A STEROID METABOLIC STUDY ON ADRENALECTOMIZED PATIENTS AFTER ADMINISTRATION OF COMPOUNDS E AND F AND REICHSTEIN'S SUBSTANCE S

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When steroid metabolic investigations are performed on patients with intact adrenals, the question always arises, how do administered steroids affect the secretion of the adrenals themselves? Hence, patients who have undergone total adrenalectomy are suitable subjects for the study of this problem. Despite the report of Laidlaw et al. (1952) that continued steroid excretion takes place after adrenalectomy, there is reason to believe that in adrenalectomized women and orchidectomized, adrenalectomized men without substitution therapy, the steroid excretion is of an order that cannot fundamentally affect the results in steroid metabolic studies. In some cases we have been able to demonstrate that as early as 48 hours after discontinuation of substitution therapy the 17-ketosteroid excretion is very small in these patients (Birke & Plantin, 1953). When it was reported by Cohen (1951) that the corticoids of the urine are conjugated to glucuronic acid and that a substantially greater yield is obtained with β-glucuronidase hydrolysis than with acid hydrolysis, a study of the effect of administered steroids on corticoid and 17-ketosteroid excretion seemed indicated, to give an idea of the quantity converted to the respective metabolites.

PRESENT INVESTIGATION

Method

Determination of the 17-ketosteroids: The urine was hydrolized by boiling with sulphuric acid. A continuous separate extraction was then performed with ethyl ether. The crude extract was separated with Girard’s reagent T. Colorimetric determination
of the 17-ketosteroids was then performed, according to Callow's modification (1938) of Zimmermann's method (1935). Chromatography of the extract was carried out according to the micromethod of Zygmuntowicz et al. (1951). To allow of identification with infrared spectrography, however, a modified method has been developed. This modification will be discussed in detail in a later paper (Birke & Plantin).

**Determination of reducing corticoids**

Sterile urine was obtained by boiling and the urine was subsequently adjusted to pH 5.0 with buffer solution. It was then incubated with β-glucuronidase from calf spleen for 42 hours at 37° C. (Cohen, 1951). Extraction was done with chloroform according to Sprechler (1950). Thereafter, colorimetric determination of reducing corticoids was carried out with phosphomolybdic acid reagent, on the ketonic fraction after separation with Girard's reagent T.

**Results**

Two patients were studied after administration of Compounds E₁ and F₂ (Kendall) and Substance S₃ (Reichstein). One patient was a 68 year old man (C O) with prostatic cancer. Orchidectomy was done in November 1950 and adrenalectomy in May 1953, because of extensive skeletal metastases. The other patient was a 62 year old woman (O Y), who had previously undergone operation for mammary cancer, and adrenalectomy in September 1953 because of relatively extensive, painful skeletal metastases. Both these patients first received substitution therapy of 50 mg. cortisol acetate administered orally. The metabolism was then studied. After five days 150 mg. Reichstein's Substance S acetate was given orally on two successive days. After the effect of this steroid had been studied for seven to eight days and it was definitely established that there was no further excretion of its metabolites in the urine, cortisol was replaced by Compound F acetate in an oral dosage of 40 mg. in the first case and of 80 mg. per day in the second. The results of steroid administration in these cases are summarized in Table 1.

It is evident from the table that the breakdown of all administered steroids to 17-ketosteroids occurs to a relatively slight extent. Moreover, it may be seen that the conversion in these two cases varied, quantitatively, particularly with respect to Compound F and Substance S. The breakdown to Compounds E and F, however, was of the same order of magnitude in the two cases. When the corticoid conversion is studied, it may be observed that a relatively large percentage of the administered steroids was converted in the case of Compounds E and F. The breakdown in the two cases was relatively similar and no notable difference between Compounds E and Compound F was observed. The excretion of Substance S as corticoids could not be studied in as great detail in

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Table 1.
The effect of Compounds E and F and Substance S on corticoid and 17-ketosteroid excretion in two adrenalectomized patients.

<table>
<thead>
<tr>
<th>Administered steroid</th>
<th>Percentage administered steroid converted to 17-ketosteroids</th>
<th>Percentage administered steroid converted to reduc. corticoids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO</td>
<td>OY</td>
</tr>
<tr>
<td>Compound E (Kendall)</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>» F</td>
<td>1.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Substance S (Reichstein)</td>
<td>0.7</td>
<td>6.3</td>
</tr>
</tbody>
</table>

these cases, as that of Compounds E and F. It was observed, however, that on the day after the final dose was administered, the 17-KS excretion rose from 8.0 mg./24 hours to 16.3 mg./24 hours in the first case and from 10.7 mg./24 hours to 42 mg./24 hours in the second.

Detailed studies about which 17-ketosteroids are obtained in the urine following administration of the different hormones were also carried out. The following results were obtained. When Compound E was administered, 11-keto-etiocholanolone and 11-hydroxy-etiocholanolone or its hydrolytic artefact, \( \Delta^9 \)-etiocholenolone, were found in both cases. Dehydroepiandrosterone, androsterone, etiocholanolone and 11-hydroxy-androsterone were not recovered in the urine during oral administration of cortisone acetate. When Substance S was administered, etiocholanolone was mainly obtained, but a small quantity of androsterone was also identified. When Compound F was subsequently administered, 11-keto-etiocholanolone and 11-hydroxy-etiocholanolone or its hydrolytic artefact were excreted as metabolites in the urine of both patients. As with cortisone no conversion to dehydroepiandrosterone, androsterone or etiocholanolone was observed. It was impossible to identify 11-hydroxy-androsterone with certainty.

**DISCUSSION**

Investigations of corticoid excretion in the urine after acid hydrolysis have shown the 24 hours excretion to be from 0.5 to 1.5 mg. in normal material (Sprechler, 1950, Heard et al., 1946, Venning et al., 1946, Daughaday et al., 1948), the values varying somewhat according to the method used. Investigations mainly by Cohen (1951), Paterson et al. (1951) and Venning et al. (1952, 1953) made it clear that an appreciably greater yield is obtained after hydrolysis with \( \beta \)-glucuronidase. Hence it appeared desirable to study the conversion
of several vital steroids in adrenalectomized patients, where the adrenals do not influence the results. This showed that a considerable part is excreted in the urine as reducing corticoids. From 18 to 32 per cent of administered steroids are converted to corticoids, a result that shows, however, either that the greater portion is not excreted in the urine or that it is converted to unknown metabolites. The breakdown to 17-ketosteroids does not represent the part not identified as corticoids. The possibility that even this hydrolytic procedure does not afford a complete yield must naturally be taken into consideration. However, the investigation shows that enzymatic hydrolysis gives a yield of such magnitude that in different clinical investigations the method may be expected, at least partially to reflect the steroid metabolism in the body. This calls for continued studies on patients with intact adrenals following stimulation with ACTH, inter alia, especially during different types of stress. Investigation of these problems has already been started. As shown in earlier investigations, the conversion of Compounds S and F and Substance S to 17-ketosteroids is relatively slight. Detailed studies of the steroid metabolism, as previously, show a marked predominance of conversion to steroids with etiocholane configuration. Only a slight excretion of androsterone was observed in both cases after administration of Substance S. Munson et al. (1953) consider that Compound E gives an increased excretion of dehydroepiandrosterone, but their investigation was not based on chemical or infrared spectrographic studies, but rather on a rise according to Allen's blue test. We have been unable to confirm this observation. It must also be regarded as improbable that a conversion from $\Delta^4$-to $\Delta^5$-steroids takes place at this stage of the steroid metabolism. On the contrary, the opposite reaction has been demonstrated by Samuels (1951), Meyer & Pincus (1953). Schneider & Mason (1948), on the other hand, found that when dehydroepiandrosterone was incubated with rabbit liver, a conversion to $\Delta^5$-androsten-3 $\beta$-17 $\beta$-diol took place, whereby the double bond was retained.

The present investigation also confirms the very recently published report of Burstein et al. (1953) that Compound F is mainly metabolized to 11-keto-etiocholanolone and 11 $\beta$-hydroxy-etiocholanolone. On the other hand, no definite proof has been obtained in this study that conversion to 11-hydroxy-androsterone also occurs in adrenalectomized patients.

**SUMMARY**

The 17-ketosteroid pattern and the corticoid excretion were studied following administration of Compounds E and F (Kendall) and Substance S (Reichstein) to two adrenalectomized patients. The 17-ketosteroids were chromatographed and identified with infrared spectrography. The results were as follows.

1. From 18 to 30 per cent of administered Compound E and Compound F was excreted in the urine as reducing corticoids.
2. The conversion to reducing corticoids is of the same order following administration of either Compound E or Compound F.

3. Substance S also causes a pronounced increase in the reducing corticoids in the urine.

4. 1.2 to 2 per cent of administered Compound E, 1.5 to 7.8 per cent of Compound F, and 0.7 to 6.3 per cent of Substance S are converted to 17-ketosteroids.

5. Compound E is excreted as 11-keto-etiocholanolone and 11-hydroxy-etiocholanolone. The metabolites of Compound F are 11-keto-etiocholanolone and 11-hydroxy-etiocholanolone. Substance S is mainly broken down to etiocholanolone and to a very slight extent to androsterone.

REFERENCES