Estimation of Hydrocortisone Secretion
Method of Calculation from Urinary-Excretion Data

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In 1938, Anderson, Haymaker, and Joseph (1) reported the finding of increased concentrations of cortin in the blood and urine of patients with Cushing's syndrome. Since then, as methods for the determination of cortical steroids have been improved, they have been applied to the urine and plasma of patients as a measure of adrenocortical activity. The reaction of 17,21-dihydroxy-20-ketosteroids with phenylhydrazine (2) has been applied to the analysis of plasma (3) and of urine (4) in Addison's disease and in Cushing's syndrome. In general, hyperactivity or hypoactivity of the adrenal cortex is reflected by increased or decreased plasma concentrations and excretion of 17,21-dihydroxy-20-ketosteroids. However, there may be conditions in which the output of the adrenal cortex is altered without demonstrable changes in the corticosteroid content of blood or urine. Therefore, it seemed desirable to devise a procedure which could be used clinically to estimate the adrenal output of hydrocortisone.

THEORETICAL CONSIDERATIONS

If a mathematical relationship between total (endogenous plus exogenous) hydrocortisone in the body and the fraction found in the urine can be established after administration of graded doses of hydrocortisone, it should be possible to extrapolate back to zero exogenous hydrocortisone and thereby determine the fraction of the endogenous hydrocortisone that is excreted. The calculation of endogenous production would then follow directly, after measurement of the basal steroid excretion in a given period of time. In theory, to accomplish this, the exogenous hydro-
cortisone should be administered slowly into the adrenal vein to mix intimately with the endogenous steroid and to share its metabolic fate in the body. This mode of administration is obviously impractical and due to the required manipulation of the subject would not necessarily reflect the normal output of the adrenal cortex. Oral administration has been found to yield surprisingly satisfactory results, with a constant fraction of the exogenous steroid detected in the urine by the procedure of Silber and Porter (5) over the dosage range studied.

MATERIAL AND METHOD

Since, by the analytic procedure employed, we find that the increase in excretion of 17,21-dihydroxy-20-ketosteroids after oral administration of hydrocortisone is essentially complete within 8 hours, this period appeared to be satisfactory for the experimental demonstration of the method. In practice a 24-hour period has also been used and may be preferred.

On 4 consecutive days 5 male laboratory workers consumed a light breakfast at 7:00 A.M., voided at 8:30 A.M., ingested 0, 10, 25 or 50 mg. of hydrocortisone (free alcohol) with about 200 ml. of water, and collected all urine until 4:30 P.M. The subjects were permitted to have lunch at 11:30 A.M. Creatinine determinations were performed on all urines to check the adequacy of the collections. Phenol was added as preservative and samples of urine were frozen until all could be treated with glucuronidase¹ and assayed simultaneously.

Effects of Drugs

One subject (No. 2) ingested additional doses of 75 and 100 mg. (Table 1). This subject was studied again several weeks later and in subsequent weeks was used to determine the effect of several drugs on adrenocortical activity. During one series of collections, 2.7 Gm. of aspirin were ingested daily in 3 equal portions at 0 time, 2½ hours, and 5 hours; in a second, 300 mg. of ascorbic acid were taken, again in 3 equal portions; in a third, 100 mg. of Pyribenzamine, in 2 equal portions, were ingested; and in a fourth, 40 units of ACTH (Armour H. P. ACTHAR Gel) was administered daily by intramuscular injection (Table 2).

¹ Due to possible changes in the enzyme preparation, we now check each lot before use and usually find that 5-10 times as much enzyme as indicated in the published method (6) is needed.
RESULTS

Four of the subjects (see Table 1) excreted 24–32 per cent of the oral hydrocortisone as 17,21-dihydroxy-20-ketosteroids. The fifth subject excreted only 13.6 per cent. By applying these percentages to the 8-hour basal excretion values the secretion of hydrocortisone by the adrenals of these subjects in 8 hours may be estimated in milligrams. However, it may be more desirable to employ an extrapolated basal value for this calculation because analytic errors are minimized after administration of the steroid. In most instances the extrapolated value and the actual basal excretion were not significantly different.

The adrenal output of these subjects averaged 13.8 mg. of hydrocortisone in the 8-hour period. When the above approach was applied to a group of 20 males (21–35 years of age) studied with Dr. Bacon Chow

Table 1. Urinary Excretion of 17, 21-Dihydroxy-20-Ketosteroids by Human Subjects

<table>
<thead>
<tr>
<th>Hydrocortisone dose (mg.)</th>
<th>Subject 1</th>
<th>Subject 2*</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.3 (4.7)</td>
<td>3.8 (2.7)</td>
<td>2.9 (2.8)</td>
<td>3.0</td>
<td>2.95</td>
</tr>
<tr>
<td>10</td>
<td>7.9</td>
<td>5.73</td>
<td>5.15</td>
<td>5.35</td>
<td>4.2</td>
</tr>
<tr>
<td>25</td>
<td>12.6</td>
<td>10.3</td>
<td>9.3</td>
<td>9.0</td>
<td>6.2</td>
</tr>
<tr>
<td>50</td>
<td>20.6</td>
<td>18.0</td>
<td>15.9</td>
<td>15.4</td>
<td>10.6</td>
</tr>
<tr>
<td>% excreted</td>
<td>31.8</td>
<td>30.4</td>
<td>25.2</td>
<td>24.0</td>
<td>13.6</td>
</tr>
<tr>
<td>Adrenal output (mg./8 hr.)</td>
<td>14.8</td>
<td>8.9</td>
<td>11.1</td>
<td>12.5</td>
<td>21.7</td>
</tr>
</tbody>
</table>

Parenthetical figures are extrapolated basal excretion values which were used in the calculations.

* After 75-mg. dose, 25.6 mg. excreted; after 100 mg. dose, 32.1 mg.

Table 2. Effect of Several Drugs on Adrenal Output of Subject 2

<table>
<thead>
<tr>
<th>Hydrocortisone dose (mg.)</th>
<th>Control</th>
<th>Aspirin (2.3 Gm.)</th>
<th>Ascorbic acid (300 mg.)</th>
<th>Pyridoxine (100 mg.)</th>
<th>ACTH (40 units)</th>
<th>Control (second)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.94 (2.9)</td>
<td>4.0</td>
<td>4.15 (4.0)</td>
<td>4.2</td>
<td>11.5</td>
<td>3.65</td>
</tr>
<tr>
<td>10</td>
<td>5.6</td>
<td>6.63</td>
<td>6.4</td>
<td>7.07</td>
<td>16.9</td>
<td>5.7</td>
</tr>
<tr>
<td>20</td>
<td>8.2</td>
<td>9.25</td>
<td>9.3</td>
<td>9.2</td>
<td>22.6</td>
<td>9.05</td>
</tr>
<tr>
<td>30</td>
<td>11.2</td>
<td>.</td>
<td>11.9</td>
<td>12.7</td>
<td>.</td>
<td>11.05</td>
</tr>
<tr>
<td>% excreted</td>
<td>27.1</td>
<td>26.3</td>
<td>25.6</td>
<td>27.3</td>
<td>54.8</td>
<td>24.1</td>
</tr>
<tr>
<td>Adrenal output (mg./8 hr.)</td>
<td>10.7</td>
<td>15.2</td>
<td>15.6</td>
<td>15.4</td>
<td>21.0</td>
<td>15.1</td>
</tr>
</tbody>
</table>

Parenthetical figures are extrapolated basal excretion values which were used in the calculations.
of Johns Hopkins (6), 24-hour urine collections were made and the adrenal output was calculated to be 21.9 ± 8.2 mg. (s.d.) per 24 hours. In this study (6) only 2 urine collections were made—one before and one after administration of 40 mg. of hydrocortisone.

Calculations

For purposes of illustration, the data obtained on subject No. 2 (see Table 2) during the first control period are shown below:

\[
\frac{\text{mg. excreted after test dose} - \text{basal excretion in mg.}}{\text{test dose in mg.}} = \text{fraction excreted}
\]

\[
\begin{align*}
\frac{5.6 - 2.9}{10} &= .27 \\
\frac{8.2 - 2.9}{20} &= .265 \\
\frac{11.2 - 2.9}{30} &= .277
\end{align*}
\]

Av. = .271 of dose excreted

Then, \(\frac{\text{basal excretion in mg.}}{\text{fraction excreted}}\) = adrenal output (mg. in 8 hours)

\[
\frac{2.9}{0.271} = 10.7 \text{ mg.}
\]

It is also possible to determine the fraction excreted by the application of simultaneous equations. (In dogs we find this procedure necessary.)

\[
a = \text{exogenous hydrocortisone, mg. (known)}
\]
\[
b = \text{a constant}
\]
\[
x = \text{fraction excreted}
\]
\[
y = \text{steroid excreted}
\]
\[
(a + b)x = y
\]
\[
(10 + b)x = 5.6
\]
\[
(20 + b)x = 8.2
\]

Then, subtracting the first from the second,

\[
10x = 2.6
\]

\[
x = 0.26 \text{ or 26%}
\]

This fraction is then applied to the analytically determined basal excretion value (as in dogs), or, if desired, to the extrapolated basal value (as in man).
DISCUSSION

The quantity of hydrocortisone secreted by the adrenal glands of human subjects has been the subject of a great deal of speculation, but relatively little pertinent data have been published. Weichselbaum (7) has found that administration of approximately 40 mg. of hydrocortisone to adrenalectomized subjects in a 24-hour period brings their plasma concentrations into the normal range. This value compares favorably with the average values reported in this paper, 13.8 mg. in the 8-hour period, or 21.9 mg. in 24 hours (6).

Comparative Evaluation of Procedures

In the dog the urinary analyses are less accurate, due in part to the low excretion of 17,21-dihydroxy-20-ketosteroids (basal about 0.2–0.3 mg. per day) but application of the procedure herein described indicates that the adrenals of a 10–12-Kg. dog secrete about 1–2 mg. per day, and after 80 units of ACTHAR daily intramuscularly this increases to 5–6 mg. Since it has been found in our laboratory that the 24-hour excretion of 17-hydroxycorticoids by adrenalectomized dogs was increased from zero to the normal range of 0.2–0.3 mg. after administration of 1 or 2 mg. of hydrocortisone, it appears that the procedure is valid and can yield satisfactory results in the dog. Analysis of blood from the adrenal vein of intact 14–20-Kg. dogs by Hume and Nelson (8) revealed an adrenal output of 0.6–2.0 μg. per minute. A dog secreting a total of 2 mg. in a 24-hour period would have an average secretion of 1.4 μg. per minute from the 2 adrenals, so the results of the direct and indirect procedures are in reasonably satisfactory agreement.

Pharmacologic Influences

The data on the effect of aspirin, ascorbic acid, and Pyribenzamine are presented here more to illustrate the method than to determine whether these materials influence adrenal output, but it does appear that these substances did not significantly influence adrenal activity. ACTH, as expected, increased adrenal output to 21 mg. in 8 hours (and 30 mg. in 12 hours). The shift of the fraction of steroid excreted from 25 or 30 per cent to 55 per cent during ACTH treatment is very interesting and suggests that ACTH may not only stimulate secretion by the adrenal cortex but may also influence hydrocortisone metabolism. When sufficient data are obtained on patients it may be found that the fraction excreted in the urine has diagnostic significance.
Absorption and Metabolism

Oral administration of the steroid appears to be justified. The normal urinary excretion of 17,21-dihydroxy-20-ketosteroids in man as measured here is about 6.6 mg. per 24 hours, with only about 5 per cent in chloroform-soluble form—that is, not conjugated (5). After oral administration of hydrocortisone the proportion of free to conjugated steroid is relatively unchanged. Furthermore, after oral or intravenous administration of 25 or 100 mg. to dogs (9), the excretion of total (free plus conjugated) 17,21-dihydroxy-20-ketosteroids is essentially the same. Peterson (10) has reported similar findings in men given hydrocortisone orally or intravenously. Therefore, it is reasonable to conclude that not only is the hydrocortisone practically quantitatively absorbed from the intestinal tract but also its metabolism is similar to that of the endogenous steroid.

CONCLUSIONS

Although one may have reservations regarding the absolute validity of results obtained with the procedure described, it nevertheless appears adequate to reveal changes in adrenal output.

The experimental approach used in this study may be applied to the study of other substances, provided certain conditions are fulfilled. Thus, if one can exclude the substance in question from the diet for several days, if an excretion product can be accurately determined in the urine, if the subject is in a reasonably steady state, and if the substance can be administered in graded doses, it may be possible to estimate the formation or synthesis of that substance in the body.

SUMMARY

A method has been described for the estimation of the secretion of hydrocortisone by the adrenals of man, based exclusively upon urinary-excretion data. In an 8-hour period (8:30 A.M.–4:30 P.M.) 5 subjects secreted an average of 13.8 mg. of hydrocortisone. In 1 subject ACTH was found to increase the adrenal output whereas aspirin, ascorbic acid, and Pyribenzamine had little, if any, effect.

The possible application of the approach to the measurement of the formation of other substances in the body has been noted.

REFERENCES