl, 10 mM Hepes, 5 mM $MgCl_2$, 0.5 mM EGTA, pH 7.5, and rotenone (4 μM). In (a), 1 mg/ml bovine serum albumin was also present, and sequential additions of 100 μM L-carnitine, 10 μM palmitoyl-CoA, 20 μM palmitoyl-L-carnitine, and 5 mM succinate are shown. Dithionite (approximately 1 mM) was added following spontaneous anaerobiosis. In (b), sequential additions of 20 μM palmitoyl-L-carnitine, 100 μM L-carnitine, 40 μM palmitoyl-L-carnitine, and 5 mM succinate are shown. Following spontaneous anaerobiosis, dithionite (approximately 1 mM) was added. Reduction is indicated by a downward deflection of the trace.

reduction which is elicited by palmitoylcarnitine arises from both acyl-CoA dehydrogenase and electron transferring flavoprotein [30, 33]. For these experiments, rotenone was added to inhibit net fatty acid oxidation by preventing the reoxidation of NADH. Under such conditions, reducing equivalents from acyl-CoA dehydrogenase can be transferred to the respiratory chain through electron transferring flavoprotein [30, 33–35]. By contrast with control mitochondria, neither palmitoylcarnitine nor palmitoyl-CoA plus L-carnitine caused flavoprotein reduction in mitochondria from rats with fatty liver (Fig. 2). Succinate-induced flavoprotein reduction, arising from the flavin of succinate dehydrogenase, was unaffected, as anticipated from the results of respiratory experiments.

State 3 oxidation rates for substrates other than fatty acids, including glutamate/malate, succinate, pyruvate/malate, and α-ketoglutarate, were not altered substantially by pentenoic acid treatment (Table 4). Mitochondria from rats with fatty liver did exhibit minor decreases in respiratory control ratios owing to small increases in State 4 respiratory rates. For example, with glutamate/malate as substrate, respiratory control ratio decreased from a control value of 4.92 ± 0.22 ($N = 11$) to 4.18 ± 0.34 ($N = 8$) in the high triglyceride group. Mitochondria from treated rats with low hepatic triglyceride levels showed no significant decrease in respiratory control ratios. However, the decline in respiratory control ratio values for mitochondria from rats with fatty liver was not observed when the mitochondria were isolated in a medium containing bovine serum albumin. Thus, this effect probably represents weak uncoupling, caused by residual fat disrupted during homogenization of the liver, and is not significant. Phosphorylation efficiencies, as represented by ADP/O ratios, were typical of coupled rat liver mitochondria and unchanged by pentenoic acid treatment in both groups.

DISCUSSION

Previous studies have established that a metabolite of pentenoic acid, most likely an intermediate in its own oxidation, is responsible for inhibition of mitochondrial β-oxidation [36–39]. Results of the present study demonstrate that fatty liver accompanied by inhibition of fatty acid oxidation can be produced by chronic administration of low doses of pentenoic acid to starved rats. The variability in the extent of fatty liver which developed in response to the treatment probably reflects pharmacokinetic differences between individual rats. Interestingly, however, the data indicate a correlation between fatty liver and a high degree of inhibition of mitochondrial fatty acid β-oxidation.

The data also indicate that starvation is required for the development of fatty liver in response to pentenoic acid treatment. Since starvation mobilizes depot fat from adipose tissue [40, 41], the latter may be the source of the hepatic triglycerides. Such a mechanism could account for the lack of fatty liver in the pentenoic acid-treated fed rats as owing to lack of mobilized fat. However, inhibition of fatty acid oxidation would still be anticipated. Since mitochondria isolated from fed rats treated with pentenoic acid did not exhibit highly inhibited fatty acid oxidation, these data suggest a possible role for nutritional or hormonal factors in disposition of pentenoic acid *in vivo* as well as in the development of fatty liver. The observations may be explained by the relatively low activity of hepatic fatty acid oxidation in the fed state [41] coupled with the requirement for activation of pentenoic acid by fatty acid oxidation in order to generate its inhibitory metabolite. Thus, it is possible that in the fed state pentenoic acid may be metabolized preferentially by non-hepatic routes [42].

At the mitochondrial level, the inhibition of octanoate as well as acyl-carnitine oxidation confirms direct inhibition of the β-oxidation pathway. The lack of reducibility of flavoproteins of the fatty acid oxidation branch of the electron transfer chain suggests that the site of inhibition is most likely at acyl-CoA dehydrogenase—the first reaction of the β-oxidation sequence. If the site of inhibition were subsequent to acyl-CoA dehydrogenase, flavoprotein reduction would be expected in spite of inhibition of oxygen uptake because transfer of reducing equivalents to electron transferring flavoprotein could still occur. Thus, although the inhibitory metabolite of pentenoic acid is probably formed by a reaction after acyl-CoA dehydrogenase in the β-oxidation sequence [36], results of the present study indicate that the effective site of inhibition by this metabolite in intact mitochondria is most likely at acyl-CoA dehydrogenase itself.

The observation that pyruvate and α-ketoglutarate oxidation were not inhibited in mitochondria from rats with fatty liver provides evidence for dissociation of the multiple effects of pentenoic acid determined from *in vitro* studies. Apparently, the *in vivo* levels of CoASH were not sufficiently altered to cause inhibition. Such a dissociation of effects confirms that the inhibition of fatty acid oxidation is not due exclusively to a depletion of CoASH [20]. The uninhibited oxidation of other respiratory substrates indicates that the electron transfer chain is unimpaired by pentenoic acid treatment.

Fatty liver is a common manifestation of toxic liver injury produced by a variety of exogenous agents [43, 44]. Although the microvesicular form of the hepatic triglyceride deposits in pentenoic acid-induced fatty liver resembles that of Reye's Syndrome [11], the ultrastructural and functional alterations of mitochondria produced by pentenoic acid treatment appear to be less pronounced than those reported in Reye's Syndrome [6, 7]. The moderate increases in serum ammonia and urea nitrogen associated with pentenoic acid-induced fatty liver that were found in the present study, while generally similar to those originally reported [11], are also less marked than those which occur in Reye's Syndrome [2, 3, 45]. Nevertheless, the results of this as well as some clinical studies [46, 47] suggest that inhibition of mitochondrial fatty acid oxidation may play a role in the pathogenesis of Reye's Syndrome. In addition, unique Reye's Syndrome serum components may disrupt mitochondrial function by other mechanisms [48].

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