The Effects of Adrenalectomy and Hydrocortisone on Rat Liver Metabolites and Glycogen Synthetase Activity

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(Received October 25, 1965)

SUMMARY

An attempt has been made to locate the defect in glycogen synthesis which results from adrenalectomy and to find the mechanism by which hydrocortisone corrects this defect. The levels of 12 metabolites believed to be associated with the pathway from lactate to glycogen were studied, and the results indicate that the major defect following adrenalectomy occurs at the step between UDP-glucose and glycogen. This was confirmed by attempts to induce glycogen formation with lactate administration. When glycogenesis was restored with hydrocortisone the changes in metabolite levels indicated that the UDP-glucose to glycogen step was greatly facilitated.

In vitro measurements of hepatic glycogen synthetase activities, together with kinetic studies of the enzyme, showed that adrenalectomy does not lower the total synthetase level, but causes a conversion of most of the enzyme to the form that is dependent on glucose-6-P for activity. Hydrocortisone, conversely, does not significantly change the total synthetase level but converts a substantial amount of the glucose-6-P dependent synthetase to the "independent" form. It is concluded that glucocorticoids permit glycogen synthesis by maintaining part of the glycogen synthetase in the form which is active even at low glucose-6-P levels.

INTRODUCTION

It was previously shown[2] (1, 2) that an early result of hydrocortisone administration is a facilitation of the step between UDP-glucose and glycogen. The evidence for this was a decrease in the levels of both UDP-glucose and glucose-6-P coincident with the onset of glycogen synthesis. Eight other metabolites of the gluconeogenic pathway were not changed from control levels. The finding of *lowered* glucose-6-P levels after hydrocortisone rules out the possibility that the facilitated use of UDP-glucose is due to activation by glucose-6-P (3–6) of glycogen synthetase (uridine diphosphate glucose-glycogen glucosyl transferase EC 4.1.11.).

The original study did not settle whether the facilitation at the synthetase step resulted from an actual increase in enzyme activity or had some other explanation, such as an improved access of UDP-glucose to the synthetase. This report describes the effect of adrenalectomy and of adrenalectomy followed by short-term hydrocortisone or lactate administration on the activity and kinetic properties of glycogen synthetase as well as on 12 metabolites related to the gluconeogenic pathway.

The results constitute evidence that (a) the increased glycogen synthesis observed after hydrocortisone administration results

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[2] A portion of this work has been reported in a preliminary communication (2).

from conversion of the glucose-6-P dependent synthetase to a form less dependent on glucose-6-P without a change in total activity and that (b) the so-called "independent" or "I-form" (7) is dependent on glucose-6-P to the extent that the Michaelis constant for UDP-glucose is lower in the presence of glucose-6-P than in its absence.

METHODS

Adrenalectomized and normal male rats (180–200 g, Holtzman strain) were obtained from the Badger Research Corporation, Madison, Wisconsin. The rats were kept 9 days before use and fed Purina Chow ad libitum. Adrenalectomized rats were given 1% NaCl for drinking water. All rats were anesthetized with sodium phenobarbital (150 mg/kg) 1.5 hr before excision of the liver sample. Where indicated, rats were starved 24 hr before experimental procedures were begun. Hydrocortisone hemisuccinate (Solu-Cortef, The Upjohn Co.), 50 mg/kg, was injected subcutaneously. Isotonic sodium lactate 0.75 g/kg or an equivalent volume of isotonic saline, was injected intraperitoneally. The body temperature was monitored with a rectal thermometer and maintained between 36° and 39° by heating when necessary with an infrared lamp.

The abdominal cavity of the anesthetized animal was opened, and the liver sample was frozen rapidly, either *in situ* or immediately after excision, using Freon-12 (CCl_2F_2) cooled to −150° with liquid nitrogen. Samples were stored at −60° until extraction. Frozen pieces (50–200 mg) were weighed at −20°, homogenized at −10° in 5 vol of 2 *M* $HClO_4$, diluted 3-fold with 1 mM EDTA, and centrifuged. The supernatant fluid was neutralized to pH 6.0–7.0 with 2 M $KHCO_3$ and centrifuged again. Neutralized extracts and standards, treated in the same manner as the samples, were stored at −60°.

Intermediates of the pathway, from lactate to glycogen, were measured by published fluorometric methods (8). When the anticipated level of a substrate was low (fructose-1,6-diP, dihydroxyacetone-P, pyruvate, P-pyruvate, and glucose-6-P), the neutralized liver extracts, reagent blanks, and extracted standards, were treated with acid-washed Florisil, (Floridin Co., Tallahassee, Florida) 60 mg/ml extract, to remove a large part of the interfering fluorescent material.

Malate was determined fluorometrically at pH 9.0 in hydrazine-HCl buffer by the production of acetylpyridine-DPNH from acetylpyridine-DPN$^+$ in the presence of malate dehydrogenase (Boehringer and Sons through California Corporation for Biochemical Research).[3]

UDP-glucose was determined fluorometrically at concentrations of 0.2 to 2 $\times 10^{-6}$ M by a modification of the spectrophotometric method of Strominger *et al.* (11). UDP-glucose dehydrogenase (Sigma Chemical Co.) was used in a final enzyme concentration of 0.3 mg/ml. Enzyme batches were tested to eliminate those containing other DPN-linked dehydrogenases. A few preparations were eliminated because they contained something which resulted in DPN$^+$ reduction in the assay system. No UDP-galactose epimerase activity was found in the UDP-glucose dehydrogenase used for these determinations. The DPN$^+$ concentration was 0.05 mM.

Glycogen was determined enzymically with phosphorylase *a*, P-glucomutase, and glucose-6-P dehydrogenase[4] (12, 13). The sensitivity and specificity of this method proved useful in measuring the low liver glycogen levels encountered after adrenalectomy.

Glycogen synthetase activity was determined fluorometrically by a modification of the method of Breckenridge and Crawford (14). Frozen liver samples were homogenized in either 25 or 50 vol of 0.4 M sucrose-5 mM EDTA neutralized to pH 7.0. Glucose-6-P "independent" synthetase activity was determined on whole homogenate

[3] M. Fleming, J. V. Passonneau and O. H. Lowry, unpublished method. A modification of the spectrophotometric technique of Hohorst (9) and Holzer and Soling (10).

[4] J. V. Passonneau, D. W. Schulz and O. H. Lowry, unpublished method.

(5 μl) equivalent to 0.2 mg of liver in 50 μl of a reaction mixture containing 50 mM Tris-acetate buffer pH 7.5, 9.5 mM UDP-glucose, 10 mM glycogen (calculated as glucose), 5 mM EDTA, and 20 mM Na_2SO_3. Rabbit liver glycogen (Mann Research Laboratories, Inc., New York) which had been dialyzed 18 hr against acetate buffer at pH 4.7, was standardized as glucose before use. It did not contain glucose-6-P. Incubation was for 30 min at 38°. Total synthetase activity was determined similarly with the equivalent of 0.1 mg of tissue in 50 mM glycylglycine buffer pH 8.0, with the addition of 10 mM glucose-6-P. Incubation was for 15 min at 38°. (The change in buffer and pH from that used without glucose-6-P was made for irrelevant reasons and has been shown to be of no consequence if the independent activity is measured in glycylglycine buffer at pH 8.0.) The reaction was stopped by heating at 100° for 2 min. The UDP produced was determined with pyruvate kinase by its reaction with P-pyruvate in the presence of lactate dehydrogenase and DPNH. The reagent contained 30 mM glycylglycine buffer pH 7.3, 0.1 mM P-pyruvate, 10 mM hydrazine HCl, 70 mM KCl, 15 mM $MgCl_2$, and 2×10^{-6} M DPNH. Hydrazine was added to decrease a reagent blank which is presumably due to keto acids. Duplicate aliquots of the incubation mixture containing between 3.5×10^{-10} and 1.4×10^{-9} moles of UDP were assayed in 1 ml of reagent. The reaction was started with 10 μg of beef heart lactate dehydrogenase (Worthington Biochemical Corporation) and 25 μg of pyruvate kinase (Boehringer and Sons). Both with and without addition of glucose-6-P, activity was linear for at least 30 min of incubation. In the absence of glucose-6-P, activity was linear with time only if a reducing agent was added (Na_2SO_3 or cysteine).

RESULTS

Liver Metabolites after Adrenalectomy and Adrenalectomy plus Hydrocortisone

Adrenalectomy produced a striking alteration in the liver metabolite profile (Fig. 1). Pyruvate, P-pyruvate, 3-P-glycerate, and UDP-glucose were markedly elevated above control levels. Most of the other metabolites measured were decreased, notably glycogen. The oxidation-reduction pairs, lactate/pyruvate- and α-glycero-P/dihydroxyacetone-P were shifted in the direction of increased oxidation (Table 1). This agrees with results of Hohorst *et al.* (15) and Henley *et al.* (16). Calculation of cytoplasmic DPN:DPNH ratios from these pairs according to the method of Hohorst *et al.* (17) gave ratios for control rats of 1390:1 and 1540:1 calculated on lactate/pyruvate and α-glycero-P/dihydroxyacetone-P, respectively. Similar calculations for adrenalectomized rats gave ratios of 2830:1 and 2250:1.

Hydrocortisone administration to adrenalectomized rats 3 hr before liver sampling increased both glycogen and glucose levels probably indicating increased gluconeogenic flux (Fig. 1). This increased flux was not associated with an increase in any of the presumptive glycogen precursors, and the level of the immediate precursor, UDP-glucose was reduced to half. It seems inescapable that the increased glycogen synthesis is associated with acceleration of the UDP-glucose to glycogen step. Some, but not all, of the more remote glycogen precursors were decreased by hydrocortisone. The most striking exceptions were P-pyruvate and 3-P-glycerate which remained at their abnormally high level. Hydrocortisone likewise failed to correct the depressed lactate:pyruvate and α-glycero-P:dihydroxyacetone-P ratios. [This agrees with Hohorst *et al.* (15), who found only a slight increase in the ratios after hydrocortisone.]

It is concluded that whereas 3 hr after hydrocortisone the defect in glycogen synthesis is repaired other changes resulting from adrenalectomy have not been reversed.

Effects of Lactate Administration

An attempt was made to induce glycogen formation in adrenalectomized animals with lactate. Although the expected large increase in glycogen was observed in control animals, little or no change occurred if the

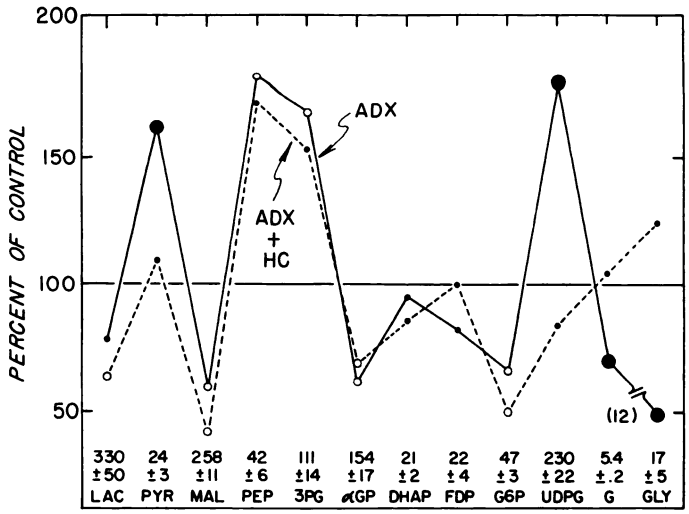

FIG. 1. *The effects of adrenalectomy and hydrocortisone treatment on 12 metabolites in liver*

The concentrations of the metabolites are expressed as percentages of the levels of starved normal control rats. These control values are recorded across the bottom of the figure and represent the means for 4 rats ± the standard errors. Values are expressed as micromoles per kilogram wet weight of liver except for glucose and glycogen, which are expressed as millimoles per kilogram. The metabolites are arranged in order along the presumptive glycogenic pathway. (Substances believed not to be on the pathway, malate, glycero-P, and glucose, are placed next to close metabolic neighbors.) Points on the graph are connected for easier visualization. There were 3 or 4 rats in each group. All rats had been starved 24 hours. Hydrocortisone was given 3 hours before samples were taken. Abbreviations used are ADX, adrenalectomized rats; HC, hydrocortisone; Lac, lactate; Pyr, pyruvate; Mal, malate; PEP, P-pyruvate; 3PG, 3-P-glycerate; αGP, α-glycero-P, DHAP, dihydroxyacetone-P; FDP, fructose diphosphate; G6P, glucose-6-P, UDPG, UDP-glucose; G, glucose; and Gly, glycogen. Values marked with an open circle are significantly different from control levels at $P < 0.05$; those with a large closed circle are significantly different at $P < 0.01$.

TABLE 1

Ratios of substrate concentrations in livers of control and adrenalectomized rats

Absolute substrate levels are given in Fig. 1. The abbreviation is adrenalectomy, ADX. Ratios are averages of 3 or 4 rats ± the standard errors of the mean.

Ratio	Control	Control + lactate	ADX	ADX + lactate	ADX + hydrocortisone	ADX + hydrocortisone + lactate
α-Glycero-P: dihydroxyacetone-P	7.3 ± 0.5	11.9 ± 4.6	5.0 ± 0.5	6.4 ± 1.1	6.0 ± 0.5	9.2 ± 1.7
Lactate: pyruvate	13.7 ± 1.5	20.2 ± 4.2	6.7 ± 0.6	19.7 ± 2.5	8.5 ± 1.4	12.5 ± 1.4

rats had been adrenalectomized (Fig. 2). This defect was overcome within 3 hr after hydrocortisone administration. By this time the glycogen response of adrenalectomized animals to lactate was even greater than in controls.

In contrast to glycogen, the glucose response to lactate was as great in the adrenalectomized animals as in the other 2 sets. Furthermore, the changes in liver metabolite profiles were similar in other respects whether the rats were untreated, or adrenalectomized, or both adrenalectomized and treated with hydrocortisone.

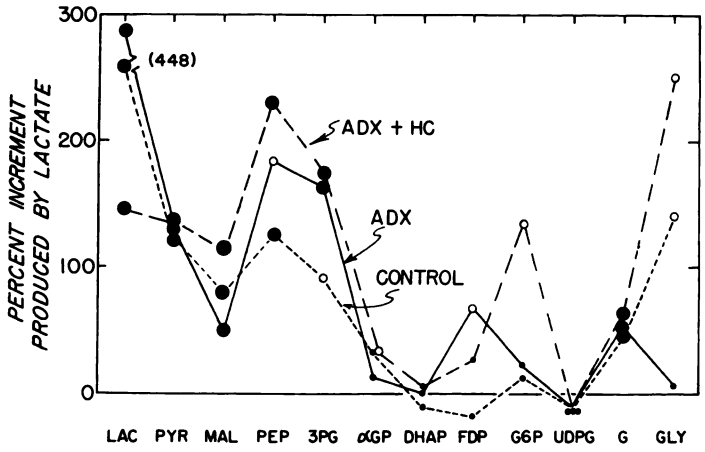

FIG. 2. *The effect of lactate on metabolites in liver from control and adrenalectomized rats and from adrenalectomized rats that had received hydrocortisone*

In order to make valid comparisons the absolute changes in levels in all three groups are compared to the prelactate levels in the control normal group. For example, the increases in malate for the control and adrenalectomized groups were 204 and 111 μmoles/kg, respectively, which represent 79% and 43% increases over the normal prelactate malate level of 258 μmoles per kilogram liver. Each group consisted of 3 or 4 rats. Lactate was given 1 hr before sampling; otherwise the groups were prepared as described for Fig. 1, and abbreviations are the same. Values marked with an open circle are significantly increased at $P < 0.05$; those with a large closed circle, at $P < 0.01$.

Exceptions were the larger increases in levels of malate and glucose-6-P in adrenalectomized rats treated with hydrocortisone, and the increase in fructose diphosphate in adrenalectomized rats. The α-glycero-P:dihydroxyacetone-P ratio showed little change in adrenalectomized rats given lactate despite the marked elevation in the lactate:pyruvate ratio (Table 1).

Kinetic Studies of Glycogen Synthetase

It seemed possible that the defect after adrenalectomy at the UDP-glucose to glycogen step could be due to a change in the amount of glycogen synthetase or to a change in the relative proportions of the two recognized forms of the synthetase: the glucose-6-P dependent "D"-form, and the glucose-6-P independent "I" form (7). (As shown below, the I-form is probably not in fact independent of glucose-6-P.) In order to make adequate measurements of these forms it seemed necessary to first examine the kinetics of synthetase as it occurs in the liver in the different endocrine states. Synthetase activity, as a function of UDP-glucose concentration, was measured in the presence of glucose-6-P (Fig. 3) and in its absence (Fig. 4). In the first instance the activity should represent the sum of both D- and I-forms, in the second instance only the I-form. Studies were made with whole homogenates from rats in four categories of glucocorticoid status: control, adrenalectomy, adrenalectomy plus hydrocortisone, and control plus hydrocortisone. (Although it is not as elegant to use whole homogenates as to use purified enzyme preparations, it is crucial in this case since the purpose is to examine the total activity, not just the activity of a possibly small fraction that might be isolated.) Linear Lineweaver-Burk plots were obtained in the presence of glucose-6-P, but not in its absence (Fig. 4). This agrees with the work of Hizukuri and Larner on a partially purified enzyme (7).

With liver from adrenalectomized rats the I-form activity was so low that studies made in the presence of glucose-6-P can be regarded as descriptive of almost pure

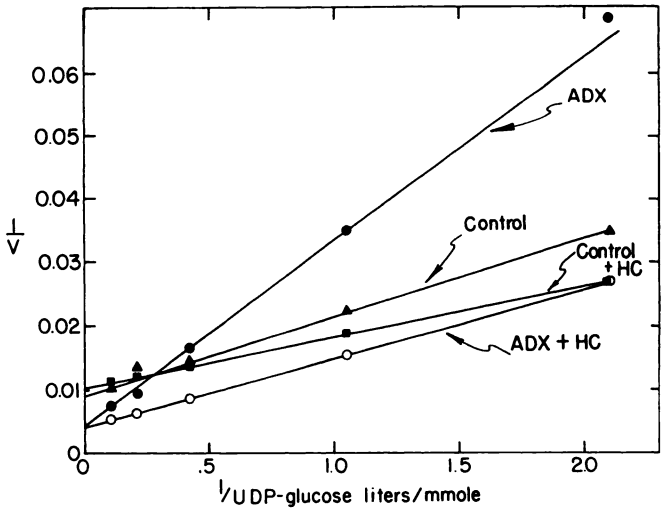

FIG. 3. *Glycogen synthetase activity in the presence of 10 mM glucose-6-P as a function of UDP-glucose concentration*

Results are given for control and adrenalectomized rats (ADX) with and without hydrocortisone administration (HC). The hydrocortisone was given 3 hr before removal of the liver. All rats had been starved. The liver dilution during incubation was 1:500. Reciprocal velocities are recorded as $(mmole)^{-1}$ kg hr.

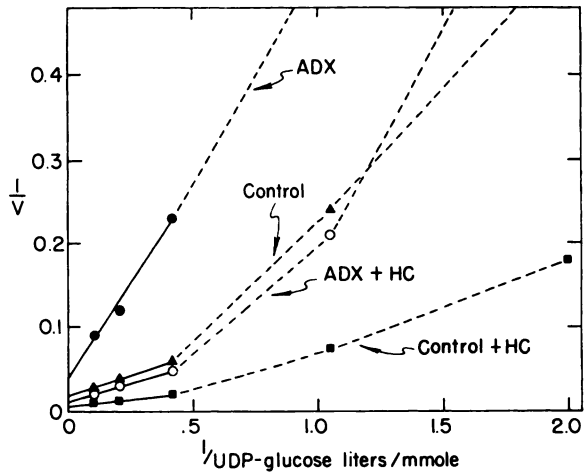

FIG. 4. *Glycogen synthetase activity in the absence of glucose-6-P as a function of UDP-glucose concentration*

The same homogenates were used as for Fig. 3. The liver dilution during incubation was 1:250. Apparent K_s values were determined from lines drawn through points of the 3 highest substrate levels. Reciprocal velocities are recorded as $(mmole)^{-1}$ kg hr.

D-form (Fig. 3). Conversely, in the case of normal (starved) animals given hydrocortisone the values for V_{max} (extrapolated velocities for infinitely high UDP-glucose concentration) were approximately the same whether glucose-6-P was present or absent. Consequently the results obtained in this case can be regarded as descriptive of almost pure I-form. By comparing the curves in Figs. 3 and 4 for a liver in this category ("control + HC") it will be seen that glucose-6-P appears to have a profound affect on the K_s for UDP-glucose. Thus with 0.5 mM UDP-glucose the rate

was 8 times faster in the presence of glucose-6-P than in its absence even though the maximum velocities were almost equal. This seems to show that the I-form is only independent of glucose-6-P at very high UDP-glucose levels—levels that are far higher than those encountered *in vivo*.

All groups, besides the adrenalectomized and the starved controls given hydrocortisone, appear to contain mixtures of D- and I-forms, in that V_{max} values were increased by glucose-6-P. Hence the apparent kinetic parameters in the presence of glucose-6-P are composite figures. A possibly adequate way to calculate the I-form K_s's for the groups with mixed D- and I-forms is given in a footnote to Table 2.

Provisionally, at least, it may be concluded (Table 2) that under the experimental conditions the K_s for UDP-glucose is about 8 mM for the D-form, 5–10 mM for the I-form in the absence of 10 mM glucose-6-P, and less than 2 mM in the presence of glucose-6-P. There is some sign that more

TABLE 2

Effect of hydrocortisone and adrenalectomy on glycogen and glycogen synthetase

Values are averages ± standard error of the mean for 3 or 4 rats except for the K_s columns, which represent individual determinations. The maximal velocities in columns 4 and 5 were calculated from velocities observed with 9.5 mM UDP-glucose and the Michaelis-Menten equation. In each case an average value of K_s, as recorded in the last 2 columns, was used for this calculation. The K_s values measured 3 hr after hydrocortisone (HC) administration were assumed to apply at 1, 2, 6 hr as well (control animals starved 24 hr). The K_s values given first in each column are from Figs. 3 and 4; those given second were determined on different livers. Glycogen is expressed as millimoles per kilogram fresh liver, enzyme activities as millimoles per kilogram fresh liver per hour.

Treatment	Hours after HC	Glycogen	Synthetase activity (V_{max}, mmoles/kg/hr)		K_s for UDP-glucose (mM)	
			− glucose-6-P	+ glucose-6-P	− glucose-6-P	+ glucose-6-P
Control						
Starved 24 hr	0	17 ± 5	52 ± 3	90 ± 8	5.6; 6.6	1.3; 1.3[a]
	1	11 ± 2	70 ± 6	89 ± 9		
	2	33 ± 9	96 ± 7	103 ± 4		
	3	60 ± 12	90 ± 6	89 ± 11	2.4; 5.7	0.75; 0.65
	6	79 ± 10	72 ± 6	85 ± 2		
Fed ad lib.	0	252 ± 12	55 ± 10	146 ± 7	9.8	2.6[a]
	3	241 ± 21	45 ± 8	119 ± 11	9.8	1.8[a]
Adrenalectomized						
Fed ad lib.	0	—	22 ± 2	131 ± 9	14	8.3
Starved 24 hr	0	2 ± 1	15 ± 4	229 ± 9	10.0; 12.5	6.7; 9.9
	3	21 ± 5	85 ± 11	187 ± 17	7.7; 8.0	2.4; 2.8[a]
1 hr after lactate	0	3 ± 2	19 ± 6	218 ± 30		
	3	64 ± 16	123 ± 13	147 ± 22		

[a] In the case of these animals an attempt was made to calculate the Michaelis constants of the pure I-form in the presence of glucose-6-P. The V_{max} for the D-form was taken as the difference between column 5 and column 4. The velocity of the D-form activity at less than saturating levels of UDP-glucose was calculated from the Michaelis-Menten equation assuming K_s = 8.3 mM. This value of K_s was the average obtained for all untreated adrenalectomized rats in which, as seen in the table, most of the activity is in the D-form. The calculated contribution of the D-form to the activity at each substrate concentration was then subtracted from the velocity determined in the presence of glucose-6-P. The differences were taken as the velocities for the I-form when measured in the presence of glucose-6-P. From these net velocities K_s values were estimated from Lineweaver-Burk plots. The values for K_s calculated in this way were 0.85, 1.7, 1.7, and 2.0 mM, respectively, for the starved control rats, fed control rats, fed control rats after hydrocortisone, and starved adrenalectomized rats after hydrocortisone.

than one I-form of synthetase can exist in liver. In the absence of glucose-6-P, the K_s values for UDP-glucose spread over a considerable range, and tended to be higher in fed rats than in those that had been starved. It is perhaps unwise to regard this as certain, however, since these data were obtained with whole homogenates, and some of the activities measured in the absence of glucose-6-P are quite small.

The Michaelis constants for glycogen were not measured. However, a few experiments were carried out with glycogen concentrations of 50 mM rather than 10 mM. At the higher concentration the K_s values for UDP-glucose were cut in half, suggesting interaction between binding sites for the two substrates.

Effect of Adrenalectomy and Hydrocortisone on Glycogen Synthetase Forms and Total Activity

From the kinetic studies it is clear that simple deduction of synthetase activity in the absence of glucose-6-P from that in its presence does not give a valid measure of the D-form, since the I-form activity is apparently increased by glucose-6-P except at very high UDP-glucose levels (Table 2). It therefore seemed preferable to first estimate the V_{max}'s (infinite UDP-glucose concentration) from the Michaelis-Menten equation, using the K_s values of the last two columns of Table 2. Comparison between V_{max}'s with and without glucose-6-P should now give more nearly adequate measures of the D- and I-forms. Hydrocortisone significantly increased the I-form in livers of starved control rats as early as 1 hr after administration. The sum of I- and D-forms was not increased. The increase in I-form was paralleled directly by the increase in glycogen after the first hour of hydrocortisone action. In contrast, hydrocortisone, administered to fed control rats, did not increase the I-form or change the sum of the two forms, nor did it affect the liver glycogen level.

Adrenalectomy decreased the I-form of synthetase in both fed and fasted rats with little change in the total activity of the fed rats, but in fasted rats the total activity actually increased by a factor of almost 2. This contrasts with the effect of fasting in control animals which was to decrease the total activity.

Hydrocortisone given to adrenalectomized rats produced large increases of I-form synthetase without an increase of total activity; there may possibly have even been a decrease.

DISCUSSION

Effects of Adrenalectomy and Hydrocortisone on Metabolite Levels

It was reported earlier (1, 2) and confirmed here not only that stimulation of glycogen synthesis by hydrocortisone is associated with a fall in UDP-glucose, but that glucose-6-P may also fall and certainly does not rise. This is a crucial point since others (3–5), having observed a *rise* in glucose-6-P after hydrocortisone, attributed the increased glycogen synthesis to stimulation of the synthetase by glucose-6-P. It seems likely that these observed increases in glucose-6-P can be ascribed to the fact that in this earlier work there was a delay in freezing the liver after cutting off its blood supply. Such delay results in a rapid increase in glucose-6-P levels, provided there is sufficient glycogen present in the liver (18).

Besides the major defect in glycogen synthesis after adrenalectomy, at the step between UDP-glucose and glycogen there are indications from changes in metabolite levels that other disturbances may be present. After adrenalectomy there were found to be increased levels of pyruvate, P-pyruvate, and 3-P-glycerate and decreased ratios of lactate to pyruvate and of glycero-P to dihydroxyacetone-P. The metabolite increases are comparable to those seen when gluconeogenic flux is increased by lactate administration to starved normal animals or observed in diabetic rats (19), but in the case of the present starved adrenalectomized animals flux is certainly not increased. Decreased activity of either P-glycerate kinase or glyceraldehyde-P dehydrogenase might explain the rise in P-pyruvate and 3-P-glycerate; the greater-

than-normal increase in these metabolites after giving lactate supports this possibility. The fact that hydrocortisone in 3 hr does not restore the abnormal metabolite ratios or metabolite levels (except in the case of pyruvate) implies that these abnormalities arise from secondary changes, or from changes that respond more slowly than glycogen synthesis to hydrocortisone, or even from other effects of adrenalectomy than glucocorticoid deficiency.

Exton and Park (20) on the basis of studies with perfused liver, concluded that after adrenalectomy there is a defect in the pathway from lactate to glucose which lies between pyruvate and triose-P. Subsequently they reported (21) that lactate perfusion through normal livers increased pyruvate, but not P-pyruvate, levels and that after adrenalectomy P-pyruvate levels were subnormal. Consequently they concluded that adrenalectomy causes a partial block at the P-pyruvate carboxykinase step. It has been seen that completely opposite results in regard to P-pyruvate levels are found *in vivo*. The reason for the difference is not apparent. Shrago *et al.* (22) found in the livers of both normal and adrenalectomized rats that fasting induces large and *equal* increases in P-pyruvate carboxykinase activity (measured *in vitro*).

It has been suggested that an important consequence of hydrocortisone action is an increase in fructose diphosphatase activity (20, 23–28). The present results do not give much support for this. There was if anything a small increase in fructose diphosphate concentration after giving hydrocortisone alone (Fig. 1), whereas a decrease would be the expected result of enhanced diphosphatase activity. On the other hand, hydrocortisone did produce a small reduction in fructose diphosphate concentration in the case of animals receiving lactate. The question is really not whether there has been an increase in fructose diphosphatase activity, but whether enhanced activity at this step is partially responsible for the accelerated rate of glucose and glycogen synthesis. The answer from present data seems to be no. Several studies (29–31) suggest that the gluconeogenesis as opposed to glycogenesis may not be seriously depressed by adrenalectomy, and Friedmann *et al.* (32) conclude that the major block in glycogen formation from either lactate or pyruvate after adrenalectomy lies between glucose-6-P and glycogen.

Effect of Adrenalectomy and Hydrocortisone on Glycogen Synthetase

It is concluded from the *in vitro* studies of synthetase kinetics and activity that hydrocortisone administered to starving or adrenalectomized animals promotes the conversion of synthetase D-form to the I-form without increasing the total. Such interconversions of glycogen synthetase have been described for purified synthetase *in vitro* by Hizukuri, Rosell-Perez, Villar-Palasi, and Friedman in collaboration with Larner (7, 33, 34, 35) and by Traut and Lipmann (36), and *in vivo* in muscle by Danforth (37).

There are several previous studies of glycogen synthetase activity in liver which might not appear to agree with the present findings. The discrepancies however, can probably be ascribed to analytical differences and to the kinetic complications described above. Thus both Steiner *et al.* (3) and Tarnowski *et al.* (4) found almost negligible amounts of I-form in liver regardless of the endocrine status. It seems reasonable that this is due in part to the fact that sulfite was not used to stabilize the I-form in the absence of glucose-6-P, and in part to the less favorable Michaelis constant for UDP-glucose when glucose-6-P is not present. Steiner *et al.* did, however, report that total synthetase activity was not increased by hydrocortisone (starved normal animals) as this report confirms. Tarnowski *et al.* found that synthetase activity, as measured in the presence of glucose-6-P and relatively low UDP-glucose concentration, was increased by hydrocortisone (adrenalectomized rats). This is what would be expected under such analytical conditions from conversion of D-form to I-form without change in total enzyme. The same explanation is offered for the report of Sie and Fishman (38)

that synthetase activity in livers of starved normal animals is increased by hydrocortisone. In agreement with the present report these authors found a decrease in synthetase activity during starvation (normal animals). Since no significant change in proportion of D- to I-form appears to take place under these circumstances it is reasonable that the analytical results should concur.

The kinetic data indicate that the I-form is not entirely independent of glucose-6-P and that glucose-6-P may play a role for the I-form that is analogous to the role of AMP for phosphorylase *a*. It has been demonstrated (39, 40) that low levels of AMP can increase the activity of phosphorylase *a* at physiological substrate concentrations through a favorable change in the Michaelis constants for glycogen and P_i. Thus conversion of D-form to I-form may represent the major control process (analogous to the conversion of phosphorylase *b* to *a*) whereas the activity of the I-form may be modulated by the glucose-6-P concentration.

ACKNOWLEDGMENT

This study was supported by NSF Grant G-18959 and USPHS Grants HD-00376 and NB-01352.

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