THE EFFECT OF THE PITUITARY ADRENOCORTICO-
TROPIC HORMONE AND OF VARIOUS ADRENAL
CORTICAL PRINCIPLES ON INSULIN HYPO-
GLYCEMIA AND LIVER GLYCOGEN

BY J. F. GRATTAN AND H. JENSEN

(From the Biochemistry Laboratory, The Squibb Institute for Medical
Research, New Brunswick)

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In a recent publication we presented experimental evidence
indicating that the anti-insulin\(^1\) (glycotropic) effect of the anterior
pituitary may be attributed to the adrenocorticotropic principle
of that gland (1). We also reported that injection of desoxycorti-
costerone acetate, in contrast to corticosterone acetate, did not
produce a significant anti-insulin effect under the same experi-
mental conditions. In order to determine which of the different
adrenal cortical principles exert a marked influence on carbo-
hydrate metabolism and which do not, various steroids chem-
ically related to corticosterone were studied with respect to their
anti-insulin effect and their influence on liver glycogen. From
the results of other investigators and our own data it appears
that quantitative and also probably qualitative differences exist
among the different adrenal cortical principles, with regard to their
effect on carbohydrate metabolism.

EXPERIMENTAL

Anti-Insulin Test—The procedure followed in determining the
anti-insulin effect of the various compounds in mice was the same
as that previously employed by Jensen and Grattan (1).

The adrenocorticotropic preparations used in the various tests
contained approximately one Moon unit per 5 to 10 mg. (2).

\(^1\) The term "anti-insulin" as employed by us refers only to the ability of
a substance to counteract the hypoglycemia subsequent upon injection of
insulin into an animal.
They were found to be free of lactogenic, thyrotropic, and gonadotropic effects at the dose levels employed in our experiments and were injected in aqueous solution at pH 7.5. The various steroid compounds were injected in corn oil at the following concentrations: corticosterone and its acetate, desoxycorticosterone and its acetate, and progesterone, 0.5 mg. per 0.2 cc.; desoxycorticosterone and its acetate, progesterone, and methyltestosterone, 2.0 mg. per 0.2 cc.; methylandrostenediol, ethynylandrostenediol, ethynyltestosterone (suspension), and α-estradiol, 2.0 mg. per 0.3 cc.; 17-hydroxycorticosterone (suspension) and 17-hydroxy-11-dehydrocorticosterone (suspension), 0.5 mg. per 0.3 cc.; ethynyltestosterone (suspension) 1.0 mg. per 0.2 cc.

### Table I

*Anti-Insulin Tests*

The steroid compounds were administered in corn oil at the following concentrations: corticosterone and its acetate, desoxycorticosterone and its acetate, and progesterone, 0.5 mg. per 0.2 cc.; desoxycorticosterone and its acetate, progesterone, and methyltestosterone, 2.0 mg. per 0.2 cc.; methylandrostenediol, ethynylandrostenediol, ethynyltestosterone (suspension), and α-estradiol, 2.0 mg. per 0.3 cc.; 17-hydroxycorticosterone (suspension) and 17-hydroxy-11-dehydrocorticosterone (suspension), 0.5 mg. per 0.3 cc.; ethynyltestosterone (suspension) 1.0 mg. per 0.2 cc.

<table>
<thead>
<tr>
<th>Preparation injected</th>
<th>Total amount per animal</th>
<th>Insulin dose per kilo</th>
<th>Total No. of animals</th>
<th>No. of convulsions</th>
<th>Per cent convulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls ............</td>
<td></td>
<td>1.5 or 2.0</td>
<td>202</td>
<td>179</td>
<td>89</td>
</tr>
<tr>
<td>Adrenocorticotropic*</td>
<td></td>
<td>1.5 or 2.0</td>
<td>128</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Corticosterone acetate†</td>
<td></td>
<td>0.5</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Corticosterone‡</td>
<td></td>
<td>0.5</td>
<td>30</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>
| 17-Hydroxycortico-
 terone‡                        | 1.0                   | 15                   | 0                    | 0                 | 0                   |
| "                  |                         | 0.5                   | 18                   | 0                 | 0                   |
| 17-Hydroxy-11-dehydrocortico-
 terone‡                        | 1.0                   | 15                   | 0                    | 0                 | 0                   |
| "                  |                         | 0.5                   | 18                   | 0                 | 0                   |
| Desoxycorticosterone§ |                       | 2.0                   | 26                   | 20                | 77                  |
| " acetate§       |                         | 2.0                   | 27                   | 20                | 74                  |
| Progesterone§      |                         | 2.0                   | 41                   | 34                | 83                  |
| α-Estradiol§       |                         | 2.0                   | 13                   | 13                | 100                 |
| Methyltestosterone§ |                       | 2.0                   | 14                   | 11                | 79                  |
| Ethynyltestosterone§ |                       | 2.0                   | 15                   | 15                | 100                 |
| Methylandrostenediol§ |                     | 2.0                   | 13                   | 12                | 92                  |
| Ethynylandrostenediol§ |                     | 2.0                   | 13                   | 11                | 85                  |

* The figures listed are the combined results obtained with various adrenocorticotropic fractions.
† Supplied by Dr. E. C. Kendall.
‡ Supplied by Dr. J. J. Pfiffner.
§ Supplied by Dr. E. Schwenk.
pletely under these conditions were administered in a uniform suspension. The preparations were generally given subcutaneously in a single dose at the onset of the 6 hour fast. Whenever it was necessary to inject more than 0.2 cc. of an oil preparation,

one-half the dose was given at either side of the body. In all instances a control group of fasted animals receiving insulin only was included in the experiments. The anti-insulin response of the various compounds is recorded in Table I.

**Table II**

*Effect of Pituitary Adrenocorticotropic Hormone and of Various Adrenal Cortical Principles on Liver Glycogen*

<table>
<thead>
<tr>
<th>Preparation injected</th>
<th>Mode of administration</th>
<th>Total No. of animals</th>
<th>Average weight per animal at onset of fast</th>
<th>Average weight loss of animal during fast</th>
<th>Average liver weight per animal</th>
<th>Liver glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjected controls</td>
<td></td>
<td>72</td>
<td>19.9</td>
<td>0.90</td>
<td>1.18</td>
<td>616</td>
</tr>
<tr>
<td>Adrenocorticotropic</td>
<td>pH 7.5</td>
<td>28</td>
<td>20.3</td>
<td>0.23</td>
<td>1.32</td>
<td>2346</td>
</tr>
<tr>
<td>Corn oil controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desoxycorticosterone acetate</td>
<td></td>
<td>1.0</td>
<td>0.2 cc. oil</td>
<td>19.8</td>
<td>0.86</td>
<td>1.13</td>
</tr>
<tr>
<td>Corticosterone acetate</td>
<td></td>
<td>0.5</td>
<td>0.2 ' '</td>
<td>18.3</td>
<td>0.98</td>
<td>1.21</td>
</tr>
<tr>
<td>Corn oil controls</td>
<td></td>
<td>0.3 ' '</td>
<td>12</td>
<td>20.8</td>
<td>0.73</td>
<td>1.16</td>
</tr>
<tr>
<td>17-Hydroxycorticosterone</td>
<td></td>
<td>0.3 ' '</td>
<td>24</td>
<td>21.3</td>
<td>1.31</td>
<td>1.43</td>
</tr>
<tr>
<td>Corticosterone acetate</td>
<td></td>
<td>0.25</td>
<td>0.15 ' '</td>
<td>15</td>
<td>19.9</td>
<td>1.12</td>
</tr>
<tr>
<td>Corn oil controls</td>
<td></td>
<td>0.8 ' '</td>
<td>24</td>
<td>21.2</td>
<td>0.30</td>
<td>1.30</td>
</tr>
<tr>
<td>Desoxycorticosterone acetate</td>
<td></td>
<td>2.0</td>
<td>0.8 ' '</td>
<td>8</td>
<td>20.3</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* Supplied by Dr. J. J. Pfaffner.
† Supplied by Dr. E. C. Kendall.
‡ Suspension.
§ Supplied by Dr. E. Schwenk.
Effect on Liver Glycogen—Since the degree of resistance of an animal to insulin is apparently intimately connected with the amount of glycogen present in the liver, we have studied the influence of some of the preparations on the formation of liver glycogen. The experiments were carried out under conditions similar to those followed in the anti-insulin test. Male mice, weighing approximately 20 gm., were injected subcutaneously with the test preparation at the onset of the 6 hour fast. Glycogen determinations were generally conducted on four mice to a group. At the completion of the fasting period, during which time the mice had access to water, the animals were killed by the administration of 0.2 cc. of nembutal (1 cc. = 1 grain) and the livers removed immediately, immersed in distilled water, and dried with a soft cloth to remove all blood. Each group of four livers was then digested on a boiling water bath for 1½ hours in 30 per cent potassium hydroxide (2 cc. per gm. of liver). 2 volumes of 95 per cent ethyl alcohol were next added and the mixture heated just to boiling. After standing overnight at 0° the digest was centrifuged, the precipitate dissolved in 10 cc. of warm water, and the glycogen reprecipitated with 2 volumes of 95 per cent ethyl alcohol. This second precipitate, after standing overnight at 0°, was removed by centrifugation, hydrolyzed with N sulfuric acid (1 cc. per gm. of liver) for 3 hours, and the solution then neutralized with sodium hydroxide, with phenol red as indicator. Duplicate glucose determinations were carried out with aliquot samples by the method of Shaffer and Hartmann, as modified by Somogyi (3).

When the amount of test material permitted, seven groups of four animals each were run on the various preparations. Control groups receiving no injection or an amount of corn oil comparable to that given the test groups were run at frequent intervals. As indicated in Table II, large doses of corn oil apparently lower the glycogen content of the liver. It is therefore advisable to administer the compounds in as small an amount of oil as possible. The effectiveness of the various preparations in promoting the deposition of liver glycogen is illustrated in Table II.

DISCUSSION

From the data presented in Tables I and II it is evident that the adrenocorticotropic factor of the anterior pituitary as well
as the adrenal cortical principles, corticosterone and 17-hydroxy-corticosterone, exert a pronounced anti-insulin effect and also markedly increase the deposition of liver glycogen under identical experimental conditions. It can also be seen that 17-hydroxy-11-dehydrocorticosterone produces a definite anti-insulin response, but unfortunately the amount of this compound available was insufficient to enable us to study its effect on liver glycogen. It is probable, however, that this cortical principle will also promote the deposition of liver glycogen.

On the other hand, the administration of desoxycorticosterone, at 4 times the dose level at which corticosterone, 17-hydroxycorticosterone, and 17-hydroxy-11-dehydrocorticosterone produce a definite anti-insulin effect and a marked increase in liver glycogen, failed to demonstrate any similar response. This observation is of importance, since it has generally been assumed that desoxycorticosterone is capable of completely alleviating the symptoms of adrenal insufficiency. It is unlikely that the difference in response is solely due to the rate of absorption. Sufficient desoxycorticosterone should have been absorbed at this comparatively high dose level to exert a definite influence on carbohydrate metabolism if the compound is at all active in this respect. Various other steroids tested for possible anti-insulin effect were also found to give a negative response.

It appears that those cortical principles which exhibit a positive anti-insulin response and produce an increase in the glycogen content of the liver will also favorably influence the work capacity of adrenalectomized rats, according to the observations of Ingle (4). Long and his associates have demonstrated that injection of corticosterone and of 11-dehydrocorticosterone into partially depancreatized rats causes an increase in glycosuria, while administration of desoxycorticosterone has little or any effect (5). Ingle recently observed that administration of 17-hydroxy corticosterone and of 17-hydroxy-11-dehydrocorticosterone will also augment the glycosuria of partially depancreatized rats (6). Recent clinical observations indicate that desoxycorticosterone acetate treatment has little or no effect in correcting the disturbance in carbohydrate metabolism which occurs in patients with Addison's disease (7). On the other hand Harrison and Harrison (8) have reported that the administration of desoxycorticosterone acetate prevents the
fall of the blood sugar in fasted adrenalectomized rats. It is of course possible that desoxycorticosterone may influence carbohydrate metabolism indirectly through its effect on the electrolyte balance. It may also be mentioned that Wells and Kendall (9) as well as Kuhlman et al. (10) have expressed the view that desoxycorticosterone is the only adrenal cortical principle capable of lowering the concentration of serum potassium, and that this factor is mainly involved in the regulation of the electrolyte balance.

These various observations seem to permit the conclusion that the different adrenal cortical principles do not affect carbohydrate metabolism, electrolyte balance, and life maintenance (adrenalectomized animals) to the same degree. Our findings and those of other investigators (11) indicate that only those adrenal cortical principles which contain either a keto or hydroxy group at C11 exert a significant influence on carbohydrate metabolism. Whether derivatives of desoxycorticosterone containing either a keto or hydroxy group at positions other than C11 or C12 will exert an influence on carbohydrate metabolism has not yet been investigated. The function of the adrenal cortex may therefore be twofold, namely regulation (a) of the electrolyte balance and (b) of carbohydrate metabolism, these effects being elicited by different cortical principles.

The anti-insulin response produced by the adrenocorticotropic hormone and the corticosterone-like compounds (substitution in Ring III) is probably due to the ability of these substances to promote the formation of liver glycogen. We believe that the anti-insulin effect of the anterior pituitary can be attributed to the adrenocorticotropic principle and is mainly mediated through the adrenal cortex. Whether the anterior pituitary can also exert a direct and immediate influence on carbohydrate metabolism of the liver is still unsettled at present.

SUMMARY

The adrenal cortical principles, corticosterone, 17-hydroxy-corticosterone, and 17-hydroxy-11-dehydrocorticosterone, exert a

2 It has not as yet been definitely established whether these groups are at C11 or C12.
pronounced anti-insulin effect and promote the deposition of liver glycogen.

Desoxycorticosterone and various other steroids exert little or no effect on insulin hypoglycemia and liver glycogen formation.

It seems that only those adrenal cortical principles substituted in Ring III (keto or hydroxy group) are principally involved in carbohydrate metabolism.

The adrenocorticotropic pituitary factor produces a definite anti-insulin (glycotropic) effect and also promotes the formation of liver glycogen.

We wish to express our appreciation to Dr. E. C. Kendall of the Mayo Clinic, to Dr. J. J. Pfiffner of Parke, Davis and Company, and to Dr. E. C. Schwenk of the Schering Corporation for supplying us with the various steroid compounds.

BIBLIOGRAPHY

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