Glucocorticosteroids and Transaminase Activity

I. INCREASED ACTIVITY OF GLUTAMIC-PYRUVIC TRANSAMINASE IN FOUR CONDITIONS ASSOCIATED WITH GLUCONEOGENESIS*

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The gluconeogenic action of glucocorticosteroids and the negative nitrogen balance induced by this class of hormones point to the involvement of these agents at some biochemical site concerned with the conversion of protein to carbohydrate (1). Increased transaminase activity would be expected to alter the rate at which precursors of protein are interchanged with carbohydrate intermediates. Recently, an effect of cortisone on the activities of certain transaminases has been observed.

Gavosto et al. (2) noted that in rats given cortisone by injection (120 mg. per kg. daily for 3 days) glutamic-oxaloacetic transaminase activity in liver was increased by 67 per cent, and glutamic-pyruvic transaminase activity, by 81 per cent. In other studies by Beaton et al. (3), daily treatment of rats with cortisone did not affect the glutamic-oxaloacetic transaminase level in liver but doubled the activity of glutamic-pyruvic transaminase. In an independent study (4) we reported that cortisone did not affect the glutamic-oxaloacetic transaminase activity of 1 ml. of undiluted enzyme being 58.4 mmoles per hour. It was unnecessary to add pyridoxal phosphate to the reaction mixture to measure the transaminase activity. In the present study the activity of glutamic-pyruvic transaminase in liver was found to be stable at -20° in the homogenates diluted 1 to 20; variations in the protein content of a purified diet were made at the expense of the sucrose. This ration contained 0 to 75 per cent of casein, 18 to 91 per cent of sucrose, 4 per cent each of salt mixture (6) and Maisola oil, 0.5 per cent of cod liver oil, and 0.2 per cent of choline chloride. Added to this ration per 100 gm. were 1 mg. each of thiamine hydrochloride, riboflavin, and pyridoxine hydrochloride, 4 mg. of niacin, 6 mg. of calcium pantothenate, 15 mg. of inositol, 20 mg. of p-aminobenzoic acid, and 20 µg. each of biotin and folic acid.

Injectable commercial preparations of cortisol acetate, hydrocortisone, and desoxycorticosterone acetate were used. Tablets of 17α-pregnadiene-17β,21-diol-3,11,20-trione (Prednisone) were powdered and suspended in 0.5 per cent carboxymethyl-cellulose. All compounds were administered daily by subcutaneous injection.

Assay Procedure—The animals were killed by exsanguination after being stunned by a blow on the head. The liver was completely removed and placed immediately on ice. The whole liver was homogenized in a Waring Blender with 19 volumes of ice-cold distilled water. Both transaminase enzymes were found to be stable at -20° in the homogenates diluted 1 to 20; however, to obtain representative sampling, it was necessary to rehomogenize the frozen preparations before analysis. Analyses were carried out on individual samples from each of five animals per group.

Protein was measured by means of the Folin phenol reagent with the use of a modified method described by Lowry et al. (7). GO-transaminase activity was determined by a fluorometric method (8). The colorimetric procedure of Nelson (9) was used for blood glucose. GP-transaminase activity was measured by an unpublished procedure of Lowry et al., which is similar in principle to that described by Wroblewski and LaDue (10) except that instead of measuring spectrophotometrically the disappearance of DPNH, the DPN formed is measured fluorometrically (11). The buffer-substrate reagent, prepared from individual stock solutions, contained the following ingredients per ml.: 86 µmoles of 2-aminoo-2-methyl-1,3-propanediol buffer (Eastman Organic Chemicals), pH 8.9; 81 µmoles of L-alanine (adjusted to a pH of 7 to 8); 0.7 µmoles of α-ketoglutaric acid (adjusted to pH of 7 to 8); 1.6 µmoles of DPNH; 19.5 µmoles of nicotinamide; 5 µl. of 10 per cent bovine plasma albumin; and 4 µl. of lactate dehydrogenase (Sigma) the activity of 1 ml. of undiluted enzyme being 58.4 mmoles per hour. It was unnecessary to add pyridoxal phosphate to the reaction mixture.
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assay system, since the activity of the enzyme was not altered by the addition of the cofactor. To obtain tissue blanks, a buffer-substrate reagent similar in composition, except that water replaced alanine, was incubated with 2 µl of the dilution of each liver sample. Incubations were carried out for 30 minutes at 38° with 50-µl volumes of reagent and 2 µl of homogenate containing 20 µg. of fresh liver from the untreated control animals or 7 µg. of liver expected to have increased GP-transaminase activity.

A modification of the colorimetric transaminase determination described by Tonhazy et al. (12) gave values comparable to those obtained with the fluorometric method (Table I). The colorimetric method was adapted as follows. Reagent A contained 12 ml. of 0.17 M 2-amino-2-methyl-1,3-propanediol buffer, pH 8.9; 2.5 ml. of 0.5 M L-alanine; 1.5 ml. of 0.1 M α-keto-glutaric acid; and 75 µl. of 10 per cent bovine plasma albumin; Reagent B for the blank was prepared similarly except that alanine was replaced with water. To 0.5 ml. of Reagent A or B, 15 µl. of tissue homogenate (1 to 40 dilution for normal rat liver, 1 to 200 dilution for livers of hydrocortisone-treated animals) were added. All samples, standards, and blanks were incubated for 30 minutes at 38°. The reaction was stopped by the addition of 0.05 ml. of 100 per cent trichloracetic acid to each tube. The color was developed with 0.5 ml. of 0.1 per cent 2,4-dinitrophenylhydrazine in 2 N HCl at 38° for 5 minutes and extracted with 1.0 ml. of water-saturated toluene. To 0.6 ml. of the toluene layer, 3 ml. of alcoholic potassium hydroxide were added. The color was read in a Beckman DU spectrophotometer at 530 mµ. The amount of pyruvate formed was determined from a standard curve obtained with 2- to 15-µl. aliquots of a 0.1 m pyruvate stock solution.

RESULTS

GP-transaminase Response to Hydrocortisone and Other Corticosteroids—The magnitude of the GP-transaminase response which occurs in the livers of rats treated with hydrocortisone, Prednisone, and cortisone acetate for 1 week is shown by the results given in Table II. A direct relationship between the GP-transaminase response and the dose of hydrocortisone was observed (Fig. 1).

DOCA lowered the activity of GP-transaminase in liver to 50 per cent of the normal value (Fig. 2). However, when DOCA was administered, by injection, in large amounts (5 mg.) and in combination with hydrocortisone (1 mg.) for 7 days, it did not inhibit the stimulating effect of hydrocortisone on this enzyme. The specific effect of glucocorticosteroids on this transaminase in contrast to DOCA and other steroids tested (13) indicates that a method of assay for this class of steroids based on changes in the activity of the enzyme may be feasible.

Effect of Dietary Protein on GP-transaminase Activity—Feeding high protein diets alone increased the GP-transaminase levels. In relation to a control group maintained for 1 week on a purified diet lacking protein, groups fed diets containing 18, 35, 50, or 75 per cent casein showed 1.6-, 2.4-, 4.1-, or 7.5-fold increases, respectively, in GP-transaminase activity based on the protein content of liver (Fig. 3). In contrast, the activity of GO-transaminase was only slightly altered (20 per cent increase) by feeding a 75 per cent protein diet for 1 week. Liver protein was related directly to the protein content of the ration and varied from 11.9 per cent in animals on the protein-free diet to 18.4 per cent in animals receiving the diet containing 75 per cent casein.

75 per cent casein. At all levels of protein intake, the activity of GP-transaminase per gm. of liver was considerably higher than the activity calculated on the basis of liver protein.

A response to hydrocortisone was obtained in animals maintained on a diet devoid of protein; administration of 2.5 mg. of hydrocortisone daily for 1 week caused an 8-fold increase in GP-transaminase activity.

Studies were carried out to determine whether the effect of dietary protein on transaminase activity in the intact animals was mediated by stimulation of the adrenal glands, or whether protein per se was primarily involved in this metabolic adaptation. Significant increases in enzyme activity occurred in adrenalectomized rats maintained on different levels of dietary protein (Table III). It should be noted, however, that in the adrenalectomized animal, the maximal response with 75 per cent casein was only slightly altered (20 per cent increase) by feeding a 75 per cent protein diet for 1 week.
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Fig. 1. Response of glutamic-pyruvic transaminase in relation to hydrocortisone dosage. The dose indicated was administered daily for 4 days; each point represents the average of individual assays on five animals per group.

Fig. 2. Effect of hydrocortisone (HC) and desoxycorticosterone (DOCA) on the glutamic-pyruvic transaminase activity of rat liver. HC (1.0 mg. per rat per day) and DOCA (5.0 mg. per rat per day) were given by subcutaneous injection for 7 days. The vertical line on each bar represents the standard deviation; five animals were used in each group.

Fig. 3. The effect of protein content of the diet on the activity of glutamic-pyruvic transaminase (GPT). * Base values: expressed as millimoles of substrate utilized per hour.

Percent protein was only about two-thirds of the activity observed in the intact rats fed the same diet. When hydrocortisone was administered to adrenalectomized rats which were fed different levels of protein, a significant but less than maximal increase in GP-transaminase activity was observed with the 0 and 25 percent protein diets, whereas with the 50 and 75 percent protein diets, a maximal response to hydrocortisone was obtained. Thus, the maximal GP-transaminase response in the liver of adrenalectomized rats appears to be attributable to an additive effect of both dietary protein and the administration of hydrocortisone.

**Effect of Diabetes on GP-transaminase Activity**—If gluconeogenesis involves an increase in this transaminase, one might expect to find high activities for this enzyme in the livers of diabetic or fasted animals. The level of this transaminase in the liver of alloxan-diabetic rats is shown in Fig. 4. The diabetic state of the rats was demonstrated by the high blood glucose values obtained on the day the animals were killed. In each animal with uncontrolled diabetes, there was a marked rise in liver GP-transaminase activity after 1 week. Considerable variation in the vigor of the severely diabetic animals seemed to be associated with different ratios of blood sugar to GP-transaminase activity. When the diabetes was controlled by the administration of 0.5 unit of insulin daily, the blood glucose values were kept within the normal range, and the activity of GP-transaminase was maintained at the level observed for untreated nondiabetic rats. In a similar experiment, the activity of GO-transaminase in the liver of rats with un-

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**Table III**

<table>
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<tr>
<th>Protein in diet*</th>
<th>GP-transaminase activity†</th>
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</thead>
<tbody>
<tr>
<td>%</td>
<td>Untreated Hydrocortisone (2.5 mg/day)</td>
</tr>
<tr>
<td></td>
<td>mmoles substrate/gm. protein/hr.</td>
</tr>
<tr>
<td>0</td>
<td>3.04 ± 0.82</td>
</tr>
<tr>
<td>25</td>
<td>4.99 ± 1.2</td>
</tr>
<tr>
<td>50</td>
<td>11.6 ± 1.5</td>
</tr>
<tr>
<td>75</td>
<td>25.9 ± 11</td>
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* The period of the experiment was seven days.
† Standard deviation is given for each value.

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**Fig. 4.** Glutamic-pyruvic transaminase (GPT) activity in the liver of diabetic rats. A single injection of alloxan (175 mg. per kg.) was given intraperitoneally. The animals were killed and the livers were analyzed on the 7th day.

**Fig. 5.** The effect of fasting on the activity of glutamic-pyruvic transaminase of rat liver. The vertical lines through each point represent the standard deviation.
controlled diabetes was in the same range as that of the non-diabetic control animals.

Effect of Fasting on GP-transaminase Activity—When food was withheld from rats for a period of 5 days, a pronounced rise in GP-transaminase activity was noted (Fig. 5). The values after 24 hours of fasting were not increased significantly above the control level in this experiment. In another study a 2-fold rise in activity was found when food was withheld for 24 hours. Residual food in the stomach probably accounts for such variation during the 1st day. The data indicate clearly, however, that between the 2nd and 5th days of fasting a marked rise in the activity of this transaminase in liver occurred. In contrast, the activity of GO-transaminase in the liver of fasted rats was only slightly elevated. During a 5-day period of starvation, the activity of GO-transaminase was 34 per cent higher on the 3rd day and 54 per cent higher on the 5th day than the corresponding nourished control rats.

DISCUSSION

An enhanced rate of gluconeogenesis and negative nitrogen balance are major physiological effects exerted by the glucocorticosteroids. The stimulation of glutamic-pyruvic transaminase activity by treatment with hydrocortisone but not by treatment with DOCA and the high gluconeogenic potency of the substrates of this enzyme (glutamate, alanine, and pyruvate) suggested that the control of hepatic levels of glutamic-pyruvic transaminase by glucocorticosteroids is importantly related to the mechanism whereby these compounds exert their gluconeogenic activity (4). In this investigation hepatic GP-transaminase activity was studied in conditions known to increase the rate of gluconeogenesis. In each of the conditions of high protein intake, diabetes, and fasting, the glutamic pyruvic transaminase activity in liver was greatly increased, whereas in contrast the activity of GO-transaminase was only slightly altered. These observations, as well as the response of GP-transaminase to hydrocortisone, support the interpretation that this enzyme is rate-limiting in gluconeogenesis.

The marked rise in hepatic GP-transaminase activity noted in four conditions, each associated with enhanced gluconeogenesis, strongly suggests the involvement of new enzyme synthesis rather than an activation of the enzyme by combination with the steroid. The parallel results obtained under these different conditions would appear to rule out the release of inhibitors or activators in liver which might affect the assay. It is not possible at this time to say whether hydrocortisone stimulates GP-transaminase activity by directly inducing synthesis of enzyme protein, or whether the increase in enzyme activity follows an initial effect of hydrocortisone at a different site. Conditions which stimulate gluconeogenesis may initially influence amino acid transport or some aspect of protein synthesis or nucleic acid metabolism which would increase the metabolic pools of amino acids resulting in substrate-induced GP-transaminase synthesis. The fact that four different conditions stimulate glutamic-pyruvic transaminase in liver to an equal extent supports the hypothesis that in enzyme synthesis follows some common effect of these treatments, each of which involves a metabolic response to stress.

Significant increases in the activity of GP-transaminase in the liver of the adrenalectomized rats, as well as in normal animals, maintained on high protein rations have been observed. Thus, the initiation of new enzyme synthesis by dietary protein is not mediated exclusively by stimulation of the adrenal glands. Attempts to induce the synthesis of increased amounts of GP-transaminase by intraperitoneal injections of the substrates of this enzyme have been unsuccessful so far. These observations are in contrast to the substrate-induced formation of tryptophan peroxidase and its induction by other amino acids (histidine and tyrosine) mediated by the adrenal glands as a result of stress (14).

An initial physiological response to treatment with glucocorticosteroids involves a rapid increase in the availability of glucose which can result in measurable glycogen deposition within several hours. A secondary response to prolonged hydrocortisone administration or stress results in utilization of tissue protein that can progress to a state of negative nitrogen balance. The maximal response of GP-transaminase to hydrocortisone treatment occurs after several days (4), and such changes can be associated with the utilization of tissue protein. In these studies maximal GP-transaminase response occurred with amounts of hydrocortisone which did not cause loss of body weight. It is of interest that subjects receiving hydrocortisone and patients with Cushing's syndrome were found to have elevated blood levels of pyruvic acid and lactic acid (15-18).

A 5-fold increase in the activity of glutamic-pyruvic transaminase represents the capacity of a liver weighing 10 gm. to metabolize 85 gm. of alanine in 8 hours. Although the amount of substrate metabolized in vivo is clearly less than such calculated capacity, it serves to emphasize that a very small increase in the activity of GP-transaminase could account for the rise in glycogen from 20 to 150 mg./10 gm. of liver observed in the bioassay for glucocorticosteroids. In several experiments of glucocorticosteroids may be of greater significance.

It is a reasonable assumption that the effective regulation of metabolism must involve relatively few rate-limiting reactions. Treatment with glucocorticosteroids increases the activity of a number of enzymes such as dipetidase (20), glucose 6-phosphatase (21), proline oxidase (22), tryptophan peroxidase (23, 24) and picolinic carboxylase (25). With the exception of the latter enzyme, for which a substrate in vivo is unknown, the changes in each case are much smaller than the several-fold increase noted with pyruvic transaminase after such treatment. Furthermore, with the exception of glucose 6-phosphatase, it is difficult to relate these enzymatic changes to the physiological effects produced by this class of hormones. GP-transaminase seems to occupy a key position in the metabolic interrelationships involving gluconeogenesis and in intermediary protein and carbohydrate metabolism. In the closely integrated systems in which pyruvic acid is a pivotal intermediate the increased availability of this substrate can be associated with multiple effects concerned directly via the Krebs' cycle with energy production as well as with the increased availability of glucose for energy or glycogen synthesis. Sayers (26) has expressed the opinion that a single energy-yielding biochemical reaction may underlie the diverse physiological responses elicited...
by the glucocorticosteroids. Since thymus gland and Walker carcinoma as well as liver, show increased GP-transaminase activity after hydrocortisone treatment, it becomes important to extend this correlation and to define the role of this transaminase in the metabolic responses to glucocorticosteroids.

SUMMARY

1. Rats treated with hydrocortisone, cortisone, or Prednisone daily for 1 week showed marked increases (6- to 13-fold) in liver glutamic-pyruvic transaminase activity, calculated per gm. of liver, per gm. of liver protein, or on the basis of the total weight of the liver.

2. The glutamic-pyruvic transaminase activity of liver was found to be directly related to the protein content of the ration. The specific activity of the enzyme in the liver of rats fed diets supplying 50 or 75 per cent protein was increased 4- and 7-fold, respectively, in comparison to control animals on a ration lacking in protein. Smaller but highly significant increases in this transaminase were observed when diets containing less than 50 per cent protein were fed. The effect of dietary protein on the activity of this enzyme was demonstrated in adrenalectomized rats.

3. In alloxan-diabetic rats, the activity of this enzyme was increased to a level equivalent to that observed in other experiments with hydrocortisone treatment or high protein feeding. This effect was prevented by administration of insulin.

4. A pronounced rise in the level of glutamic-pyruvic transaminase of rat liver occurred when food was withheld. After 48 hours there was a 2-fold increase, whereas a 5-fold increase occurred after 120 hours of fasting.

5. Glutamic-pyruvic transaminase activity in liver was 5 to 7 times higher than normal in rats which were fasted, fed high protein diets, or made diabetic by treatment with alloxan. In contrast, glutamic-oxaloacetic transaminase was only slightly altered under these conditions. The significance of an increase in glutamic-pyruvic transaminase activity is discussed in relation to this metabolic response to stress.

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