INCORPORATION OF MALONYL CoA INTO FATTY ACIDS BY LIVER IN STARVATION AND ALLOXAN-DIABETES*

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Received October 27, 1960

In previous preliminary communications from this laboratory (Gibson, et al., 1960 a,b) it was reported that the specific enzyme activity of a soluble fatty acid synthesizing system from rat liver supernatant was markedly depressed if the preparation was obtained from alloxan-diabetic or from starved rats. The activity was normal or above normal if the diabetic rats were pretreated with insulin or the starved rats refed. These results were obtained with both the whole supernatant from liver homogenates and the derived dialyzed 0-40% ammonium sulfate fraction.

As in previous studies with avian liver (Gibson, et al., 1958), the purified rat liver system required supplementation with separate acetyl CoA and TPNH generating systems. Since the supply of acetyl CoA and TPNH was not limiting, the diminished activities observed in diabetes and starvation could therefore be attributed to a defect in the enzymes directly concerned with the synthesis of fatty acids from acetyl CoA. Other factors or enzymes that may influence fatty acid formation by intact cells or even by undialyzed liver supernatant (eg. endogenous TPNH generation) could be excluded by using

*This investigation was supported by Public Health Service Grant # H-4219; by American Heart Association Grant-in-Aid #59-G-21; and by American Cancer Society grants # P-178A and P-244.

**This work was performed during the tenure of an Established Investigatorship of the American Heart Association, Inc.

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this assay system. Acetyl CoA carboxylase activity (Wakil, 1958; Formica and Brady, 1959; Lynen, 1959) was not impaired in diabetes or starvation. Indeed, specific activity levels greater than normal were observed (Gibson, et al., 1960).

In the present study the rate of incorporation of 2-C^{14}-malonyl CoA into fatty acids was measured simultaneously with the rate of malonyl CoA-dependent oxidation of TPNH using the dialyzed 0-40% ammonium sulfate sub-fraction prepared from diabetic and starved rats. The evidence presented in Tables I and II indicates that, in the liver fractions obtained from alloxan-diabetic rats and from rats starved for a brief period (48 hours), the specific enzyme activity both of malonyl CoA incorporation into fatty acids and of simultaneous TPNH oxidation was greatly diminished. After injecting insulin into the alloxan-diabetic rats (which were fed throughout the experiment), or refeeding the starved rats (for 48 hours), the specific activity measure-ments were at normal or greater than normal levels.

The observed defect could not be caused by a dialyzable inhibitor, nor could the diminished concentration of enzyme be explained in terms of a change in the fractionation characteristics of the liver constituents. The yield of enzyme per liver in the 0-40% ammonium sulfate fraction is virtually the same whether derived from homogenates prepared in phosphate buffer (0.1 M, pH 7.5) or in unbuffered sucrose solution (0.25 M), irrespective of the nutritional state of the animal.

In the rat liver system, as in avian liver systems (Wakil and Ganguly, 1959; Steberl, et al., 1960), acetyl CoA is necessary for optimal incorporation of malonyl CoA into fatty acids and for TPNH oxidation. Butyryl CoA can completely replace acetyl CoA. This is observed in liver preparations from both the diabetic and insulin-treated diabetic rats (Table III). Thus, in this system, butyryl CoA does not uniquely restore activity to the impaired diabetic preparation (Shaw, et al., 1957).

Under the conditions of these assays (employing the 0-40% fraction, TPNH, 2-C^{14}-malonyl CoA, acetyl CoA at pH 6.5) normal microsomes (prepared

Table I

Malonyl CoA Incorporation into Fatty Acids by Liver Enzyme Fraction from Normal, Alloxan-Diabetic, and Insulin-Treated Diabetic Rats

Type	Specific Enzyme Activity *		Enzyme Yield **		Ave. Liver Weight (gm)
	TPNH	Incorp.	TPNH	Incorp.	
Series A					
Normal	60.	19.	15.	4.8	12.9
Alloxan	13.	2.	1.8	0.3	6.5
Insulin	71.	15.	19.	4.1	10.2
Series B					
Normal	69.	19.	15.	4.0	9.9
Alloxan	31.	7.8	4.9	1.2	5.8
Insulin	135.	39.	31.	8.8	9.4

*Specific Enzyme Activity: -Δ mμmoles TPNH per 5 min. per mg protein in 0-40% fraction; and mμmoles 2-C^{14} malonyl CoA incorporated into fatty acids per 5 min. per mg protein in 0-40% fraction

**Enzyme Yield: -Δ μmoles TPNH per 5 min. (in the 0-40% fraction) per liver; and μmoles 2-C^{14} malonyl CoA incorporated into fatty acids per 5 min (in the 0-40% fraction) per liver.

78 mμmoles of 2-C^{14}-malonyl CoA (prepared by the method of Trams and Brady) were incubated with 20 mμmoles of acetyl CoA, 100 mμmoles of TPNH, 20 μmoles of potassium phosphate buffer (pH 6.5) and 50 to 500 μg of enzyme in a final volume of 0.40 ml at 38°. Oxidation of TPNH was followed continuously at 340 mμ (with an incubation mixture containing no malonyl CoA as blank). The reaction was stopped after five minutes with alcholic KOH. Following saponification and acidification of the mixture, fatty acids were extracted into pentane, plated and counted. Protein was determined by the biuret reaction.

Rats were made diabetic by a single intravenous injection of alloxan (4.0 mg alloxan per 100 gm body weight). After 7 days rats having high blood glucose levels were designated the diabetic group. From these a number were randomly selected for insulin treatment (average: 4 units of protamine-zinc insulin per 100 gm body weight per day). After four additional days the diabetic, the insulin-treated diabetic and normal control rats were killed.

The fatty acid synthesizing enzyme fraction was prepared by homogenizing freshly extirpated liver with 3 volumes of 0.1 M potassium phosphate buffer (pH 7.5) in a teflon-glass homogenizer for a period of less than one minute. The homogenate was centrifuged at 70,000 g for 100 minutes. The clear supernatant was removed by syringe to avoid contamination with the overlying fatty layer or with the sediment. The 0-40% ammonium sulfate subfraction of the supernatant was dialyzed and cleared of any insoluble matter before enzyme analysis.

Table II

Malonyl CoA Incorporation into Fatty Acids by Liver Enzyme Fraction from Normal, Starved and Refed Rats

Type	Specific Enzyme Activity *		Enzyme Yield *		Ave. Liver Weight (gm)
	TPNH	Incorp.	TPNH	Incorp.	
Series A					
Normal	66.	17.	10.	2.6	10.4
Starved	13.	1.4	1.6	0.18	4.9
Refed	86.	27.	13.	4.2	8.8
Series B					
Normal	69	14.	4.0	0.81	7.5
Starved	18	2.7	0.90	0.14	4.4
Refed	96	27.	7.0	2.0	7.0

* Assay conditions the same as in Table I. A group of rats previously fed a balanced stock diet (Wayne Lab-Blox, Allied Mills, Inc.) were starved for 2 days. Part were killed for analysis. The remainder were refed the stock diet for an additional 2 day period after which time they were killed along wit the control series.

Table III

Comparison of Acetyl and Butyryl CoA

	Specific Enzyme Activity*		TPNH / Incorp. Ratio
	TPNH	Incorp.	
Alloxan			
2-C^{14}-Mal CoA	8.6	2.0	4.3
2-C^{14}-Mal CoA + Acetyl CoA	31.	7.8	4.0
2-C^{14}-Mal CoA + Butyryl CoA	35.	11.	3.2
Insulin			
2-C^{14}-Mal CoA	60.	19.	3.2
2-C^{14}-Mal CoA + Acetyl CoA	135.	39.	3.4
2-C^{14}-Mal CoA + Butyryl CoA	172.	46.	3.7

* -Δ mμmoles TPNH per 5 min per mg protein (0-40% fraction); and mμmoles 2-C^{14}-malonyl CoA incorporated into fatty acids per mg protein. Same assay conditions as in Table I. Where indicated, 63 mμmoles of butyryl CoA were added.

in 0.25 M sucrose) did not stimulate malonyl CoA incorporation into fatty acids. This is in keeping with previous observations with avian liver that (microsome-bound) enoyl reductase is not a component in this biosynthetic system (Gibson, et al., 1958). Consequently, the reported reduction of enoyl reductase activity in alloxan-diabetes (Matthes, et al., 1960), is probably not limiting malonyl CoA incorporation into fatty acids.

Summary. Diabetes and starvation are associated with a marked enzymic defect in the direct conversion of malonyl CoA to long chain fatty acids by liver. The enzyme impairment is restored by pretreating the diabetic animal with insulin or by refeeding the starved animal.

Acknowledgements. The authors wish to acknowledge the competent technical assistance of D. Allmann, G. McLain, and D. Mitchell. They are also indebted to Dr. J. Ashmore and Mr. C. Love for preparing the alloxan-diabetic rats.

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