*DEPENDENCE ON INSULIN OF THE APPARENT HYDROCORTISONE ACTIVATION OF HEPATIC GLYCOGEN SYNTHETASE**

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The early stimulation by adrenal corticoids of hepatic glycogen deposition in the rat has recently been attributed to a steroid-induced activation of glycogen synthetase (UDP-glucose: α-1,4-glucan-α-4-glucosyl transferase [E.C. 2.4.1.11]).[1, 2] The stimulation at this step derives from a conversion of the enzyme from a glucose-6-phosphate (G-6-P) dependent (D) to a G-6-P independent (I) form.[3] However, from numerous reports demonstrating that the glycogenic response to adrenal steroids follows from their more direct stimulatory effects on the gluconeogenic process,[4, 5] the questions that arise are (1) how essential adrenal steroids are *per se* in the activation of the glycogenic process proper, and (2) to what extent and by what mechanism the products of steroid-enhanced gluconeogenesis might affect the rate of glycogen deposition in the liver. The increased levels of plasma and hepatic glucose which ultimately result after steroid treatment are generally considered to promote glycogenesis through a kinetic or mass action effect. However, another possibility is that the steroid-induced hyperglycemia leads to a release of insulin, and it is the stimulation of hepatic glycogenesis by this hormone which is responsible for the elevation of tissue glycogen concentrations found after steroid administration.

This hypothesis would be consistent with the failure to observe a stimulation of glycogenesis by adrenal steroids *in vitro*[6] whereas such a response has been obtained *in vitro* with insulin.[7] Furthermore, the capacity of insulin to promote a rapid conversion of G-6-P dependent synthetase to the I form in dog liver has recently been established.[8]

In this report it is demonstrated that administered hydrocortisone or large amounts of glucose have no measurable effect on the rate of early glycogenesis in alloxan-diabetic animals. The apparent stimulation of hepatic glycogen deposition and conversion of synthetase D to I resulting from the administration of hydrocortisone or glucose in nondiabetic rats appears to be dependent on the action of insulin.

Materials and Methods.—Chemicals: UDP-glucose and UDP-glucose dehydrogenase were obtained from the Sigma Chemical Co. Other substrates, cofactors, and enzymes were obtained from either Sigma Chemical Co. or Boehringer and Sons. Hydrocortisone, purchased from the Sigma Chemical Co., was suspended in 1% saline containing 0.2 ml of Tween-80 per 100 ml of suspension. Alloxan monohydrate was obtained from Eastman Organic Chemicals and was dissolved in cold 1% saline just prior to use. Glucagon-free amorphous pork insulin (Lilly lot no. 192-235B-188; 20 units per mg) was dissolved in cold, acidified (3 mM HCl) 1% saline to achieve a concentration of 5 units per ml.

Experimental procedures: Male rats (140–160 gm, Holtzman strain) were used in all experiments. Bilateral adrenalectomy was performed under pentobarbital sodium anesthesia. Acute diabetes was produced in animals fasted 16 hr by the intravenous administration of alloxan (40 mg/kg) as described by Morgan and Lazarow.[9] Diabetic-adrenalectomized animals were prepared by treating with alloxan at the time of adrenalectomy. Experiments were conducted 2 or

3 days after the injection of alloxan, on animals exhibiting a marked glucosuria. Drugs were administered to animals after a specified period without food and the rats killed by cervical dislocation at selected times after treatment. Livers were rapidly removed and frozen in Freon-12 cooled to −150°C with liquid nitrogen. The tissues were stored at −80°C until they could be prepared for substrate or enzyme analysis.

Plasma glucose was determined in samples of blood collected from the tail vein just prior to sacrificing the animals. Acid extracts of liver were prepared and acid-soluble intermediates measured by the procedures described by Lowry *et al.*[10] UDP-glucose was estimated fluorometrically using UDP-glucose dehydrogenase. Glycogen synthetase activity was determined by a modification of the method described by Hornbrook *et al.*[1] G-6-P independent synthetase activity was estimated by incubating 10 μl of a 1:20 tissue homogenate with 100 μl of a reaction mixture containing: 50 mM Tris·HCl, pH 7.8; 10 mM UDP-glucose; 50 mM dialyzed glycogen; 5 mM EDTA; and 20 mM sodium sulfite. Samples were incubated for 15 min at 38°C. Total synthetase activity was determined similarly except with 10 mM G-6-P present in the reaction mixture. The reaction was stopped by heating at 100°C for 2 min. The UDP produced was determined fluorometrically using pyruvate kinase and lactate dehydrogenase to catalyze the disappearance of NADH.

Results.—Glucose administration to nondiabetics: After the administration of a single dose of glucose to adrenalectomized animals in an amount known to increase circulating insulin-like activity,[11] hepatic glucose levels increase rapidly, within 30

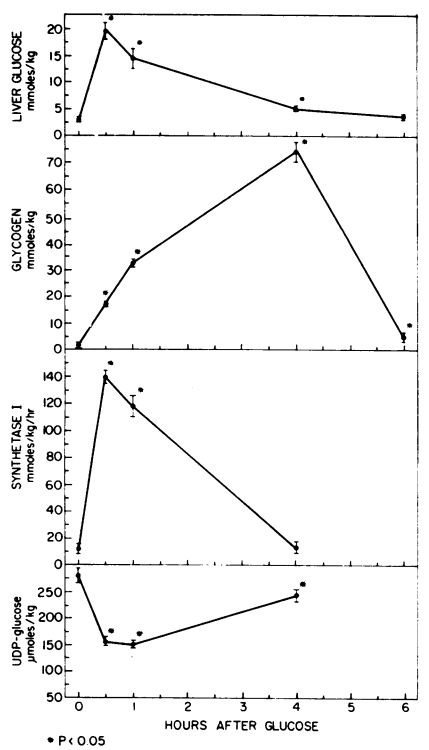

FIG. 1.—Effect of glucose administration in adrenalectomized rats. Adrenalectomized rats were injected with glucose (2.5 gm/kg, i.p.) 12–14 hr after the removal of food and sacrificed at the specified times. Values are averages ± standard error of the mean (SEM) for four or five rats.

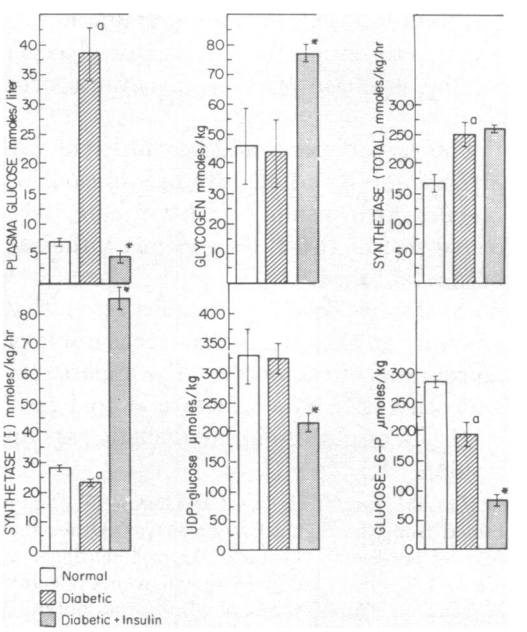

FIG. 2.—Effect of insulin administration in alloxan-diabetic rats.

a, $P < 0.05$ as compared with normal control.

*, $P < 0.05$ as compared with diabetic control.

Diabetic rats were injected with glucagon-free insulin (6 U/kg, i.p.) $2\frac{1}{2}$ hr after the removal of food and sacrificed 75 min after insulin injection. Values are averages ± SEM for three or four rats.

minutes, and decline exponentially to almost control values by six hours (Fig. 1). There is a rapid deposition of glycogen which continues linearly for at least 60 minutes and is accompanied by a marked increase in glycogen synthetase I activity (from 12 to 140 mmoles/kg liver/hr). Although not shown, no changes occur in total (+G-6-P, D + I form) synthetase activity. The 50 per cent decrease in heptatic UDP·glucose concentrations at 30 and 60 minutes is believed to be indicative of a facilitation at the glycogen synthetase step.[1]

Insulin administration to diabetics: Within 75 minutes after the administration of insulin to alloxan-diabetic rats (Fig. 2), the elevated levels of plasma glucose associated with diabetes return to values of control nondiabetic animals. Surprisingly, hepatic glycogen levels are not significantly diminished in the acute diabetics. The per cent of glycogen synthetase I activity decreases from 17 to 9 per cent in diabetic animals but, more important, synthetase activity in the D form increases 50 per cent in the diabetics while the I activity changes are small. After insulin administration, glycogen concentrations increase along with the activity of glycogen synthetase in the I form (fourfold). There is no evidence of new enzyme synthesis after insulin since "total" synthetase activity does not increase beyond that found in the diabetic controls. The stimulated glycogenesis following insulin is also reflected by a 50 per cent decrease in hepatic UDP-glucose. G-6-P levels are significantly lower in diabetic rats, a change that might be expected on the basis of the known dependence of hepatic glucokinase on insulin.[12]

Steroid stimulation of glycogenesis: The apparent stimulatory action of steroids on the glycogenic mechanism is illustrated in Figure 3. Two hours after the ad-

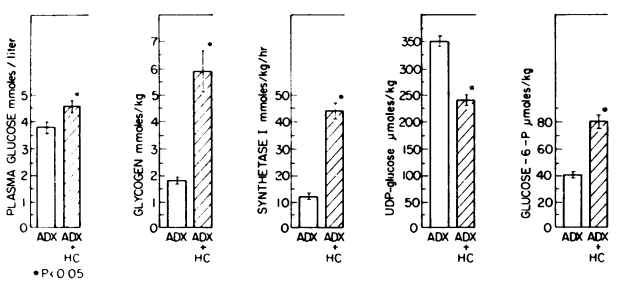

FIG. 3.—Effect of hydrocortisone (HC) administration in adrenalectomized rats. After 16 hr without food, adrenalectomized rats were injected with hydrocortisone (10 mg/kg s.c.) and sacrificed 2 hr later. Values are averages ± SEM for eight or nine rats.

ministration of hydrocortisone to fasted-adrenalectomized rats, the levels of plasma glucose, hepatic glycogen, G-6-P, and glycogen synthetase I activity increase while UDP-glucose concentrations fall. The glycogenic response to steroid and/or glucose is, however, completely lost in diabetic adrenalectomized animals (Fig. 4). No changes consistent with a stimulation of glycogen deposition occur in any of the components of the glycogenic process except perhaps the increase in glucose after glucose administration or the increase in G-6-P when steroid is given to the diabetic-adrenalectomized animals. Apparently neither of these changes in themselves provides an adequate stimulus for the system. On the other hand, insulin administration under the same conditions results in an eightfold increase in syn-

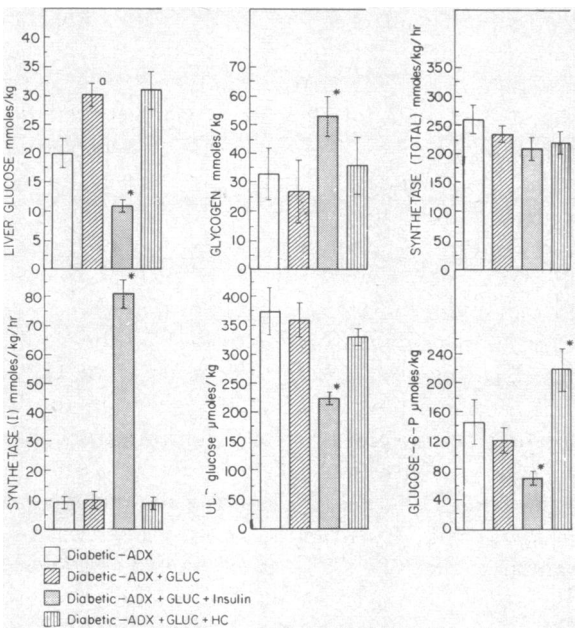

FIG. 4.—Effect of insulin or hydrocortisone and/or glucose administration in diabetic-adrenalectomized rats.

a, $P < 0.05$ as compared with diabetic-ADX control.

*, $P < 0.05$ as compared with diabetic-ADX + glucose control.

Animals were deprived of food for 4 hr and given glucose (5 gm/kg orally) 2 hr before sacrificing.

Hydrocortisone (10 mg/kg, s.c.) was injected at the same time as glucose.

Insulin (6 U/kg, i.p.) was injected 75 min prior to sacrifice.

Each value is the average ± SEM for four rats.

thetase I activity, a net deposition of glycogen, a characteristic decrease in UDP-glucose, and a fall in tissue G-6-P concentrations.

Discussion.—The inability of hydrocortisone to promote glycogenesis or effect a conversion of glycogen synthetase to the I form in the diabetic animal demonstrates the dependence of these processes on insulin. From these data it may be concluded that the early stimulation of hepatic glycogen deposition by adrenal steroids is probably mediated through the action of insulin rather than by any more direct effect of the steroid on the glycogen-synthesizing mechanism. Whether the insulin release derives from a direct action of the steroid on pancreatic tissue or indirectly as a result of the hyperglycemia induced by the gluconeogenic action of the hormone remains to be determined.[11]

Since elevating tissue glucose levels by administration of the sugar fails to stimulate glycogen deposition or activate glycogen synthetase in the diabetic rat, it becomes more apparent that a mass action or "push" effect is less likely to promote rapid glycogenesis than is the "pull" mechanism resulting from the activation of glycogen synthetase in the presence of insulin. The major dependence of rapid glycogenesis on the conversion of glycogen synthetase to the I form rather than the activation of the D form by increasing tissue G-6-P levels is evident from the lack of correlation between G-6-P levels and the rate of glycogenesis. In fact, it can be calculated from published *Km* values for the activation of glycogen synthetase by G-6-P and activities with UDP-glucose for both forms of synthetase from liver that after glucose administration (in nondiabetics) at least 90 per cent of the glycogen synthetic capacity can be accounted for by the activity of glycogen synthetase in the I form.

The role of steroids in the glycogenic process appears to be primarily in supplying glucosyl units necessary to sustain glycogen production. The elevation of tissue

G-6-P levels following steroid administration could constitute a stimulatory factor for glycogenesis by activating the D form of glycogen synthetase or lowering the *Km* of synthetase I[3] for UDP-glucose. However, the small absolute increases in G-6-P levels observed in these experiments and the failure of other investigators to observe any increases in G-6-P levels[1] during steroid-induced glycogenesis minimizes the importance of this effect, at least as it relates to steroid action.

The increase in synthetase D activity which occurred in diabetic animals has also been observed by Steiner and King,[13] and might represent a compensatory *de novo* enzyme synthesis in response to a prolonged hyperglycemia and an inability to "activate" synthetase in the absence of insulin. On the other hand, there is no evidence in the early period after insulin administration that the increase in glycogen synthetase activity arises from a *de novo* synthesis of the enzyme (e.g., an increase in total enzyme) as reported by Steiner and King.[13]

Summary.—The role of hydrocortisone in stimulating hepatic glycogen deposition by either a direct "activation" of glycogen synthetase or by a more indirect mechanism stemming from its gluconeogenic action was investigated. It was found that the increased rate of hepatic glycogen deposition following steroid or glucose administration to nondiabetic animals is accompanied by increases in the activity of the G-6-P independent (I) form of glycogen synthetase but that hydrocortisone or exogenous glucose has no effect on the rate of glycogen deposition or synthetase activity in diabetic animals. Insulin does, however, promote enhanced glycogenesis and synthetase "activation" in the absence of endogenous steroid. It is concluded that the apparent steroid or glucose stimulation of hepatic glycogen deposition and conversion of synthetase to the I form is dependent upon the action of insulin and that the activation of synthetase is a major requirement in the early phase of stimulated glycogenesis.

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Abbreviations: ADX, adrenalectomized; EDTA, ethylenediaminetetraacetate; gluc, glucose; G-6-P, glucose-6-phosphate; HC, hydrocortisone; tris, tris(hydroxymethyl)aminomethane; UDP-glucose, uridine diphosphate glucose.

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