Studies on Experimental Diabetes

II. CARBON DIOXIDE FIXATION*

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Experimental diabetes has been characterized as a metabolic state involving both overproduction and underutilization of glucose. Although these changes have been recognized for many years, the specific biochemical alterations resulting in increased hepatic glucose production have not been adequately described. The adrenal cortical steroids are known, however, to have an important role in the regulation of this process (1). Previous studies from this laboratory have demonstrated that in alloxan diabetes there is an increase in the incorporation of C¹⁴ from labeled pyruvate and alanine into blood glucose (2). It would appear from our present knowledge of intermediary metabolism that the incorporation of labeled carbon atoms from 3 carbon glucose intermediates such as lactate, pyruvate, propionate, or alanine into hexose by hepatic tissue would require CO₂ fixation (3-6). Furthermore, under conditions where increased hepatic glucose production would be expected, e.g., prior treatment with cortisone, incorporation of C¹⁴ from both pyruvate-2-C¹⁴ and C⁴O₂ into blood glucose is increased to a comparable extent (7).

The present studies report observations on C⁴O₂ fixation in vivo and in vitro and the influence of insulin insufficiency induced by alloxan and anti-insulin serum on this process.

EXPERIMENTAL PROCEDURE

Animals—Male albino rats of the Wistar strain weighing between 100 and 150 g were used. They were fed ad libitum on Purina laboratory chow. Alloxan diabetes was produced by intravenous injection of alloxan monohydrate (40 mg per kg of body weight) in the manner described by Renold et al. (8). Adrenalectomized diabetics were produced by bilateral adrenalectomy of alloxan diabetic rats, and these animals were maintained on 0.9% NaCl for 4 or 5 days before use.

Anti-insulin serum was prepared as described previously (9). Acute insulin insufficiency was produced by the administration of 2 ml of anti-insulin serum, which would neutralize the activity of 2 to 2.5 units of beef insulin. This quantity of anti-insulin serum would increase the blood sugar of normal rats to 180 to 220 mg per 100 ml within 30 minutes.

Animals treated with cortisol received 5 mg of the steroid suspended in NaCl by subcutaneous injection twice a day for a period of 5 days.

Studies with Intact Rats—All animals received an intraperi-

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Fig. 1. Radioactivity recovered in blood glucose, expressed as counts per minute per ml of blood, has been plotted as a function of time after injection of C\textsuperscript{14}-bicarbonate. Each curve represents the mean of four or more animals, and total spread of points is indicated.

**TABLE I**

**Incorporation of C\textsuperscript{14} from radioactive C\textsuperscript{14}O\textsubscript{2} into tissue proteins**

Each figure is the mean for three values, with the mean deviation as indicated.

<table>
<thead>
<tr>
<th>Type of rat</th>
<th>Liver</th>
<th>Kidney</th>
<th>Diaphragm</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3710 ± 210</td>
<td>2720 ± 200</td>
<td>107 720 ± 120</td>
<td></td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>3227 ± 247</td>
<td>2103 ± 143</td>
<td>960 ± 360 ± 125</td>
<td></td>
</tr>
<tr>
<td>Normal + anti-insulin serum</td>
<td>4130 ± 600</td>
<td>1820 ± 210</td>
<td>1640 ± 1200 ± 240</td>
<td></td>
</tr>
<tr>
<td>Normal + normal serum</td>
<td>3453 ± 513</td>
<td>2310 ± 490</td>
<td>1920 ± 1900 ± 280</td>
<td></td>
</tr>
</tbody>
</table>

Differences in incorporation of CO\textsubscript{2} into tissue proteins were observed, except in the alloxan diabetic rats. In this case, C\textsuperscript{14} incorporation into muscle proteins was slightly reduced.

The studies on the incorporation of C\textsuperscript{14}O\textsubscript{2} in liver slices are summarized in Table II. Addition of pyruvate or propionate, 40 mM, to the incubation medium did not significantly increase the incorporation of C\textsuperscript{14}O\textsubscript{2} into glucose by rat liver slices. However, 40 mM lactate added to the incubation medium increased C\textsuperscript{14} incorporation from 8,250 c.p.m. per g in the absence of added substrate to 23,600 c.p.m. per g in the presence of lactate.

The incorporation of C\textsuperscript{14}O\textsubscript{2} into glucose was increased in liver slices from alloxan diabetic rats. Increased CO\textsubscript{2} fixation in the diabetic was evident in the absence of added substrate or in the presence of added pyruvate, lactate or propionate. Such an increase was not observed in livers from animals treated with anti-insulin serum. No significant differences in C\textsuperscript{14}O\textsubscript{2} incorporation into glucose were observed between animals previously treated with normal guinea pig serum and those given anti-insulin serum.

Pyruvate isolated from the incubation medium in experiments in which unlabeled pyruvate had previously been added was found to have appreciable radioactivity. The incorporation of C\textsuperscript{14}O\textsubscript{2} into pyruvate by liver slices from alloxan diabetic rats was increased to approximately the same extent as C\textsuperscript{14}-glucose; however, the amount of C\textsuperscript{14} recovered in pyruvate was only one-third that found in glucose.

No marked differences between normal and alloxan diabetic rats in incorporation of C\textsuperscript{14}O\textsubscript{2} into liver glycogen were observed in vivo. Less glycogen was found in livers from anti-insulin serum-treated animals than in livers from normal serum-treated rats.

**DISCUSSION**

Both insulin and the adrenal steroids appear to be involved in the regulation of CO\textsubscript{2} fixation observed in intact rats. In insulin insufficiency, whether produced by alloxan or by prior treatment with anti-insulin serum, an increase in C\textsuperscript{14}-glucose was observed in the blood after administration of radioactive
bicarbonate. Absence of insulin, per se, does not appear to be
responsible for the increased CO2 fixation observed in the diabete,
since adrenalectomy of alloxan diabetic rats markedly
reduces the amount of C14-glucose formed after C14O2 adminis-
tration. Further evidence for the particination of adrenal corti-
sone into glucose carbon into glucose most likely proceeds via
pyruvate or propionate and subsequent conversion of the malate
or succinate formed to phosphoenolpyruvate and glucose forma-
tion by the reversal of glycolysis (3). Further, the incorpora-
tion of pyruvate carbon into glucose was observed. It is probable that most of the C14-glucose formation from C14O2.

When radioactive bicarbonate and anti-insulin serum were
administered simultaneously to rats, very little increase in C14-
glucose was observed. It is probable that most of the C14O2
diabetes is not due to the presence of endogenous sub-
strates. Of the unlabeled substrates added, only lactate re-
sulted in an increase in C14-glucose formation, and this increase
was observed in liver slices from diabetic as well as normal rats.

To that added lactate, but not pyruvate, will increase C14-glucose formation from radioactive CO2 in rat liver slices suggests that
hydrogen atoms from the non-reduced substrate may contrib-
ute to C14O2 incorporation. Thus, CO2 "fixation" and gluco-
neogenesis in hepatic tissue may be regulated in part by the
availability of TPNH for malate formation from pyruvate and
CO2. The increase in CO2 fixation observed with lactate might
also further involve the enzymatic transfer of hydrogen from DPN
to TPN. Such a mechanism has been suggested as a possible
point of action of adrenal cortical steroids in the regulation of
gluconeogenesis (14).

It is tempting to speculate that the observed increase in CO2
fixation in diabetic liver may be due in part to a diversion of
TPNH, formerly used for fatty acid synthesis, for malate forma-
tion from pyruvate. In addition, reduced coenzyme formed by
fatty acid oxidation and increased ketone body formation in the
diabetic may contribute to reductive carboxylation of pyruvate.
Previous experiments (15) have demonstrated that a decrease in
pyruvate-2-C14 incorporation into fatty acids occurs at the
same time that an increased incorporation of pyruvate carbon
into glucose is observed in the induction of experimental diabete.
The idea that reduced coenzyme is necessary for gluco-
neogenesis is not new. Fitch and Chaikoff (16) have suggested
that TPNH produced by glucose oxidation via the pentose phos-

<table>
<thead>
<tr>
<th>Added substrate</th>
<th>No. of observations</th>
<th>Glycogen</th>
<th>Glucose</th>
<th>Pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µmoles/g</td>
<td>c.p.m./g</td>
<td>µmoles/g</td>
</tr>
<tr>
<td>Normal control:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>94 ± 21</td>
<td>529 ± 34</td>
<td>83 ± 3.7</td>
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<tr>
<td>Pyruvate, 40 mm</td>
<td>6</td>
<td>88 ± 33</td>
<td>502 ± 73</td>
<td>94 ± 4.1</td>
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<tr>
<td>Lactate, 40 mm</td>
<td>6</td>
<td>70 ± 10</td>
<td>804 ± 76</td>
<td>88 ± 3.2</td>
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<tr>
<td>Alloxan diabetic:</td>
<td></td>
<td>50 ± 8.3</td>
<td>150 ± 23</td>
<td>114 ± 7.7</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
<td>32 ± 4.7</td>
<td>383 ± 41</td>
<td>66 ± 6.1</td>
</tr>
<tr>
<td>Pyruvate, 40 mm</td>
<td>6</td>
<td>33 ± 5.8</td>
<td>395 ± 73</td>
<td>82 ± 5.6</td>
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<tr>
<td>Lactate, 40 mm</td>
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<td>27 ± 7</td>
<td>464 ± 56</td>
<td>61 ± 4.8</td>
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<tr>
<td>Propionate, 40 mm</td>
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<td>15 ± 3</td>
<td>120 ± 11</td>
<td>35 ± 2.7</td>
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<td>Normal serum:*</td>
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<td></td>
<td></td>
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<tr>
<td>Glucose, 5.5 mm</td>
<td>4</td>
<td>119 ± 8.0</td>
<td>150 ± 11</td>
<td>131 ± 7.5</td>
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<tr>
<td>Propionate, 40 mm</td>
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<td>87 ± 7.4</td>
<td>150 ± 22</td>
<td>91 ± 2.1</td>
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<tr>
<td>Lactate, 40 mm</td>
<td>4</td>
<td>93 ± 12</td>
<td>650 ± 82</td>
<td>105 ± 7.1</td>
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<tr>
<td>Anti-insulin serum:*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, 5.5 mm</td>
<td>4</td>
<td>58 ± 6.3</td>
<td>128 ± 12</td>
<td>137 ± 3.4</td>
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<tr>
<td>Propionate, 40 mm</td>
<td></td>
<td>64 ± 7.0</td>
<td>470 ± 53</td>
<td>83 ± 5.1</td>
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<tr>
<td>Lactate, 40 mm</td>
<td>4</td>
<td>70 ± 3.0</td>
<td>600 ± 47</td>
<td>110 ± 3.6</td>
</tr>
</tbody>
</table>

* The animals were treated with normal or anti-insulin guinea pig serum 1½ hours before death.
phate pathway may be used to drive the pyruvate to malate reaction and thus contribute to increased glucose formation in the diabetic liver.

Studies on the incorporation of radioactivity from CO₂ into protein show no marked difference in various tissues examined. Only a small decrease was observed in muscle and diaphragm proteins from alloxan diabetic rats. These observations are in agreement with previous studies (2).

**SUMMARY**

Carbon dioxide fixation has been studied both in vivo and in vitro in experimental animals with various treatments. Alloxan diabetic rats showed a 7- to 8-fold increase in incorporation of radioactive CO₂ into glucose in vivo and a 2- to 3-fold increase in vitro. Anti-insulin serum- and cortisol-treated rats also exhibited an increase in C¹⁴-glucose formation from C¹⁴O₂. CO₂ fixation in vivo occurs in the following decreasing order: alloxan diabetic, anti-insulin serum-treated, cortisol, normal unfed, adrenalectomized diabetic, normal with normal serum, and normal. Increased CO₂ fixation was also observed in liver slices from alloxan diabetic rats both in the absence of added substrate or in the presence of added pyruvate, lactate, or propionate. Alloxan diabetic rats showed a 50% decrease in the incorporation of C¹⁴ from bicarbonate into muscle proteins, with no alterations in liver or kidney proteins.

**REFERENCES**

Studies on Experimental Diabetes: II. CARBON DIOXIDE FIXATION
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