Effects of Insulin, Glucagon, and Glucocorticoids upon Hepatic Glycogen Synthesis from Uridine Diphosphate Glucose*

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Impaired ability to store glycogen in the liver in various forms of experimental diabetes has frequently been observed (1-4). Until recently it has been assumed that this results from a decreased amount of hexose monophosphate available in the diabetic liver, as verified by measurements of hepatic glucose-6-phosphate concentration. However, within a few hours after the administration of insulin to diabetic rats, glycogen accumulates rapidly in the liver, and during this time it is not possible to demonstrate an increase in the glucose-6-pool. On the contrary, a slight decrease occurs (4). It has been postulated, therefore, that insulin in vivo may in some manner affect reactions directly concerned with glycogen synthesis in the liver.

The recent description by Leloir and Cardini (5) of an enzyme in liver which catalyzes the transfer of glucoseyl units from uridine diphosphate glucose to a glycogen primer has provided the basis for the current view that glycogen synthesis can occur in animal tissues without participation of the classical phosphorylase system. Villar-Palasi and Larner (6), and Robbins et al. (7) have demonstrated this pathway in skeletal muscle, and we have observed similar activity in heart muscle preparations. Leloir and Goldenberg (8) have studied the properties of partially purified enzyme preparations from rat liver and have called the enzyme "glycogen synthetase."

In the present experiments, glycogen synthetase activity and glucose-6-P concentration of homogenates of livers from diabetic animals have been examined before and after treatment of the animals with insulin. The effects in normal animals of glucagon and glucocorticoids, hormones which are known to influence the metabolism of glycogen in the liver, have also been studied.

Experimental Procedure

ATP, UTP, UDP-glucose, glucose-6-P, fructose-6-P, yeast hexokinase (grade III), and glucose-6-P dehydrogenase were obtained from the Mann Chemical Company. 2-Deoxy-

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1 D. F. Steiner, and V. Rauda, unpublished observations.

2 Unpublished method of Dr. Y. D. Halsay.
at 700 × g for 10 minutes to sediment nuclei and unbroken cells. The supernatant, which contained all the activity, was used for assay after appropriate dilution. Glycogen was determined immediately after preparation of homogenates with the use of the modified anthrone reagent of Roe (14) after digestion by the method of Good et al. (15). For determination of glucose-6-P, 11 ml of iced homogenate were immediately added to 5 ml of ice-cold 20% trichloroacetic acid and the extraction procedure followed as described previously (4). The glucose-6-P dehydrogenase assay system was modified by addition of 50 μM sodium azide to inhibit glutathione reductase (16), which promoted the reoxidation of TPNH by small amounts of glutathione remaining in the extracts after preparation. This addition permitted the accurate estimation of known amounts of glucose-6-P in combination with small amounts of glutathione, with lots of glucose-6-P dehydrogenase having much higher glutathione reductase activity than was encountered in the preparations used for previous work.

Assay System—Incubations were carried out at 37°C in Tris buffer at pH 7.4. The system included 6.0 μmoles of glycogen, 0.15 μmole of uniformly labeled UDP-glucose-C14, 10 μmoles of Tris buffer (pH 7.4), and 25 or 50 μl of enzyme preparation. The total volume of the reaction mixture was 150 μl. To provide maximal stimulation of activity in certain experiments, 1.0 μmole of glucose-6-P was added to the above mixture. When glucose-6-P was included, the homogenate was diluted three- to four-fold before assay. After incubation, the reaction was stopped by addition of 1 ml of 30% KOH. Carrier glycogen (4 mg) was then added and the tubes heated for 5 minutes at 100°C. After cooling in an ice bath, 2 ml of 95% ethanol were added with vigorous stirring to initiate glycogen flocculation. Tubes were warmed in a 60°C water bath for 5 minutes to promote flocculation, cooled, and centrifuged at 5°C; the supernatant was discarded. The glycogen was dissolved in 1 ml of water and again precipitated with 2 ml of 95% ethanol. Losses of glycogen in this isolation procedure did not exceed 1 to 2%, as determined by the anthrone method. The precipitated glycogen was dissolved in 1 ml of 5% HCl and heated to 100°C for 20 minutes to hydrolyze the glycogen partially. The hydrolysates were evaporated to dryness in a vacuum and dissolved in 0.7 ml of water, and an 0.5-ml aliquot was transferred to a glass counting vial containing 10 ml of Polyether 611 (17). Counts were recorded in a Packard liquid scintillation counter with approximately 52% counting efficiency. It was necessary to count samples within 60 minutes after addition of the 0.5-ml aliquot to the Polyether 611 because of slow loss of counts because of precipitation from the phosphor. A small initial loss in counting efficiency was corrected by appropriate standard blanks. More prolonged hydrolysis of the glycogen samples did not appear to improve the counting efficiency. Results were expressed in μmoles of glucose-C14 incorporated per g of wet weight liver per hour.

UDP-glucose disappearance was measured by substituting nonlabeled UDP-glucose in the assay system described above. The reaction was terminated by heating the tubes for 1 minute at 100°C. UDP-glucose was determined with UDP-glucose dehydrogenase.

RESULTS

Characteristics of Assay System—When homogenate were diluted appropriately before assay, the rate of incorporation of radioactive glucose from UDP-glucose-C14 into glycogen was characteristic of a first order reaction to levels as high as 40 to 45% incorporation, and the rate was linearly proportional to the amount of the homogenate added. The values for rate of disappearance of UDP-glucose were considerably higher than the corresponding values for rate of incorporation of radioactivity into glycogen. The reasons for this were not investigated in detail. However, the assay system was examined for artifacts which could arise from rapid release of labeled glucose from glycogen formed during the incubation period. The loss of counts during the incubation period from a glycogen primer which had been previously labeled with glucose-C14 amounted to less than 4% of the total radioactivity of the glycogen sample. Amylase activity of the enzyme preparations, measured under the same conditions as those employed for the assay by the method of Stein and Fisher (16), was too small to account for this slight degradation. Phosphorylation of glycogen in the presence of small amounts of inorganic phosphate present in the homogenates was probably responsible for this loss, and was eliminated by dialysis of the homogenates before assay. However, dialysis, even for only 4 hours, caused a marked loss of synthetase activity in both the presence or absence of EDTA, and activity could not be restored by addition of glutathione, cysteine, or metal ions to the reaction mixture. It was necessary, therefore, to use nondialyzed preparations for reliable assay of tissue enzymatic activity, and to ignore the relatively minor error arising from glycogenolysis.

Maximal rates of incorporation as high as 130 μmoles per hour per g of wet weight of liver were obtained, exceeding the estimated normal rate of glycogen deposition in liver in vivo after glucose administration. Fractionation of the microsomal material, which contained approximately 85% of the synthetase activity, revealed a striking correlation of the enzymatic activity of each fraction with its content of glycogen, in accord with observations by Leloir and Goldenberg (8) of binding of this enzyme to glycogen or glycogen-containing particles.

Effect of Glucose-6-P and 2-Deoxyglucose-6-P upon Enzymatic Activity—We have confirmed the stimulation of synthetase activity by glucose-6-P and certain analogues with both liver and heart muscle preparations. This effect was first observed in muscle preparations by Leloir et al. (19). Larner et al. (20) reported similar findings regarding the enzyme from muscle, and Leloir and Goldenberg (8) have recently noted this effect with partially purified enzyme preparations from rat liver. In our experiments, glucose-6-P effected increased incorporation of radioactivity into glycogen, as well as increased disappearance of UDP-glucose from the reaction mixture. However, as noted by Leloir et al. (19), no radioactivity appeared in glycogen when incubations were carried out with glucose-C14,6-P and nonlabeled UDP-glucose.

Activation by glucose-6-P, fructose-6-P, and glucoseamine-6-P was competitively inhibited by 2-deoxyglucose-6-P as illustrated in Fig. 1, and when glucose-6-P was not added to the reaction mixture, 2-deoxyglucose-6-P virtually abolished the activity of either homogenates or microsomal preparations. To determine whether activation might require only a brief exposure of the enzyme to a high concentration of glucose-6-P, experiments were carried out in which TPN was added to the reaction mixture in amounts sufficient to promote rapid oxidation of the glucose-6-P added to the mixture by glucose-6-P dehydrogenase present in the homogenates. These results, illustrated in Fig. 2, clearly indicate that removal of glucose-6-P from the reaction mixture...
after activation has occurred results in marked reduction of incorporation of radioactivity into glycogen. These findings and those of Leloir et al. indicate that glucose-6-P and several analogues may serve as essential cofactors for the glycogen synthetase reaction. Further purification of the enzyme will undoubtedly permit clarification of this important point.

Glycogen Synthetase Activity of Livers from Normal and Diabetic Rats—Data from several experiments, summarized in Table I, indicate that the glycogen synthetase activity of diabetic livers was significantly higher than the activity of normal livers when measured after maximal activation by addition of excess glucose-6-P to the assay system. Since the diabetic liver is enlarged relative to the body weight (4), the total enzymatic activity of the organ is actually increased considerably more than these data indicate. On the other hand, the enzymatic activity without glucose-6-P addition was significantly lower in the diabetic group (Table I). This difference may reflect the lower concentration of glucose-6-P in the homogenates from diabetic livers (Table I). However, the enzyme preparations were diluted approximately 30-fold in the process of assay, so that the final concentration of glucose-6-P was often less than \(10^{-5} M\) in the assay mixture. There was no correlation of the variations in activity with the glycogen content of the homogenates, and such a relationship would not be anticipated, since a considerable excess of glycogen was included in the assay system. Although it is tempting to speculate that the observed activity without added glucose-6-P may closely represent the activity in vivo, the possibility must also be considered that multiple artifacts were introduced by the process of homogenization, dilution, and assay. Nevertheless, a decrease in activation of this pathway in vivo, rather than a deficiency of glycogen synthetase per se, could account for the decreased storage of glycogen in the diabetic liver.

![Fig. 1. Competitive inhibition by 2-deoxyglucose-6-P of the activation of glycogen synthetase by glucose-6-P.](http://www.jbc.org/)

![Fig. 2. The effect of addition of TPN to the reaction mixture upon incorporation of glucose-C\(_{14}\) from UDP-glucose-C\(_{14}\) into glycogen. Excess TPN was added to promote rapid oxidation of added glucose-6-P via the glucose-6-P dehydrogenase pathway. The arrow indicates the time of addition of TPN. The incubation system is described under "Experimental Procedure."](http://www.jbc.org/)

![Table I](http://www.jbc.org/)

**Effect of Insulin in Diabetic Rats**—Fig. 3 demonstrates the profound effect of insulin injection upon glycogen storage in the livers of rats with alloxan diabetes. It is of interest that glycogen did not begin to increase until 1 or 2 hours after injection of glucagon-free insulin, in contrast to the immediate effect of insulin upon glucose uptake and glycogen deposition in other tissues such as muscle or adipose tissue. The data in Table II indicate that glycogen synthetase activity increased significantly at 2 and 4 hours after the injection of insulin. Since the maximal activity, i.e. the activity with excess glucose-6-P, was increased significantly, it may be postulated that insulin causes an increase in the total amount of active enzyme present in the cells. How
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Insulin might result from glucagon contamination of the insulin in order to exclude the possibility that the changes observed with the administration of glucagon to diabetic rats, which amounted to only approximately 60%. Moreover, the concentration of glucose-6-P remained essentially unchanged in the homogenates prepared from livers after insulin injection (Table II), as reported previously (4). Thus the possibility of stimulation of enzymatic activity in vitro by an increased concentration of glucose-6-P appears to be ruled out. Perhaps activating substances other than glucose-6-P are formed in increased amounts in the diabetic liver after insulin treatment.

Insulin added in vitro did not affect synthetase activity except for slight inhibition at high concentrations. Tolbutamide in concentrations as high as 5 × 10⁻³ M was also without effect upon the synthetase system in vitro. Control experiments were also carried out in which glucagon was administered to diabetic rats, in order to exclude the possibility that the changes observed with insulin might result from glucagon contamination of the insulin preparations. No significant change in synthetase activity was noted (Table II).

Effect of Glucocorticoids in Normal Rats—The important role of the glucocorticoids of the adrenal cortex in maintaining hepatic glycogen stores during fasting is now generally accepted (22), and it has been demonstrated that increased fasting levels of glycogen can be produced by the administration of glucocorticoids to normal animals (23). Moreover, Hyde has shown that the rise in liver glycogen begins 3 to 4 hours after administration of radioactive hydrocortisone, and at this time the liver no longer contains detectable amounts of the radioisotope (24).

Several experiments were carried out to determine whether glucocorticoids affect glycogen synthetase activity. Normal fasted rats were injected with 1 mg of prednisolone phosphate 5 hours before they were killed. As shown in Table III, part A, in a typical experiment a significant increase in blood glucose concentration and liver glycogen occurred, but there was no alteration in the activity of glycogen synthetase. There was a marked increase in the concentration of glucose-6-P in the homogenates, as might be anticipated, since it is generally accepted that glucocorticoids increase gluconeogenesis from protein precursors (25). When cortisone acetate was administered over a period of 4 days before the rats were killed, a 30% increase in glycogen synthetase activity also appeared (Table III, part A).

However, increased glycogen deposition clearly preceded this slight increase in activity, which therefore must be regarded as a secondary effect, similar in some respects to the adaptive rise in hepatic glucose 6-phosphatase activity which occurs with prolonged administration of glucocorticoids (26, 27).

Effect of Glucagon in Normal Rats—Both glucagon and epinephrine have been demonstrated to activate the phosphorylase mechanism (28, 29) and thereby to acutely lower liver glycogen and increase blood glucose concentration. Twenty-four hours after glucagon administration, liver glycogen levels are super-normal (30) and chronic administration of glucagon leads to negative nitrogen balance, hyperglycemia, and glycosuria—effects similar to those of glucocorticoid excess (31). It was therefore of particular interest to examine the effect of glucagon upon the UDP-glucose pathway. Table III, part B, illustrates the lack of effect of glucagon upon synthetase activity 2 hours after injection into fasted normal rats. Chronic administration for 3 days produced a 43% rise in synthetase activity, which was quite comparable to the effect observed with prolonged treatment with cortisone acetate.

### Table II

Results of insulin or glucagon administration to rats with alloxan diabetes

Animals were treated by subcutaneous injection with glucagon-free insulin (Novo) or glucagon in the dosages indicated. Preparation of homogenates and assay conditions were as described under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Injections</th>
<th>Time after injection</th>
<th>No. of animals</th>
<th>Rate of incorporation of glucose-43C into glycogen</th>
<th>Glucose-6-P concentration</th>
<th>Glycogen concentration in homogenate</th>
<th>Blood glucose concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>hrs</td>
<td>µmoles/g liver/hr*</td>
<td>µmoles/g liver/hr*</td>
<td>µmoles/g liver*</td>
<td>mg glucose/g liver*</td>
<td>mg/100 cc</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>1.5 ± 0.2†</td>
<td>137 ± 11.3†</td>
<td>0.20 ± 0.01</td>
<td>23.6 ± 3.4</td>
<td>428</td>
</tr>
<tr>
<td>Insulin, 4 units</td>
<td>2</td>
<td>10.0 ± 0.9†</td>
<td>236 ± 14.6†</td>
<td>0.16 ± 0.03</td>
<td>55.9 ± 11.1</td>
<td>209</td>
</tr>
<tr>
<td>Glucagon, 0.2 mg</td>
<td>2</td>
<td>2.0 ± 0.4</td>
<td>151 ± 16.7</td>
<td>0.18 ± 0.02</td>
<td>10.1 ± 3.8</td>
<td>304†</td>
</tr>
<tr>
<td>Saline</td>
<td>4</td>
<td>2.4 ± 0.3</td>
<td>135 ± 8.3</td>
<td>0.13 ± 0.01</td>
<td>17.0 ± 1.7</td>
<td>307</td>
</tr>
<tr>
<td>Insulin, 4 units</td>
<td>4</td>
<td>12.0 ± 1.2</td>
<td>205 ± 11.6</td>
<td>0.14 ± 0.01</td>
<td>41.5 ± 1.5</td>
<td>176</td>
</tr>
</tbody>
</table>

* † Standard error of the mean.  | p < 0.001.
TABLE III
Results of injection of glucocorticoids or glucagon into normal rats

<table>
<thead>
<tr>
<th>Injections</th>
<th>Time after injection (hr)</th>
<th>No. of animals</th>
<th>Rate of incorporation of glucose-C14 into glycogen</th>
<th>Glucose-6-P concentration in homogenate</th>
<th>Glycogen concentration in homogenate</th>
<th>Blood glucose concentration mg/100 cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Glucocorticoids</td>
<td>5</td>
<td>4</td>
<td>2.4</td>
<td>97</td>
<td>0.13</td>
<td>7.7</td>
</tr>
<tr>
<td>Saline†</td>
<td>5</td>
<td>4</td>
<td>± 0.5</td>
<td>± 0.4</td>
<td>± 0.7</td>
<td>± 0.4</td>
</tr>
<tr>
<td>Prednisolone†</td>
<td>5</td>
<td>4</td>
<td>3.7</td>
<td>100</td>
<td>0.32</td>
<td>20.6</td>
</tr>
<tr>
<td>Saline†</td>
<td>90</td>
<td>4</td>
<td>2.2</td>
<td>93</td>
<td>0.00</td>
<td>4.7</td>
</tr>
<tr>
<td>Cortisone-acetate†</td>
<td>96</td>
<td>4</td>
<td>5.8</td>
<td>121</td>
<td>0.23</td>
<td>14.5</td>
</tr>
<tr>
<td>Without added glucose-6-P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With excess glucose-6-P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Glucagon</td>
<td>2</td>
<td>4</td>
<td>3.0</td>
<td>106</td>
<td>0.04</td>
<td>11.5</td>
</tr>
<tr>
<td>Saline†</td>
<td>2</td>
<td>4</td>
<td>± 0.6</td>
<td>± 0.4</td>
<td>± 2.4</td>
<td>± 0.4</td>
</tr>
<tr>
<td>Glucagon†</td>
<td>2</td>
<td>4</td>
<td>2.7</td>
<td>96</td>
<td>0.00</td>
<td>1.2</td>
</tr>
<tr>
<td>Saline</td>
<td>75</td>
<td>5</td>
<td>4.4</td>
<td>89</td>
<td>0.41</td>
<td>56.6</td>
</tr>
<tr>
<td>Glucagon</td>
<td>76</td>
<td>6</td>
<td>5.3</td>
<td>127</td>
<td>0.44</td>
<td>25.8</td>
</tr>
<tr>
<td>† Fasted 24 hours.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‡ p &lt; 0.001.</td>
<td></td>
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</tr>
</tbody>
</table>

SUMMARY

1. The glycogen synthetase activity of homogenates of rat liver has been studied by means of a microassay which is based upon measurements of the rate of incorporation of glucose-C14 from uridine diphosphate glucose-C14 into glycogen.

2. Stimulation of glycogen synthetase activity by glucose 6-phosphate, fructose 6-phosphate, and glucosamine 6-phosphate was inhibited competitively by 2-deoxyglucose 6-phosphate. In the absence of added glucose 6-phosphate, enzymatic activity was markedly reduced by addition of this inhibitor. Removal of glucose 6-phosphate by addition of triphosphopyridine nucleotide to assay mixtures also decreased incorporation of radiolabel into glycogen. This and other evidence suggests that glucose 6-phosphate or certain analogues may be essential cofactors for this reaction.

3. Hepatic synthetase activity of rats with alloxan diabetes was higher than that of normal control animals when glucose 6-phosphate was added to the assay mixture in excess. Without added glucose 6-phosphate, activity was significantly lower in the diabetic group. Treatment of diabetic rats with insulin resulted in a marked rise in hepatic synthetase activity within 2 hours after injection, and coincident with rapid deposition of glycogen in the liver. Enzymatic activity was increased both in the phosphorylase system, it is well established that glucagon and epinephrine profoundly influence enzymatic activity. Evidence presented here indicates that the synthetic pathway also may be regulated by metabolic and endocrine factors, further emphasizing its physiological importance.
in the presence or absence of added glucose 6-phosphate 2 and 4 hours after insulin, but the concentration of glucose 6-phosphate found in homogenates did not change. Addition of insulin in vitro was without effect.

4. Administration of prednisolone to fasted normal rats 5 hours before they were killed resulted in a marked rise both in hepatic glycogen in vivo and glucose 6-phosphate concentration in liver homogenates. The synthetase activity was not changed. After daily injections of cortisone acetate for 4 days, a small but significant increase in enzymatic activity also appeared.

5. Glucagon did not alter the synthetase activity of normal or diabetic rat livers when injected 2 hours before the rats were killed. Administration of this substance to normal rats for 3 days resulted in a small but significant rise in synthetase activity.

6. The data presented indicate that synthesis of glycogen from uridine diphosphate glucose may be regulated in part by hormonal influences which may affect either the amount of active enzyme or the concentration of important activating substances such as glucose 6-phosphate.

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REFERENCES
24. Hyde, P. M., Endocrinology, 61, 774 (1957).
Effects of Insulin, Glucagon, and Glucocorticoids upon Hepatic Glycogen Synthesis from Uridine Diphosphate Glucose
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