STUDIES ON PYRUVATE CARBOXYLASE ACTIVITY IN ALLOXAN DIABETIC AND NORMAL ANIMALS *

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Previous studies from this laboratory have shown that in alloxan diabetic rats there is an increased incorporation of C^{14} from labeled bicarbonate, pyruvate, alanine and glutamate into glucose (Wagle and Ashmore; 1961, 1963). Further observations showed that phosphoenolpyruvate carboxykinase (EC 4.1.1.32) activity was found to be increased three to five fold in livers of alloxan diabetic rats (Wagle and Ashmore; 1963). An increase in this enzyme activity alone could not account for increased glucose formation as observed in diabetes; therefore, the activity of pyruvate carboxylase, involved in oxaloacetate formation was investigated. Recently, Keech and Utter (1963) have described the properties of pyruvate carboxylase (EC 6.4.1.1) in rat and chicken liver preparations. Henning et al. (1963) have reported that pyruvate carboxylase activity is increased in rat liver following cortisol administration.

The enzymatic activity of pyruvate carboxylase in normal and diabetic animals was measured as reported by Keech and Utter (1963). Normal and alloxan diabetic rats, prepared as described previously (Wagle and Ashmore; 1961) were fed ad libitum on Purina

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Chow, killed by decapitation and livers removed and homogenized in 0.25 M sucrose.

Liver homogenates were spun at 105,000 x g for one hour. The supernatant fraction was incubated for ten minutes with 5 μc of radioactive bicarbonate (Specific Activity 1 μc/μMole) and various co-factors, (Table I). Incubations were terminated by addition of 0.5 ml of 10 per cent trichloroacetic acid and proteins removed by centrifugation. The supernatant fluid was gassed for ten minutes with CO_2 to remove any excess of $C^{14}O_2$ and an aliquot was counted in an anthracene-packed cuvette in a Packard Scintillation Counter before and after the treatment with aniline hydrochloride (Barkulis and Lehninger 1951). The radioactivity lost after aniline hydrochloride treatment was presumed to be due to C^{14} incorporation into oxaloacetate. The results of this study are summarized in Table I.

A three fold increase in pyruvate carboxylase activity was observed in a supernatant of diabetic liver. Further, it was observed that all of the $C^{14}O_2$ fixed was lost when treated with aniline hydrochloride indicating $C^{14}O_2$ fixed was in oxaloacetate. Avidin inhibited whereas DPN and TPN stimulated CO_2 fixation in supernatant of normal rat livers. The increased $C^{14}O_2$ fixed in the normal preparation was not lost on treatment with aniline hydrochloride and avidin was less effective in inhibiting CO_2 fixation. These observations would suggest that this radioactivity was in malate, fumarate or succinate and not in oxaloacetate. This increase in carboxylation observed in diabetic preparations may be due to the defect in the removal of oxaloacetate formed. In order to exclude this possibility the removal of oxaloacetate was studied in 105,000 x g liver supernatant. The liver supernatant from diabetic and normal liver preparations were incubated for ten minutes with various concentrations of oxaloacetate and with co-factors as present in pyruvate carboxylase assay systems. At the end of ten minute incubation unused oxaloacetate was measured by the procedure of Tonhazy et al. (1950). The

TABLE I

PYRUVATE CARBOXYLASE ACTIVITY OF RAT LIVER

All values are expressed of cpm C^{14} incorporated/g wet liver during a ten minute incubation

		NORMAL	DIABETIC
(1)	C.S.*-pyruvate-ATP-CoA-Mg	570 ± 80++	620 ± 90
(2)	C.S.-pyruvate-CoA-Mg	850 ± 80	720 ± 60
(3)	C.S.-CoA-Mg	960 ± 60	840 ± 40
(4)	C.S.-pyruvate-ATP-CoA	560 ± 30	860 ± 40
(5)	C.S.-Acetyl-CoA	1,200 ± 200	1,400 ± 300
(6)	C.S.	20,800 ± 2,500	61,600 ± 8,300
(7)	C.S.+An.HCl.**	6,400 ± 500	2,800 ± 300
(8)	C.S.+Avidin***	4,370 ± 430	6,500 ± 650
(9)	C.S.+DPN****+TPN****	37,400 ± 3,200	62,300 ± 6,050
(10)	C.S.+DPN+TPN+An.HCl.**	19,600 ± 800	3,600 ± 400
(11)	C.S.+DPN+TPN+Avidin	16,400 ± 1,200	12,800 ± 1,300

* Complete System (C.S.) contained 0.5 ml of 105,000 x g supernatant 20 µMoles of K pyruvate, 50 µMoles of $NaHCO_3$ (50 µc); 3.3 µMoles $MgCl_2$ 1.25 µMoles ATP; 0.38 µMoles Acetyl-CoA and 50 µMoles of Tris HCl (pH 7.4) in a total volume of 1 ml.

** Aniline Hydrochloride was added after the incubation.

*** Avidin added 2 units per incubation mixture.

**** DPN and TPN added was 2 µMoles each per incubation mixture.

++ Each figure is an average of four values

oxaloacetate removed was 48.2 ± 6.8 µMoles/g for diabetic and 23.6 ± 3.2 µMoles/g for normal liver preparation in ten minutes. These observations are in agreement with previous studies reported (S. R. Wagle, and J. Ashmore,

1963) where it was observed a three fold increase in the conversion of pyruvate and CO_2 to glucose in diabetic liver slice preparations. The present studies indicate that in diabetic livers there is three fold increase in oxalacetate formation from pyruvate and also an increase in utilization of this oxaloacetate formed. It is suggested that the increase in gluconeogenesis observed in diabetic preparations may be in part due to the increase formation and utilization of oxaloacetate.

Another important observation made in the present study is that pyruvate carboxylase is very stable at room temperature and that enzymatic activity in 105,000 x g supernatant was only observed if homogenization and centrifugation was carried out at room temperature. Similar observations have been made by Utter (Personal Communication). The mitochondrial fraction obtained from homogenates prepared at room temperature did not exhibit pyruvate carboxylase activity. These results indicate that pyruvate carboxylase may be extracted from mitochondria when homogenized at room temperature but not in chilled preparations.

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