ELEVATION IN ACETOACETATE FORMATION FROM ACETOACETYL-CoA IN DIABETIC RAT LIVER PREPARATIONS*

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Biochemical mechanisms proposed for the ketosis of diabetes have generally been based upon a postulated accumulation of precursors of acetoacetic acid, such as acetoacetyl-CoA and acetyl-CoA, presumed to result from a defect in the utilization of these intermediates by other pathways. For example, the hypothesis has recently been put forward that the excessive rate of ketone body formation in diabetes is a result of an impairment in the ability of the diabetic liver to convert acetoacetyl-CoA to fatty acids (Siperstein and Fagan 1957; Siperstein 1958).

We have obtained evidence for an alternative mechanism of ketosis based upon the observation of an increase in diabetic rat liver preparations in the activity of the enzyme system(s) *per se* concerned with the formation of acetoacetate from acetoacetyl-CoA.

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Liver homogenates were prepared in 9 volumes of 0.25 M sucrose in a Servall Omni-mixer and were divided into a mitochondrial and supernatant fraction by centrifugation at 10,000 x g for 10 minutes. The pellet was resuspended to its original volume in 0.25 M sucrose by means of a teflon hand homogenizer. Incubations were performed at 37° for 20 minutes in a volume of 1.0 ml. containing 100 μmoles of Tris buffer, pH 7.9, 5 μmoles of GSH, 10 μmoles of Na_2S, 5 μmoles of $MgCl_2$, 0.5 mg. of CoA, 30 μmoles of acetyl phosphate, 0.1 ml. of liver fraction, and 0.05 ml. of Cl. kluyveri extract as a source of phosphotransacetylase and β-keto thiolase (Lynen et al. 1958). The reaction was terminated by the addition of 1.0 ml. of 1.0 M $HClO_4$, precipitated protein was centrifuged off, and an aliquot of the supernatant solution was taken for acetoacetate analysis by the method of Walker (Walker 1954). Amounts of acetoacetate formed under these conditions were approximately linear with respect to time and enzyme concentration and independent of further increases in the amount of Cl. kluyveri extract. The activity, as determined by this method, was distributed approximately equally between the mitochondrial and supernatant fractions. Upon freezing and thawing the resuspended mitochondria, an approximately 6-fold increase in activity was obtained. The results from a group of 8 normal livers are presented in Table I.

Similar measurements were made with fractions from alloxan-diabetic rats and diabetic rats treated with insulin for 2 or 3 days (2 units protamine zinc insulin per 100 g.

TABLE I

Rate of formation of acetoacetate from acetoacetyl-CoA in liver fractions

Activities are expressed as mμmoles of acetoacetate formed in 20 minutes per mg. of liver, plus or minus the standard error.

Fraction	Activity
Homogenate	7.1 ± 0.9
Supernatant	5.2 ± 0.3
Resuspended mitochondria	4.1 ± 0.5
Frozen-thawed mitochondria	23.8 ± 1.4

twice a day). Mitochondria prepared from diabetic livers and activated by the freezing and thawing procedure were found to possess an activity approximately double that of the normal preparation. Further freezing and thawing produced no additional increase in activity with either the normal or diabetic preparations. Administration of insulin to the diabetic animals as indicated above restored to normal the activity of activated mitochondria. The results are summarized in Table II. No significant differences from the normal were found in the homogenates, or supernatant or unfrozen mitochondrial fractions of diabetic livers. Administration of prednisolone (1 mg. per day for 3, 6, or 9 days) to normal rats produced no change in

TABLE II

Activity of "activated" mitochondria from livers of normal, diabetic, and insulin-treated rats

Numbers in parentheses under "Status" refer to the number of animals in each group. Activity data are given as means plus or minus standard error. "p" values refer to significance of differences from normal. Protein was determined by the method of Lowry et al. (Lowry et al. 1951).

Status	Activity		
	mμmoles per mg. equivalent of liver	mμmoles per mg. of protein	μmoles per 100 gm. of body weight
Normal (8)	23.8 ± 1.4	220 ± 15	94 ± 7
Diabetic (7)	45.5 ± 4.9 ($p < 0.005$)	312 ± 32 ($p < 0.02$)	245 ± 25 ($p < 0.001$)
Diabetic plus insulin (7)	18.9 ± 2.0	219 ± 20	176 ± 18

the activities of any of the liver fractions.

Two pathways have been proposed for the formation of acetoacetate from acetoacetyl-CoA in liver. In one case the evidence was interpreted to indicate that the reaction occurs via condensation of acetoacetyl-CoA with acetyl-CoA to form β-hydroxy, β-methyl-glutaryl-CoA, followed by cleavage to acetoacetate and acetyl-CoA (Lynen et al. 1958), while in the other, a hydrolytic cleavage of acetoacetyl-CoA to form acetoacetate directly was suggested from the data (Drummond

and Stern 1960). The experiments reported here give no indication of the nature of the pathway which is elevated, or if the same pathway is operative in the separate fractions.

These results suggest that an elevation in the activity of the acetoacetate-forming enzyme(s), rather than an accumulation of acetoacetyl-CoA due to a decrease in its utilization for fatty acid synthesis, may be the cause of the increased ketone body production in diabetes. This view is in harmony with current information on the mechanism of fatty acid synthesis which indicates that acetoacetyl-CoA is not an intermediate in the pathway of de novo fatty acid synthesis in the particle-free supernatant system of liver (Wakil and Ganguly 1959).

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