INCREASE IN LIVER ACETYL - COENZYME A DURING KETOSIS *

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Received January 25, 1963

It is generally presumed that ketosis occurs when the liver content of acetyl-CoA*** is increased as a result of excessive fatty acid oxidation,but there is no clear cut evidence to support this assumption.We have therefore determined acetyl-CoA in the livers of ketotic rats.Our experiments show that in the ketoacidoses accompanying both decompensated diabetes and alimentary fat loading there is a considerable accumulation of liver acetyl-CoA.

METHODS

In order to produce ketoacidosis,Sprague-Dawley albino rats were injected with alloxan (160 mg/kg,subcutaneously) and than received twice-daily injections of 2.8 - 3 units of long-acting insulin (Depot-Insulin,Farbwerke Hoechst,Germany) for several weeks whereupon the insulin treatment was stopped.Ketosis occured within 24 - 72 hrs.and was accentuated by injection of adrenalcorticoid hormone during the insulin-free period (Scow and Chernick,1960).In a group of normal rats ketosis was induced by feeding a diet of smoked pork fat.The rise in blood aceto-

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* Supported by the Deutsche Forschungsgemeinschaft,Bad Godesberg,Germany.

** Part of the Doctor's Thesis of L.Weiss at the Medical Faculty of the University of Munich,1963.

*** Abbreviations: CoA = coenzyme A; CCE = citrate condensing enzyme; DPN = diphosphopyridine nucleotide; DPNH = reduced diphosphopyridine nucleotide; MDH = malate dehydrogenase; tris = tris(hydroxymethyl)-aminomethane.

acetate as related to the duration of fat feeding is shown in Table I.

TABLE I

Ketoacidosis induced by fat feeding

	Acetoacetate in blood (mg%)[a]			
	Rat I	Rat II	Rat III	Rat IV
Standard diet[b]	0.2[c]	0.2	0.2	0.2
Fat diet, 30 hrs.	8.1 (11.5)[d]	6.1 (7.0)	5.2 (8.4)	3.7 (7.5)
" " , 55 hrs.	7.8 (9.0)	7.3 (6.3)	4.1 (3.9)	3.6 (2.6)
" " , 80 hrs.	12.4 (6.7)	10.0 (4.8)	7.8 (2.6)	7.9 (7.2)

a Determined in 0.2 ml samples of tail vein blood by the colorimetric procedure of Walker (1954).

b Altromin R,Altrogge Co.,Lage,Lippe,Western Germany.

c Mean values for animals kept on standard diet.

d Food intake,gm,in brackets.

The rats were anesthetised with ether and portions of the liver were excised using forceps which had been pre-cooled in liquid oxygen (Hohorst et al.,1959).The frozen tissue was homogenized with 6% perchloric acid in a glass homogenizer.The tissue to perchloric acid ratio was adjusted from 1:4 to 1:10 depending on the amount of liver.After high speed cenrtifugation of the homogenates at 0° C.,the clear supernatants were made to pH 6.8 with 2 N KOH using a glass electrode.The solutions were than allowed to stand for 15 - 30 min.in an ice bath and the precipitate of $KClO_4$ was removed by a short centrifugation.

Acetyl-CoA was determined spectrophotometrically on an aliquot of the clear supernatant using the assay system described for the measurment cf CCE activity by Ochoa et al. (1951).The

reaction is based on the enzymatic condensation of acetyl-CoA and oxalacetate to form citrate (eq.1) in combination with the DPN-dependent oxidation of malate by MDH (eq.2).As indicated in reaction (eq.3) one molecule of acetyl-CoA gives rise to one molecule of DPNH the absorbance of which is measured at 366 mμ.

(1) $$\text{acetyl-CoA} + \text{oxalacetate} + H_2O \xrightleftharpoons{\text{CCE}} \text{citrate} + \text{CoA}$$

(2) $$\text{malate} + DPN^+ \xrightleftharpoons{\text{MDH}} \text{oxalacetate} + DPNH + H^+$$

(3) $$\text{acetyl-CoA+malate+}DPN^+ + H_2O \rightleftharpoons \text{citrate+CoA+}DPNH+H^+$$

Fig.1 shows the kinetics of the formation of DPNH in the presence of a stoichiometric amount of acetyl-CoA.Fig.2 illustrates the linear relation of DPNH formation with increasing concentrations of acetyl-CoA in this test system.In control experiments 95 % recovery was obtained when acetyl-CoA was added to the acid liver homogenate.

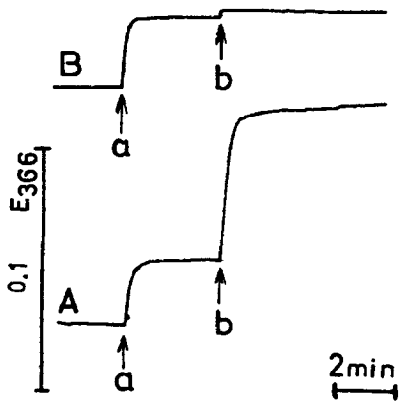

Fig.1 Spectrophotometric determination of acetyl-CoA in liver.Original recording,Eppendorf-photometer with Siemens-recorder.Curve A: The assay system contained,in a final volume of 2.25 ml tris-HCl buffer,pH 7.0,150 μM; potassium malate,10 μM; DPN, 2 μM; MDH,50 μg (C.F.Boehringer u.Soehne,Mannheim,Germany); CCE, 20 μg (crystallized according to Srere et al.(1961);liver extract,pH 6.8, 2 ml.After addition of MDH (at a) DPNH is formed until the reaction has come to equilibrium.DPNH formation after adding CCE (at b) is due to acetyl-CoA. Curve B: Same components as in A; the liver extract was incubated prior to the assay for 30 min. at pH 13 and 20° C.to hydrolyze acetyl-CoA.The slight increase in optical density at b corresponds to the absorption of the added enzyme.Light path 1 cm ; wave length 366 mμ.

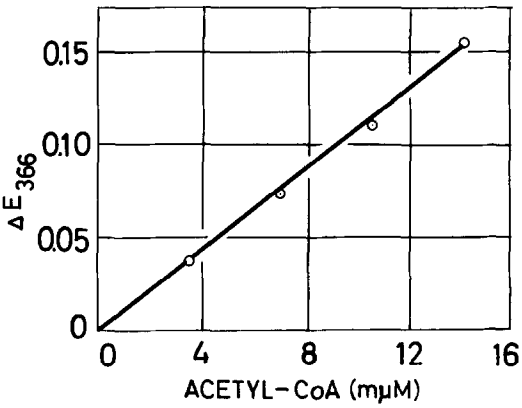

Fig.2 Formation of DPNH with varying amounts of acetyl-CoA. The assay system contained,in a final volume of 1.4 ml,tris-HCl buffer,pH 7.0, 100 μM; potassium malate,15 μM; DPN, 2 μM; MDH, 50 μg; acetyl-CoA (prepared by reaction of acetic anhydride and CoA according to Simon and Shemin,1953), as indicated; CCE, 20 μg; the reaction was started by adding CCE.Light path 2 cm; wave length 366 mμ.Acetyl-CoA was calculated from the extinction coefficient of DPNH $\varepsilon_{366} = 3.3 \times 10^6 (cm^2/Mol)$(Hohorst,1957).

RESULTS

Table II summarizes the acetyl-CoA levels which were found in the livers of normal and ketotic rats.There was a marked increase of liver acetyl-CoA in both the diabetic cortisol-treated animals and in the animals made ketotic by fat feeding.In some cases (also shown in Table II) the changes in acetyl-CoA before and after fat feeding were demonstrated in the same animal.

In Fig.3 the acetyl-CoA levels are plotted against the levels of acetoacetate in blood.There is a definite relationship between the liver acetyl-CoA content and the degree of ketonemia.This seems most pronounced in the fat fed animals.

DISCUSSION

Our experiments clearly demonstrate an accumulation of acetyl-CoA in the liver under conditions leading to enhanced ketone body formation.As can be seen in Fig.3 the rise of liver acetyl-CoA which was observed during severe ketoacidosis was

TABLE II

Acetyl-CoA in the livers of ketoacidotic rats [a]
(mμMol acetyl-CoA/gm wet weight)

Controls	Diabetic cortisol treated [b]	Fat fed
11.2	62.4	59.0
14.7	41.4 [c]	58.0
25.0	68.5 [d]	72.4
27.5	51.2	85.2
26.9	51.0	39.0 (25.5) [f]
10.3	29.5	34.3 (18.7)
26.4	40.1	58.7 (20.5)
19.0	28.6	
27.0	55.6 [e]	
10.9	40.7	
$\bar{x}$ 19.9 ∓ 7.45	46.9 ∓ 13.4	58.1 ∓ 17.7

a) minimum blood acetoacetate level 4 mg %.

b) 100 mg of dexamethasone on the first day after insulin withdrawal; rats were sacrificed on the fourth day.

c) cortisol (Scherisolon), 1 mg, on the second day.

d) cortisol (Scherisolon), 1 mg each on the second and third day.

e) no treatment with adrenal corticoid hormones.

f) changes in the same rat; the figures in brackets give the levels before fat feeding as determined in biopsy specimens.

not found with lower concentrations (less than 4 mg %) of blood acetoacetate. This finding helps to clarify our former observations in which an elevation of acetyl-CoA was not detected in the diabetic liver (Wieland et al., 1960). In the earlier experiments the liver acetyl-CoA levels were measured at a later stage of the diabetes when blood acetoacetate levels had decreased to less than 1 mg %. A slight increase of acetyl-CoA in the livers of chronic alloxan diabetic rats has been described by Buehring and Kühnau (1960) but no data pertaining to the degree of ketosis was reported.

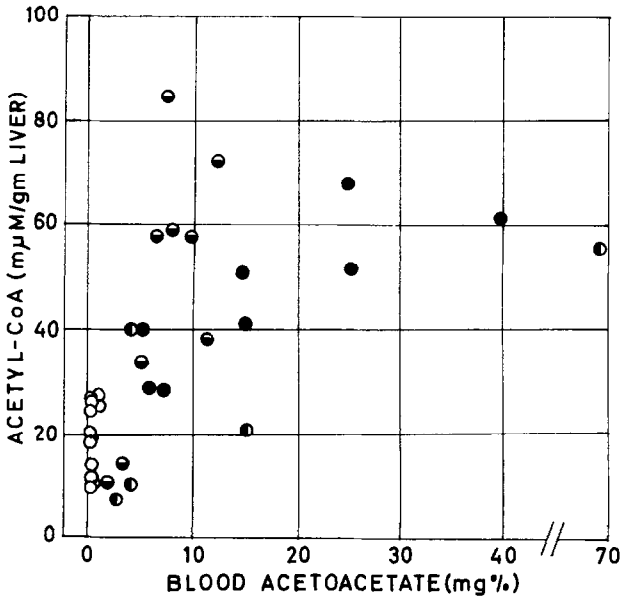

Fig.3 Correlation between acetyl-CoA content of liver and degree of ketoacidosis.The values of Table II are plotted against the corresponding acetoacetate blood levels. ○ controls, ◒ fat fed, ● diabetic cortisol treated, ◐ diabetic.

SUMMARY

The level of acetyl-CoA in the livers of alloxan diabetic rats rendered ketotic by withdrawal of insulin and administration of adrenal corticoid hormones and of rats made ketotic by fat feeding is 2.5 - 3 times higher than in untreated controls. This finding is taken as evidence in support of the theory that ketosis occurs as a result of over-production of acetyl-CoA in the liver.

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