Liver Microsomal Glucose 6-Phosphatase, Inorganic Pyrophosphatase, and Pyrophosphate-Glucose Phosphotransferase

IV. EFFECTS OF ADRENALECTOMY AND CORTISONE ADMINISTRATION ON ACTIVITIES ASSAYED IN THE ABSENCE AND PRESENCE OF DEOXYCHOLATE*

ROBERT C. NORDLIE, WILLIAM J. ARION,† AND ERIC A. GLENDE, JR.‡

From the Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota Medical School, Grand Forks, North Dakota

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Levels of liver microsomal glucose 6-phosphatase (Reaction 1) are significantly stimulated in the diabetic or glucocorticoid-treated animal, and they are depressed after insulin administration or adrenalectomy (see, for example, References 1 to 8). This hydrolytic activity may play a physiologically significant role in the regulation of blood glucose and liver glycogen levels (1, 2, 9). Recent studies reported from our laboratory (10, 11) and by Stetten (12) and Stetten and Taft (13) indicate that inorganic pyrophosphatase hydrolysis (Reaction 2) and glucose 6-phosphate synthesis via the pyrophosphate-glucose phosphotransferase reaction (Reaction 3) appear to be catalyzed by this same microsomal enzyme. The presence of nucleoside triphosphate- and nucleoside diphosphate-glucose phosphotransferase activities (Reactions 4 and 5) in deoxycholate-treated rat liver microsomes and kidney homogenates is documented in Paper III of this series (14). Levels of these microsomal phosphotransferases have been found to rise in alloxan diabetes and to decrease after insulin administration (14).

Enzymic Assays—Sources of chemicals and analytical procedures were those employed by us in previous studies (10, 11, 14). Homogenates were thawed at 0°C just prior to assay. Specific composition of assay mixtures is described below. Linearity of reactions with time and with protein concentration under these conditions (pH 5.5) employed for phosphotransferase activity assay was also demonstrated.

Assay reaction mixture volumes were 1.5 ml in all instances. Incubations were carried out for 10 min at 30 ± 0.1°C in a shaking, thermostatically regulated water bath. Specific composition of assay mixtures for experiments described in the indicated
Enzymic activity per mg of protein

Average mg of protein per g of liver.

Average body weight (g).

Number of animals.

Average liver weight (g).

Average mg of protein per g of liver.

Enzymic activity* per mg of protein

PP1-glucose phosphotransferase

Deoxycholate absent.

Deoxycholate present.

Inorganic pyrophosphatase

Deoxycholate absent.

Deoxycholate present.

Glucose 6-phosphatase

Deoxycholate absent.

Deoxycholate present.

RESULTS

Effects of Hormonal Alterations on Enzymic Activities

Experiment I—Tables I and II depict the results of an experiment in which PP1-glucose phosphotransferase, inorganic pyrophosphatase, and glucose 6-phosphatase levels were measured in liver homogenates prepared from control, cortisone-treated control, adrenalectomized, and cortisone-treated adrenalectomized rats. The animals averaged 236 g at the initiation of the experiment. Assays were carried out in duplicate in the absence and presence of deoxycholate. Final deoxycholate concentration in homogenates was 0.2%, w/v (5.0 mM), in all instances, since this concentration was found to give maximal activation of the PP1-glucose phosphotransferase activity (see Fig. 1). Table I depicts results of measurements of the various enzymic activities in assay mixtures of constant composition for each reaction. All results were submitted to statistical analysis (19), and they are expressed in terms of average activity per mg of protein ± standard deviation. The t test of significance (19) was applied to average activity values for the variously treated groups of animals, and p values (the probability of observed differences being due purely to chance) are recorded in this and other tables. Differences in averages for the groups of animals for which p < 0.05 are considered highly significant statistically. Enzymic activities, determined with reaction mixtures of fixed composition (Table I), were depressed slightly after adrenalectomy, and were elevated significantly by the

**TABLE I**

Effects of adrenalectomy and cortisone administration on liver PP1-glucose phosphotransferase, inorganic pyrophosphatase, and glucose 6-phosphatase levels

<table>
<thead>
<tr>
<th>Experimental animal</th>
<th>Control + cortisone</th>
<th>Control</th>
<th>Adrenalectomized</th>
<th>Adrenalectomized + cortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Average body weight (g)</td>
<td>203 ± 9e, b</td>
<td>274 ± 22e</td>
<td>257 ± 21b</td>
<td>209 ± 26</td>
</tr>
<tr>
<td>Average liver weight (g)</td>
<td>11.1 ± 0.9a</td>
<td>11.0 ± 0.9b</td>
<td>14.5 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Average mg of protein per g of liver</td>
<td>130 ± 9a</td>
<td>145 ± 8</td>
<td>153 ± 6</td>
<td>134 ± 6</td>
</tr>
<tr>
<td>Enzymic activity* per mg of protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP1-glucose phosphotransferase</td>
<td>0.31 ± 0.03a</td>
<td>0.17 ± 0.014</td>
<td>0.15 ± 0.02b</td>
<td>0.31 ± 0.04</td>
</tr>
<tr>
<td>Deoxycholate absent</td>
<td>0.58 ± 0.11</td>
<td>0.59 ± 0.16</td>
<td>0.59 ± 0.09</td>
<td>0.62 ± 0.06</td>
</tr>
<tr>
<td>Deoxycholate present</td>
<td>0.70 ± 0.08a</td>
<td>0.67 ± 0.20</td>
<td>0.65 ± 0.07</td>
<td>0.65 ± 0.10</td>
</tr>
<tr>
<td>Inorganic pyrophosphatase</td>
<td>0.64 ± 0.15</td>
<td>0.54 ± 0.04</td>
<td>0.70 ± 0.09</td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>Glucose 6-phosphatase</td>
<td>0.73 ± 0.10a</td>
<td>0.73 ± 0.13</td>
<td>0.73 ± 0.10a</td>
<td>0.73 ± 0.13</td>
</tr>
</tbody>
</table>

* Standard deviation, σ = \( \sqrt{\frac{e^2 - (\bar{e})^2}{N}} \), N - 1
b p < 0.01.
c p > 0.05, unless noted otherwise.
d p = 0.01 to 0.05. p notation applies between footnoted value and value in succeeding column to the right.

a Activities in this, and all other tables, are expressed in terms of micromoles of substrate hydrolyzed, or micromoles of glucose-6-P formed, per 10-min incubation.
Michaelis constants and activities based on extrapolated $V_{max}$ or apparent $V_{max}$ values

<table>
<thead>
<tr>
<th>Experimental animal</th>
<th>Deoxycholate absent</th>
<th>Deoxycholate present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adrenalectomized</td>
<td>Adrenalectomized +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cortisone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adrenalectomized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ cortisone</td>
</tr>
<tr>
<td>Michaelis constants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{PPi}$, glucose phosphotransferase</td>
<td>$12 \pm 3^a$</td>
<td>$11 \pm 2$</td>
</tr>
<tr>
<td>$K_{PPi}$, glucose phosphotransferase</td>
<td>$87 \pm 17$</td>
<td>$93 \pm 13$</td>
</tr>
<tr>
<td>Inorganic pyrophosphatase: $K_{PPi}$</td>
<td>$10 \pm 0.3$</td>
<td>$11 \pm 1$</td>
</tr>
<tr>
<td>Glucose 6-phosphatase: $K_{glucose-6-P}$</td>
<td>$6.2 \pm 0.6$</td>
<td>$6.8 \pm 0.2$</td>
</tr>
</tbody>
</table>

Activity per mg of protein

<table>
<thead>
<tr>
<th></th>
<th>PPi-glucose phosphotransferase; PPi concentration varied</th>
<th>Glucose 6-phosphatase</th>
<th>PPi-glucose phosphotransferase; glucose concentration varied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$0.23 \pm 0.03^a$</td>
<td>$0.63 \pm 0.04^a$</td>
<td>$0.50 \pm 0.09$</td>
</tr>
<tr>
<td></td>
<td>$0.26 \pm 0.06^a$</td>
<td>$0.58 \pm 0.09$</td>
<td>$1.1 \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>$0.65 \pm 0.04^a$</td>
<td>$0.93 \pm 0.10$</td>
<td>$0.93 \pm 0.09$</td>
</tr>
<tr>
<td></td>
<td>$0.62 \pm 0.08^a$</td>
<td>$0.82 \pm 0.09$</td>
<td>$0.82 \pm 0.09$</td>
</tr>
</tbody>
</table>

$^a$ All Michaelis constants are expressed as millimolar values.

$^b$ $p > 0.05$, unless noted otherwise.

$^c$ $p < 0.01$.

$^d$ $p = 0.01$ to 0.05. $p$ notation applies between footnoted value and value in succeeding column to the right.

administration of cortisone to control or adrenalectomized animals when assays were carried out in the absence of deoxycholate. However, no statistically significant change was observed when the assays were conducted in the presence of added deoxycholate.

An increase in apparent Michaelis constant, as well as total glucose 6-phosphatase activity, has been noted in diabetic rats; the effect was reversed by insulin administration to diabetic animals (3). Because of the possibility of the effects of hormonal states on Michaelis constants, our standard assay mixtures (Table I) were designed to contain relatively high concentrations of substrates. However, to investigate further the possibility of changes in Michaelis constants after hormonal alterations, we carried out kinetic analyses of enzymic activities with those preparations employed in the studies described in Table I. Preparations from four animals in each of the adrenalectomized and cortisone-treated adrenalectomized groups were utilized for kinetic analyses, since the largest variations in activity levels were observed after cortisone-treatment of adrenalectomized animals. Reaction mixtures employed are described under “Experimental Procedure.” Five different concentrations of substrate, in the ranges described above, were utilized in each instance. For the PPi-glucose phosphotransferase system, studies were carried out both with glucose concentrations maintained constant and varied PPi levels, and vice versa. Michaelis constants$^1$ and maximal reaction velocity ($V_{max}$) or apparent $V_{max}$ values were calculated from Lineweaver-Burk (20) plots of data obtained. Results are presented in Table II. No significant change in Michaelis constants due to cortisone treatment of adrenalectomized animals was observed. However, the addition of deoxycholate resulted in a decrease in $K_{PPi}$ in both phosphotransferase and pyrophosphatase reactions to approximately 33% of values observed in the absence of the bile salt, and an approximately 50% decrease in $K_{glucose-6-P}$ for the phosphotransferase reaction was not appreciably altered. Activities calculated on the basis of extrapolated $V_{max}$ (pyrophosphatase and glucose 6-phosphatase) and extrapolated apparent $V_{max}$ (phosphotransferase) values showed the same

![Fig. 1. Effects of deoxycholate (DOC) concentration of PPi-glucose phosphotransferase activity in liver homogenates from adrenalectomized (O-O) and cortisone-treated adrenalectomized (●-●) rats. Treatment of animals and preparation of homogenates was as described in the text. Various concentrations of sodium deoxycholate solution were added to freshly prepared homogenates, which were then allowed to stand for 30 min at 0°, and the phosphotransferase activity was then assayed. The assay reaction mixture composition was as described in the text for the experiment depicted in Table I. Results are averages for two animals in each group. Maximal activation was also obtained with 0.0 mm of deoxycholate with homogenates from untreated control animals.](http://www.jbc.org/content/3481/2/1)

$^1$ Previous kinetic studies (11) have indicated that for the PPi-glucose phosphotransferase reaction, Michaelis constants for either substrate are independent of the concentration of the second substrate.
patterns of change due to hormonal administration as did those activities determined with assay mixtures of constant composition (Table I).

Experiment I. The second major experiment, the results of which are described in Table III, was performed (a) to include activity assays with isolated microsomes as the enzyme source, (b) to include assays of CDP-glucose phosphotransferase activity which was discovered (14) in microsomes after completion of the earlier hormonal experiment (Experiment I), and (c) to provide for assay of glucose-6-P hydrolysis at pH 6.5, which is more characteristic (21) of microsomal glucose 6-phosphatase than is pH 5.5. PPi-glucose phosphotransferase and pyrophosphatase activities were assayed in homogenates both to confirm earlier findings and to provide a basis for comparison of results obtained with microsomal preparations. Since the most dramatic changes in the earlier experiments were those due to cortisone treatment of adrenalectomized rats, cortisone was administered to four of a group of ten adrenalectomized animals. In this instance, the initial average body weight was 174 g. The results obtained with both microsomes and homogenates were quite similar to those observed in the earlier experiment (compare results in Tables I and II, Experiment I, with those in Table III, Experiment II). That is, significant increases in all of the activities were noted after cortisone administration, when assays were carried out in the presence of deoxycholate. These differences were, however, due in part to lack of growth or actual loss of weight in those animals receiving the glucocorticoid (see "Body Weights" in Table I).

To determine whether cortisone added in vitro would stimulate enzymic activity, the following experiment was carried out. Cortisone was dissolved in 50% v/v, aqueous propylene glycol and added in varying amounts to homogenates just as was done routinely with deoxycholate in the earlier experiments discussed above. Vehicle concentration was maintained constant. Under these conditions, cortisone (1.25 \( \mu \)M, 1.25 \( \times 10^{-5} \) M, 1.25 \( \times 10^{-4} \) M, or 6.25 \( \times 10^{-4} \) M in homogenates) was without effect, although 1.25 mM hormone stimulated phosphotransferase or glucose 6-phosphatase activity approximately 10%. Similar results were obtained whether control, adrenalectomized, or cortisone-treated adrenalectomized animals were employed. Clearly, the hormone must be administered in vivo to produce the observed effects described in Tables I to III.

Effects of Hormonal Alterations on Lipid and Lipid Phosphorus Levels

Krahl (23) has proposed as a working hypothesis that insulin (deprivation or administration to diabetic animals) may exert its effects on glucose metabolism by affecting the state or amount of insulin receptors in the membrane of the cell. If this were true, one would expect a change in the functional state of the receptor sites caused by hormonal changes. The changes in histology described by Krahl, however, are not consistent with the concept of microsomal activity-structure relationships proposed by Ernst, Siekevitz, and Palade (22).

The lesser apparent recovery of homogenate activity in isolated microsomes, when assays were performed in the absence of deoxycholate, suggests that the isolated, frozen fragments of the endoplasmic reticulum undergo a change in physical form which in turn makes unavailable many active enzymic sites. The addition of deoxycholate, or to some extent the presence of certain cellular constituents in the homogenates, either reverses or may partially modify this process, thus unmasking the catalytic sites. Such a modification of activity by alterations in the physical state of the particulate body with which the enzyme is associated is consistent with the concept of microsomal activity-structure relationships proposed by Ernst, Siekevitz, and Palade (22).

agreement with the findings of Segal and Washko (3) with glucose 6-phosphatase. The parallelism of microsomal enzymic activity variations with responses observed with whole liver homogenates indicates that the effects observed did not involve simply some factor or factors present in the extramicrosomal portion of the liver.

All of the activities recorded in Tables I to III were also reevaluated, in supplementary calculations not otherwise presented in this paper, on the basis of total liver activity per g of body weight. With the data expressed in this fashion, statistically significant differences in activity levels after cortisone administration to adrenalectomized animals persisted even when assays were carried out in the presence of deoxycholate. These differences were, however, due in part to lack of growth or actual loss of weight in those animals receiving the glucocorticoid (see "Body Weights" in Table I).

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of lipid or lipoprotein associated with, for example, microsomal glucose 6-phosphatase. The work of Beaufay and de Duve (24), and more recently Ganoza and Byrne (25, 26), has revealed that certain lipids are necessary for this enzymic activity, and that the nature of the phospholipid associated with this enzyme affects the specificity of the reaction catalyzed. We (14) recently found a significant decrease in liver lipid phosphorus content in alloxan-diabetic rats, which was elevated to above untreated control levels after administration of insulin to these animals. The differences in relative levels of enzymic activities noted in the present experiments, when assays were performed in the absence as compared with presence of deoxycholate, a well known "dispersing" agent, also suggested the involvement of lipids or phospholipids in the cortisone response mechanism. Hence, aliquots of both liver homogenates and microsomal preparations were assayed for total lipid and lipid phosphorus content. Homogenates assayed were those used for the investigations depicted in Tables I and II, while the microsomal preparations were those employed in the activity studies described in Table III. Results of these analyses are presented in Table IV. Most significantly, the administration of cortisone to adrenalectomized animals produced a decrease in homogenate lipid phosphorus levels. Both microsomal total lipid and lipid phosphorus per g of microsomal protein were markedly depressed in hormone-treated animals. However, in no instance was a change greater than 18% observed in homogenate total lipid content.

### TABLE IV

**Total lipid and lipid phosphorus concentrations in liver homogenates and microsomes**

<table>
<thead>
<tr>
<th>Experimental animal</th>
<th>Homogenates</th>
<th>Microsomes</th>
<th>Homogenates</th>
<th>Microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg total lipid/g protein</td>
<td>umoles lipid phosphorus/g protein</td>
<td>mg total lipid/g protein</td>
<td>umoles lipid phosphorus/g protein</td>
</tr>
<tr>
<td>Control + cortisone</td>
<td>350 ± 76</td>
<td>220 ± 15</td>
<td>340 ± 76</td>
<td>210 ± 15</td>
</tr>
<tr>
<td>Control</td>
<td>310 ± 24</td>
<td>260 ± 17</td>
<td>300 ± 24</td>
<td>250 ± 17</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>280 ± 39</td>
<td>410 ± 32</td>
<td>270 ± 12</td>
<td>400 ± 39</td>
</tr>
<tr>
<td>Adrenalectomized + cortisone</td>
<td>280 ± 57</td>
<td>210 ± 32</td>
<td>200 ± 29</td>
<td>210 ± 31</td>
</tr>
</tbody>
</table>

* p > 0.05, unless otherwise noted.

### DISCUSSION

The similarity of responses of levels of glucose 6-phosphatase, inorganic pyrophosphatase, and PP1-glucose phosphotransferase to adrenalectomy and cortisone administration provides additional evidence supporting the contention (10-13) that these three activities are catalyzed by the specific liver microsomal glucose 6-phosphatase. Various microsomal nucleoside diphosphate and triphosphate-glucose phosphotransferase activities have also been found to be similar in many respects to the microsomal PP1-glucose activity (14). It is significant to note that CDP-glucose phosphotransferase levels also responded in these studies in a manner similar to that of the other three activities.

The pattern of results obtained in the absence of added deoxycholate is consistent with observations on the effects of adrenalectomy and gluocorticoid administration on levels of both glucose 6-phosphatase (1-8) and acid inorganic pyrophosphatase (27). The absence of any effect due to adrenalectomy or cortisone on apparent $K_m$ values for phosphate substrates, as well as the lowering of these values in the presence of deoxycholate, is also in agreement with previous reports concerning microsomal glucose 6-phosphatase (3, 8). However, the unexpected finding, that statistically significant differences in specific activity levels in the adrenalectomized and cortisone-treated animals disappeared when the assays were conducted in the presence of deoxycholate, raises the question of whether the response to glucocorticoid treatment observed in the usual, detergent-free assay system employed for glucose 6-phosphatase represents an absolute increase in new enzyme protein, or whether cortisone produces some change in the already existing enzyme which results
in the elevation of activity measurable in the conventional assay system. The data suggest that the latter may be the principal effect. Further supporting this idea is the observation that stimulation of activity by deoxycholate was greatest with preparations from untreated adrenalectomized animals and least with cortisone-treated control animals.

The role of lipid in glucose 6-phosphatase activity has been mentioned under "Results." In view of the known phospholipid requirement of this enzyme (24-26), it is difficult to correlate the observed hormonally-induced apparent elevation of enzymic activities observed in the absence of deoxycholate with the marked depression of microsomal lipid and lipid phosphorus content noted after cortisone administration. Similar relationships between lipid phosphorus and enzymic activity levels were observed in earlier alloxan diabetes-insulin studies (14).

However, in contrast with the noted effects of cortisone, elevation of phosphatase and phosphotransferase activity levels in diabetic animals persisted in the presence of deoxycholate (14). Ashmore and Nesbett (28) have reported similar observations with glucose 6-phosphatase from control and diabetic rats. It appears tenable from the present studies that cortisone may exert its effect on glucose 6-phosphatase (and associated phosphatase and phosphotransferase activities) by regulating the synthesis of unknown enzymes which in turn function to alter in nature or amount a compound, possibly a phospholipid, intimately associated with the catalytic mechanism of this phosphatase. Such an effect would be prevented by concurrent administration of actinomycin D or puromycin with the hormone, a treatment which has been shown (4) to suppress the response of this enzyme to glucocorticoid treatment. Alternatively, our observations also are consistent with the possibility of a selective inhibition by deoxycholate of that glucose 6-phosphatase phosphotransferase newly synthesized in response to cortisone therapy. Whatever the ultimate explanation for the observed differences, it is clear that, as Segal and Washko (3) have stated, "the effect of cortisone administration in increasing glucose 6-phosphatase activity appears to involve a different mechanism from that of insulin deprivation . . . " This conclusion can now be extended to include the recently-characterized inorganic pyrophosphatase, PPi-glucose phosphotransferase, and nucleotide-glucose phosphotransferase activities associated with this enzyme.

**Summary**

The effects of adrenalectomy and cortisone administration on rat liver pyrophosphatase glucose phosphotransferase, inorganic pyrophosphatase, glucose 6-phosphatase, and cytidine diphosphate-glucose phosphotransferase activities have been studied. All activities were depressed moderately after adrenalectomy, and they were significantly elevated by administration of cortisone, when assays were carried out in the absence of added deoxycholate. However, when deoxycholate was added to homogenates, to a final concentration of 5.0 mM, before assay, no statistically significant differences in specific activity levels in the variously-treated groups of animals were observed. Similar patterns of results were obtained when activities were calculated from apparent maximal reaction velocity values or when isolated microsomes rather than homogenates were employed as enzyme source. Michaelis constants for phosphate substrates (but not glucose) were reduced to 33 to 50% of original values when deoxycholate was present, but they were not altered significantly by hormonal manipulations, whether calculated from activity values based on assays conducted in the absence or presence of the bile salt. These findings support the common identity of these liver microsomal phosphatase and phosphotransferase activities, and they indicate basic differences in the mechanism of responses to cortisone administration and experimental diabetes.

**REFERENCES**

Liver Microsomal Glucose 6-Phosphatase, Inorganic Pyrophosphatase, and Pyrophosphate-Glucose Phosphotransferase: IV. EFFECTS OF ADRENALECTOMY AND CORTISONE ADMINISTRATION ON ACTIVITIES ASSAYED IN THE ABSENCE AND PRESENCE OF DEOXYCHOLATE


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