EFFECT OF 4-PENTENOIC ACID ON COENZYME A METABOLITES IN RAT LIVER *

J. R. Williamson, M. H. Fukami, M. J. Peterson, S. G. Rostand and R. Scholz
Johnson Research Foundation, University of Pennsylvania
Philadelphia, Pennsylvania

Received June 16, 1969

4-Pentenoic acid has been shown to have a hypoglycemic effect on mice in vivo (1) and to inhibit gluconeogenesis and lactate oxidation in pigeon liver homogenates (2). Gluconeogenesis from alanine or pyruvate is inhibited by 4-pentenoic acid in perfused rat liver (3,4), and glucose production from pyruvate and succinate is decreased in rat kidney slices (5). 4-Pentenoic acid also inhibits the oxidation of fatty acids in a variety of systems (1-8). Since fatty acids are known to stimulate glucose production by the liver (9, 10), the impairment of gluconeogenesis has been attributed to a suppression of fatty acid oxidation. It has been suggested that inhibition of fatty acid oxidation is mediated by lack of free CoA and carnitine as a result of the accumulation of non-metabolizable CoA and carnitine esters formed during the oxidation of 4-pentenoic acid (2,6). However, no detailed studies on possible changes of CoA intermediates in tissues have been reported. In the present work, the kinetics of the effects of 4-pentenoic acid on adenine and pyridine nucleotides, CoA and carnitine derivatives have been determined in the perfused rat liver. These studies, and data obtained with intact and sonicated mitochondria show that the metabolic deficiencies observed in liver are caused both by inhibition of β-oxidation, and by depletion of CoA and acetyl-CoA.

Methods. Livers from male Holtzman rats (200-230 g), fasted 30-36 hours were perfused with Krebs bicarbonate buffer containing 4°/o (w/v) defatted albumin, as previously described (10,11). Techniques for measuring flavin and pyridine nucleotide redox changes by surface fluorometry, and the oxygen uptake of the perfused liver have been reported (12). Rat liver mitochondria were prepared according to Johnson and Lardy (13). Metabolic intermediates were measured in neutralized perchloric acid extracts by fluorometric enzyme methods (14).

Results. At relatively low concentrations 4-pentenoic acid is oxidized by the liver, as shown by the increased oxygen uptake and formation of ketone bodies (Fig. 1). The ratio of the extra oxygen consumed to the amount of 4-pentenoic acid added was 3.4 and 2.5, respectively, for the first and second

* Supported by grants from the U. S. Public Health Service (GM 12202) and the American Heart Association.

additions. These values may be compared with the theoretical 2 μatoms oxygen required, per μmole of 4-pentenoyl-CoA, for β-oxidation to acrylyl-CoA, plus an additional μatom of oxygen needed to form 4-pentenoyl-CoA (assuming a P/O ratio of 2). The stoichiometry of ketone body formation to 4-pentenoic acid added was approximately 1:2, as expected if one mole of acetyl-CoA were formed per mole of 4-pentenoic acid undergoing β-oxidation. With concentrations greater than 0.5 mM, oxidation of 4-pentenoic acid was incomplete.

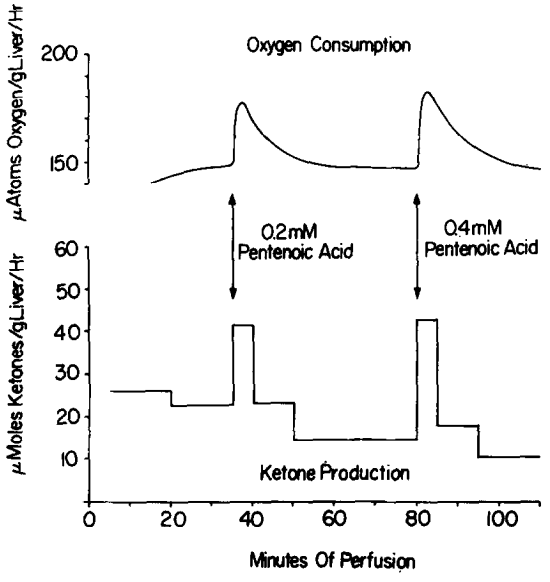

Figure 1. Oxidation of 4-pentenoic acid in perfused rat liver. Liver from a 30-hour fasted rat was perfused with 100 ml of albumin buffer containing no added substrate.

Addition of 4-pentenoic acid to liver mitochondria incubated in the presence of ADP and rutamycin[1] caused a reduction of flavin and pyridine nucleotides, which was similar to that produced by octanoate (Fig. 2). No redox changes were seen with fully uncoupled mitochondria unless ATP was present. These studies support the suggestion of Corredor et al. (1), that 4-pentenoic acid is metabolized by β-oxidation.

Fig. 3 shows that in perfused rat liver 4-pentenoic acid caused a reduction of flavin nucleotides coincident with an increased oxygen uptake. The flavin redox changes are derived predominantly from the mitochondria (12), while the pyridine nucleotide fluorescence reflects changes in both the mitochondrial and cytosolic spaces. About 1 minute after 4-pentenoic acid addition, a rapid oxidation of both flavin and pyridine nucleotides occurred. The

[1] This compound has similar actions to oligomycin.

pyridine nucleotide oxidation coincided with a decrease in the β-hydroxybutyrate/acetoacetate ratio from 0.8 to 0.4 (Fig. 4). The delayed oxidation phase is interpreted as indicating an inhibition of pyruvate dehydrogenase. The ATP/ADP ratio remained constant during the 10-minute perfusion period after 4-pentenoic acid addition (Fig. 4), but with longer perfusion times, both the ATP/ADP ratio and oxygen consumption decreased.

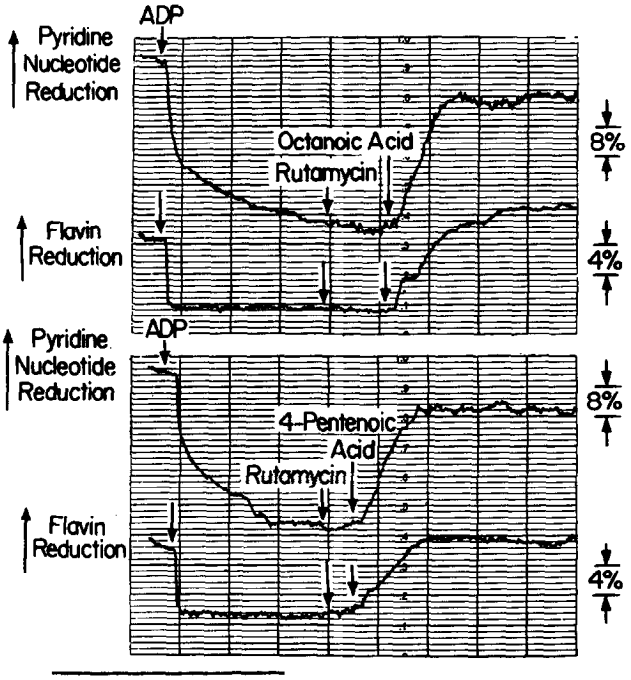

Figure 2. Comparison of the effects of octanoic and 4-pentenoic acids on flavin and pyridine nucleotide reduction in rat liver mitochondria. The incubation medium was 100 mM KCl, 50 mM sucrose, 20 mM Tris-Cl and 5 mM Tris-PO_4 containing 2-3 mg/ml mitochondrial protein. Rutamycin (10 μg/ml) and octanoic acid (100 μM) or 4-pentenoic acid (100 μM) were added as noted by the arrows. Redox changes were followed by using 366 mμ excitation, 450 mμ measurement (pyridine nucleotides) and 436 mμ excitation, 570 measurement (flavins).

During the first minute after 4-pentenoic acid addition to perfused liver, the CoA content fell rapidly while acid soluble acyl-CoA derivatives increased (Fig. 5). The content of long chain, acid insoluble, acyl-CoA derivatives increased only slightly during the first 10 minutes after 4-pentenoic acid addition, but acetyl-CoA levels fell by 50°/o. However, after 45 minutes, acid insoluble fatty acyl-CoA increased three-fold, while acetyl-CoA levels decreased to 20°/o of control values. The free carnitine content decreased rapidly (Fig. 5), whereas acid soluble acyl carnitine other than acetyl-CoA increased from 38 ± 3 mμmoles/g dry wt to 300 ± 25 mμmoles/g dry wt.

Experiments with rat liver mitochondria showed that 20 μM 4-pentenoic acid caused an 80°/o decrease of free CoA, and an increase of both acid soluble and acid insoluble fatty acyl-CoA (Table I). A ten-fold higher concentration of 4-pentenoic acid had little further effect. Palmitate stimulated respiration five-fold, decreased free CoA by 40°/o, and increased acetyl-CoA and acid insoluble fatty acyl-CoA (15). However, in the presence of 200 μM

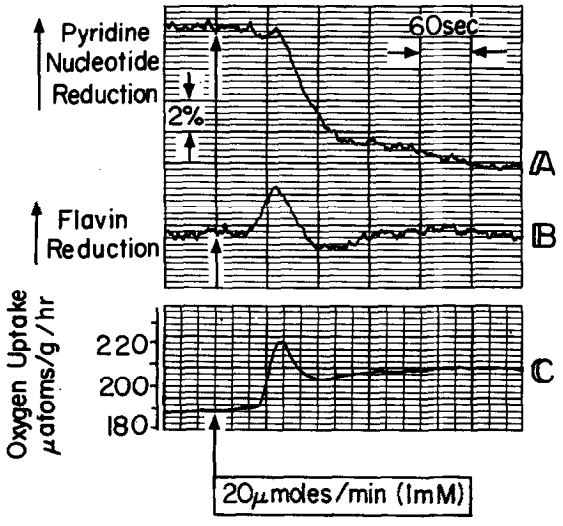

Figure 3. Effect of 4-pentenoic acid on flavin and pyridine nucleotide redox changes in perfused rat liver. The perfusion medium contained approximately 2 mM pyruvate.

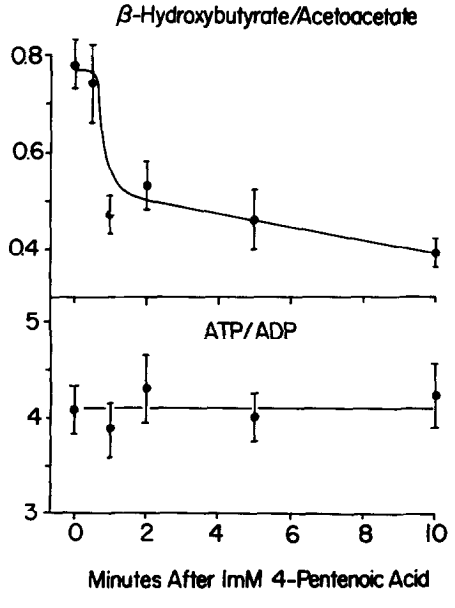

Figure 4. Effect of 4-pentenoic acid on tissue β-hydroxybutyrate/acetoacetate and ATP/ADP ratios in perfused rat liver. Rat livers were perfused for 30 minutes in the presence of 1.5 to 2 mM pyruvate. Perfusion was terminated by rapidly freezing livers with tongs cooled in liquid N_2. Values shown are means ± standard error of the mean of 6 to 8 livers.

4-pentenoic acid, palmitate oxidation was completely inhibited, free CoA and acetyl-CoA fell, while short and long chain acyl-CoA derivatives increased.

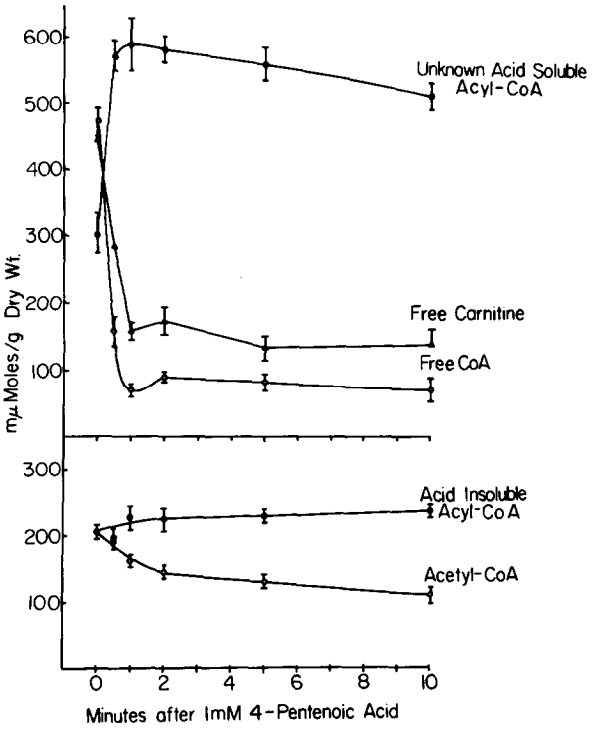

Figure 5. Effect of 4-pentenoic acid on tissue contents of CoA derivatives and carnitine in perfused rat liver. The experimental conditions were the same as those of Fig. 4.

Table I

Effects of 4-Pentenoic Acid (PA) on Isolated Liver Mitochondria

The incubation conditions were similar to those of Fig. 2. Palmitate was added 2 minutes after 4-pentenoic acid.

Additions to buffer + 1 mM ADP	Oxygen uptake (initial rate)	CoA	Acetyl-CoA	Unknown acid soluble CoA	Acid insoluble acyl-CoA
	mμatoms/ mg/min	mμmoles/mg protein			
None	7	1.6	0.2	1.1	0.7
20 μM PA	20	0.3	0.5	1.3	1.2
200 μM PA	24	0.2	0.2	1.6	1.2
40 μM Palmitate	36	0.9	0.5	1.2	0.9
Palmitate + 200 μM PA	5	0.2	0.1	1.5	1.5

The increase of acid insoluble acyl-CoA after 4-pentenoic acid addition suggests that inhibition of β-oxidation occurs prior to CoA depletion. Exper-

iments with sonicated mitochondria (Fig. 6) show that the oxidation of palmityl-CoA was inhibited by 4-pentenoyl-CoA. The addition of free CoA did not reverse the inhibition. 4-Pentenoic acid itself was not activated in this uncoupled preparation and had no effect on fatty acid oxidation. Since both substrate and inhibitor were added to the sonicated mitochondria as CoA derivatives, these experiments establish that fatty acid oxidation is inhibited by a 4-pentenoic acid metabolite.

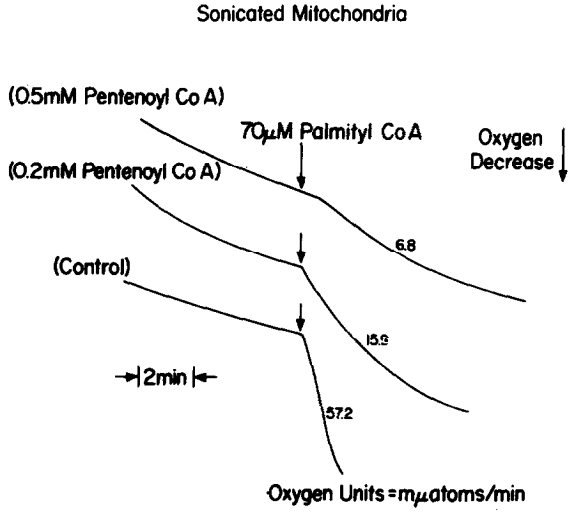

Figure 6. Effect of 4-pentenoyl-CoA on palmityl-CoA oxidation in sonicated mitochondria. Sonicated mitochondria were incubated at 28° in 50 mM potassium phosphate, pH 7.2, with 4-pentenoyl-CoA for 18 minutes before the addition of palmityl-CoA.

Discussion. The present work establishes that 4-pentenoic acid is metabolized by β-oxidation after prior activation to 4-pentenoyl-CoA. However, regeneration of CoA is incomplete and short chain fatty acyl-CoA derivatives accumulate. The chemical identity of the acyl-CoA compounds formed upon addition of 4-pentenoic acid to perfused liver and liver mitochondria has not been ascertained. The most obvious product of the β-oxidation of 4-pentenoic acid would appear to be acrylyl-CoA. However, Rendina and Coon observed that acrylyl-CoA is hydrated by enoyl hydrase to give β-hydroxypropionyl-CoA. This compound is a substrate for acetyl carnitine transferase, and may be the acid soluble acyl-CoA which accumulates upon 4-pentenoic acid oxidation. On the other hand, the inhibitor of fatty acid oxidation itself may well be acrylyl-CoA. Robinson and coworkers (17) found that some 2,3-enoyl-CoA derivatives, among them acrylyl-CoA, inhibited fatty acid synthesis. They suggested that an interaction between the unsaturated bonds of these compounds and enzyme sulfhydryl groups was responsible for the inhibition. Two of the four enzymes

of the β-oxidation cycle which are inhibited by sulfhydryl reagents and which, therefore, may be inhibited by an interaction with acrylyl-CoA are enoyl hydrase (18) and β-ketoacylthiolase (19). Presumably, oxidation of both medium and long chain fatty acids is affected since 4-pentenoic acid has been shown to inhibit its own oxidation (6).

The fall of free carnitine and increase of acyl carnitine esters in the liver after 4-pentenoic acid addition confirms earlier work (1), and shows that carnitine depletion was almost as rapid as CoA depletion. However, it is not clear whether inhibition of fatty acid oxidation in the intact liver occurs primarily through lack of CoA and carnitine, or by a more specific inhibition of an enzyme of its β-oxidation system as discussed above. Inhibition of pyruvate oxidation is probably mediated by CoA lack, while inhibition of gluconeogenesis is caused by a combination of influences; namely, a fall of acetyl-CoA resulting in diminished pyruvate carboxylase activity (20), an oxidation of the NAD redox state, and a fall of ATP.

References

1. Corredor, C., Brendel, K. and Bressler, R., Proc. Nat. Acad. Sci. U. S. A., 58, 2299 (1967).
2. Corredor, C., Brendel, K. and Bressler, R., J. Biol. Chem., 244, 1212 (1969).
3. Ruderman, N., Shafrir, E. and Bressler, R., Life Sciences, 7, 783 (1968).
4. Williamson, J. R., Peterson, M., Rostand, S. and Scholz, R., Fed. Proc., 28, 411 (1969).
5. Senior, A. E. and Sherratt, H. S. A., Biochem. J., 110, 499 (1968).
6. Brendel, K., Corredor, C. F. and Bressler, R., Biochem. Biophys. Res. Commun., 34, 340 (1969).
7. Senior, A. E., Robson, B. and Sherratt, H. S. A., Biochem. J., 110, 511 (1968).
8. Fukami, M., Fed. Proc., 28, 852 (1969).
9. Struck, E., Ashmore, J. and Wieland, O., Biochem. Z., 343, 107 (1965).
10. Williamson, J. R., Kreisberg, R. A. and Felts, P. W., Proc. Nat. Acad. Sci. U. S. A., 56, 247 (1966).
11. Williamson, J. R., Browning, E. T., Scholz, R., Kreisberg, R. A. and Fritz, I. B., Diabetes, 17, 194 (1968).
12. Scholz, R., Thurman, R. G., Williamson, J. R., Chance, B. and Bücher, T., J. Biol. Chem., 244, 2317 (1969).
13. Johnson, D. and Lardy, H. A., in Methods of Enzymology, Vol. 10, (R. W. Estabrook and M. E. Pullman, editors), Academic Press, New York (1967), p. 94.
14. Williamson, J. R. and Herczeg, B. E., in Methods of Enzymology, Vol. 13, (J. M. Lowenstein, editor), Academic Press, New York (1969), p. 434.
15. Garland, P. B., Shepherd, D. and Yates, D. W., Biochem. J., 97, 587 (1965).
16. Rendina, G. and Coon, M. J., J. Biol. Chem., 225, 523 (1957).
17. Robinson, J. D., Brady, R. O. and Bradley, R. M., J. Lipid Res., 4, 144 (1963).
18. Wakil, S. J. and Mahler, H. R., J. Biol. Chem., 207, 125 (1954).
19. Lynen, F. and Ochoa, S., Biochim. Biophys. Acta, 12, 299 (1953).
20. Keech, D. B. and Utter, M. F., J. Biol. Chem., 238, 2603 (1963).