SERUM LEVELS OF UNCONJUGATED AETIOCHOLANOLONE, ANDROSTENEDIONE, TESTOSTERONE, DEHYDROEPIANDROSTERONE, ALDOSTERONE, PROGESTERONE AND OESTROGENS DURING THE NORMAL MENSTRUAL CYCLE

By

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ABSTRACT

The results of daily determination of the levels of gonadotrophins, oestradiol, oestrone, progesterone, aldosterone, dehydroepiandrosterone, androstenedione, testosterone, and aetiocholanolone in the serum of 6 normal, ovulating women are reported and discussed. A pre-ovulatory aldosterone peak and rising values in the luteal phase of the cycle were found. Androstenedione, testosterone, and aetiocholanolone levels were significantly elevated from 3 days before until 3 days after ovulation. Since the mean androstenedione/aetiocholanolone ratio in the individual cycles in this period was similar to the ratio found during the rest of the cycle, we think it unlikely that aetiocholanolone is produced by the ovaries. No correlation was found between the aetiocholanolone patterns and the basal body temperature. In a case of conception followed for 20 days after ovulation, the steroid patterns remained unchanged until the presumed day of implantation, after which the aldosterone, androstenedione, testosterone, and aetiocholanolone levels started to rise. The mean androstenedione/aetiocholanolone ratio during the 10 days after implantation did not differ from the values obtained in the foregoing periods, so direct aetiocholanolone production by the ovaries after implantation seems unlikely.
Systematic names of steroids

Aldosterone: \(1\beta,21\text{-dihydroxy-3,20\text{-dioxo-4-pregnene-18-al}}\).
Androstenedione: \(4\text{-androstene-3,17\text{-dione}}\).
\(11\alpha\text{-OH-androstenedione: }11\alpha\text{-hydroxy-4-androstene-3,17\text{-dione}}\).
Androsterone: \(3\alpha\text{-hydroxy-5\alpha\text{-androstan-17\text{-one}}}\).
Cortisol: \(11\beta,17,21\text{-trihydroxy-4-pregnene-3,20\text{-dione}}\).
Cortisone: \(17,21\text{-dihydroxy-4-pregnene-3,11,20\text{-trione}}\).
Corticosterone: \(11\beta,21\text{-dihydroxy-4-pregnene-3,20\text{-dione}}\).
Dehydroepiandrosterone: \(3\beta\text{-hydroxy-5\text{-androsten-17\text{-one}}}\).
5\alpha\text{-Dihydrotestosterone: }17\beta\text{-hydroxy-5\alpha\text{-androstan-3\text{-one}}}.
5\beta\text{-Dihydrotestosterone: }17\beta\text{-hydroxy-5\beta\text{-androstan-3\text{-one}}}.
Deoxycorticosterone: \(21\text{-hydroxy-4-pregnene-3,20\text{-dione}}\).
Aetiocholanolone: \(3\alpha\text{-hydroxy-5\beta\text{-androstan-17\text{-one}}}\).
\(11\alpha\text{-OH-aetiocholanolone: }3\alpha,11\alpha\text{-dihydroxy-5\beta\text{-androstan-17\text{-one}}}\).
6-Keto-oestradiol: \(1,3,5(10)\text{-oestratriene-3,17\text{-diol-6\text{-one}}}\).
\(11\alpha\text{-Methyltestosterone: }11\alpha\text{-methyl-17\beta\text{-hydroxy-4-androsten-3\text{-one}}}\).
Oestradiol: \(1,3,5(10)\text{-oestratriene-3,17\beta\text{-diol}}\).
Oestradiol-17\alpha: \(1,3,5(10)\text{-oestratriene-3,17\alpha\text{-diol}}\).
Oestrone: \(3\text{-hydroxy-1,3,5(10)\text{-oestratrien-17\text{-one}}}\).
Pregnenolone: \(3\beta\text{-hydroxy-5-pregnen-20\text{-one}}}\).
Progesterone: \(4\text{-pregnene-3,20\text{-dione}}\).
\(11\alpha\text{-OH-progesterone: }11\alpha\text{-hydroxy-4-pregnene-3,20\text{-dione}}\).
\(16\alpha\text{-OH-progesterone: }16\alpha\text{-hydroxy-4-pregnene-3,20\text{-dione}}\).
\(17\alpha\text{-OH-progesterone: }17\alpha\text{-hydroxy-4-pregnene-3,20\text{-dione}}\).
Testosterone: \(17\beta\text{-hydroxy-4-androsten-5\text{-one}}}\).

Several investigations have been published on the serum levels of oestrogens (Baird 1974; Baird & Fraser 1974), aldosterone (Sundsfjord & Aakvaag 1973; Katz & Romfh 1972), dehydroepiandrosterone (DHEA), androstenedione, and testosterone (Abraham et al. 1973; Abraham & Chakmakjian 1973; Abraham 1974; Baird et al. 1974; Judd & Yen 1973) during the normal menstrual cycle. These have shown that androstenedione and testosterone are produced by the adrenals and the active ovary with a minor contribution from the contralateral ovary. Around ovulation, the androstenedione and testosterone levels increase due to production of these steroids by the follicle, the stroma of the active ovary, and the corpus luteum. DHEA is secreted mainly by the adrenals, and no cyclic ovarian production of DHEA could be detected (Lloyd et al. 1971). Aldosterone patterns were investigated by Sundsfjord & Aakvaag (1973) and by Katz & Romfh (1972) who found higher levels in the luteal phase of the cycle than in the follicular phase. This difference is explained by the Na-diuretic effect of progesterone, which is compensated for by an aldosterone increase (Sundsfjord 1971). Sundsfjord (1971) found a pre-ovulatory aldosterone peak in non-fasting ambulant women, coinciding with the pre-ovulatory 17-OH-progesterone peak (Abraham et al. 1972), but this find-
ing was not confirmed by Katz & Romfh (1972) who investigated fasting supine women.

As far as we know, no data have been published on aetiocholanolone patterns during the menstrual cycle. Normal levels in women have been reported recently (Youssefnejadian et al. 1974), but only for female plasma pools. Although progesterone has been found to raise the body temperature (review by Tausk 1971), the mechanism underlying this thermogenic effect has not yet been fully elucidated. Because aetiocholanolone is known to increase the body temperature too (Bondy et al. 1965; Bodel et al. 1968), we measured the aetiocholanolone levels during the menstrual cycle to investigate a possible role in the post-ovulatory temperature elevation. For an understanding of the physiological interrelationships during the menstrual cycle, the simultaneous measurement of the various steroid patterns is critical. Investigations in six normal cycles and one early phase of pregnancy are presented.

MATERIALS AND METHODS

Subjects

Seven healthy young women with a history of regular menses of 24–30 days duration were studied. Blood samples were taken daily between 9 and 11 a.m. during the cycle, under non-fasting, ambulant conditions. The LH, FSH, oestradiol, oestrone, progesterone, aldosterone, DHEA, androstenedione, testosterone, and aetiocholanolone levels were measured in each serum sample by radioimmunoassay. Cycles were considered to be ovulatory on the basis of a biphasic basal body temperature and plasma progesterone levels. For the interpretation of the data, the cycles were centered around the midcycle gonadotrophin peak (day 0). Because of the variation in the length of the individual cycles, we present hormone levels from 10 days before ovulation until 10 days thereafter. One of the women conceived during the study; in this case hormone levels were followed until day +20.

Glycoproteins

LH and FSH were measured by radioimmunoassay by Dr. R. M. Lequin, Radboudziekenhuis, Nijmegen (Smals et al. 1974).

Aldosterone and C-19 steroids

Four ml serum was extracted with 40 ml dichloromethane after addition of 2000 DPM of [1,2-3H]aldosterone, [1,2-3H]testosterone, [7-3H]DHEA, and [1,2-3H]androstenedione as internal standards. Aldosterone, testosterone, DHEA, and androstenedione were separated on Whatman-1 chromatography paper in a Bush system (heptane/methanol 80%/v 1/1, Bush 1952) for 11 h at 28°C. Androstenedione and aetiocholanolone are not separated in this system, but because of the specificity of the antisera against androstenedione and aetiocholanolone, both steroids could be detected from the same spot on the chromatogram. Table 1 shows the cross-reacting steroids with a similarity in structure that had a percentage cross-reaction higher than 0.01%. In the system we used, androsterone, which can interfere in the aetiocholanolone
Table 1. 
Cross-reactions in %, determined at 50 % displacement.

<table>
<thead>
<tr>
<th></th>
<th>Antiserum against</th>
<th>Aldosterone</th>
<th>Testosterone</th>
<th>DHEA</th>
<th>Aetiocholanolone</th>
<th>Androstenedione</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aldosterone</strong></td>
<td></td>
<td>100</td>
<td>&lt; 0.01</td>
<td></td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
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<td>&lt; 0.01</td>
<td></td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td></td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>17-OH-progesterone</td>
<td></td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td><strong>Testosterone</strong></td>
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<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Androstenedione</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>0.15</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>5α-dihydrotestosterone</td>
<td></td>
<td>11</td>
<td>&lt; 0.01</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>5β-dihydrotestosterone</td>
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<td>10</td>
<td>8</td>
<td></td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>11α-methyltestosterone</td>
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<td></td>
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</tr>
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<tr>
<td><strong>Aetiocholanolone</strong></td>
<td></td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>100</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>11α-OH-aetiocholanolone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td></td>
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<td>Androsterone</td>
<td></td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Dehydroepiandrosterone</strong></td>
<td></td>
<td>&lt; 0.01</td>
<td>100</td>
<td>1.0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>3β,20β-dihydroxy-5-pregnene</td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β,20α-dihydroxy-5-pregnene</td>
<td></td>
<td>12</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pregnenolone</td>
<td></td>
<td>8</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Dehydroepiandrosterone-sulphate</td>
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</tr>
</tbody>
</table>

assay, has a higher RF value than androstenedione/aetiocholanolone. To avoid blanks, 10 000 DPM tritiated steroids were used for location purposes (Fröhlich et al. 1974). The amounts of aldosterone, DHEA, androstenedione, testosterone, and aetiocholanolone were measured by radioimmunoassay.

In the calculations for aetiocholanolone the same recovery values were used as for androstenedione. We checked the validity of this practice by a series of recovery experiments with known amounts of androstenedione and aetiocholanolone added to the same sample. The mean recoveries after chromatography were 85 % for aldosterone, 72 % for testosterone, 68 % for DHEA, and 52 % for androstenedione/aetiocholanolone.

Antiserum against androstenedione, donated by Dr. W. Schopman, Bergwegziekenhuis, Rotterdam, was produced in rabbits by injecting them intracutaneously with a conjugate of 11α-hydroxy-4-androstene-3,17-dione-11α-hemisuccinate and bovine serum albumin (BSA), K = 0.95 × 10⁹ l/mol, dilution 1/2000. Antiserum against aldosterone was prepared in our laboratory by intracutaneous injection of rabbits with
aldosterone-18,21-diacetate-3(-0-carboxy-methyl)-oxim coupled to BSA, K = 3.2 x 10^9 l/mol, dilution 1/1000. Antisera against DHEA, testosterone, and aetiocholanolone were similarly obtained with a conjugate of BSA and dehydroepiandrosterone-17-(0-carboxy-methyl)-oxim K = 0.83 x 10^9 l/mol, dilution 1/500; a conjugate of BSA and testosterone-3-(0-carboxy-methyl)-oxim K = 2.2 x 10^9 l/mol, dilution 1/2000 and a conjugate of BSA and aetiocholanolone-17-(0-carboxy-methyl)-oxim K = 4.1 x 10^9 l/mol, dilution 1/1500. The lowest detectable amounts in the radioimmunoassay were aldosterone: 27 pg (P < 0.05), DHEA: 40 pg (P < 0.05), androstenedione: 30 pg (P < 0.05), and aetiocholanolone: 8 pg (P < 0.05). Recovery experiments confirmed the accuracy of the assays. The intra-assay variability of the different assays ranged from 10.1 to 12.3 %, the inter-assay variability from 12.5 to 14.7 %.

**Oestrogens**

Two ml serum was extracted twice with 6 ml ethylacetate after the addition of 5000 dpm [6,7-3H]oestradiol and [6,7-3H]oestrone as internal standards. The extract was evaporated to dryness and transferred to a column of 1.5 g Sephadex LH-20 (Pharmacia, Uppsal). Oestrone, oestradiol, and oestriol were separated by batch-wise elution with 6 ml benzene/methanol 95/5 (oestrone fraction) and 6 ml benzene/ethylacetate/methanol 80/15/5 (oestradiol fraction), each preceded by 4 ml washings. Cross-contamination, as checked with radioactive oestrogens, was below 2 %. Eluates were washed once with 1 ml 0.05 n HCl, evaporated to dryness, and dissolved in buffer. The mean recoveries were 76 % oestradiol and 84 % oestrone. Antisera against oestradiol and oestrone were produced in rabbits with a conjugate of BSA and 6-keto-oestradiol-17β-6-(0-carboxy-methyl)-oxim K = 1.2 x 10^10 l/mol, dilution 1/3000, and a conjugate of BSA and oestrone-17-(0-carboxy-methyl)-oxim K = 3.0 x 10^10 l/mol, dilution 1/6000. The lowest detectable amounts were for oestradiol: 4 pg (P < 0.05) and

<table>
<thead>
<tr>
<th>Antiserum against</th>
<th>Oestrone</th>
<th>Oestradiol</th>
<th>Progesterone</th>
</tr>
</thead>
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<tr>
<td><strong>Progesterone</strong></td>
<td>&lt; 0.03</td>
<td>&lt; 0.03</td>
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</tr>
<tr>
<td>17-OH-progesterone</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>11α-OH-progesterone</td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>16α-OH-progesterone</td>
<td></td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td></td>
<td></td>
<td>5.5</td>
</tr>
<tr>
<td>Corticosterone</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Oestrone</strong></td>
<td>100</td>
<td>1.3</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td><strong>Oestradiol</strong></td>
<td>27</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>6-keto-oestradiol</td>
<td>&lt; 0.03</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Oestriol</td>
<td>6</td>
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</tr>
<tr>
<td>Oestradiol-17α</td>
<td>4.4</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Cross-reactions in %, determined at 50 % displacement.
for oestrone: 6 pg ($P < 0.05$). Relevant cross-reactions are shown in Table 2; non-phenolic steroids showed no cross-reaction. Intra-assay variability was 8% for oestradiol and oestrone. Inter-assay variability was 16% for oestradiol and 17% for oestrone.

**Progesterone**

One and a half ml serum was extracted with 7 ml heptane after the addition of 5000 DPM $[1,2-^3H]$progesterone as internal standard. The extract was evaporated to dryness and dissolved in buffer. The mean recovery was 60%. Antiserum was produced in rabbits with a conjugate of BSA and $11\alpha$-hydroxy-progesterone-$11\alpha$-hemisuccinate $K = 4.4 \times 10^5$ l/mol, dilution $1/2400$. Relevant cross-reactions are shown in Table 2. The intra-assay variability was 9%, the inter-assay variability 12%, and the lowest detectable amount 14 pg ($P < 0.05$).

**Chemicals and tritiated steroids**

All chemicals used were of analytical grade. All organic solvents were re-distilled shortly before use, if necessary after pre-treatment with KMnO$_4$, to avoid blank values in the radioimmunoassay. Unlabelled steroids were obtained from Ikapharm, Ramat Gan, Israel. $[1,2-^3H]$Aldosterone (S. A. 53 Ci/mmol), $[6,7-^3H]$oestradiol (S. A. 56 Ci/mmol), $[6,7-^3H]$oestrone (S. A. 46 Ci/mmol) and $[1,2-^3H]$progesterone (S. A. 48 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, U. K. $[7-^3H]$Dehydroepiandrosterone (S. A. 21 Ci/mmol), $[1,2-^3H]$androstenedione (S. A. 45.9 Ci/mmol), $[1,2-^3H]$testosterone (S. A. 50 Ci/mmol), and $[1,2-^3H]$aetiocholanolone (S. A. 48 Ci/mmol) were purchased from NEN-Chemicals, Boston, USA. The purity of all tritiated steroids was at least 98%.

**RESULTS**

The mean serum levels of LH, FSH, oestradiol, oestrone, and progesterone, measured during six normal cycles, are presented in Fig. 1, which also shows the mean basal body temperatures. All of these values are in accordance with the data presented by Abraham et al. (1972). The mean serum levels of aldosterone, DHEA, androstenedione, testosterone, and aetiocholanolone are given in Fig. 2. Aldosterone shows a marked peak on the day before ovulation. In the luteal phase of the cycle the aldosterone levels rise to about twice the follicular values. DHEA fluctuates widely, without any consistent pattern. Androstenedione, testosterone, and aetiocholanolone levels start to rise 3 days before ovulation and remain elevated for 7 days. On day +4, pre-ovulatory levels are reached again. On the basis of these trends, we divided the cycles into three periods: period I covering day −10 to day −3, period II days −3 to +4, and period III days +4 to +10. The mean androstenedione levels during period II are significantly higher than during period I (Student's $t$-test $P < 0.0125$) or period III ($P < 0.05$). There is no significant difference between periods I and III ($P < 0.25$). For testosterone there is
Fig. 1.
Mean basal body temperature and mean values of LH, FSH, progesterone, oestradiol and oestrone in serum from 6 women during ovulatory cycles. Vertical bars represent one se of the mean.
Fig. 2.
Mean values of aldosterone, DHEA, androstenedione, testosterone, and aetiocholanolone in serum from 6 women during ovulatory cycles. Vertical bars represent one SE of the mean.
no significant difference between periods I and II \((P < 0.3)\) or between I and III \((P < 0.1)\), but the difference between II and III is significant \((P < 0.025)\). Aetiocholanolone shows a highly significant difference between the mean levels measured during periods I and II \((P < 0.005)\) and II and III \((P < 0.005)\). No significant difference could be demonstrated between periods I and III \((P < 0.45)\).

The ratios between the mean androstenedione levels and the mean aetiocholanolone levels of each subject in periods I, II, and III are given in Table 3. The individual ratios vary widely, but during one cycle no differences were found in the androstenedione/aetiocholanolone ratio.

One of the women became pregnant during the study. Her gonadotrophin, oestradiol, oestrone, and progesterone levels, measured from day \(-10\) until day \(+20\), and her basal temperature values are shown in Fig. 3. Fig. 4 gives the aldosterone, DHEA, androstenedione, testosterone, and aetiocholanolone values. From day \(-10\) to day \(+9\), the steroid patterns are similar to those seen in the normal cycles. On day \(+9\), 2 days after the presumed day of implantation, the aldosterone, androstenedione, testosterone, and aetiocholanolone levels started to rise again. The androstenedione/aetiocholanolone ratio in periods I, II, and III and in the period from day \(+9\) to day \(+20\) (IV) are given in Table 3. There are no significant differences between these ratios.

The mean aetiocholanolone levels of six normal cycles and the mean basal body temperature in the subjects are presented in Fig. 5. The aetiocholanolone levels started to rise 4 days before the post-ovulatory temperature elevation.

**Table 3.**

Individual androstenedione/aetiocholanolone ratios*.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Period:</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days:</td>
<td>-10/-3</td>
<td>-3/+4</td>
<td>+4/+10</td>
<td>+10/+20</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>1.98</td>
<td>1.62</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>10.60</td>
<td>8.76</td>
<td>9.65</td>
<td></td>
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<tr>
<td>E</td>
<td></td>
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<td>3.42</td>
<td>3.18</td>
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<tr>
<td>G</td>
<td></td>
<td>3.47</td>
<td>3.26</td>
<td>3.19</td>
<td></td>
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<tr>
<td>K</td>
<td></td>
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<td>1.02</td>
<td>1.00</td>
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<tr>
<td>L</td>
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<td>2.35</td>
<td>2.05</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>(early pregnancy)</td>
<td>4.41</td>
<td>4.14</td>
<td>3.52</td>
<td>3.57</td>
</tr>
</tbody>
</table>

* The values are means for the indicated periods.
Fig. 3.
Basal body temperature and LH, FSH, oestrone, oestradiol, and progesterone levels in the serum of a woman, shortly before and after conception.
Aldosterone, DHEA, androstenedione, testosterone, and aetiocholanolone levels in the serum of a woman shortly before and after conception.
DISCUSSION

As is known from previous studies (Abraham et al. 1973; Horton & Tait 1966; Tait & Horton 1965; Strott et al. 1970), the metabolic clearance rates (MCR) of DHEA, androstenedione, and testosterone do not show marked fluctuations during the menstrual cycle. Thus, if we assume the ratio of conjugated steroid to free steroid to be constant, the measured serum levels can be considered to reflect the production of these steroids by the adrenals and the ovaries as well as the peripheral interconversion. The production of DHEA, androstenedione, and testosterone by the adrenals is nearly constant during the cycle (Abraham 1974). In our investigation DHEA, which is secreted mainly by the adrenals (Baird 1974), does not show a well-defined pattern during the normal menstrual cycle.

Androstenedione and testosterone levels are elevated in period II of the cycle (Fig. 2), probably due to the augmented steroid production by the Graafian follicle, the stroma of the active ovary, and the corpus luteum (Abraham 1974; Baird et al. 1974; de Jong et al. 1974), and additionally by the interconversion of androstenedione and testosterone (Bardin & Lipsett 1967; Horton & Tait 1966). It is noteworthy that the increase in the serum androgen levels coincides with the increase in the serum LH levels. It seems probable that the pre-ovulatory increase in androgen production is caused by ovarian LH
stimulation, and that androgens possibly play a role in the mechanism underlying ovulation, as postulated for rats by Gaarenstroom & de Jong (1946).

The aetiocholanolone levels are also increased during period II of the cycle (Fig. 2). As far as we know, aetiocholanolone is mainly produced by the reduction of androstenedione in the liver. In an attempt to determine whether this is the sole source of aetiocholanolone, the mean androstenedione/aetiocholanolone ratios in each of the 6 women in the three periods of the cycle were calculated (Table 3). Comparison of the results for periods I, II, and III showed no significant differences. This suggests that aetiocholanolone is produced by the 5β-reductase system of the liver, without a direct contribution from the active ovary during period II.

The mean post-ovulatory temperature elevation of 0.4°C in the 6 normal women appeared 4 days (day +1) after the aetiocholanolone levels rose (day -3) to 45–50 ng/100 ml serum (Fig. 5). The temperature elevation of 0.6–1.1°C after an infusion of aetiocholanolone, where levels of between 1.7 and 4.8 μg/100 ml serum were reached, appeared after 4 to 6 h (Bondy et al. 1965). In view of the difference in serum levels and the lag-time before the rise in temperature occurs, it seems unlikely that aetiocholanolone is the direct trigger for the post-ovulatory temperature elevation.

The marked aldosterone peak on day -1 and the post-ovulatory elevation of the aldosterone levels (Fig. 2) were also described by Sundsfjord & Aakvaag (1973) in non-fasting ambulant women. In a group of fasting supine women no pre-ovulatory aldosterone peak was measured (Katz & Romfh 1972).

It seems possible that the pre-ovulatory 17-OH-progesterone elevation (Abraham et al. 1972) triggers a pre-ovulatory aldosterone peak although it is known that 17-OH-progesterone has no Na-diuretic effect (Landau et al. 1958).

In the case of pregnancy in this series, no differences in the aldosterone and C-19 patterns could be detected as compared with the normal cycles up to day +9 (Fig. 4) as already mentioned. On day +9, 2 days after the presumed implantation, the androstenedione, testosterone, and aetiocholanolone levels started to rise. DHEA remained at the same level. The aldosterone level also increased from this day on, presumably due to the Na-diuretic effect of progesterone (Fig. 3) (Sundsfjord & Aakvaag 1973) and the stimulatory effect of 17-OH-progesterone. The increase in androstenedione and testosterone levels starting on day +9 can be explained by stimulation of the steroid secretion of the corpus luteum graviditatis by HCG (Baird et al. 1974).

As is known (Mishell et al. 1974) HCG production starts on day +8 and in our investigation HCG was detectable in the LH assay from day +13 (Fig. 3) because of cross reaction.

Aetiocholanolone also started to rise on day +9 (Fig. 4). The mean androstenedione/aetiocholanolone ratios in periods I, II, and III and in the period
between day +9 and day +20 (IV) in the case of conception (Table 3) are not divergent. The reduction of androstenedione to aetiocholanolone by the 5β-reductase in the liver seems to be the main source of aetiocholanolone. During the time covered by the study, no evidence was found for direct ovarian secretion of aetiocholanolone after implantation.

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