THE EFFECT OF CORTICOTROPHIN AND CORTISONE ON NEUTRAL 17-KETOSTEROID METABOLISM AS DEMONSTRATED BY MICROCHROMATOGRAPHIC FRACTIONATION OF URINARY EXTRACTS FROM TWELVE TREATED PATIENTS

By

M. Helen Pond

While it is generally recognised that corticotrophin and cortisone increase and diminish respectively the urinary excretion of neutral 17-ketosteroids (17-KS), the precise identity of the steroids, the excretion of which is mainly affected by these hormones, is still controversial (Forsham et al., 1948; Copeman et al., 1952; Louchart & Jailer, 1953). Dingemanse & Huis in't Veld (1950) and van Creveld et al. (1951) showed an increase of 11-oxy-17-ketosteroids, etiocholanolone and to a lesser extent dehydroisoandrosterone after corticotrophin therapy, using a chromatographic method of analysis. Ronzoni (1952) also demonstrated an increase of dehydroisoandrosterone using a digitonin separation and »Allen's reaction«, but Migeon & Gardner (1952) could not demonstrate any change by similar methods in patients with hypercorticalism. With infra-red spectrophotometry, Dobriner (1954) found variable increases in the different fractions in normal persons receiving corticotrophin. The fractions usually increased were the 11-oxy-17-ketosteroid fraction and etiocholanolone.

It is thus difficult to obtain any standard pattern of neutral 17-KS excretion in these patients, as individual variation is so large, and the methods of investigation often widely different.

The behaviour of cortisone treated patients is more constant, and a depression of the total neutral 17-KS excretion has been observed by most workers in patients with a normal or initially high excretion (Copeman et al., 1952; Louchart & Jailer, 1953). A specific depression of the β-fraction of a few patients with adrenal hyperplasia, and idiopathic hirsutism, both with and without elevated neutral 17-KS excretion, has been occasionally demonstrated during
the administration of cortisone (Venning et al., 1952; Gardner, 1953; Louchart & Jailer, 1953). In patients with a normal total output of neutral 17-KS most investigators find a depression of all neutral 17-KS except the 11-oxy-17-ketosteroids, which may be breakdown products of the cortisone itself (Sprague et al., 1949).

PRESENT INVESTIGATION

The present material consists of the results from 12 patients treated with cortisone or corticotrophin, in whom neutral 17-KS excretion has been followed before, during and after therapy, by the estimation and microchromatographic fractionation of the urinary neutral 17-KS (Pond, 1951). Discussion of the significance of the eight fractions has been previously published (Pond, 1954).

As far as possible patients were selected in whom adrenal function could be studied and where gonadal function was minimal. Two patients had Simmonds' disease or a partial form of it (Martin & Pond, 1954) (Cases 1 and 2). Two had Addison's disease (3 & 4); four had rheumatoid disease (5–8) in which neutral 17-KS excretion happened to be low, and two were cases of dystrophia myotonica with testicular atrophy (9 & 10). Two patients in whom there was no demonstrable endocrine abnormality (11 & 12) were treated with cortisone. In all, four were treated with cortisone, seven with corticotrophin and one case was investigated with both hormones. All patients had an initial total neutral 17-KS excretion either low or within normal limits. Wherever possible, estimations were carried out at intervals throughout treatment and after cessation of treatment.

RESULTS

The results are shown in Table 1 and for brevity, only those which demonstrate some change are shown.

Patients 1 and 2, whose adrenal function was seriously diminished, showed a marked response in total output of neutral 17-KS to corticotrophin, with increases of 5 and 8 times the initial level. These patients presumably had an inactive but potentially normally functioning adrenal cortex. Patient 3 also showed a moderate response; she was a case of Addison's disease of mild severity, maintained on salt only, in whom there appeared to be enough active adrenocortical tissue to respond to corticotrophin.

The effect of corticotrophin on the excretion of neutral 17-KS in patients free from endocrine disease was more variable (6–10). All these patients showed a combined rise of all fractions with selective rises of some. In no case was the $\beta$-fraction raised outside the normal limits. In Case 10, the $\beta$-fraction increased
from 12–20% during therapy but returned to only 9% after cessation of treatment. There was little selective rise of the 11-oxy-17-ketosteroid fraction except in Case 9 and to a lesser extent 8. Cases 6 and 7 showed a relative rise of etiocholanolone compared with androsterone.

The use of cortisone in patient 4 showed that adequate replacement therapy was taking place, with a rise of total neutral 17-KS, the rise being largely of the 11-oxy-17-ketosteroid fraction. The results of the endocrinologically normal patients so treated show that there is a depression of all fractions save the 11-oxy-17-ketosteroid fraction. The β-fraction was not differentially depressed (11 & 12).

**DISCUSSION**

In the patients in whom the initial adrenal function was greatly diminished, the effect of corticotrophin was striking and affected the two fractions commonly associated with activity of the adrenal cortex, i.e., the β-fraction and the 11-oxy-17-ketosteroid fraction. This agrees with the findings of previous workers, using patients with more normal adrenal function (*Dingemanse & Huis in't Veld, 1950; van Creveld et al., 1951*). It also demonstrates that ACTH does not affect all the activities of the adrenal cortex equally, but is more closely associated with a glucocorticoid stimulus and with the β-fraction, the physiological function of which is as yet unknown, but its output is generally regarded as an indication of the activity of the parent gland. Similar neutral 17-KS patterns have been demonstrated in patients with Cushing's syndrome (*Dingemanse & Huis in't Veld, 1950; Pond, 1954*).

In patients in whom the initial level of neutral 17-KS excretion was normal, the effect of corticotrophin was variable, in agreement with previous workers. A rise of the β-fraction or the 11-oxy-17-ketosteroid fraction was demonstrable in only a few cases, and was not large and did not occur in the same patient at the same time. Gonadal function was absent or minimal in all these patients, and therefore such effects as were demonstrable are likely to have been of adrenal origin. It appears that the effect of corticotrophin on neutral 17-KS metabolism must be largely determined by the degree and type of activity of the adrenal cortex before administration. This may be related to the diminished variability in adrenal responsiveness, in relation to neutral 17-KS production in advanced debilitating disease (*Gordon, Horwitt & Segaloff, 1954*). *Simpson* (1952) suggests that there may also be a stimulation of androgens by corticotrophin, but except in the few findings of elevated β-fraction, this could not be demonstrated conclusively. The rise of etiocholanolone relative to androsterone would suggest that the androgenic activity of the extract would not be increased. This was demonstrated in two cases, but had previously been shown.
Table 1.
Fractionation of 17-ketosteroid extracts from patients' urine.

<table>
<thead>
<tr>
<th>No. of case and diagnosis</th>
<th>Dosage of drug per day</th>
<th>Age</th>
<th>Sex</th>
<th>17-KS mg./24 hrs.</th>
<th>Fractionation results – mg./24 hrs.</th>
<th>Fractionation results – percent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I     II    III   IV   V    VI   VII  VIII</td>
<td>I     II    III   IV   V    VI   VII  VIII</td>
<td></td>
</tr>
<tr>
<td>1. a. Simmonds' disease</td>
<td>ACTH 100 mg.</td>
<td>50</td>
<td>F</td>
<td>1.1   0.1  tr  0  0.4  0.3  0.3  0  0</td>
<td>7     3     0    35  25  30  0    0</td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td>Cortisone 25 mg.</td>
<td></td>
<td></td>
<td>5.8   0.5  0.7  0.7  1.0  1.7  1.5  0  0.2</td>
<td>4     11    11   17  28  21  3    4</td>
<td></td>
</tr>
<tr>
<td>c.</td>
<td>(1c only)</td>
<td></td>
<td></td>
<td>1.0   not fractionated</td>
<td></td>
<td>* For explanation &amp; footnote see end of table.</td>
</tr>
<tr>
<td>2. a. Partial Simmonds' disease</td>
<td>ACTH 80 mg.</td>
<td>37</td>
<td>F</td>
<td>2.0   0.1  0   0  0.6  0.4  0.8  0  tr</td>
<td>5     0     0    32  18  42  0    3</td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td>Cortisone 25 mg.</td>
<td></td>
<td></td>
<td>3.2   0.1  0.1  0.1  0.8  1.2  1.2  0  0.1</td>
<td>5     3     4    25  21  37  3    3</td>
<td></td>
</tr>
<tr>
<td>3. a. Addison's disease (mild)</td>
<td>ACTH 80 mg.</td>
<td>51</td>
<td>F</td>
<td>1.0   0.1  0.1  0.3  0.2  0.2  0.2  0  0.1</td>
<td>14    9     10   17  8   18  3    12</td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td>Cortisone 25 mg.</td>
<td></td>
<td></td>
<td>0.7   0.1  0   0  0.3  0.2  0.2  0  0.2</td>
<td>8     0     0    37  30  25  0    0</td>
<td></td>
</tr>
<tr>
<td>4. a. Addison's disease</td>
<td>Cortisone 50 mg.</td>
<td>52</td>
<td>F</td>
<td>2.4   0.3  0.3  0.2  0.8  0.8  0.3  0  tr</td>
<td>11    14    7    20  33  13  0    2</td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td>Cortisone 50 mg.</td>
<td></td>
<td></td>
<td>1.6   0.1  0.2  0.1  0.2  0.3  0.4  0  0.2</td>
<td>7     16    9    10  17  23  10   8</td>
<td></td>
</tr>
<tr>
<td>5. a. Rheumatoid arthritis</td>
<td>Cortisone 100 mg.</td>
<td>54</td>
<td>F</td>
<td>2     0.1  tr  0  0.7  0.5  0  0.1</td>
<td>5     2     1    31  34  24  0    3</td>
<td></td>
</tr>
<tr>
<td>b.</td>
<td>Sjögren's disease with rheumatoid features</td>
<td>47</td>
<td>F</td>
<td>1     0.1  0  0.3  0.3  0.3  0  0.1</td>
<td>13    0     0    25  25  25  0    12</td>
<td></td>
</tr>
<tr>
<td>c.</td>
<td>ACTH 100 mg.</td>
<td></td>
<td></td>
<td>4     0.4  0.1  0  0.9  1.7  0.7  0  0.2</td>
<td>11    3     0    22  42  18  0    4</td>
<td></td>
</tr>
<tr>
<td>d.</td>
<td>Cortisone 25 mg.</td>
<td></td>
<td></td>
<td>6     0.4  0.5  0  1.3  2.1  1.4  0  0.3</td>
<td>7     9     0    22  34  23  0    5</td>
<td></td>
</tr>
<tr>
<td>e.</td>
<td>Cortisone 25 mg.</td>
<td></td>
<td></td>
<td>2     0.2  tr  0  0.4  0.8  0.5  0  0.2</td>
<td>9     1     0    18  39  24  0    9</td>
<td></td>
</tr>
</tbody>
</table>

* For explanation & footnote see end of table.
8. a. » » ACTH 36 F 3 0.1 0.2 0 0.5 1.4 0.6 0 0.2 3 8 0 15 48 19 0 7
   b. 100 mg. 5 0.6 0.2 0 0.9 1.7 1.2 0 0.5 11 3 0 17 34 26 0 9
   c. 9 0.5 0 0 2.7 4.1 1.6 0 0.3 5 0 0 30 44 18 0 3
   d. 100 mg. 9 0.3 0.3 0 0.8 0.6 0.3 0.6 0.2 9 9 0 25 20 9 19 0 9

9. a. Dystrophia myotonica ACTH 32 M 3 0.4 0.1 0 1.0 1.2 0.4 0 0.1 14 2 0 34 41 9 0 0
   b. with testicular atrophy 100 mg. 3 0.2 0.1 tr 0.9 1.0 0.7 0.1 0 5 3 1 31 34 22 4 0
   c. 7 0.5 0.1 0 1.9 2.3 1.5 0.3 0.4 7 2 2 27 34 21 4 5
   d. 5 0.6 0.1 0.2 2.1 1.4 0.5 0 0.2 12 2 3 42 28 10 0 3

10. a. » » ACTH 48 M 4 0.2 0.2 0.1 1.3 1.3 0.8 0 0.1 5 5 2 33 32 21 0 2
    b. 100 mg. 8 0.7 0.4 0.5 1.9 2.4 1.8 0 0.4 9 5 6 21 32 22 0 5
    c. 5 0.5 0 0 1.3 2.2 1.1 0 0 9 0 0 26 44 21 0 0

11. a. Non-endocr. disorder Cortisone 38 F 6 0.6 0.4 0 1.7 2.3 1.0 0 0.1 10 6 0 28 39 16 0 1
    b. (iridocyclitis) 50 mg. 2 0.1 tr tr 0.3 0.5 1.0 0 tr 7 2 2 14 27 47 0 1
    c. 2 0.1 0.1 0 0.2 0.6 0.9 0 0.1 6 4 0 11 28 47 0 4

12. a. Non-endocr. disorder Cortisone 29 F 4 0.2 0.1 0.2 1.0 1.5 0.8 0 0.2 5 3 5 25 37 20 0 5
    b. (iridocyclitis) 50 mg. 2 0.1 0.1 0 0.2 0.7 0.8 0 0.1 3 3 0 12 35 40 0 7
    c. 3 0.1 0.1 0.3 0.5 1.1 1.1 0 0 3 2 9 15 35 36 0 0

* Fractions of 17-ketosteroid urinary extracts:

The main constituents of the various fractions are as follows:


Explanation of nomenclature of specimen:

Before drug treatment = all specimens marked a and 1b.

After » » of 1–2 days' duration = specimens 1c, 2b, 3b and 1c.
» » » 4 days' duration = 6b, 7b, 8b, 9b, 10b, 11b and 1d.
» » » 1 weeks' duration = 6c, 7c, 8c, 9c, 11c and 12b.
» » » » 2 or more week's duration = 6d, 9c.
» cessation of treatment for at least one week = 6e, 7d, 8d, 9d, 10c and 12c.
tr = trace = less than 0.1 mg.

Results in brackets may be omitted.
more frequently by Dingemanse & Huis in't Veld (1950) and Dobriner (1954). But in this series it occurred only in patients with long standing debilitating disease, and could actually be demonstrated in one case before therapy (8). It has been described in other non-endocrine conditions, and may therefore be unrelated to corticotrophin (Robinson & Goulden, 1949).

The effect of cortisone on neutral 17-KS excretion confirmed the findings of Copeman et al. (1952) and Louchart & Jailer (1953) that all fractions are depressed save that of the 11-oxy-17-ketosteroid fraction. Again this is evidence that cortisone may itself be excreted in part as a neutral 17-ketosteroid (Sprague et al., 1949). The $\beta$-fraction was not depressed relative to the other fractions as was shown by the work of Venning et al. (1952) and Gardner (1953). This may be because the initial level of the $\beta$-fraction was either normal or lowered, and therefore such a change, if present could not be demonstrated. The main part of the depression of the neutral 17-KS was borne by the androsterone and etiocholanolone fraction, since they commonly constitute about two-thirds of the total extract.

Further investigation is needed to elucidate the significance of these varied responses of steroid metabolism to endocrine stimuli, and for more correlation of the results of the various analytical methods, since variability in results may also be due to differences in method.

**SUMMARY**

The effect of corticotrophin and cortisone on the neutral 17-ketosteroid excretion has been investigated in 12 patients in whom adrenal function was either previously normal or very much reduced. Corticotrophin produced a marked effect on the $\beta$-fraction and the 11-oxy-17-ketosteroid fraction in patients with low adrenocortical activity. In those with a normal adrenal function, the results were neither so striking nor so well marked, and confirmed the variability shown by previous workers. The effect of cortisone was also similar to that previously described, and consisted of a general depression of neutral 17-ketosteroid excretion with a rise of the 11-oxy-17-ketosteroid fraction, suggesting that cortisone may in part be metabolised to this compound. The $\beta$-fraction was not differently depressed.

**ACKNOWLEDGMENTS**

I wish to thank Professor C. H. Gray for invaluable help and facilities for these experiments; also to acknowledge the collaboration of the physicians of King's College Hospital under whose care the patients were treated; and for technical assistance I am indebted to Miss Jane Cowan and Miss Marion Edwards.
This work was carried out during the tenure of a William Gibson Research scholarship of the Royal Society of Medicine, London, and of a grant from the endowment fund of King's College Hospital, to the Governors of which I am indebted.

REFERENCES


