PLASMA GLYCOPEPTIDES SYNTHESIS IN ALLOXAN-DIABETIC RATS

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Experiments were carried out to determine whether alloxan diabetes in rats altered: (1) the level of plasma seromucoid (a small carbohydrate-rich fraction), (2) the perchloric acid (PCA)-insoluble protein, making up the remainder of the plasma protein, and (3) the ability of the animal to incorporate [14C]glucosamine and [14C]leucine into these plasma fractions.

Diabetes was produced in 20 male Wistar rats (250–275 g) by the following procedure. Rats, fasted for 16 h, were lightly anaesthetized with ether and injected i.v. with alloxan (70 mg/kg) in 0·9 % NaCl solution acidified to pH 3·5. They were then given protamine zinc insulin s.c. at a daily dose of 2 u. for 7 days, 1 u. for 5 days and 0·5 u. for 5 days. Experiments were carried out 3 days after the last dose of insulin. In the 24 h preceding the experiments, urinary output of glucose per rat was 667 ± 46 (S.E.M.) mg. Control Wistar rats were matched by weight.

Ten diabetic and ten control rats were injected i.v. with 5 µCi [1-14C]glucosamine/220 g body weight. The remaining ten diabetic and ten control rats were similarly injected with 10 µCi [14C]leucine/220 g body weight. Blood (1 ml) was withdrawn from the jugular vein every hour for 4 h after the injection. Seromucoid and PCA-insoluble protein were separated (Winzler, Devor, Mehl & Smyth, 1948) and the protein of each fraction assayed (Lowry, Rosebrough, Farr & Randall, 1951). Radioactivity of each of these fractions and of the protein-free supernatant was measured on a Nuclear Chicago gas-flow low-background counter. Specific activity was calculated on each sample of protein. The values obtained at 4 h were analysed statistically because they had reached a plateau by this time.

Total plasma protein levels in the diabetic rats were not changed significantly but a significant depression ($P < 0·001$) occurred in the PCA-insoluble fraction (19494 ± 414 v. 22 843 ± 363 µg/ml blood in the controls). The seromucoid levels in the diabetic rats, normally 1/4 of the total plasma protein of the normal rat, remained unchanged ($564 ± 20$ v. $529 ± 20$ µg/ml blood in the control animals). One hour after the injection of isotope, less than 0·2 % of the injected radioactivity was left in the circulation in any experiment, indicating rapid incorporation and/or excretion of both glucosamine and leucine.

Four hours after the injection of [1-14C]glucosamine the specific activity of the seromucoid in the diabetic rats exceeded that of the control group ($P < 0·025$). The

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amount of seromucoid was the same in both groups and this difference is reflected in the absolute counts (Table 1). No significant difference was found in the incorporation of [1-14C]glucosamine into PCA-insoluble protein. The amount of PCA-insoluble protein was found to be slightly depressed, the absolute counts paralleling this depression.

Table 1. Specific activity as c.p.m./mg protein and absolute c.p.m. of plasma fractions/ml blood 4 h after isotope injection into rats (means ± S.E.M. for 10 rats in each group)

<table>
<thead>
<tr>
<th>Material injected</th>
<th>Group</th>
<th>Specific activity</th>
<th>Absolute counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PCA-insoluble</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>protein</td>
<td></td>
</tr>
<tr>
<td>[1-14C]Glucosamine</td>
<td>Diabetic</td>
<td>5333 ± 430</td>
<td>2965 ± 339</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4208 ± 192</td>
<td>2336 ± 261</td>
</tr>
<tr>
<td>[14C]Leucine</td>
<td>Diabetic</td>
<td>262 ± 13</td>
<td>143 ± 15</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>392 ± 13</td>
<td>219 ± 20</td>
</tr>
</tbody>
</table>

PCA = perchloric acid.

Four hours after injection of [14C]leucine the specific activity of the seromucoid was depressed by 1/3 in the diabetic rats ($P < 0.001$) (Table 1) and the specific activity of the PCA-insoluble fraction was halved ($P < 0.001$) compared with control values. Absolute counts reflect these differences. Although the PCA-insoluble protein had fallen in the diabetic rats, its decrease was slight compared with the large fall in leucine incorporation in this fraction.

The liver of the intact rat appears to be the primary source of serum glucosamine (Spiro, 1959a), about 0.9 µmol being synthesized/h in a 250 g rat. Although the synthesis of glucosamine is unimpaired in the alloxan-diabetic rat (Spiro, 1959b) these preliminary results suggest that complicated changes have probably taken place in the synthesis of plasma glycoprotein.

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REFERENCES


