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 C\(^{14}\) 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 glucoseogenic intermediates such as lactate, pyruvate, propionate, or alanine into hexose by hepatic tissue would require CO\(_2\) fixation (3-6). Furthermore, under conditions where increased hepatic glucose production would be expected, e.g., prior treatment with cortisone, incorporation of C\(^{14}\) from both pyruvate-2-C\(^{14}\) and C\(^{14}\)O\(_2\) into blood glucose is increased to a comparable extent (7).

The present studies report observations on C\(^{14}\)O\(_2\) 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 200 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 C¹⁴-bicarbonate. Each curve represents the mean of four or more animals, and total spread of points is indicated.

**Table I**

_Incorporation of C¹⁴ from radioactive C¹⁴O₂ 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>2730 ± 200</td>
<td>1746 ± 107</td>
<td>720 ± 120</td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>3327 ± 247</td>
<td>2103 ± 243</td>
<td>143 ± 960</td>
<td>120 ± 125</td>
</tr>
<tr>
<td>Normal + anti-insulin serum</td>
<td>4130 ± 600</td>
<td>1820 ± 210</td>
<td>1640 ± 200</td>
<td>1200 ± 240</td>
</tr>
<tr>
<td>Normal + normal serum</td>
<td>3453 ± 513</td>
<td>2310 ± 490</td>
<td>1920 ± 280</td>
<td>1200 ± 260</td>
</tr>
</tbody>
</table>

differences in incorporation of CO₂ into tissue proteins were observed, except in the alloxan diabetic rats. In this case, C¹⁴ incorporation into muscle proteins was slightly reduced.

The studies on the incorporation of C¹⁴O₂ 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 C¹⁴O₂ into glucose by rat liver slices. However, 40 mM lactate added to the incubation medium increased C¹⁴ 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 C¹⁴O₂ into glucose was increased in liver slices from alloxan diabetic rats. Increased CO₂ 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 C¹⁴O₂ 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 C¹⁴O₂ into pyruvate by liver slices from alloxan diabetic rats was increased to approximately the same extent as C¹⁴-glucose; however, the amount of C¹⁴ recovered in pyruvate was only one-third that found in glucose.

No marked differences between normal and alloxan diabetic rats in incorporation of C¹⁴O₂ into liver glycogen were observed _in vitro_. 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 CO₂ fixation observed in intact rats. In insulin insufficiency, whether produced by alloxan or by prior treatment with anti-insulin serum, an increase in C¹⁴-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 diabetic, since adrenalectomy of alloxan diabetic rats markedly reduces the amount of C⁴-glucose formed after C⁴O₂ administration. Further evidence for the participation of adrenal steroids in the regulation of CO₂ fixation is that prior treatment of rats with cortisone (7) or cortisol will increase C⁴-glucose formation from C⁴O₂. When radioactive bicarbonate and anti-insulin serum were administered simultaneously to rats, very little increase in C⁴-glucose was observed. It is probable that most of the C⁴O₂ fixation occurs within the first 1/2 hour after injection. Although anti-insulin serum results in a prompt increase in blood glucose (9, 13), it is possible that the full effects of insulin insufficiency are not obtained until several minutes after the serum is administered. Therefore, in a second series of experiments, anti-insulin serum was given to rats 30 minutes before the C⁴O₂ was injected. In this case, a marked increase in C⁴-glucose was observed.

The distribution of C⁴ in glucose formed by hepatic tissue incubated with C⁴O₂ would be consistent with CO₂ fixation by pyruvate or propionate and subsequent conversion of the malate or succinate formed to phosphoenolpyruvate and glucose formation by the reversal of glycolysis (3). Further, the incorporation of pyruvate carbon into glucose most likely proceeds via oxaloacetate as an intermediate, rather than by direct reversal of glycolysis (4). Therefore, an increase in CO₂ fixation would be expected in diabetes to the extent that 3 carbon intermediates contribute to increased hepatic glucose formation. It is probable that an increase in CO₂ fixation in diabetic rats is due in part to an increase in protein catabolism, leading to an increased formation of metabolic intermediates capable of adding CO₂. Decreased glucose utilization in the absence of insulin would also be expected to contribute to the accumulation of C⁴-glucose in the blood. However, in experiments with liver slices, possible contributions of extrahepatic tissues are removed. In this case, it was found that livers from anti-insulin serum-treated and alloxan diabetic rats formed different amounts of C⁴-glucose.

Although anti-insulin serum results in an increase in C⁴-glucose in vivo, no change in C⁴O₂ incorporation into glucose was obtained in liver slices from anti-insulin serum-treated rats. However, increased CO₂ fixation was observed in liver slices from alloxan diabetic rats as well as in the intact animal. It would appear that the increased C⁴-glucose formation observed in the alloxan diabetic is not due to the presence of endogenous substrates. Of the unlabeled substrates added, only lactate resulted in a marked increase in C⁴-glucose formation from C⁴O₂.

The data presented in Table II indicate that the increase in CO₂ fixation observed with lactate might be due in part to a diversion of TPNH produced by glucose oxidation via the pentose phospho-

<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 ± 14</td>
<td>292 ± 34</td>
<td>83 ± 3.7</td>
</tr>
<tr>
<td>Pyruvate, 40 mM</td>
<td>6</td>
<td>88 ± 13</td>
<td>502 ± 73</td>
<td>94 ± 6.1</td>
</tr>
<tr>
<td>Lactate, 40 mM</td>
<td>6</td>
<td>70 ± 9</td>
<td>804 ± 76</td>
<td>88 ± 5.1</td>
</tr>
<tr>
<td>Propionate, 40 mM</td>
<td>3</td>
<td>50 ± 3.3</td>
<td>150 ± 23</td>
<td>114 ± 7.7</td>
</tr>
<tr>
<td>Alloxan diabetic:</td>
<td></td>
<td></td>
<td></td>
<td></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 ± 5.6</td>
<td>355 ± 73</td>
<td>82 ± 5.6</td>
</tr>
<tr>
<td>Lactate, 40 mM</td>
<td>6</td>
<td>27 ± 7</td>
<td>464 ± 55</td>
<td>64 ± 8.8</td>
</tr>
<tr>
<td>Propionate, 40 mM</td>
<td>6</td>
<td>16 ± 3</td>
<td>120 ± 11</td>
<td>35 ± 2.7</td>
</tr>
<tr>
<td>Glucose, 5.5 mM</td>
<td>4</td>
<td>119 ± 8.0</td>
<td>150 ± 11</td>
<td>131 ± 7.5</td>
</tr>
<tr>
<td>Propionate, 40 mM</td>
<td>4</td>
<td>87 ± 7.4</td>
<td>150 ± 22</td>
<td>91 ± 2.1</td>
</tr>
<tr>
<td>Lactate, 40 mM</td>
<td>4</td>
<td>93 ± 12</td>
<td>650 ± 82</td>
<td>105 ± 7.1</td>
</tr>
<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>
</tr>
<tr>
<td>Propionate, 40 mM</td>
<td>4</td>
<td>64 ± 7.0</td>
<td>470 ± 53</td>
<td>83 ± 5.1</td>
</tr>
<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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