METABOLISM OF EXOGENOUS DEHYDROEPiANDROSTERONE IN MAN

By

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ABSTRACT

To study the metabolism of dehydroepiandrosterone, 3 normal subjects ingested 50, 300, and 1000 mg of dehydroepiandrosterone for 3 day periods. Although the fraction of the administered dehydroepiandrosterone that appeared in the urine decreased with increasing doses, the amount of dehydroepiandrosterone excreted approached that seen in adrenal cancer. The excretion of etiocholanolone was greater than that of androsterone at the higher dehydroepiandrosterone levels suggesting increased activity of the enzyme, Δ4-5β-reductase. Conjugation of the C19O2-ketosteroid metabolites of dehydroepiandrosterone was examined at each dose level. The excretion of dehydroepiandrosterone glucuronoside remained a small, constant fraction of the total dehydroepiandrosterone excretion. Similarly, the excretion of androsterone sulfate and etiocholanolone sulfate was a fairly constant but minor fraction of the total excretion of androsterone and etiocholanolone.

Twenty-five years ago dehydroepiandrosterone was shown to be a significant fraction of the urinary 17-ketosteroids in patients with adrenal cortical cancer (Callow 1936). Since then, this steroid has been found in small amounts in the urine of most normal adults. Vande Wiele & Lieberman (1960) have demonstrated, however, that the normal adult secretes as much as 15–25 mg of dehydroepiandrosterone daily so that the urinary dehydroepiandrosterone is a minor fraction of the amount secreted. A comparison of the secretion rate

Trivial names have been used for the following steroids:
5α-androstane-3α-ol-17-one – androsterone
5β-androstane-3α-ol-17-one – etiocholanolone
androst-5-en-3β-ol-17-one – dehydroepiandrosterone
5α-androstane-3β-ol-17-one – epiyandrosterone
of dehydroepiandrosterone with its excretion rate (Lipsett 1961) revealed that if dehydroepiandrosterone were metabolized with the same efficiency by the patient with adrenal cancer as by the normal subject, then lesser amounts of dehydroepiandrosterone should be excreted in the urine. It thus seemed pertinent to reexamine the hypothesis proposed by Mason & Kepler (1947) to explain the high levels of urinary dehydroepiandrosterone in adrenal cancer: namely, that the enzyme systems necessary for the metabolism of dehydroepiandrosterone are saturated by the load presented.

Mason (1948) summarized the results of administration of large amounts of dehydroepiandrosterone to 4 subjects and concluded that he had not proved his hypothesis. Yet in 2 of the studies, more dehydroepiandrosterone was isolated from the urine than either androsterone or etiocholanolone. Miller et al. (1950) in 2 similar studies recovered only 0.5% and 0% of 1350 mg of dehydroepiandrosterone acetate as dehydroepiandrosterone.

Since both groups utilized hot acid hydrolysis and successive crystallizations to measure dehydroepiandrosterone excretion, the values presented were minimal. Improvements in the methods for measuring several urinary 17-ketosteroids have made it possible to extend the previous work. Accordingly, we have examined the excretion of dehydroepiandrosterone, androsterone, and etiocholanolone and their partition into sulfates and glucuronosides after increasing oral loads of dehydroepiandrosterone.

METHODS

One-tenth of a day’s urine was treated with beef liver β-glucuronidase (Ketodase), 500 units/ml for 3 days, and in another 1/10 day aliquot, the sulfates were solvolyzed by the method of Burstein & Lieberman (1958). The ether extracts of the β-glucuronidase hydrolysate and the ethyl acetate from the solvolysis were washed with sodium hydroxide, neutralized and dried over sodium sulfate. The following 17-ketosteroids (17-KS), androsterone* (Andro), etiocholanolone (Etio) and dehydroepiandrosterone (DHEA) in each aliquot were then measured by gas-liquid chromatography (GLC).

In brief, the method used consisted of preliminary purification of each extract by thin layer chromatography, elution of the 3 17-KS from a single zone, formation of the trimethylsilyl ethers, and quantitation by GLC, using the stationary phase, QF-1. The details and reliability of this method have been reported (Kirschner & Lipsett 1963). Figures 1 and 2 are typical GLC records obtained from the glucuronoside and sulfate fractions respectively. Total crude 17-KS were measured by a modification of the method of Peterson & Pierce (1960) and 17-hydroxycorticoids by the method of Wilson & Lipsett (1963).

EXPERIMENTAL

Three normal men, ages 25–40, ingested 50 mg, 300 mg, and 1000 mg of DHEA daily in divided doses for 3 days at each level. The subjects were ambulatory and on
a normal diet. Twenty-four hour urine collections were made and the 17-KS measured on the third day of DHEA administration at the 50 and 300 mg level. Each day's urine was separately analyzed at the 1000 mg level. In a subsequent study, 2 subjects received 1000 mg DHEA by mouth in divided doses for 5 days, and the 17-KS were measured daily.

RESULTS AND COMMENT

The analytical data are presented in Table 1 and the derived data in Table 2. The urinary levels of Andro, Etio, and DHEA measured by this method were

| Table 1. Excretion of Androsterone, Etiocholanolone and Dehydroepiandrosterone. |
|--------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Subject (1)                        | Andro (2) | Etio (3) | DHEA (4) | Andro (5) | Etio (6) | DHEA (7) | Andro (8) | Etio (9) | DHEA (10) | Crude total 17-KS (11) |
| Control Period                     | 2.9 | 2.6 | 1.0 | 2.7 | 2.5 | 0.2 | 0.2 | 0.05 | 0.8 | 2.0 | 1.7 | 0.1 | 0.7 | 0.1 | 0.4 | 2.0 | 1.7 | **<0.1 | 1.5 | 1.4 | **<0.1 | 11.0 |
| 50 mg DHEA daily                   | 9.2 | 9.6 | 0.75 | 0.7 | 0.4 | 6.0 | 9.9 | 10.0 | 6.8 | 28 |
| 300 mg DHEA daily                  | 7.4 | 8.0 | 0.3 | 0.5 | 0.2 | 6.7 | 7.9 | 8.2 | 7.0 | 33 |
| 1000 mg DHEA daily                 | 7.4 | 13.2 | 0.5 | 0.5 | 0.6 | 1.8 | 7.9 | 13.8 | 2.3 | 35 |

* All values are expressed as mg/24 h.

** A small peak or shoulder was noted that could not be quantitated.
comparable to those tabulated by Vestergaard & Clausen (1962). One subject, »c«, excreted less than 0.1 mg of DHEA during the control period. The excretion of 17-hydroxycorticoids was normal during the control period and was unchanged by any dose of DHEA.

Fraction of crude 17-KS measured as Andro, Etio and DHEA

The total of the excretion of these 3 17-KS averaged 40–65 % of the crude 17-KS during the control period. This is somewhat above the percentages recently reported (Goldzieher & Axelrod 1962) for normal women using paper chromatography for separation of the individual 17-KS. This percentage increased with increasing doses of DHEA, so that at the 1000 mg level, the sum of the 3 17-KS approached 90 % of the total crude 17-KS. This demonstrates that at the 1000 mg level, 17-KS other than Andro, Etio, and DHEA constitute a minor amount of the total 17-KS metabolites of DHEA.

![GLC tracing of β-glucuronoside fraction of urine. Conditions: QF-1 1 %, Column Temp. 187°C, 22 psi, flow = 25 ml/min, 850 V. Shaded marker is Androstane-17-one.](image-url)
Fig. 2.
GLC tracing of solvolysis fraction of urine. Conditions: Same as Fig. 1.

Percentage of administered DHEA excreted as Andro, Etio and DHEA

Although the excretion of the crude total 17-KS and of the individual 17-KS increased with increasing doses of DHEA, the percent of the administered DHEA accounted for as the 3 individual 17-KS diminished. When 50 mg of DHEA were taken, 44% was excreted as the 3 C₁₉O₂-17-KS (Table 2, Col. 6). At the 1000 mg level, the percentage recovery had decreased to 22%. With increasing doses of DHEA the percent recovered as Andro and DHEA decreased (Table 2, Cols. 3 and 5), whereas the percent of DHEA metabolized to Etio remained fairly constant (Table 2, Col. 4). At the higher doses of DHEA, Etio became the major C₁₉O₂-17-KS metabolite.

Since the excretion of the C₁₉O₂-17-KS constituted a smaller fraction of the load at the higher dose levels, the factor of absorption was considered. The changing pattern of the metabolites suggested that the increased load of DHEA reached the liver. However, to test for absorption of DHEA, one subject ingested 1000 mg of DHEA labeled with 7α-³H-DHEA. Since the urinary excretion of radioactivity in 48 hours was 91% of the administered dose, we have concluded that absorption of even the largest amount of DHEA was
<table>
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<tr>
<th>Subject (1)</th>
<th>Etio/Andro (2)</th>
<th>% Administered DHEA Recovered as Sum</th>
<th>Andro % Total Andro (3)</th>
<th>Etio % Total Etio (4)</th>
<th>DHEA % Total DHEA (5)</th>
<th>(3 + 4 + 5) % (6)</th>
<th>Andro Gluc % (7)</th>
<th>Etio Gluc % (8)</th>
<th>DHEA sulfate % (9)</th>
<th>17-KS sulfate % (10)</th>
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* Day 3 of 5-day study.
relatively complete. Therefore the decrease in the percent of DHEA recovered as C₁₉O₂-17-KS was not due to decreased absorption and these differences must be attributed to the metabolism of DHEA either to products other than 17-KS or to conjugates not hydrolyzable by β-glucuronidase or solvolysis. Schneider & Lewbart (1959) have demonstrated the existence of 17-KS other than sulfates and glucuronosides after administration of a large dose of DHEA.

Changes in DHEA excretion

With increasing oral loads, DHEA excretion increased to levels comparable to those seen in adrenal cancer (Table 1, Col. 10). The amounts of DHEA metabolized, however, increased with the increasing dose, as shown by the decreasing percentage of administered DHEA excreted as DHEA (Table 2, Col. 5). Thus, although hepatic metabolism of DHEA increased with the load presented, this increase was insufficient to prevent the excretion of large amounts of DHEA.

Excretion of Andro and Etio and E/A ratio

Although the excretion of Andro plus Etio increased almost in proportion to the DHEA load, this was not due to equal increases in the 2 metabolites since the E/A ratios rose progressively (Table 2, Col. 2). During the control periods, the E/A ratio was slightly less than 1.0; at the 300 mg level, the ratio averaged 1.6, and at the 1000 mg level, 2.8. The increase in ratio was due to a major increase in Etio excretion and but a slight increase in Andro excretion. These data suggested that not only was the E/A ratio influenced by the DHEA load, but also that this ratio increased with time at a high constant load of DHEA. To further examine these temporal changes 2 subjects took 1000 mg DHEA by mouth for 5 days, and their urines were similarly analyzed. There was a uniform increase in the ratio of E/A during these periods (Fig. 3). In Fig. 4, the serial changes in the proportions of Etio and Andro excreted can be appreciated from the GLC records. The large relative increase in Etio excretion is evident by the third day of DHEA administration. The E/A ratios returned to normal 3 days after discontinuation of the DHEA.

Conjugation of metabolites of DHEA

DHEA glucuronoside

The GLC record of the glucuronoside fraction had a small peak or shoulder with a retention time that coincided with that of DHEA. It was essential to show that this peak represented DHEA and that DHEA had actually been conjugated as the glucuronoside.

To prove that the material giving this peak was DHEA, aliquots of the glucuronoside fraction taken after thin layer chromatography were treated with digitonin. DHEA was subsequently identified in the precipitated 3β-
Fig. 3.
Ratio of urinary Etiocholanolone to Androsterone during 1000 mg DHEA daily.

hydroxysteroid fraction by its Rf on paper in the Bush A system, its reaction in the Allen »blue« test, its spectral characteristics after treatment with the cold Allen reagent (Wilson 1960), and the retention time of the trimethylsilyl ether derivative on GLC. Furthermore the DHEA peak was absent from the GLC record of the »α« fraction obtained by treatment with digitonin.

Fig. 4.
Serial GLC tracings demonstrating changing Etio/Andro ratios during and after 1000 mg DHEA daily.

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In order to show that the DHEA found after incubation with $\beta$-glucuronidase arose at least in part from a glucuronoside, the following study was performed. Urines containing the presumptive DHEA-glucuronoside were extracted twice with ether. No unconjugated DHEA was found. The sulfates were then cleaved by solvolysis, and the glucuronosides extracted from the ethyl acetate with aqueous alkali. After incubation of this fraction with $\beta$-glucuronidase, DHEA was identified in the neutral extract. Since only unhydrolyzed glucuronosides would be extracted from ethyl acetate with alkali, this result strongly suggests that DHEA existed initially as the glucuronoside.

**Effect of DHEA load on mode of conjugation**

Since the 17-KS sulfates and glucuronosides were separately analyzed the effect of oral dosage on mode of conjugation could be examined. During the control period small amounts of DHEA-glucuronoside were found in the urine of 2 of the 3 subjects (Table 1, Col. 4). Although the amounts of DHEA-glucuronoside increased with increasing doses of DHEA, the fraction of urinary DHEA excreted as the glucuronoside remained unchanged.

Andro sulfate and Etio sulfate were present (Table 1, Cols. 5 and 6) in the control urines in amounts comparable to those reported by others (Kellie & Wade 1957; Baulieu et al. 1961 a, b). At all dose levels, Etio sulfate comprised less than 4% of the total Etio (Table 2, Col. 8). Andro sulfate, likewise remained a fairly constant fraction of the total Andro excreted (Table 2, Col. 7). Although the percentage of Andro sulfate was higher than the percentage of Etio sulfate at increasing levels of DHEA intake, the DHEA sulfate made up most of the 17-KS sulfate excreted and the total of the 17-KS sulfates remained a constant fraction of the total of the excretion of Andro, Etio, and DHEA (Table 2, Col. 10).

**Epiandrosterone sulfate**

Since epiandrosterone has been found in normal urine, we attempted to establish the identity of the small peak or shoulder following the DHEA peak in the solvolysis fractions. This peak had the same retention time as epiandrosterone. The material comprising this peak was shown to precipitate with digitonin, to have the same Rf as epiandrosterone in Bush A system, and, after paper chromatography to give a single peak on GLC with the same retention time as authentic epiandrosterone. This constituted presumptive identification of the epiandrosterone peak.

This steroid never became a significant metabolite of DHEA and even at the 1000 mg level, its excretion was less than 4.0 mg daily. The level of urinary excretion has not been reported at other levels since measurement of these peak areas was subject to large errors.
DISCUSSION

The initial objective of this study was to determine whether the presence of large amounts of DHEA in the urine of most patients with adrenal carcinoma may be a consequence simply of the load presented to the liver for metabolism or whether other mechanisms must be postulated. The derived data clearly show that urinary DHEA increased to levels comparable to those seen in adrenal cancer when the oral load is increased, in spite of the fact that the amount of DHEA metabolized likewise increased with increasing doses.

In an earlier study, Lipsett (1961) compared the DHEA excretion with the DHEA secretion rate in 4 patients with adrenal cancer. The urinary DHEA was a much higher fraction of the secreted DHEA than was the urinary DHEA in this study. Direct comparison of the 2 studies is difficult, however, since the metabolism of endogenous DHEA may differ from that of oral DHEA. Nevertheless, both studies demonstrate that urinary DHEA increases with load.

The excretion of much larger amounts of Etio than Andro has been a common feature of the urinary steroid pattern in adrenal cancer. The increasing E/A ratios seen both with increasing loads of DHEA and with time of administration of DHEA suggested that either DHEA or one of its metabolic products was selectively altering the activity of the hepatic C₁₉-5α and 5β-Δ₄ reductases.

It has been shown that the activity of these reductases can be altered separately by such states as hyperthyroidism (Hellman et al. 1959) and Cushing's syndrome (James 1961). The increase in the relative proportion of the 5β-reductase following ingestion of large amounts of DHEA has not been previously reported although high E/A ratios have been seen in several studies with other C₁₉O₂ steroids (Dorfman 1954). The simplest hypothesis that will explain our results is induction of the hepatic 5β-reductase as a result of the DHEA load, per se. Lieberman (1960) in a discussion of a similar hypothesis, pointed out that changing substrate concentrations could increase the velocity of one enzymatic reaction and thus simulate enzyme induction. This proposal, however, would not adequately account for the progressive increase in E/A ratios with time when the load was constant. We have therefore tentatively concluded that this phenomenon results from induction of the 5β-Δ₄-reductase resulting from increasing concentrations of DHEA or one of its metabolites.

These data suggest that one of the causes of the high E/A ratios in adrenal cancer is the secretion of increased amounts of DHEA. There may be other factors as well. Vande Wiele et al. (1958) proposed that there was a greater conversion of C₂₁-precursors to C₁₉O₂-17-KS thus leading to an excess of Etio excretion and this suggestion was supported by studies using labelled DHEA (Lipsett 1961). It has been shown also (Wilson et al., unpublished) that the
administration of corticoids increases the E/A ratio. Thus there are several factors leading to an excess of Etio.

The pattern of conjugation of C₁₉O₂-17-KS, i.e., excretion of DHEA chiefly as a sulfate and excretion of Andro and Etio chiefly as glucuronosides, has been observed in many studies. The excretion of small amounts of DHEA as the glucuronoside, however, has not been frequently reported. Although small amounts of DHEA-glucuronoside were found in the urine during the control period in 2 of the 3 subjects, other workers have not detected DHEA-glucuronoside in the urine of normal subjects (Malassis et al. 1957; Staib et al. 1960) or of women with Stein-Leventhal syndrome (Mauvais-Jarvis & Baulieu 1962). However, DHEA-glucuronoside has been isolated from the urine of a woman with an adrenal tumour (Baulieu et al. 1961 a, b) and after administration of large amounts of DHEA (Schneider & Lewbart 1959). More recently, its isolation has been reported from the urine of normal subjects (Lieberman et al. 1962) in amounts comparable to those noted here. There seems little doubt therefore that DHEA can be conjugated as the glucuronoside and that this happens to a small extent in the normal subject.

The question of whether the DHEA isolated after treatment with β-glucuronidase actually arises from a glucuronoside was raised by Burstein & Dorfman (1962) and Vestergaard (1962) who reported that DHEA sulfate in urine was hydrolyzed during incubation with β-glucuronidase at pH 5. We have confirmed these observations by showing that 10–15% of DHEA sulfate was hydrolyzed by incubation in urine at 37°C even without addition of β-glucuronidase. Parenthetically, it should be noted that when a similar amount of DHEA sulfate was incubated in water with acetate buffer and β-glucuronidase, less than 1% of the DHEA was released.

Consequently, a portion of the DHEA recovered by us from the glucuronoside fraction may have been excreted as the sulfate. Since we have shown that DHEA is also excreted as the glucuronoside, it seems probable that the DHEA measured after the usual incubation with β-glucuronidase is derived from both conjugates, the sulfate and the glucuronoside.

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REFERENCES

Endocr. 19 (1959) 936.
Lieberman S. In: Biologic Activities of Steroids in Relation to Cancer, Ed. G. Pincus
(Paris) 151 (1957) 447.
Mason H. L.: Recent Progr. Hormone Res. 3 (1948) 103.
Peterson R. S. & Pierce C. E. In: Lipids and the Steroid Hormones in Clinical Medi¬
158.
(N. Y.) 99 (1958) 520.
Vande Wiele R. & Lieberman S. In: Biologic Activities of Steroids in Relation to
Vestergaard P.: Acta endocr. (Kbh.) Suppl. 64 (1962) 50.

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