URINARY EXCRETION OF DIFFERENT CORTICOSTEROID-METABOLITES IN ORAL CONTRACEPTION AND PREGNANCY

By

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ABSTRACT

In two groups of patients the urinary excretion of numerous metabolites of cortisol, 11-deoxycortisol (compound S), corticosterone, 17-hydroxyprogesterone and pregnenolone were determined according to a modification of the method of Cost & Vegter (1962). The first group consisted of 7 women with severe primary dysmenorrhoea. Their ages ranged from 17 to 23 years, and all of them received cyclical treatment with a daily dose of 5 mg of 6-methyl-6-dehydro-17α-acetoxyprogesterone (megestrol acetate) + 0.1 mg of 17α-ethynyl-oestradiol-3-methylether (mestranol) until the excretion of total pituitary gonadotrophins was completely suppressed, and the excretion of 17-ketogenic steroids (17-KGS) had decreased to a constant level. This occurred after treatment for 18 to 24 weeks. In all patients the excretion of individual corticosteroid-metabolites was determined before treatment started and again at the end of the treatment period. At the same times the pituitary function was evaluated by means of the metyrapone-test. The second group consisted of 5 normal pregnant women aged 21 to 28. In these women, the excretion of individual corticosteroid-metabolites was determined once during the second trimester of pregnancy. In the first group, the average excretion of allo-tetrahydrocortisol, tetrahydrocortisone, allo-tetrahydrocorticosterone, and pregnanetriol was decreased during treatment, and the decrease in the allo-compounds was significantly greater than the decrease in the other metabolites. The excretion of non-hydrogenated cortisol increased during treatment. Furthermore, the metyrapone-test was found to be normal in all 7 patients both before and during treatment.
In the second group, a decrease was found in the excretion of allo-tetrahydrocortisol, allo-tetrahydrocorticosterone, and $\Delta_5$-pregnenetriol, while the excretion of Reichstein's substance U, non-hydrogenated cortisol, tetrahydro-11-dehydrocorticosterone, and non-hydrogenated 11-dehydrocorticosterone was increased.

The increase observed in non-hydrogenated cortisol, corresponded to the high plasma cortisol levels found in both groups. These changes were ascribed to the oestrogenic component in the preparation administered to the patients in group I, and to the high oestrogen level in the pregnant women (group II).

The decrease in the excretion of the 5α-hydrogenated compounds, allo-tetrahydrocortisol and allo-tetrahydrocorticosterone, indicates that there is an inhibition of the 5α-reductase activity in pregnancy as well as during treatment with megestrol acetate + mestranol. However, the data allow of no conclusion as to whether the inhibition is a competitive or non-competitive effect.

Cyclical treatment with various oral contraceptives causes a decrease in the urinary excretion of 17-hydroxycorticosteroids (Brichant et al. 1958; Brooks & Prunty 1957; De Moor et al. 1963; Kaiser 1959; Layne & Meyer 1965; Starup et al. 1966; Vermeulen & Ferin 1963; Østergaard et al. 1966 among others). The fact that a normal response was obtained both to the administration of corticotrophin and metyrapone during such treatment, indicates the capacity of the adrenal cortex and the corticotrophin releasing system to react to normal stimuli (Starup et al. 1966).

It seems reasonable therefore to look for a change in the extra-adrenal metabolism of the corticosteroids to explain the decrease in the adrenocortical function.

The purpose of the present study was to investigate the urinary excretion of a large number of different corticosteroid-metabolites during treatment with an oral contraceptive. Furthermore, these results were compared with those obtained in pregnant women during the second trimester of pregnancy.

**MATERIALS AND METHODS**

The first group consisted of 7 women with severe primary dysmenorrhoea. Their ages ranged from 17 to 23, and all of them received a daily dose of 5 mg of megestrol acetate + 0.1 mg of mestranol cyclically from day 5 to day 24 of the cycle. The observation period was 18 to 24 weeks.

In all 7 patients the excretion of 17-ketogenic steroids (17-KGS) and total pituitary gonadotrophins was determined twice before the treatment started and then every 4 weeks during the treatment period. In every case the treatment was continued until the excretion of gonadotrophins was undetectable (less than 3 MUU/d), and the excretion of 17-KGS had decreased to a constant level. The urinary excretion of different corticosteroid-metabolites was determined in all patients before treatment started.
and again just before treatment was discontinued. At the same times the pituitary function was evaluated by means of the metyrapone-test using exactly the same procedure as described previously (Starup et al. 1966).

The second group consisted of 5 normal pregnant women aged 21 to 28. In these 5 women, the urinary excretion of different corticosteroid-metabolites was determined once during the second trimester of pregnancy.

The excretion of 17-KGS was determined in 24-hour urine specimens by the method of Jørgensen (1957). In the age group represented, the normal range of 17-KGS is 4-14 mg/d. The excretion of total pituitary gonadotrophins was determined in 24-hour urine specimens according to the method described by Johnsen (1958).

The concentration of cortisol and compound S in plasma was determined using a modification of the specific isotope dilution principle of Bojesen (1956). The normal range of plasma cortisol is 4.5-19.5 μg/100 ml and of compound S, 0.6-2.3 μg/100 ml.

Determination of the urinary excretion of different corticosteroids, pregnantriol and Δ₅-pregnenetriol was carried out by a modification of the method of Cost & Vegter (1962).

One fifth of a 24-hour urine specimen was hydrolyzed at 37°C for 48 hours with 2000 units/ml urine of β-glucuronidase and 1000 units/ml urine of sulphatase from the digestive juice of Helix Pomatia. The urine was extracted with chloroform (3 × 1/3 volume) and reincubated for 24 hours at 37°C without the addition of further enzyme. The urine was reextracted with chloroform (3 × 1/3 volume), acidified to pH = 1, and extracted with chloroform 3 times during 1 hour, at room temperature. The combined chloroform extracts were washed and evaporated to dryness as described by Cost & Vegter (1962).

Preliminary fractionation into 11 fractions by paper chromatography was performed in toluol/propyleneglycol according to Cost & Vegter (1962). Further separation of the steroids THF, allo-THF, THE, comp. U, F, E, THS, THB, allo-THB, THA, B, A, P-triol, and Δ₅-P-triol was carried out on Whatman paper no. 1 in the following systems:

**THF, allo-THF, THE:** Toluol-methanol-water (400:75:25) at 22°C for 17 hours. Under these conditions THF travelled 14 cm, allo-THF 16 cm, and THE 20 cm.

**F, comp. U:** Isooctan-tert. butanol-water (300:150:270) (E₂B) at 30°C for 5 hours. Under these conditions compound U travelled 12 cm, and F 16 cm.

**E, THS, THB:** Isooctan-tert. butanol-methanol-water (300:150:40:270) at 30°C for 3 1/2 hours. Under these conditions E travelled 12.7 cm, THS 29.0 cm, and THB 22.2 cm.

**THB, allo-THB, THA, B, A:** Toluol-petroleum ether (60-80°C)-methanol-water (5:5:7:3) (Bush B₂). The conditions for this system were as described by Cost & Vegter (1962).

Identification and quantitative determinations of these steroids were carried out by the blue-tetrazolium reaction and/or alkaline fluorescence as described by Cost & Vegter (1962).

Δ₅-P-triol, P-triol: Δ₅-P-triol has an Rₚ-value in the toluol/propyleneglycol system equivalent to THB and allo-THB. Appropriate dilutions of these fractions were chromatographed in the system: Isooctan-toluol-methanol-water (50:150:160:40) at 30°C for 3 hours. Under these conditions Δ₅-P-triol and P-triol travelled 15.5 cm and 18.5 cm, respectively.

The quantitative determination was carried out after reaction at 100°C with trichloroacetic acid (dipping of the paper in a 25 per cent ethanol solution). Standards of known amounts (1IV, 2IV, and 5 μg) of the pure steroids were run together with the unknown fractions. The amount of steroid was determined by visual comparison
of the fluorescence developed according to the same principle as that of Cost & Vegter (1962) for the blue-tetrazolium reaction. Normal values were obtained from 40 non-hospitalized subjects 18 to 50 years of age. The average standard deviation of determinations in duplicate was 16 per cent.

The following abbreviations and trivial names are used

THF: tetrahydrocortisol; 3α,11β,17,21-tetrahydroxy-5β-pregnan-20-one.
Allo-THF: allo-tetrahydrocortisol; 3α,11β,17,21-tetrahydroxy-5α-pregnan-20-one.
THE: tetrahydrocortisone; 3α,17,21-trihydroxy-5β-pregnan-11,20-dione.
Reichsteins substance U: 17α,20β,21-trihydroxy-pregn-4-ene-3,11-dione.
F: cortisol; 11β,17,21-trihydroxy-pregnan-4-ene-3,20-dione.
E: cortisone; 17,21-dihydroxy-pregnan-4-ene-3,11,20-trione.
THS: tetrahydro-11-deoxycortisol; 3α,17,21-trihydroxy-5α-pregnan-20-one.
Compound S: 11-deoxycortisol; 17,21-dihydroxy-pregnan-4-ene-3,20-dione.
THB: tetrahydrocorticosterone; 3α,11β,21-trihydroxy-5β-pregnan-20-one.
Allo-THB: allo-tetrahydrocorticosterone; 3α,11β,21-trihydroxy-5α-pregnan-20-one.
THA: tetrahydro-11-dehydrocorticosterone; 3α,21-dihydroxy-5β-pregnane-11,20-dione.
B: corticosterone; 11β,21-dihydroxy-pregnan-4-ene-3,20-dione.
P-triol: pregnanetriol; 5β-pregnane-3α,17,20α-triol.
Δ5-P-triol: pregnenetriol; 3β,17,20α-trihydroxy-pregn-5-ene.
Megestrol acetate: 6-methyl-6-dehydro-17α-acet oxyprogesterone.
Mestranol: 17α-ethynyl-oestradiol-3-methyl ether.

RESULTS

Figs. 1 and 2 show the urinary excretion of 17-KGS and the plasma concentration of cortisol and compound S in response to administration of metyrapone in the 7 patients, before and during treatment with megestrol acetate + mestranol. Judged from the usual criterions the metyrapone-test was normal in all patients both before and during treatment.

Table 1 shows the daily excretion of the various 17-hydroxy-corticosteroid-metabolites in group I, and Table 2 the daily excretion of the corticosterone-metabolites, pregnanetriol and Δ5-pregnenetriol in group I. All the pretreatment values were within the normal range (Tables 3 and 4). The excretion of the two 5α-hydrogenated compounds, allo-THF and allo-THB during treatment showed a decrease in all cases. The average reduction in these two metabolites was 81 and 65 per cent of the pretreatment values. The average excretion of THE and pregnenetriol was also reduced significantly. The average excretion of non-hydrogenated cortisol was significantly increased during treatment. The excretion of some of the other metabolites showed tendencies towards a change during treatment, but none of these were significant.
Urinary excretion of 17-KGS in response to administration of metyrapone in 7 patients before and during cyclical treatment with megestrol acetate + mestranol.

The average excretion of the same metabolites found in the 5 pregnant women is shown in Tables 3 and 4. The values were compared to those found in 40 normal subjects. Just as in the patients treated with megestrol acetate + mestranol, the average excretion of the 5α-hydrogenated compounds, allo-THF and allo-THB, was significantly reduced. Similarly the excretion of non-hydrogenated cortisol was significantly increased. The excretion of compound U was significantly increased in group II, whereas the excretion in group I only showed a tendency towards an increase. The average excretion of THA and A was significantly increased. The average excretion of Δ5-pregnenetriol was reduced as compared to normal subjects, whereas the excretion of pregnanetriol was unchanged. The last-mentioned finding was in contrast to the significant decrease found in group I during treatment.

Table 5 summarizes the changes observed in the two groups. The excretion of allo-THF, F and allo-THB showed similar changes in groups I and II, but for THE, THA, pregnanetriol, and Δ5-pregnenetriol, the changes were not consistent in the two groups.
Fig. 2.
Total plasma cortisol and plasma compound S in response to administration of metyrapone in 7 patients before and during cyclical treatment with megestrol acetate + mestranol.

DISCUSSION

The average excretion of allo-THF, allo-THB, THB and pregnanetriol was decreased, while that of cortisol was increased during treatment with megestrol acetate + mestranol. With the exception of the reduction in the excretion of THE, the main changes were similar to those seen in pregnant women.

The changes observed might be caused by megestrol acetate alone, by mestranol alone, or by both substances. Arends (1963) investigated the effect of megestrol acetate alone on the adrenal function in rats. In hypophysectomized female rats, a marked adrenal atrophy was found during treatment, indicating a direct extra-pituitary effect on the adrenal glands. In contrast to this, Lebech & Nordentoft (1967) found no significant changes in the excretion of 17-KGS in 15 elderly male patients during treatment with a daily dose of 20 mg of megestrol acetate for a period of 3 months. The last-mentioned investigation indicates that it is the oestrogenic component in the combined preparation used, which is responsible for the reduction in the urinary excretion of the corticosteroids.

It is known that in the presence of high oestrogen levels, as found during
Table 1.
The excretion of the various 17-OH-corticosteroid-metabolites in mg per day before (1) and during (2) treatment with megestrol acetate + mestranol.

<table>
<thead>
<tr>
<th></th>
<th>THF 1)</th>
<th>THF 2)</th>
<th>allo-THF 1)</th>
<th>allo-THF 2)</th>
<th>THE 1)</th>
<th>THE 2)</th>
<th>Comp. U 1)</th>
<th>Comp. U 2)</th>
<th>F 1)</th>
<th>F 2)</th>
<th>E 1)</th>
<th>E 2)</th>
<th>THS 1)</th>
<th>THS 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.9</td>
<td>0.7</td>
<td>0.4</td>
<td>0.09</td>
<td>2.6</td>
<td>1.8</td>
<td>0.03</td>
<td>0.05</td>
<td>0.04</td>
<td>0.07</td>
<td>0.05</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Standard</td>
<td>0.28</td>
<td>0.20</td>
<td>0.14</td>
<td>0.055</td>
<td>0.90</td>
<td>0.63</td>
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</tbody>
</table>

Significance of difference (P-value)

|               |        |        |             |             |        |        |            |             |      |      |      |      |        |        |
|               | N.S.   | < 0.01 | < 0.05      | N.S.        | < 0.01 | N.S.   | N.S.      | N.S.        |      |      |      |      |        |        |
Table 2.
The excretion of corticosterone-metabolites, pregnanetriol and \( \Delta_5 \)-pregnenetriol in mg per day before (1) and during (2) treatment with megestrol acetate + mestranol.

<table>
<thead>
<tr>
<th></th>
<th>THB 1)</th>
<th>THB 2)</th>
<th>allo-THB 1)</th>
<th>allo-THB 2)</th>
<th>THA 1)</th>
<th>THA 2)</th>
<th>B 1)</th>
<th>B 2)</th>
<th>A 1)</th>
<th>A 2)</th>
<th>P-triol 1)</th>
<th>P-triol 2)</th>
<th>( \Delta_5 )-P-triol 1)</th>
<th>( \Delta_5 )-P-triol 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.12</td>
<td>0.12</td>
<td>0.25</td>
<td>0.08</td>
<td>0.11</td>
<td>0.13</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>1.0</td>
<td>0.3</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Standard</td>
<td>0.03</td>
<td>0.07</td>
<td>0.07</td>
<td>0.02</td>
<td>0.05</td>
<td>0.07</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.36</td>
<td>0.2</td>
<td>0.13</td>
<td>0.07</td>
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<tr>
<td>deviation</td>
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</tr>
<tr>
<td>Significance of difference (P-value)</td>
<td>N. S.</td>
<td>&lt; 0.001</td>
<td>N. S.</td>
<td>N. S.</td>
<td>N. S.</td>
<td>&lt; 0.01</td>
<td>N. S.</td>
<td></td>
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Table 3.
The excretion of 17-OH-corticosteroid-metabolites in mg per day in 40 normal subjects (N) and in 5 pregnant women (P) in the second trimester of pregnancy.

<table>
<thead>
<tr>
<th></th>
<th>THF N</th>
<th>P</th>
<th>allo-THF N</th>
<th>P</th>
<th>THE N</th>
<th>P</th>
<th>Comp. U N</th>
<th>P</th>
<th>F N</th>
<th>P</th>
<th>E N</th>
<th>P</th>
<th>THS N</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Mean</td>
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<td>0.8</td>
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<td>0.15</td>
<td>2.3</td>
<td>2.8</td>
<td>0.02</td>
<td>0.09</td>
<td>0.04</td>
<td>0.11</td>
<td>0.05</td>
<td>0.11</td>
<td>0.02</td>
<td>0.08</td>
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<tr>
<td>Standard deviation</td>
<td>0.56</td>
<td>0.27</td>
<td>0.57</td>
<td>0.12</td>
<td>0.91</td>
<td>0.92</td>
<td>0.01</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
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<td>0.08</td>
<td>0.02</td>
<td>0.05</td>
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<tr>
<td>Significance of difference (P-value)</td>
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<td>N.S.</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
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<td>N.S.</td>
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</table>
Table 4.
The excretion of corticosterone-metabolites, pregnanetriol and \( \Delta_5 \)-pregnenetriol in mg per day in 40 normal subjects (N) and in 5 pregnant women (P) in the second trimester of pregnancy.

<table>
<thead>
<tr>
<th></th>
<th>THB</th>
<th>allo-THB</th>
<th>THA</th>
<th>B</th>
<th>A</th>
<th>P-triol</th>
<th>( \Delta_5 )-P-triol</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>Mean</td>
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<tr>
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<tr>
<td>Significance</td>
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<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>N. S.</td>
<td>= 0.05</td>
<td>N. S.</td>
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<tr>
<td>of difference</td>
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<tr>
<td>(P-value)</td>
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Table 5.
Summary of the changes in excretion of various metabolites of the corticosteroids and some of their precursors during treatment with megestrol acetate + mestranol compared to pretreatment values (Group I), and in pregnant women in the second trimester of pregnancy compared to the values found in normal subjects (Group II).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Significance of difference P-value</th>
<th>Significance of difference P-value</th>
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</thead>
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<td>Group I Change</td>
<td>Group II Change</td>
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<tr>
<td>allo-THF</td>
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<tr>
<td>THE</td>
<td>↓&lt; 0.001</td>
<td>↓&lt; 0.01</td>
</tr>
<tr>
<td>Comp. U</td>
<td>↓&lt; 0.05</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>↑&lt; 0.01</td>
<td>↑&lt; 0.01</td>
</tr>
<tr>
<td>allo-THB</td>
<td>↓&lt; 0.001</td>
<td>↓&lt; 0.05</td>
</tr>
<tr>
<td>THA</td>
<td>—</td>
<td>↑&lt; 0.05</td>
</tr>
<tr>
<td>A</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-triol</td>
<td>↓&lt; 0.01</td>
<td>—</td>
</tr>
<tr>
<td>Δ5-P-triol</td>
<td>↓N.S.</td>
<td>↓&lt; 0.01</td>
</tr>
</tbody>
</table>

pregnancy or treatment with oestrogenic compounds, the plasma cortisol level increases (Cohen et al. 1958; Gemzell 1953; Layne et al. 1962; Mills et al. 1960; Peterson et al. 1960; Robertson et al. 1959). High plasma cortisol levels have also been found in the patients in the present study during treatment with megestrol acetate + mestranol. This results in an increased excretion of cortisol. The pregnant women also show an increased urinary excretion of cortisol.

As to the question of an altered metabolism of the corticosteroids during treatment with megestrol acetate + mestranol, the conversion to 5α-metabolites of cortisol and corticosterone is significantly lower than before treatment. A similar low percentage of the 5α-metabolites is, however, also found in the 5 pregnant women. The cause of the decreased capacity to 5α-reduction observed in these two conditions is not known, but it might be ascribed to a direct effect on the liver enzymes of the steroids administered. Since the same change is also found in pregnancy, it is most probably caused by the oestrogenic component.

The enzymes responsible for 5α-reduction are the Δ4-5α-reductases. These enzymes have been isolated from rat liver, and they are associated with the liver microsomes (Dorfman & Ungar 1965). An effect on these liver enzymes might be due to competition between the steroids administered and cortisol or corticosterone, but the data allow of no conclusion as to whether the inhibition is a competitive or non-competitive effect.
The degradation of cortisol might also take place through processes other than the normally predominant pathways, for instance by a hydroxylolation at C6 or C20. The procedure used in this study utilizing a chloroform extraction medium is not suitable for the extraction of these very polar substances. However, the metabolites of cortisol as well as the cortols and the cortolones are determined as 17-KGS.

The present study indicates that the decrease in the excretion of 17-KGS observed during treatment with megestrol acetate + mestranol might be due to both a general reduction in the synthesis and the secretion of corticosteroids in the adrenal cortex and to a change in the extra-adrenal metabolism of the corticosteroids, probably caused by a direct effect on the liver enzymes, particularly those necessary for the formation of the 5α-metabolites those necessary for the formation of the 5α-metabolites of both cortisol and corticosterone. Further studies with tracer amounts of labelled steroids will be necessary to solve the problem.

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REFERENCES


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