THE EFFECT OF CHLORMADINONE ACETATE ON PROGESTERONE SECRETION AND METABOLISM

By
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ABSTRACT

The effect of chlormadinone acetate (6-chloro-17α-hydroxypregna-4,6-diene-3,20-dione-17-acetate) on the secretion and metabolism of progesterone has been investigated in six healthy women. Twenty-four hour urine samples were collected daily throughout pre-treatment cycles and again throughout the second cycle of treatment with 0.5 mg/day chlormadinone acetate. In all urine specimens LH, pregnanediol, oestrone, 17β-oestradiol, and oestriol were determined. Progesterone metabolism was assessed in terms of the transformation of labelled progesterone administered intravenously nine days after the urinary LH peak and progesterone production was calculated from the conversion of labelled hormone to urinary 5β-pregnane-3α,20α-diol glucuroniside and the mass of urinary metabolite of endogenous origin. The results suggested that although the LH peak was markedly suppressed in all subjects (P 0.05 – 0.02; t test), the ovarian response could be divided into two categories. In three individuals, a low but definite premenstrual rise in LH excretion persisted and this was associated with normal or only moderately suppressed progesterone production rates. In addition, there was only a minimal decrease in the level of pregnanediol during the luteal phase of the cycle and in the cyclical excretion of urinary oestrogens. These results are consistent with the luteinisation of a developing follicle without ovulation having necessarily occurred. In the remaining three subjects, the premenstrual rise in LH was not detectable and progesterone production was markedly suppressed. There was no evidence of a rise in pregnanediol in the second half of the cycle in two patients and in the third the level was markedly reduced. In addition, in two of those cycles there was suppression of urinary oestrogen excretion. In treated cycles, there was a
significantly lower incorporation of radioactivity into products liberated by solvolysis (\( P \text{0.02} - \text{0.01}; t \text{ test} \)). Furthermore, in subjects in whom the progesterone production rate was not markedly suppressed, there was a significant decrease in the conversion of administered progesterone to 5\( \beta \)-pregnane-3\( \alpha \),20\( \alpha \)-diol (\( P \text{0.01} - \text{0.001}; t \text{ test} \)) and some increase in the conversion to 5\( \beta \)-pregnan-3\( \alpha \)-ol-20-one (\( P \text{0.02} - \text{0.01}; t \text{ test} \)).

The oral administration of chlormadinone acetate (0.5 mg/day) has been shown to be an effective contraceptive agent (Martinez-Manautou et al. 1967). However, the means by which this effect is mediated is incompletely understood and is probably the result of many interrelated factors. Thus, the presence of corpora lutea and of secretory endometria suggested that ovulation may not be inhibited in a high proportion of subjects (Martinez-Manautou et al. 1967). Furthermore, studies involving the determination of various steroids and luteinising hormone (LH) (Elstein 1969; Jaffe & Midgley 1969; Geller 1969; Hammerstein 1969; Diczfalusy et al. 1969; Larsson-Cohn et al. 1970) have indicated a considerable individual variation in response.

On the other hand, relatively constant effects upon the physical and biochemical characteristics of cervical mucus have been demonstrated (Elstein 1969) and these are associated with the presence of few sperm of low motility in post coital tests (Gutierrez et al. 1969; Elstein 1969). These effects might result from changes in the concentration or metabolism of ovarian steroids in the endometrium, or from a direct action of chlormadinone acetate on various enzyme systems which are associated with the production of cervical mucus.

The purpose of the present study was to investigate the effect of daily administered chlormadinone acetate on the metabolism of progesterone (pregn-4-ene-3,20-dione) as indicated by the transformation of labelled progesterone administered intravenously nine days after the urinary LH peak; and on the secretion of progesterone as reflected in the level of urinary 5\( \beta \)-pregnane-3\( \alpha \),20\( \alpha \)-diol and calculation of the urinary production rate.

**EXPERIMENTAL**

**Subjects**

Six women without obvious endocrine dysfunction and with regular menstrual cycles volunteered to participate in this study. All had cycles of normal length (27–31 days), and were aged 21–28 years.

**Plan of study**

Twenty-four hour urine samples from each subject were collected for one complete cycle. Chlormadinone acetate (0.5 mg/day) was then taken continuously and a similar series of urine samples collected for the entire second cycle of treatment. All specimens were analysed for LH, pregnanediol, oestrone, 17\( \beta \)-oestradiol, and oestriol. In addition, nine days after the LH peak 1 \( \mu \text{Ci} \) of progesterone was administered intra-
venously. The spectrum of urinary metabolites was examined and the urinary production rate of progesterone calculated.

Materials

A preparation of $\beta$-glucuronidase from beef liver (5000 units/ml) was obtained from General Diagnostics Dept., William R. Warner and Co. Ltd., Eastleigh, Hants.

Thin-layer plates (20 cm $\times$ 20 cm) of silica gel F$_{254}$ were obtained from E. Merck A.G., Darmstadt, and prewashed in methanol by ascending chromatography. The liquid phase for gas-liquid chromatography (cyclohexane dimethanol succinate) and the support (Gas Chrom Q 100/120 mesh) were obtained from Applied Science Laboratories, Mc. State College, Pennsylvania.

Preparation of labelled progesterone

[4-$^{14}$C] Progesterone (S. A. 58.5 mCi/mm) was obtained from the Radiochemical Centre, Amersham, Bucks. One $\mu$Ci in ethanol was dried in a tube under nitrogen at 40°C and redissolved in 1 ml of 90\% ethanol in saline. Immediately before intravenous injection a further 19 ml of saline was drawn into the syringe to give a final concentration of 5\% ethanol in saline. After each injection, the residual radioactivity in the syringe and needle was determined.

Methods

Urinary LH was determined by a double antibody radioimmunoassay based on the method of Wilde et al. (1967). Urinary oestrone, 17$\beta$-oestradiol and oestriol were determined by the method of Brown et al. (1957) and urinary pregnanediol by the method of Klopper et al. (1955). The conversion of intravenously administered progesterone to urinary metabolites was determined according to the following procedure. Urine was collected daily for three consecutive days; the total radioactivity excreted on each day was determined and the mass and radioactivity associated with metabolites in various subfractions were calculated. The urinary production rate of progesterone was calculated from the total percentage conversion of labelled progesterone to 5$\beta$-pregnane-3a,20a-diol glucuroniside over three consecutive days and from the average mass of this compound over the same period of time.

Determinations of radioactivity

Duplicate samples of 0.5 ml from each 24 hour urine sample were counted in 10 ml of NE 250 (Nuclear Enterprises Ltd., Edinburgh) to which 4\% CAB-O-SIL (Packard Instrument Company Inc., Ill.) had been added to form a thixotropic gel. At each subsequent stage in the procedure 5\% aliquots were removed, transferred to counting vials, dried and redissolved in 8 ml of toluene containing 3 g per litre of 2,5-diphenyloxazole (PPO). Each sample was stabilised in an automatic liquid scintillation counter for 2 hours at 2°C, and a counting time selected to give a coefficient of variation of less than 1.5\%. The counting efficiencies were determined from appropriate calibration curves for an external standard channels ratio method using a $^{133}$Ba source.

Extraction

Two-hundred ml of urine were extracted with 2 $\times$ 100 ml diethyl ether. The pooled extracts were washed with 30 ml of 0.3 n sodium hydroxide and 2 $\times$ 30 ml distilled water. The ether was evaporated in a round bottomed flask.

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**β-glucuronidase hydrolysis**

The volume of the aqueous fraction was measured and the pH adjusted to 4.5 with 0.1 N hydrochloric acid. The solution was buffered by the addition of acetate buffer pH 4.5 (10% of total volume) and sufficient β-glucuronidase added to give a final concentration of 500 units/ml of urine. Five mg of benzyl penicillin were added to each flask. The hydrolysis was allowed to proceed overnight at 37°C. The pH was then readjusted to 10.5 and the liberated steroids extracted with 2 × 100 ml diethyl ether. The pooled ether extracts were washed with sodium hydroxide solution and distilled water as in the extraction of untreated urine.

**Solvolysis**

The volume of residue was measured and solvolysis performed according to the method of Burstein & Lieberman (1958). The pH was adjusted to 1.0 with normal sulphuric acid and 1.75 g of sodium chloride added for each 10 ml of solution. The mixture was warmed until the solid had dissolved. An equal volume of ethyl acetate was added, the flask shaken, and left in an incubator for 48 hours at 37°C. The ethyl acetate extract was filtered, separated from the aqueous layer, and evaporated to dryness under vacuum in a rotary evaporator.

**Thin-layer chromatography and formation of derivatives**

The neutral extracts were dissolved in chloroform-methanol 1:1 (v/v) and transferred quantitatively to a thin-layer plate coated with silica gel. Radioactive and non-labelled standards were applied to either side of the extracts. The unknown steroids and labelled standards were located by overnight autoradiography using Kodirex non-screen X-ray films. Using this technique 1000 dpm are readily detected. The non-labelled standards (5 μg) were located by spraying the plate with a 5% solution of phosphomolybdic acid in ethanol and heating at 120°C for 15 min.

The first thin-layer chromatogram (TLC) developed in chloroform-methanol 99:1 (v/v) effectively separated pregnan-3α-ol-20-one and the C₃ stereoisomers of pregnane-3α,20α-diol. Each region was aspirated on a No. 3 filter disc and eluted with ethanol. The eluates were dried and acetylated overnight at room temperature with pyridine and acetic anhydride 1:1 (v/v).

After acetylation, the various subfractions were subjected to a second TLC on silica gel in the system hexane-ether 8:2 (v/v). No additional compounds were visible after autoradiography overnight. Each acetylated derivative was eluted from the plate and dried in a pointed tube.

**Gas-liquid chromatography**

A Pye-model 84 Series 104 gas chromatograph with a flame ionisation detector was used. Glass columns (144 cm × 2 mm) were packed with 1% CDMS on Gas Chrom Q 100/120 mesh. A solid injection technique was employed. An appropriate aliquot (from 0.5% to 10%) was transferred onto the inner needle of a Hamilton solid injection syringe (SS-60 with 8.5 cm outer needle) and applied directly through the injection port to the top of the column. The temperature of the oven containing the column was maintained at 230°C to obtain suitable elution times. Argon was used as carrier gas and the flow rates of the flame gases were adjusted to give satisfactory sensitivity and base-line stability. Each sample was run in triplicate and the peak areas measured by an electronic digital integrator (Infotronics, CRS-10HB).
Calculation of results

The percentage of initial radioactivity in various urinary metabolites and their masses were determined by a procedure which corrects for experimental losses from a single injection of labelled steroid. A scheme of the method is shown in Fig. 1. At each stage in the isolation procedure an aliquot is removed for liquid scintillation counting and the amount corrected to 100% recovery in terms of the preceding counts. The corrected percentage of dpm in the 24 hour urine sample in compound $P_{11}$ is given by the equation

$$ P_{11} \% = \frac{100 \beta_{11} \cdot A_{11} \cdot A_1 \cdot (X_0 - X_1)}{(A_{1R} + n \ A_{1j}) (A_R + n \ A_j) \ X_0} $$

and the corrected mass of compound $M_{11}$ as measured by gas-liquid chromatography is given by equation (2)

$$ M_{11} = \frac{(X_0 - X_1) \ A_1 \cdot \gamma_{11}}{(A_{1R} + n \ A_{1j}) (A_R + n \ A_j)} $$

Fig. 1.

Scheme of method for the isolation and quantitative determination of radiometabolites.
where \( \gamma_{11} \) is the radiochemical purity as determined by recrystallisation studies; \( A_{11} \) the dpm in area \( A_{11} \), containing compound \( P_{11} \) on the second TLC and \( A_1 \) the dpm in area \( A_1 \) on the first TLC; \( X_n \) is the dpm in the total 24 hour urine and \( X_1 \) the dpm remaining in the aqueous fraction after ether extraction; \( A_R \) and \( A_{1R} \) are the cpm in the residues on the first and second TLC respectively; \( A_i \) and \( A_{1i} \) are the cpm in the aliquots taken from areas \( A_i \) and \( A_{1i} \) respectively; finally \( \gamma_{11} \) is the mass of steroid in \( \mu g \) as determined by gas-liquid chromatography using flame ionisation detection.

The metabolites liberated by \( \beta \)-glucuronidase hydrolysis and solvolysis are determined in a similar manner.

The urinary production rate \( (Pu) \) is given by the following equation

\[
Pu = \frac{I}{Ir} \cdot M \cdot \frac{1}{t}
\]

where \( I \) is the total dpm injected, \( Ir \) the total dpm and \( M \) the mass in mg of 5\( \beta \)-pregnane-3\( \alpha \),20\( \alpha \)-diol; \( t \) is the collection time in days.

**Evaluation of procedure**

The procedure has been evaluated in terms of the total random theoretical percentage error by calculating the errors on each term in equations (1) and (2). If all aliquots are counted for a total of 10,000 disintegrations, then the overall theoretical error on the determination of the percentage of total radioactivity in any metabolite is approx. 7\% and in the determination of mass approx. 15\%.

An evaluation of the procedure in terms of replicate analysis of mixtures of authentic steroids has proved impossible due to the lack of labelled metabolites of progesterone. However, similar studies with regard to C\( \text{H}_{19} \) steroids and their urinary metabolites have indicated that the determination of the percentage of initial radioactivity in any metabolite may be determined with a mean accuracy of 85\% and a coefficient of variation ranging from 10–15\% (Hennam et al., in preparation).

With regard to specificity the \( R_f \) values of pregnanolone and pregnanediol isomers on thin-layer chromatography as their free and acetylated derivatives are shown in Table 1. In addition, the relative retention times of the acetate derivatives on the gas-chromatograph are recorded. Recrystallisation studies have been performed on one sample of 5\( \beta \)-pregnan-3\( \alpha \)-ol-20-one and 5\( \beta \)-pregnane-3\( \alpha \),20\( \alpha \)-diol. The purities of these compounds were 92 and 95\% respectively.

**RESULTS**

**Urinary LH**

In five women, the peak of LH excretion in the control cycles was in the normal range and was markedly suppressed by clormadinone acetate. In the sixth subject, the peak was low in both cycles (21 and 23 IU/24 h respectively). The mean (± sd) values for all cycles are shown in Fig. 2. On days -1 to -14 the concentration in cycle I was 16.3 ± 5.8 (mean ± sd) and in cycle III 19.8 ± 1.9. The corresponding values for days +1 to +13 were 21.7 ± 10.4 and 21.6 ± 2.6 respectively. Thus, there is no statistically significant difference in the values during these phases of the control and treated cycles. On the other hand, the extent to which the LH peak was suppressed by clormadinone
Table 1.
Relative retention values of pregnanolone and pregnanediol isomers on thin-layer chromatography (TLC) and relative retention times to progesterone on gas-liquid chromatography (GLC).

<table>
<thead>
<tr>
<th>Steroid</th>
<th>1st TLC Chloroform/ Methanol 99:1</th>
<th>2nd TLC after acetylation Hexane/Ether 8:2</th>
<th>GLC 1% CDMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>5α-pregnan-3α-ol-20-one</td>
<td>0.64</td>
<td>0.48</td>
<td>0.44</td>
</tr>
<tr>
<td>5α-pregnan-5β-ol-20-one</td>
<td>0.54</td>
<td>0.43</td>
<td>0.56</td>
</tr>
<tr>
<td>5β-pregnan-3α-ol-20-one</td>
<td>0.68</td>
<td>0.03</td>
<td>0.48</td>
</tr>
<tr>
<td>5β-pregnan-3β-ol-20-one</td>
<td>0.73</td>
<td>0.37</td>
<td>0.42</td>
</tr>
<tr>
<td>5α-pregnan-20α-ol-5-one</td>
<td>0.61</td>
<td>0.06</td>
<td>0.80</td>
</tr>
<tr>
<td>5α-pregnan-20β-ol-3-one</td>
<td>0.84</td>
<td>0.30</td>
<td>0.67</td>
</tr>
<tr>
<td>5β-pregnan-20α-ol-3-one</td>
<td>0.59</td>
<td>0.33</td>
<td>0.67</td>
</tr>
<tr>
<td>5β-pregnan-20β-ol-3-one</td>
<td>0.74</td>
<td>0.35</td>
<td>0.62</td>
</tr>
<tr>
<td>5α-pregnan-3α,20α-diol</td>
<td>0.36</td>
<td>0.53</td>
<td>0.49</td>
</tr>
<tr>
<td>5α-pregnan-3α,20β-diol</td>
<td>0.34</td>
<td>0.48</td>
<td>0.46</td>
</tr>
<tr>
<td>5α-pregnan-3β,20β-diol</td>
<td>0.30</td>
<td>0.47</td>
<td>0.57</td>
</tr>
<tr>
<td>5α-pregnan-3β,20α-diol</td>
<td>0.27</td>
<td>0.45</td>
<td>0.61</td>
</tr>
<tr>
<td>5β-pregnan-3α,20α-diol</td>
<td>0.13</td>
<td>0.47</td>
<td>0.54</td>
</tr>
<tr>
<td>5β-pregnan-3α,20β-diol</td>
<td>0.16</td>
<td>0.51</td>
<td>0.50</td>
</tr>
<tr>
<td>5β-pregnan-3β,20β-diol</td>
<td>0.34</td>
<td>0.53</td>
<td>0.44</td>
</tr>
<tr>
<td>5β-pregnan-3β,20α-diol</td>
<td>0.42</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
</tbody>
</table>

acetate is statistically significant (P 0.05–0.02; t test). In cycle I there was evidence for a pre-menstrual rise in all subjects whereas in cycle III this was limited to subjects J. C., L. D. and S. F.

**Progesterone metabolism**

The amount of radioactivity excreted daily in three consecutive 24 hour urine specimens after the intravenous administration of labelled progesterone nine days after the urinary LH peak is shown in Table 2. The results are expressed as a percentage of the administered dose. There was no statistically significant difference in the daily or total excretion of radioactivity between the untreated cycles and the cycles during chlormadinone acetate administration.

The amount of radioactivity subsequently extracted with diethyl ether from untreated urine and after β-glucuronidase hydrolysis of the aqueous residue is shown in Table 3. These results are expressed as a percentage of the total radioactivity in the 24 hour urine samples. There was no statistically sig-
The concentration of urinary LH (mean ± sd) in 6 subjects before (cycle 1) and after (cycle 3) oral administration of chlomadinone acetate (0.5 mg/day). The values are expressed in IU of the 2nd IRP-HMG.

Table 2.
The amount of radioactivity excreted in three consecutive 24 hour urine specimens after the intravenous administration of labelled progesterone. The results are expressed as a percentage of the administered dose.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day 1</th>
<th></th>
<th>Day 2</th>
<th></th>
<th>Day 3</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle 1</td>
<td>Cycle 3</td>
<td>Cycle 1</td>
<td>Cycle 3</td>
<td>Cycle 1</td>
<td>Cycle 3</td>
<td>Cycle 1</td>
</tr>
<tr>
<td>A. B.</td>
<td>30.7</td>
<td>19.1</td>
<td>11.3</td>
<td>5.2</td>
<td>4.8</td>
<td>3.7</td>
<td>46.8</td>
</tr>
<tr>
<td>M. C.</td>
<td>41.1</td>
<td>37.9</td>
<td>11.3</td>
<td>9.6</td>
<td>5.7</td>
<td>5.9</td>
<td>58.1</td>
</tr>
<tr>
<td>J. C.</td>
<td>19.7</td>
<td>30.5</td>
<td>5.3</td>
<td>5.1</td>
<td>2.6</td>
<td>3.6</td>
<td>27.6</td>
</tr>
<tr>
<td>S. L.</td>
<td>23.6</td>
<td>35.0</td>
<td>8.6</td>
<td>12.8</td>
<td>3.3</td>
<td>7.8</td>
<td>35.5</td>
</tr>
<tr>
<td>L. D.</td>
<td>30.8</td>
<td>34.5</td>
<td>7.3</td>
<td>9.1</td>
<td>4.1</td>
<td>6.3</td>
<td>42.2</td>
</tr>
<tr>
<td>S. F.</td>
<td>27.9</td>
<td>23.9</td>
<td>5.9</td>
<td>18.9</td>
<td>2.6</td>
<td>3.3</td>
<td>35.4</td>
</tr>
</tbody>
</table>

| Mean    | 28.9   | 30.2  | 8.3    | 10.1 | 3.9    | 5.1  | 40.9   | 45.4  |
| ± sd    | ± 7.3  | ± 7.3 | ± 2.6  | ± 5.2| ± 1.2  | ± 1.8| ± 10.6 | ± 10.3|

 significant difference in the percentage of administered radioactivity in either of these fractios. However, when the amounts of radioactivity liberated by solvolysis were compared, urine from the first cycle contained significantly more radioactivity in this fraction ($P \ 0.02-0.01; t$ test).
Table 3.
The amount of radioactivity associated with various subfractions in urine during the first 24 hours after the administration of labelled progesterone. The results are expressed as a percentage of the total radioactivity in the urine.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Ether extractable</th>
<th>β-Glucuronidase hydrolysable</th>
<th>Solvolysable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle 1</td>
<td>Cycle 3</td>
<td>Cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cycle 3</td>
</tr>
<tr>
<td>A. B.</td>
<td>2.36</td>
<td>0.88</td>
<td>73.28</td>
</tr>
<tr>
<td>M. C.</td>
<td>1.03</td>
<td>0.35</td>
<td>42.60</td>
</tr>
<tr>
<td>J. C.</td>
<td>0.70</td>
<td>0.33</td>
<td>66.45</td>
</tr>
<tr>
<td>S. L.</td>
<td>0.89</td>
<td>2.57</td>
<td>64.08</td>
</tr>
<tr>
<td>L. D.</td>
<td>1.60</td>
<td>0.43</td>
<td>63.24</td>
</tr>
<tr>
<td>S. F.</td>
<td>2.51</td>
<td>1.24</td>
<td>62.23</td>
</tr>
<tr>
<td>Mean</td>
<td>1.52</td>
<td>0.97</td>
<td>61.98</td>
</tr>
<tr>
<td>± sd</td>
<td>± 0.77</td>
<td>± 0.8</td>
<td>± 10.28</td>
</tr>
</tbody>
</table>

The principal metabolite extractable with diethyl ether was 5β-pregnane-3α,20α-diol. The percentage of total radioactivity (mean ± sd) in the first 24 hour urine sample associated with this compound was 0.55 ± 0.29%. The concentration (mean ± sd) was 68.2 ± 98.4 μg/24 h. The amounts of radioactivity in 5β-pregnanolone (5βP-3α-ol-20-one), 5α-pregnandiol (5αP-3α,20α-diol) and 5β-pregnane diol (5βP-3α,20α-diol) after β-glucuronidase hydrolysis are shown in Table 4. There was no significant difference in the mean percentages of

Table 4.
The amount of radioactivity in various metabolites liberated by β-glucuronidase hydrolysis. The values are expressed as a percentage of the total radioactivity excreted in urine during the first 24 hours after administration of labelled progesterone.

<table>
<thead>
<tr>
<th>Subject</th>
<th>5βP-3α-ol-20-one</th>
<th>5αP-3α,20α-diol</th>
<th>5βP-3α,20α-diol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle 1</td>
<td>Cycle 3</td>
<td>Cycle 1</td>
</tr>
<tr>
<td></td>
<td>Cycle 1</td>
<td>Cycle 3</td>
<td>Cycle 3</td>
</tr>
<tr>
<td>A. B.</td>
<td>N. D.</td>
<td>2.74</td>
<td>6.26</td>
</tr>
<tr>
<td>M. C.</td>
<td>1.60</td>
<td>2.48</td>
<td>7.66</td>
</tr>
<tr>
<td>J. C.</td>
<td>6.61</td>
<td>9.54</td>
<td>5.06</td>
</tr>
<tr>
<td>S. L.</td>
<td>3.03</td>
<td>3.23</td>
<td>5.82</td>
</tr>
<tr>
<td>L. D.</td>
<td>5.17</td>
<td>5.39</td>
<td>4.09</td>
</tr>
<tr>
<td>S. F.</td>
<td>4.42</td>
<td>7.03</td>
<td>5.01</td>
</tr>
<tr>
<td>Mean</td>
<td>4.21</td>
<td>5.07</td>
<td>5.60</td>
</tr>
<tr>
<td>± sd</td>
<td>± 1.9</td>
<td>± 2.81</td>
<td>± 1.2</td>
</tr>
</tbody>
</table>

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radioactivity in all three compounds. However, the percentage of radioactivity in 5β-pregnanolone was statistically higher ($P \leq 0.02$; $t$ test) and the conversion to 5β-pregnane-diol significantly lower ($P \leq 0.01$; $t$ test) in the treatment cycles of subjects J.C., L.D. and S.F. The concentrations of these compounds are shown in Table 5. With one exception, the levels of all three compounds were from 5-10 fold lower in the third cycle from subjects A.B., M.C. and S.L. The principal metabolite in the fraction liberated by solvolysis was 5β-pregnane-3α,20α-diol. The percentage of the total radioactivity in the first 24 hour urine sample associated with this component (mean ± sd) was $1.72 ± 1.92\%$ and the mean concentration $147 ± 175\mu g/24\ h$.

**Progesterone production**

The urinary production rates of progesterone before and after daily administration of chlormadinone acetate are shown in Table 6. In the control cycles the mean $P_u$ was $29.4 ± 7.5\ mg/24\ h$. In three of the treatment cycles the values were markedly suppressed and in the remainder (J.C., L.D. and S.F.) the mean value was $21.8 ± 5.4$.

**Urinary pregnanediol**

In all control cycles there was a definite rise in the urinary pregnanediol excretion in the luteal phase. The maximum value in four subjects was in the range 4.7-7.0 $mg/24\ h$. In subject S.L. the peak was low (2.8 $mg/24\ h$) and was high in subject L.D. (11.1 $mg/24\ h$). In all treatment cycles there was evidence for suppression of the normal luteal rise by chlormadinone acetate. This was very marked in three subjects (A.B., S.L. and M.C.) in whom the

<table>
<thead>
<tr>
<th>Subject</th>
<th>5βP-3α-ol-20-one</th>
<th>5αP-3α,20α-diol</th>
<th>5βP-3α,20α-diol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>Cycle 3</td>
<td>Cycle 1</td>
<td>Cycle 3</td>
</tr>
<tr>
<td>A. B.</td>
<td>N.D.</td>
<td>0.007</td>
<td>0.441</td>
</tr>
<tr>
<td>M. C.</td>
<td>0.173</td>
<td>0.026</td>
<td>0.071</td>
</tr>
<tr>
<td>J. C.</td>
<td>0.423</td>
<td>0.463</td>
<td>0.189</td>
</tr>
<tr>
<td>S. L.</td>
<td>0.247</td>
<td>0.053</td>
<td>0.411</td>
</tr>
<tr>
<td>L. D.</td>
<td>0.837</td>
<td>0.289</td>
<td>0.492</td>
</tr>
<tr>
<td>S. F.</td>
<td>0.516</td>
<td>0.582</td>
<td>0.433</td>
</tr>
<tr>
<td>Mean</td>
<td>0.439</td>
<td>0.439</td>
<td>5.97</td>
</tr>
<tr>
<td>± sd</td>
<td>±0.261</td>
<td>±0.154</td>
<td>±2.84</td>
</tr>
</tbody>
</table>

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Table 6.  
The urinary production rates of progesterone before and after daily administration of chlormadinone acetate.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cycle 1</th>
<th>Cycle 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. B.</td>
<td>25.2</td>
<td>2.5</td>
</tr>
<tr>
<td>M. C.</td>
<td>26.9</td>
<td>3.7</td>
</tr>
<tr>
<td>J. C.</td>
<td>23.7</td>
<td>20.2</td>
</tr>
<tr>
<td>S. L.</td>
<td>37.6</td>
<td>5.2</td>
</tr>
<tr>
<td>L. D.</td>
<td>40.1</td>
<td>17.4</td>
</tr>
<tr>
<td>S. F.</td>
<td>22.9</td>
<td>27.9</td>
</tr>
</tbody>
</table>

urinary pregnanediol excretion showed a steady plateau throughout the cycle or a slight rise (0.5 mg/24 h in subject M. C.). In the remaining three subjects, there was a rise in excretion during the second half of each cycle. In subject L. D. the maximum value was 4 mg/24 h; in subject J. C. 2.8 mg/24 h. In subjects L. D. and J. C. the maximum values were approximately 50% of the control cycles whereas in subject S. F. the suppression was minimal.

Urinary oestrone, 17β-oestradiol and oestriol

In the control cycles the levels were variable but all showed a normal biphasic pattern of excretion. In subjects A. B. and S. L. there was no evidence of a cyclical pattern in the treatment cycles but in subject M. C. lower values in the first half of the cycle were followed by a late rise in the excretion of the three oestrogens. In the remaining three subjects (L. D., J. C. and S. F.) there was no consistent evidence for suppression by chlormadinone acetate.

DISCUSSION

The results obtained by the determination of LH, pregnanediol, oestrone, 17β-oestradiol and oestriol in twenty-four hour urine samples collected daily throughout the control cycles afford indirect evidence for the occurrence of ovulation in all cycles. In addition, the ranges and patterns of excretion suggest that pituitary and ovarian function was normal, in so far as endogenous hormone production can be assessed from urinary excretion. With regard to progesterone production it is recognised that calculations based upon the conversion of labelled progesterone to urinary 5β-pregnane-3α,20α-diol glucuronoside may overestimate. Thus, pregnenolone (3β-hydroxy-pregn-5-en-20-one)
and 20α- or 20β-hydroxy-pregn-4-en-3-one may contribute to urinary pregnanediol glucuroniside (Arcos et al. 1964; Romanoff et al. 1966). The blood production rate (Pb) calculated from the metabolic clearance rate and the mean plasma progesterone concentration is more specific. A mean Pb of 22.1 mg/24 h has been calculated for the luteal phase of the menstrual cycle (Little et al. 1966; van der Molen & Aakvaag 1967) as compared with the Pb of 29.4 ± 7.5 mg/24 h on the 9th day after the LH peak in the present series. The urinary production rate was chosen to obviate additional sampling from the volunteers and because the main purpose was to compare two cycles in the same individuals.

The most striking feature of the investigation is that despite almost complete suppression of the LH peaks in all treatment cycles, this was associated with two distinct types of response with regard to progesterone production and urinary steroid excretion. Thus, in three subjects (A. B., M. C. and S. L.), the production rate was reduced to less than 5.2 mg/24 h associated with a plateau of urinary pregnanediol excretion, whereas, in the remainder, the mean Pb was only moderately suppressed (21.8 mg/24 h) and there was a significant rise in urinary pregnanediol in the second half of each cycle. In view of the calculated contribution of precursors of pregnanediol other than progesterone (van der Molen & Aakvaag 1967) it is extremely unlikely that this finding reflects an effect of chlormadinone acetate on these precursors rather than upon the secretion of the hormone.

These observations raise the question as to the minimal concentration of LH which may be associated either with follicular development, ovulation or ovarian steroidogenesis. It is probable that ovulation did not occur in the treatment cycles of the three subjects in whom there was no pre-menstrual rise in urinary LH, a minimal LH peak and a low production rate of progesterone. The possibility may also be considered that in the remainder there was luteinisation of a follicle without ovulation or that the latter may occur following an almost undetectable LH peak. Furthermore, a cyclical pattern of urinary oestrogen excretion occurred in the treatment cycles of four of the subjects despite the suppression of the LH peak. Thus, in one subject (M. C.) there was a dissociation between the effect upon progesterone production and oestrogen excretion.

With regard to changes in the extent to which radioactivity was incorporated in various fractions and metabolites, there is evidence that less radioactivity was released by solvolysis of urine from the treated cycles. Harkness et al. (1969) compared the metabolism of labelled progesterone combined with 25 or 300 mg of unlabelled hormone in two adrenalectomised ovariectomised women and reported an increase in the sulphate fraction at the higher dose of hormone. The present finding may reflect the mean decrease of progesterone production in the treated cycles. Finally, chlormadinone acetate has an effect
which was statistically significant upon the relative incorporation of radioactivity in two metabolites — a low incorporation in 5β-pregnanediol associated with a higher conversion to 5β-pregnanolone. This finding was limited to the three subjects in whom progesterone production or pregnanediol excretion were minimally suppressed. The shift in metabolism would influence the Pu value but, in fact, the lower incorporation is associated with higher Pu values. Accordingly, the results suggest that apart from individual alterations in pituitary and ovarian function, chlormadinone acetate may have an effect upon the reductive metabolism of progesterone. There is a suggestion therefore that chlormadinone acetate may influence the reductive metabolism of progesterone in those subjects in whom the urinary production rate persists at a relatively high level. For this reason, the determination of plasma progesterone should be a better index of luteal function than urinary pregnanediol. In a recent study, Larsson-Cohn et al. (1970) reported that in the second cycle of treatment with chlormadinone acetate, three subjects had total progesterone values which were lower than those observed in control cycles.

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The authors are grateful to Dr. Hilary Orr and Dr. K. D. Bagshawe, Edgar Laboratory, Fulham Hospital for the LH assays; to Mr. G. Goka, Mr. G. Baffoe, and other technicians who performed the urinary steroid assays under the supervision of Dr. M. I. Stern; to Mr. Ian Craft who performed the injections and arranged for the collection of urine specimens and to Syntex Pharmaceuticals Ltd. and The Population Council, New York for financial support.

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