ACETYL CoA LEVEL IN PERFUSED RAT LIVER DURING GLUCONEOGENESIS AND KETOGENESIS

L.A. Menahan,* B.D. Ross, and O. Wieland**

Klinisch-Chemisches Institut
Schwabinger Krankenhaus
Munich, Germany

Received December 1, 1967

In the perfused rat liver the maximum rate of gluconeogenesis from three-carbon precursors can be increased by addition of long-chain fatty acids (Struck, Ashmore, and Wieland, 1965), glucagon (Struck et al., 1965; Exton and Park, 1965; Ross, Hems, and Krebs, 1967) or 3'5' cyclic AMP (Exton and Park, 1966). The role of acetyl CoA as an activator of pyruvate carboxylase in vitro, one of the possible rate limiting enzymes of gluconeogenesis, has focused attention on this intermediate as an effector in the intact cell (Utter, Keech, and Scrutton, 1964). Krebs, Speake, and Hems (1965) observed an increase in the acetyl CoA level in kidney slices in which gluconeogenesis had been stimulated with short chain fatty acids.

In the present studies, of which a more detailed account is to be published (Menahan, Ross, and Wieland, in press), acetyl CoA levels were determined in the liver at various times during a perfusion to which lactate together with oleate, acetate or glucagon was added. Contrary to expectations, dramatic increases in both gluconeogenesis and keto-

*NATO Post-doctoral Fellow, 1966-1967.

**Supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg

genesis could be effected without alteration in the tissue level of acetyl CoA.

Materials and Methods

Male albino rats (Sprague-Dawley, Gassner, München), 120-200 g., were fasted for 24 hrs before use. The apparatus and technique of perfusion were as previously described (Struck et al. 1966; Teufel, Menahan, Shipp, Böning, and Wieland, 1967). Krebs-Henseleit saline was substituted for Tyrode solution in the perfusion medium to give a final HCO_3^- content of 25 mM and pH 7.4 (Hems, Ross, Berry, and Krebs, 1966). Glucagon and substrates, with the exception of oleate, were given in a single dose after 40 min of pre-perfusion. (Glucagon was a gift of Eli Lilly, Indianapolis USA or from Farbwerke Hoechst, Frankfurt Germany, and was suspended in 1.3% $NaHCO_3$). Na-L-lactate (Schuchardt) was used as substrate. In experiments with oleate, a dose of 110 µmoles oleic acid emulsion and 100 µmoles DL-carnitine was given at 40 min and an infusion of 100 µmoles oleic acid per hr was continued throughout the perfusion. The oleic acid was prepared in a solution of 12% bovine serum albumin in Krebs-Henseleit buffer using a high-speed homogeniser.

Samples of perfusion medium were taken at 20, 40 and 43 min and thereafter at 10 min intervals. Liver samples were taken by the rapid freezing technique of Wollenberger, Ristau and Shoffa (1960) and extracted with frozen $HClO_4$. Changes were recorded in the same liver by taking samples at 40 min, before substrate and then at 60 and 80 min, after the addition of substrate. Acetyl CoA was determined fluorimetrically, using the coupled assay of Stern, Ochoa, and Lynen (1952) in the Eppendorf photometer with fluorimeter

and compensating voltage attachments. The results have been corrected for the altered MDH equilibrium (Buckel and Eggerer, 1963) and correlated with an internal standard. Glucose, lactate, acetoacetate and β-hydroxybutyrate were determined enzymatically (Teufel et al., 1967).

Results and Discussion

The rate of glucose synthesis from 10 mM Na-L lactate was 1.06 ± 0.09 μmoles/min/g fresh liver, which agrees closely with the result of Hems et al. (1966). As indicated in

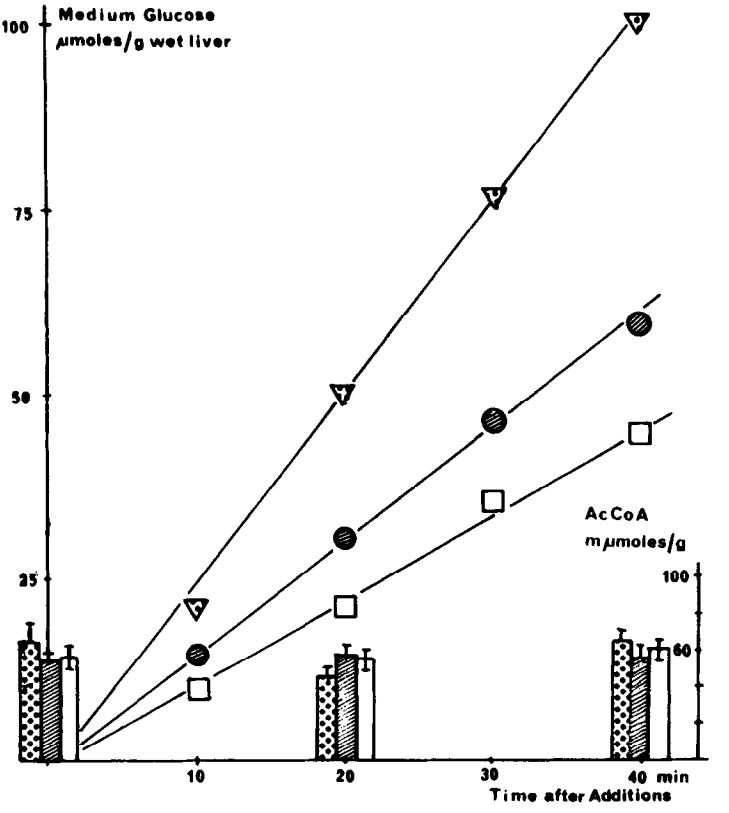

Fig. 1. Acetyl CoA Levels in Perfused Rat Liver during Stimulated Gluconeogenesis

Livers of rats starved 24 hrs were perfused as described in Methods. At 40 min were added 10 mM lactate (□—□), 10 mM lactate + 5 mM acetate (⊘—⊘) or 10 mM lactate + glucagon (200 μg/100 ml) (▽—▽).

Fig. 1, the formation of glucose was linear during the 40 min experimental period. The rate of glucose synthesis was increased by the addition of 5 mM acetate (1.45 ± 0.08 μmole/min/g), oleate (1.35 ± 0.08 μmole/min/g) [not shown in Fig. 1] or glucagon (2.64 ± 0.09 μmole/min/g). The ratio of lactate removed/glucose formed was in each case close to 2:1 which excludes the "sparing effect" of fatty acid on lactate oxidation. The glycogen content of the liver at 40 min was less than 2 μmoles/g and did not therefore contribute significantly to the observed increases in medium glucose.

The acetyl CoA content of perfused liver was identical with that of the non-perfused liver of rats starved 24 hrs (Table 1) and remained practically constant in perfusions with and without lactate (Fig. 1 and Table 1). As shown in Fig. 1, the acetyl CoA level did not alter significantly even when glucose synthesis was markedly stimulated by acetate or glucagon. Similarly, in perfusions with lactate and oleic acid, the figures for acetyl CoA were 68 ± 8, 51 ± 8 and 51 ± 8 at 40, 60 and 80 min respectively.

In a similar series of experiments the correlation between acetyl CoA and the rate of ketogenesis was studied (Table 1). Endogenous ketogenesis was linear during the period studied and could be increased by 150% by the addition of acetate (5 mM). Even greater increases followed perfusion with glucagon or oleate. As expected, ketogenesis could be significantly suppressed by the addition of lactate, and in the case of acetate, this suppression was almost 100% (Menahan, Ross, and Wieland, 1967). No significant increase in the level of acetyl CoA

Table 1

Acetyl CoA Levels in Perfused Rat Liver during Stimulated Ketogenesis

Livers of rats starved 24 hrs were perfused without lactate as described in Methods. Ketone body formation is expressed as μmoles acetoacetate + β hydroxybutyrate/min/g fresh liver. In the 40 min period following addition of substrate, ketone body formation was linear.

Addition	No. of expts.	Acetyl CoA (nmole/g fresh liver) Before	after substrate addition		Ketone-body Formation μmole/min/g liver
		40 min	60 min	80 min	(40-80 min)
Nil	3	61 ± 5	54 ± 3	54 ± 5	0.31 ± 0.05
Oleate	5	63 ± 4	56 ± 6	60 ± 5	2.00 ± 0.16
Acetate (5 mM)	4	62 ± 7	51 ± 7	56 ± 6	0.77 ± 0.16
Glucagon (200 μg)	6	61 ± 9	75 ± 7	79 ± 6	1.07 ± 0.15
Liver not perfused	8	62 ± 5			

was observed under any of these conditions (Table 1). This makes it improbable that the rate of ketogenesis is controlled by the acetyl CoA level in the whole liver of starved rats. The possibility remains that the acetyl CoA content of individual cell compartments alters independently.

The observation that gluconeogenesis also may be stimulated without change in the acetyl CoA level suggests that this stimulation occurs without a change in pyruvate carboxylase activity, as proposed by Struck et al. (1965) or that the activity of pyruvate carboxylase can be increased without alteration of acetyl CoA. The rate limiting character of pyruvate carboxylase for the formation of glucose from C3 precursors must be further questioned since its activity has been found to be 10.3 ± 0.9 μmoles/min/g (I.Böttger, personal communication) about 5 times that of phospho-enolpyruvate carboxykinase.

Willms (in press) has shown that the initially lower acetyl CoA level in liver of fed rats can be increased in perfusion by the addition of caprylate. Together, these results are in accord with the finding of Wieland and Weiss (1963) in diabetic rats *in vivo*, that the acetyl CoA level and blood acetoacetate level increase in parallel to a plateau, but that thereafter, increases in ketogenesis occur without further change in acetyl CoA.

References

Buckel,W. and Eggerer,H. (1963) Biochem. Z. 343, 29.
Exton,J.H. and Park,C.R. (1965) J.Biol. Chem. 240, 955.
Exton,J.H. and Park,C.R. (1966) Pharm. Revs. 18, 181.
Hems,R., Ross,B.D., Berry,M.B. and Krebs,H.A. (1966) Biochem.J. 101, 284.
Krebs,H.A., Speake,R.N. and Hems,R. (1965) Biochem.J. 94, 712.

Menahan,L.A., Ross,B.D. and Wieland,O. (in press). In Stoffwechsel der isoliert perfundierten Leber. 3. Konferenz der Gesellschaft für Biologische Chemie. Oestrich,Germany.

Ross,B.D., Hems,R. and Krebs,H.A. (1967) Biochem.J. 102 942.

Stern,J.R., Ochoa,S. and Lynen,F., (1952) J.Biol.Chem. 198, 313.

Struck,E., Ashmore,J. and Wieland,O. (1965) Biochem.Z. 343, 107.

Struck,E., Ashmore,J. and Wieland,O. (1966). In Advances in Enzyme Regulation (ed. by G.Weber) Pergamon Press, New York, Vol. 4, p.219.

Teufel,H., Menahan,L.A., Shipp,J.C., Böning,S. and Wieland,O. (1967) Europ.J.Biochem. 2, 182.

Utter,M.F., Keech,D.B. and Scrutton,M.C. (1964). In Advances in Enzyme Regulation (ed. by G.Weber) Pergamon Press, New York, Vol. 2, p.49.

Wieland,O. and Weiss,L. (1963) Biochem.Biophys.Res.Commun. 13, 333.

Willms,B. (in press). In Stoffwechsel der isoliert perfundierten Leber. 3. Konferenz der Gesellschaft für Biologische Chemie. Oestrich, Germany.

Wollenberger,A., Ristau,O. and Schoffa,G. (1960) Pflüg. Arch.ges.Physiol. 270, 399.