GLUCOSE-6-PHOSPHATASE AND PHOSPHORYLASE ACTIVITIES IN MICE BEARING CORTICOTROPIN-SECRETING TUMORS

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The activity of hepatic glucose 6-phosphatase, the enzyme catalyzing the conversion of glucose-6-phosphate to glucose, has been measured under normal conditions as well as in a number of situations in which carbohydrate metabolism is disturbed. Cori and Cori (1) found an almost complete absence of this enzyme in the livers of two fatal cases of von Gierke's (glycogen storage) disease and levels below normal in four surviving subjects. Ashmore et al. (2) and Langdon and Weakley (3) have demonstrated that the activity of glucose-6-phosphatase is greatly increased in the livers of rats made diabetic by alloxan. The activity of this enzyme could be brought toward normal by the administration of insulin in vivo but not by the addition of this hormone in vitro. Both of these groups of workers, as well as Weber and Cantero (4), reported an increase in this enzyme per gm. of tissue in the fasted animal, a condition in which the carbohydrate metabolism is similar to that in the diabetic state. In subsequent experiments by Weber et al. (5) and Ashmore et al. (6) the hormonal regulation of glucose-6-phosphatase has been studied. Weber and colleagues found that the administration of cortisone to rats in vivo caused a 49 per cent increase in the hepatic glucose-6-phosphatase per unit weight of wet liver. Ashmore and coworkers found that diabetic, adrenalectomized rats had normal liver glucose-6-phosphatase activity. The administration of 17-hydroxycorticosterone in vivo increased, while insulin administration decreased the activity of this enzyme. In contrast, Langdon and Weakley observed only unimpressive increases in hepatic glucose-6-phosphatase in rats treated with cortisone or adrenal cortical extracts.

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Because of the important position occupied by glucose-6-phosphatase in the regulation of blood sugar it would be of value to assay the effect of the normally secreted adrenocortical hormones on the activity of this enzyme. It would also be of interest, in view of the reported effects of exogenous adrenocortical hormones on glycogen storage, to assay the effects of the endogenous adrenal corticoids on the levels of liver glycogen and phosphorylase. The experiments performed on mice grafted with corticotropin (ACTH)-secreting tumors were designed with this purpose in mind. Mice which bear transplanted pituitary tumors which were radiation-induced were first described by Furth and colleagues (7, 8). These workers found that three transplantable tumor strains of the chromophobe type secrete ACTH. Mice grafted with such tumors display greatly enlarged adrenals, involution of the thymus, lymphopenia, and frequently increased weight. Endocrine organs other than the adrenals do not show enlargement even in hypophysectomized hosts. Recent studies performed on these mice by Mayer et al. (9) indicate that even when the mice bear minute tumors and have body weights similar to their normal controls they contain twice as much extrahepatic fat. Liver fat as well as both carcass and liver cholesterol were also found to be elevated in the tumor-bearing mice.

**Materials and Methods**

Male mice of the LAFl strain were used in these experiments and were approximately 17 weeks of age at the time of sacrifice. One-half of the animals at 10 weeks were grafted with radiation-induced pituitary tumors subcutaneously in the right thigh muscles by injection of a saline suspension of viable tumor tissue. The untreated mice served as controls. At the time of study the tumor-bearing mice weighed 31.3 ± 3.5 gm. and the control animals 30.2 ± 2.0 gm. The tumors at this time averaged 1 cm. in diameter. The control and tumor-bearing mice were allowed commercial laboratory chow ad libitum. The water of both groups of animals contained animal formula Terramycin HCl through the courtesy of Dr. Gilbert M. Shull.

Glucose-6-Phosphatase and Phosphorylase Determinations

Glucose-6-phosphatase was assayed by a modification of the method of phosphate release of Swanson (10) which has previously been described (11). Phosphorylase was assayed as previously described (11), with the exception that the liver was homogenized in water without added fluoride.
since both glucose-6-phosphatase and phosphorylase determinations were performed on the same homogenates. Each phosphorylase assay was made in duplicate, one with and one without added fluoride. In general, the activities of the duplicates were similar. The results in Table I represent the mean between these duplicate determinations.

**Table I**

*Phosphorylase and Glucose-6-phosphatase Activities of Six Normal and Six Tumor-Bearing Mice*

Enzymic activities are expressed as millimoles of glucose-1-phosphate or glucose-6-phosphate split in 30 minutes at 30°C.

<table>
<thead>
<tr>
<th>Type</th>
<th>Phosphorylase activity per gm.*</th>
<th>Glucose-6-phosphatase activity per gm.*</th>
<th>Phosphorylase total activity*</th>
<th>Glucose-6-phosphatase total activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, LAF₁</td>
<td>0.470 ± 0.042</td>
<td>0.244 ± 0.016</td>
<td>0.787 ± 0.118</td>
<td>0.406 ± 0.033</td>
</tr>
<tr>
<td>Tumor-bearing, LAF₁</td>
<td>0.565 ± 0.075</td>
<td>0.354 ± 0.045</td>
<td>0.859 ± 0.090</td>
<td>0.540 ± 0.068</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

**Table II**

*Blood Glucose and Liver Glycogen Levels of Six Normal and Six Tumor-bearing Mice*

<table>
<thead>
<tr>
<th>Type</th>
<th>Feeding status</th>
<th>Liver glycogen* per cent</th>
<th>Blood glucose* mg. per cent</th>
<th>Total glycogen* mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, LAF₁</td>
<td>Ad libitum</td>
<td>4.61 ± 1.11</td>
<td>128 ± 3</td>
<td>76.6 ± 18.9</td>
</tr>
<tr>
<td></td>
<td>24 hr. fast</td>
<td>0.096 ± 0.045</td>
<td>77 ± 8</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Tumor-bearing, LAF₁</td>
<td>Ad libitum</td>
<td>3.83 ± 0.28</td>
<td>108 ± 10.5</td>
<td>58.5 ± 6.7</td>
</tr>
<tr>
<td></td>
<td>24 hr. fast</td>
<td>1.37 ± 0.46</td>
<td>115 ± 13.4</td>
<td>16.1 ± 5.9</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

**Blood Glucose and Liver Glycogen Determinations**

The method of Good et al. (12) was used for extraction and hydrolysis of the glycogen. Glucose determinations on the glycogen hydrolysates and on blood were carried out by the method of Somogyi (13), using the colorimetric procedure of Nelson (14).

**Results**

In Table I are recorded the total activities and the activities per gm. of wet liver for glucose-6-phosphatase and phosphorylase in tumor-bearing and normal LAF₁ mice. Comparison of the activities per gm. of liver of the tumor-bearing mice with those of the normal mice indicated that the
glucose-6-phosphatase activity of the former group was greater ($p < 0.001$) than that of the latter group. The phosphorylase activity of the tumor-bearing group was significantly greater than the control group ($p < 0.05$). Comparisons of the total glucose-6-phosphatase activities indicated that, while the difference in activity between the two groups was not as high as that between the specific activities, a high degree of significance still obtained ($p < 0.01$). However, the total phosphorylase activity of the tumor-bearing mice was not notably different from that of the control mice at the 5 per cent level.

Table II lists the blood glucose and liver glycogen levels of the tumor-bearing and normal LAFl mice. The blood glucose and liver glycogen levels of the fed animals in these two groups were not remarkably different. However, the fasting values for these two constituents differed markedly in the two groups. In the tumor-bearing group, fasting did not cause a reduction in the blood glucose level while it did in the normal controls. Similarly, the tumor-bearing mice retained approximately 14 times more liver glycogen after a 24 hour fast than their controls.

**DISCUSSION**

The striking increase in glucose-6-phosphatase activity per gm. of liver observed in the tumor-bearing mice indicates that the activity of this enzyme *in vivo* is probably controlled, at least in part, by the adrenal cortical hormones. This corroborates and extends findings of Ashmore *et al.* (6) and Weber *et al.* (5). Ashmore and colleagues reported glucose-6-phosphatase values of 0.157 and 0.210 mm glucose-6-phosphate split per 30 minutes per gm. of liver, respectively, for normal rats and normal rats treated over a 48 hour period with 20 mg. of 17-hydroxycorticosterone. Weber and colleagues found a 49 per cent increase in glucose-6-phosphatase activity in rats treated with cortisone. It is of interest to note that the 45 per cent increase in glucose-6-phosphatase activity observed in the tumor-bearing mice was probably produced largely by corticosterone. Bahn and others have found that these tumor-bearing mice excrete 0.5 $\gamma$ per day total neutral C$_{21}$ steroids in the urine of which corticosterone comprised 0.3 $\gamma$. Only traces of Compounds E and F and no aldosterone activity were demonstrated. The fact that the significance of the difference in total liver glucose-6-phosphatase activity of tumor-bearing mice and controls is less than that of the specific activity is undoubtedly due to the smaller functional liver mass of the tumor-bearing mice. The decrease in liver mass was probably produced by the well known protein catabolic effect of the adrenal corticosteroids.

The significantly greater amounts of glycogen found in the livers of the tumor-bearing mice compared to their controls are not surprising in view of the known effects of adrenal cortical hormones on carbohydrate metabolism. The classical experiments of Long et al. (15) demonstrated that fasted rats treated with adrenocortical extracts had considerably more liver glycogen than their untreated controls. The higher blood glucose levels in the fasted tumor-bearing mice compared to their controls also agree with the findings of Long and coworkers, who reported higher blood and tissue glucose levels in their fasted animals treated with cortical extracts. The normal blood glucose levels maintained by the fed tumor-bearing mice are consistent with the findings of Shull and Mayer (16) on the effects of single large doses of cortisone on the blood glucose of normal fed mice. Parenteral administration of 10 mg. of this hormone did not significantly change the blood glucose levels of these mice. Similarly, in the rat, consistent hyperglycemia after adrenal cortical treatment is obtained only in animals forcibly fed a high carbohydrate diet (17) or in animals in which the pancreas is partially reduced (15).

The significant increase in liver phosphorylase observed in the tumor-bearing mice compared to their controls is of interest. The work of Sutherland and Cori (18) indicates that both epinephrine and glucagon in vivo increase the amount of active phosphorylase in the liver. The results of the present study indicate that the adrenal corticosteroids may also increase the amount of active liver phosphorylase, although the fact that the increase is relatively small suggests that this may be a secondary (adaptive) phenomenon.

**SUMMARY**

The hepatic glucose-6-phosphatase activity per gm. of tissue in mice bearing corticotropin (ACTH)-secreting tumors was considerably greater than in normal mice of the same strain. A small but significant increase in hepatic phosphorylase activity per gm. of liver was also found in the tumor-bearing mice.

The liver glycogen and blood sugar levels of the tumor-bearing and control mice were similar under fed conditions. However, under fasting conditions the amounts of liver glycogen and blood sugar were significantly greater in the mice bearing ACTH-secreting tumors.

**BIBLIOGRAPHY**

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