THE EFFECT OF CRYSTALLINE ADRENAL CORTICAL STEROIDS, DL-THYROXINE, AND EPINEPHRINE ON THE ALKALINE AND ACID PHOSPHATASES AND ARGINASE OF THE LIVER AND KIDNEY OF THE NORMAL ADULT RAT*

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(Received for publication, May 7, 1948)

Aqueous adrenal cortical extract (Upjohn), but not desoxycorticosterone acetate, produces a remarkable increase in the "alkaline" phosphatase of the liver of the adrenalectomized rat (1-3). This effect is apparently related to glyconeogenesis from endogenous protein (4). The extract, however, contains in addition to the C11 steroids many other substances including epinephrine1 (less than 1:800,000), which is able to increase liver glycogen with lactic acid as a precursor. It became important, therefore, to determine whether this enzyme phenomenon was a specific property of C11 adrenal cortical steroids and also to obtain information as to in what phase in the glyconeogenic process this enzyme was involved. As a means to these ends, a comparison has been made of the effect of aqueous adrenal cortical extract with a crystalline mixture of hog adrenal cortical steroids (Upjohn's lipoextract), 2 11-dehydrocorticosterone acetate (Merck, synthetic), 3 and epinephrine. Furthermore, a study with thyroxine has been included because it is known to accelerate glycogenolysis due to an increase in energy demands.

At the same time the arginase activity of the liver was studied in order to obtain further information as to the apparent discrepancy between the increase observed by Fraenkel-Conrat et al. (5) and the lack of increase noted in this laboratory (4) after administration of adrenal cortical extracts to adrenalectomized rats.

Procedure

Male rats of the Sprague-Dawley strain were placed in individual cages in an air-conditioned room at 25.5-26.6° and fed 10 gm. per day of a pre-

* This investigation was aided by grants from the Josiah Macy, Jr., Foundation
1 Personal communication, Dr. D. J. Ingle, The Upjohn Company.
2 The lipoextract (Research No. 8120) was provided by Dr. M. H. Kuizenga of The Upjohn Company on June 21, 1943, and was kept at room temperature. The experiments in this paper were carried out on February 16, 1948.
3 The synthetic 11-dehydrocorticosterone acetate was provided by Merck and Company on December 19, 1947, and dissolved at 5 mg. per ml. in sesame oil containing 10 per cent benzyl alcohol.
pared diet composed of casein 16.7, sucrose 61.2, hydrogenated vegetable oil 7.4, yeast (Fleischmann's 2019) 9.2, Cellu flour 1.8, Wesson's salt mixture 3.7 (6), and as a daily supplement 1 drop of cod liver oil and 1 drop of a 34 per cent tocopherol concentrate of wheat germ oil diluted 10-fold with Wesson oil. The rats were kept 3 to 4 weeks on this regimen before the experiments were carried out.

The adrenal cortical hormones were administered according to the procedure of Reinecke and Kendall (7). The epinephrine was injected 1 hour before autopsy as a single 0.05 ml. subcutaneous dose of a 1:1000 commercial preparation of the hydrochloride (Parke, Davis and Company). The dl-thyroxine (Roche-Organon, synthetic) was dissolved in a small amount of 0.02 N sodium hydroxide and then made to 2.5 mg. per ml. with water. The rats lost weight rapidly with the initial dose during the first 3 days; therefore, the amount was reduced to 0.25 mg. per day for the remaining 2 days. The last injection was made 24 hours before autopsy.

At the end of the experiments the animals were anesthetized by the intraperitoneal injection of 0.3 ml. of dial-urethane, and the liver was removed and rapidly weighed on a Roller Smith torsion balance. The left segment of the median lobe was used for the enzyme studies (2, 4). The remainder was placed immediately into 5 ml. of hot 30 per cent potassium hydroxide and analyzed for glycogen by the Good-Kramer-Somogyi technique (8), except that the hydrolyzed glycogen was neutralized to phenolphthalein (9). The recently modified Somogyi method (10) was used to determine the reducing substance and the results are expressed as glucose.

The kidneys were removed, weighed, and the left one used for the enzyme studies.

The nitrogen content of the organs of the rats treated with dl-thyroxine and epinephrine was determined by the micro-Kjeldahl technique on aliquots of the enzyme homogenates.

Results

Adrenal Cortical Hormone (Table I)—There was the expected formation of liver glycogen under the stimulation of these hormone preparations. The most effective material in the doses used was the lipoextract, then the aqueous extract, and finally the synthetic 11-dehydrocorticosterone acetate. The "alkaline" phosphatase of the liver was greatly increased and to approximately the same extent for each adrenal cortical preparation. There was no parallelism with the degree of glycogenesis.

The arginase activity and also the "acid" phosphatase of the liver were not significantly altered.

4 The wheat germ oil concentrate was provided by Distillation Products, Inc., through the courtesy of Dr. P. L. Harris.

6 The dial-urethane was provided by Ciba Pharmaceutical Products, Inc.
Epinephrine (Table II)—The injection of epinephrine produced within 1 hour a tremendous deposition of glycogen in the liver of rats fasted for 18 hours, which was accompanied by a proportionate decrease in the per cent but not total nitrogen (protein) content.

The arginase and “alkaline” phosphatase were not significantly altered.

TABLE I

Effect of C11 Adrenal Cortical Steroids on Composition and Enzymes of Livers of Male Rats (250 to 259 gm.)

Each group consisted of four rats.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Liver Glycogen total</th>
<th>&quot;Alkaline&quot; phosphatase*</th>
<th>Arginase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>mg.</td>
<td>units per gm. per cent†</td>
</tr>
<tr>
<td>Control</td>
<td>6640</td>
<td>22</td>
<td>(3.3)</td>
</tr>
<tr>
<td>Adrenal cortical extract‡</td>
<td>8 X 1 ml. per hr.</td>
<td>7440</td>
<td>118 +164</td>
</tr>
<tr>
<td>Lipoextract§</td>
<td>8 X 0.5 ml. per hr.</td>
<td>7060</td>
<td>154 +161</td>
</tr>
<tr>
<td>11-Dehydrocorticos-</td>
<td>8 X 1 mg. per hr.</td>
<td>6270</td>
<td>46 +171</td>
</tr>
<tr>
<td>terone acetate (syn-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thetic)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* No effect on “acid” phosphatase of liver or enzymes of kidney.
† Per cent change from control values which are given in parentheses.
‡ Aqueous, Upjohn.
§ Lipoextract = Upjohn’s adrenal cortical extract. 1 ml. is equivalent to 2 mg. of 11-dehydro-17-hydroxycorticosterone.
‖ Dissolved at 5 mg. per ml. in sesame oil containing 10 per cent benzyl alcohol.

TABLE II

Effect of Epinephrine and Thyroxine on Composition and Enzymes of Livers of Male Rats

Each group consisted of five rats.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Body weight</th>
<th>Liver</th>
<th>Glycogen total</th>
<th>Nitrogen total</th>
<th>&quot;Alkaline&quot; phosphatase*</th>
<th>Arginase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g.m.</td>
<td>mg.</td>
<td>mg.</td>
<td>per cent</td>
<td>units per gm. per cent†</td>
<td>units per gm. per cent†</td>
</tr>
<tr>
<td>Control</td>
<td>287</td>
<td>8230</td>
<td>120</td>
<td>208 3.63</td>
<td>(3.0)</td>
<td>(11,310)</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.05 mg.†</td>
<td>286</td>
<td>9510</td>
<td>613</td>
<td>279 2.95</td>
<td>-5</td>
</tr>
<tr>
<td>(3 x 0.5 mg. per day)</td>
<td>288$</td>
<td>710</td>
<td>7</td>
<td>284 3.71</td>
<td>-33</td>
<td>+3</td>
</tr>
<tr>
<td>(2 x 0.25 mg. per day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Per cent change from controls which are given in parentheses.
† The autopsy was performed 1 hour after injection.
‡ The body weight was 278 gm. at autopsy.
DL-Thyroxine (Table II)—The animals injected with thyroxine lost 10 gm. of their body weight with a slight but not significant increase in urinary nitrogen. The liver glycogen was extremely low but the nitrogen (protein) content was comparable with that of the control rats.

The "alkaline" phosphatase was somewhat decreased, but the arginase was not affected.

The weight of the kidney as was expected (cf. (11)) increased 22 per cent. Part of this increase was due probably to fat deposition (12). The kidneys were putty-colored. It is of interest that the kidneys increased in size while the rats were on a constant food intake. In previous observations (11) the rats were fed ad libitum.

In all of the above experiments there was no change in the "acid" phosphatase activity of the liver or any of the enzymes of the kidney.

DISCUSSION

The similar increases in "alkaline" phosphatase of the liver of the rat after administration of aqueous adrenal cortical extract, lipoextract, and synthetic 11-dehydrocorticosterone acetate indicate that this phenomenon is a property of the S hormones of the adrenal cortex.

The inability of either epinephrine or thyroxine to influence the level of activity of this enzyme in the liver in spite of their marked glycogenic and glycogenolytic properties provides indirect evidence that the enzyme may be concerned with the endogenous protein or amino acid phase of glyconeogenesis. This hypothesis gains further support from the fact that neither a high protein nor a high carbohydrate diet will produce comparable increases in the "alkaline" phosphatase of the liver (3).

The inability of any of the C_11 steroid preparations to change significantly the arginase level of the liver of the normal rat supports the results obtained in the adrenalectomized rat (3, 4). These data, therefore, provide further evidence that a change in liver arginase level is not essential for glyconeogenesis from protein under the stimulation of the C_11 steroids as suggested by Fraenkel-Conrat et al. (5, 13). Therefore, the decrease in the level of this enzyme after adrenalectomy (5, 4, 14) and hypophysectomy (13) must be due to some other factor or group of factors (3, 4). It is of immediate interest that the decrease in liver arginase after hypophysectomy occurs in spite of an enhanced protein catabolism. Furthermore, intense glycosuria and glyconeogenesis during alloxan diabetes are not accompanied by any change in the arginase activity of the rat liver.

6 The addition of aqueous adrenal cortical extract to the homogenate of normal rat liver does not produce an increase in the "alkaline" phosphatase activity (preliminary experiments).

7 Unpublished.
The failure of thyroxine to affect liver arginase is in agreement with the negative results obtained by Lightbody and Kleinman (15). It is of interest that Fraenkel-Conrat et al. (13) found that the thyrotropic hormone was ineffective in short term experiments but produced a small decrease in hypophysectomized rats after 10 days of treatment. Similar results were obtained with thyroxine.

The failure of any of the C\textsubscript{11} steroids to influence the enzymes of the kidney of the normal rat is not surprising. The small changes observed in the adrenalectomized rat (2, 4, 14) were in all probability restorative in nature.

**Summary**

Aqueous (beef) adrenal cortical extract, lipoextract (hog adrenals), and 11-dehydrocorticosterone acetate produced very marked increases in the "alkaline" (pH 9.8) phosphatase of the liver of fasted rats when injected eight times at hourly intervals. The increase in enzyme activity did not parallel the degree of glyconeogenesis. Thyroxine produced a marked depletion of liver glycogen and a decrease in the enzyme. Epinephrine produced a tremendous deposition of liver glycogen but did not affect the activity of the enzyme.

In none of the above treatments were the activities of the arginase and "acid" (pH 5.4) phosphatase of the liver or the enzymes of the kidney altered.

**Bibliography**

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