Preliminary Notes

PN 1281

Studies on carbon dioxide fixation in normal and alloxan-diabetic animals

Experimental diabetes has been characterized as a metabolic state involving both overproduction and underutilization of glucose; however, the enzymatic mechanisms by which increased hepatic glucose production has been achieved have not been elucidated. Previous studies from this laboratory have shown that in alloxan-diabetic rats there is an increased incorporation of ^{14}C from labeled bicarbonate, pyruvate, alanine, and glutamate into blood glucose[1,2]. An increased incorporation of $^{14}CO_2$ into glucose has also been observed in liver slices from alloxan-diabetic rats[2]. The present studies report further observations on CO_2 fixation by homogenates of liver from normal and alloxan-diabetic rats. Normal and alloxan-diabetic rats prepared as described previously[1], fed *ad libitum* on Purina Chow, were killed by decapitation and the livers removed and homogenized (1 g wet liver per 2.5 ml 0.154 M KCl) containing nicotinamide (1 mg/ml). Homogenates were incubated for 30 min with 5 μC of radioactive bicarbonate (specific activity 1 μC/μmole) and various co-factors. Incubations were terminated by addition of 0.5 ml of 10% trichloroacetic acid and the proteins removed by centrifugation. The supernatant fluid was gassed for 15 min with CO_2 to remove the excess of $^{14}CO_2$; an aliquot of the solution was dried on a planchet and radioactivity determined in a gas flow counter. The results of these experiments are summarized in Table I. Homogenates fortified with NADP and lactate showed an increase in CO_2 fixation as compared with unfortified homogenates or those containing only lactate or lactate plus NAD. That addition of methylene blue, 10^{-4} M, markedly reduced CO_2 fixation provides further evidence that a re-

TABLE I

$^{14}CO_2$ FIXATION counts/min/100 mg WET LIVER*

1 ml of homogenate was incubated with 20 μmoles $^{14}CO_2$ (5.0 μC), 0.15 M potassium phosphate buffer (pH 7.4), 40 μmoles lactate, 2 μmoles NAD, 2 μmoles NADP, 40 μmoles pyruvate, 2.5 μmoles ATP and 20 μmoles oxaloacetate. Each value represents the mean ± S.E. of 6 observations.

	Normal	*Diabetic*
Homogenate only	1310 ± 550	3250 ± 950
Homogenate + lactate	1400 ± 310	3790 ± 740
Homogenate + NAD^+ + lactate	1020 ± 350	2500 ± 700
Homogenate + NADP + lactate	1850 ± 400	4180 ± 1150
Homogenate + methylene blue + lactate	170 ± 40	201 ± 32
Homogenate + pyruvate + ATP + oxaloacetate*	1980 ± 110	9060 ± 780
Homogenate + pyruvate + ATP + oxaloacetate treated with aniline hydrochloride*	930 ± 50	1310 ± 180

* These incubations were carried out for 10 min. All other incubations were for a period of 30 min.

ductive process is involved. In all cases, homogenates of diabetic liver showed a marked increase in CO_2 fixation.

Incorporation of $^{14}CO_2$ into oxaloacetate by whole liver homogenates was determined by incubation in the presence of pyruvate and ATP. An aliquot of the supernatant fluid was counted in anthracene-packed cuvettes in a Packard Scintillation Counter before and after treatment with aniline hydrochloride[3]. The difference in radioactivity was presumed to be due to ^{14}C incorporation into oxaloacetate. In homogenates of diabetic livers 7750 ± 780 counts/min were incorporated as compared with 1050 ± 110 counts/min in homogenates of normal liver.

Phosphopyruvate carboxylase (EC 4.1.1.32) was assayed in livers of normal and alloxan-diabetic rats by the method of UTTER AND KURAHASHI[4]. Livers were homogenized in 0.154 M KCl and centrifuged at 105 000 × *g* for 60 min 0.1 ml of the supernatant fluid equivalent to 0.033 g of fresh liver was incubated with 5 μC of [^{14}C]bicarbonate, 20 μmoles of oxaloacetate; 2 μmoles of $MnCl_2$ and with or without ITP (2 μmoles). At the end of a 10-min incubation solutions were deproteinized with trichloroacetic acid and gassed with CO_2. An aliquot of the filtrate was counted in a Packard Scintillation Counter. Phosphopyruvate carboxylase activity was found to be increased 300 ± 25% when expressed per g of wet liver in alloxan-diabetic animals as compared to normal.

These results indicate that in the liver of alloxan-diabetic rats there is an increase in CO_2 fixation and phosphopyruvate carboxylase activity. It is suggested that the increase in gluconeogenesis in the diabetic may be in part due to an increased conversion of lactate and pyruvate to phosphoenolpyruvate.

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The iodide pool of the thyroid studied by means of the isotopic equilibrium method and double labelling with ^{125}I and ^{131}I

The amount of iodide present in the thyroid is very low. It is renewed in two ways: by uptake from the plasmatic iodide and by deiodination of the iodotyrosines (recycling). In the present work, the amount of iodide present in the rat thyroid has been measured by the isotopic equilibrium method[1-3] without any pharmacological intervention. By combining this method with double labelling it has been