CHAPTER SIX

CLINICAL STUDIES ON POSITIVE FEEDBACK
Introduction

The studies described in this chapter will deal exclusively with conditions in which the negative feedback effects of gonadal steroids on gonadotrophin secretion are preserved but the positive feedback of oestrogen is absent. In women of reproductive age, such "isolated" failures of positive feedback (Van Look, 1976) give rise to persistent anovulation.

Anovulatory cycles may occur in normal women at any time during reproductive life (Döring, 1969) and are probably due to a temporary failure of hypothalamic-pituitary function resulting in defective follicular development and/or failure to release LH in response to the increasing ovarian oestrogen secretion at midcycle. In adolescent girls, as well as in women approaching the menopause however, anovulation is far more common than at any other stage of reproductive life (Döring, 1969) and, more important, is often of persistent nature. This latter characteristic suggests a more profound, permanent abnormality of hypothalamic-pituitary function, the cause of which however is unknown.

In order to gain some insight into the underlying pathophysiology of the ovulatory failure in these instances, ovarian steroid secretion and hypothalamic-pituitary function were studied in adolescents and perimenopausal women presenting with menstrual histories suggestive of persistent anovulation, and the results obtained compared to those of normal women. Since it seemed likely that the failure to ovulate in the patients was due to a failure to release LH in response to oestrogen, it seemed appropriate to compare this pathological failure of positive feedback with physiological positive feedback failure. Accordingly, hypothalamic responsiveness to oestrogen was also studied in genetic males and these results are presented first.
6.1 Studies on positive feedback in normal men

6.1.1 Background

The evidence derived from animal studies with respect to the sexual dimorphism of the mechanisms involved in the control of gonadotrophin secretion has been reviewed in Chapter 1. As pointed out in that chapter, considerable controversy exists as to whether data obtained in lower mammals such as rodents or sheep are relevant to the situation in primates. It was therefore decided to re-investigate this question in the intact human male.

6.1.2 Design of the study

A total of 8 normal men with ages ranging from 24 to 39 years participated in 2 separate experiments. All men were in good health and without evidence of endocrine disease. In the first experiment (6 subjects, 01-06) an oestrogen provocation test using ethinyloestradiol (200 µg a day for 3 days) was performed as described in Chapter 4. Five of these men as well as two additional volunteers (013 and 17) subsequently participated in a second experiment during which the daily dose of ethinyloestradiol was increased to 500 µg. Blood sampling was as on the first occasion except that the frequent, 15 minute interval sampling on days 4 and 6 of the test was omitted.

Six normal women, aged 23-45 years, served as controls. All women were of proven fertility (parity-range, 2-5), had a history of regular menstrual cycles (26-30 days) and were free of obvious endocrine disease. Serial measurements of urinary total oestrogen and pregnanediol excretion during the months prior to and following the test confirmed the ovulatory character of their cycles. The oestrogen
provocation test, performed as described in Chapter 4, was started between days 1 and 4 of the cycle (day 1 being the first day of menstrual bleeding).

6.1.3 Results
(a) Normal women

Daily hormonal changes
Mean (+ S.E.M.) plasma FSH, LH, 17β-oestradiol and 17α-ethinyl-oestradiol levels are illustrated in Figure 6.1 and FSH and LH concentrations in individual subjects are presented in Table 6.1.

Basal FSH and LH levels (days 1 to 4) were within the normal range for the early follicular phase of the cycle although mean LH levels tended to be somewhat higher than those previously observed in our laboratory (Figure 1.2).

Ethinyloestradiol administration rapidly and significantly suppressed FSH and LH to 60, respectively 50 per cent of the initial level 24 hours after the initiation of treatment (p < 0.005, paired t-test). FSH levels remained low during the remainder of the treatment period and then gradually returned to pre-treatment levels. Both during and following oestrogen administration peripheral FSH concentrations, expressed as the percentage change from baseline levels, were inversely correlated with circulating 17α-ethinyloestradiol levels (y = 107.2 - 62.7 log x where y = percentage change in FSH from mean basal value on days 1-4 and x = peripheral 17α-ethinyloestradiol concentration; r = -0.5575; p < 0.01).

In contrast to FSH, plasma LH levels showed a pronounced biphasic pattern, characterised by a brief (less than 48 hours) initial period of suppression followed by a dramatic rise to a peak value on day 9 of
FIGURE 6.1: Mean (± S.E.M.) plasma FSH, LH, 17β-oestradiol and 17α-ethinyloestradiol concentrations before, during and after administration of ethinyloestradiol (4 x 50 μg a day on days 5-7) to six normal women in the early-mid follicular phase of the cycle. Values which are significantly (p < 0.05) different from mean basal levels are indicated by an asterisk.
TABLE 6.1: Daily changes in plasma FSH and LH (in mU/ml) during an oestrogen provocation test in 6 normal women

<table>
<thead>
<tr>
<th></th>
<th>Day of the oestrogen provocation test</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>1.8</td>
<td>1.9</td>
<td>1.6</td>
<td>0.9</td>
<td>0.9</td>
<td>3.7</td>
<td>5.5</td>
<td>2.9</td>
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35.0 ± 5.5 (mean ± S.E.M.) mU/ml, an increase of about 250% above pre-treatment levels (p<0.01, paired t-test). Twenty-four hours later, at the end of the observation period, LH values were still significantly higher than basal levels (p<0.025, paired t-test). In terms of magnitude and duration, the ethinyloestradiol-induced LH surge was not significantly different from the spontaneous midcycle LH-peak (Figure 1.2). In 5 out of 6 women LH levels reached their maximum on day 9 (Table 6.1) while in the remaining subject (©01) LH was highest 2½ hours earlier. The magnitude of the induced LH-surges ranged from 23.8 to 58.0 mU/ml with a mean ± S.E.M. of 36.1 ± 4.8 mU/ml. In contrast to FSH, peripheral LH levels were not correlated with 17β-ethinyl-oestradiol levels.

Before oestrogen treatment, the pattern of plasma 17β-oestradiol was typical of that seen during the early to midfollicular phase of the menstrual cycle. Peripheral levels of this hormone rose gradually from a value of 45.3 ± 4.5 pg/ml (mean ± S.E.M.) on day 1 to 92.2 ± 21.5 pg/ml on day 5 of the test. Following the start of oestrogen treatment, 17β-oestradiol concentrations declined in an exponential fashion to very low values which in 3 out of 6 subjects fell below the limit of sensitivity of the assay (< 10 pg/ml) on day 7. In 5 of the 6 women plasma 17β-oestradiol remained low throughout the remainder of the test (mean ± S.E.M.: day 9, 30.2 ± 7.9 pg/ml; day 10, 23.2 ± 5.7 pg/ml). In the sixth woman (©01) on the other hand 17β-oestradiol levels rapidly increased after stopping the oestrogen to reach a value of 319 pg/ml on day 10. Ovulation however did not occur as evidenced by the absence of a rise in urinary pregnanediol excretion in the samples collected after completion of the test. It is of interest to note in
this respect that the mean pre-treatment $17\beta$-oestradiol concentration $75.3 \pm 18.7$ pg/ml, mean $\pm$ S.E.M.) in this woman was significantly higher than that in the other five (mean $\pm$ S.E.M., $60.0 \pm 4.6$ pg/ml; p $< 0.05$, one-tailed t-test) and, as mentioned earlier, that the oestrogen-induced LH peak also occurred 24 hours earlier in this subject.

The profile of peripheral $17\alpha$-ethinyloestradiol levels, illustrated in Figure 6.1, represents the mean ($\pm$ S.E.M.) of 5 out of the 6 women since the samples from one subject (g02) were accidentally lost. As can be seen in Figure 6.1, $17\alpha$-ethinyloestradiol concentrations were maintained at a fairly stable level of about 200 pg/ml for a period of at least 4.8 hours. Although circulating levels of the hormone on the morning of day 8 (i.e. approximately 12 hours after ingestion of the last 50 µg tablet) had slightly decreased as compared to the previous day, the difference was not statistically significant and could certainly not account for the significant rise (p $< 0.005$, paired t-test) in plasma LH over that period. The half-life of $17\alpha$-ethinyloestradiol, calculated from the difference in plasma concentrations of the hormone between day 8 and 9

$$T_{1/2} = \frac{e^{\log 2 \times t}}{e^{\log N_0}}$$

(Scientific Tables, Documenta Geigy, p. 217)

where $T_{1/2} =$ half-life (in minutes)

$N_0$ and $N_t = 17\alpha$-ethinyloestradiol levels on days 8 and 9 respectively

$t = 1.40$ min (i.e. 24 hours)

was 945 $\pm$ 116 minutes (15 hours 45 min $\pm$ 1 hour 56 min; mean $\pm$ S.E.M.).
Episodic gonadotrophin release

In order to assess the effect of exogenous oestrogen administration on pulsatile gonadotrophin release, blood samples for FSH and LH measurements were collected at 15 minute intervals for a period of 3 hours on day 4 and day 6 (i.e. 24 hours before and 24 hours after the start of treatment), and these results are summarised in Tables 6.2 and 6.3.

The transverse mean (± SD) gonadotrophin levels during both collection periods are listed in Table 6.2 which illustrates that FSH and LH levels were significantly suppressed in all subjects except 0.05 in whom FSH, but not LH, remained virtually unchanged. A similar differential feedback effect of 17α-ethinyloestradiol on FSH and LH secretion was not present in any of the other five women, and both gonadotrophins in these women were always suppressed to a similar degree.

The data with respect to episodic gonadotrophin secretion before and during oestrogen are summarised in Table 6.3. In the analysis of individual gonadotrophin patterns for pulsatile release the following arbitrary criteria were used:

(1) an increase in peripheral gonadotrophin concentrations was considered to represent a "peak" if

(a) the concentration in at least 2 consecutive samples (the "peak-samples") was higher than the mean of the previous 2 samples (the "baseline") (or the previous sample if the increase occurred in the 2nd and 3rd sample)

and if (b) the increase in at least one of the peak samples was significantly (p<0.05) higher than might be expected on the basis of within-assay variation (in our laboratory
TABLE 6.2: Transverse means (± SD) (in mU/ml) and percentage change of peripheral gonsadotrophin levels in 6 normal women sampled at 15 minute intervals for 3 hours before (day 14) and during (day 6) ethinyloestradiol administration.

<table>
<thead>
<tr>
<th></th>
<th>Day 14</th>
<th>Day 6</th>
<th>Percentage change*</th>
</tr>
</thead>
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<tr>
<td></td>
<td>FSH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>♀ 01</td>
<td>5.66 ± 0.42</td>
<td>4.43 ± 0.25</td>
<td>-21.7</td>
</tr>
<tr>
<td>02</td>
<td>3.10 ± 0.28</td>
<td>2.05 ± 0.32</td>
<td>-33.9</td>
</tr>
<tr>
<td>03</td>
<td>15.25 ± 2.04</td>
<td>6.68 ± 1.22</td>
<td>-56.2</td>
</tr>
<tr>
<td>04</td>
<td>5.75 ± 0.52</td>
<td>3.33 ± 0.30</td>
<td>-42.1</td>
</tr>
<tr>
<td>05</td>
<td>4.58 ± 0.40</td>
<td>4.72 ± 0.82</td>
<td>+ 3.1</td>
</tr>
<tr>
<td>06</td>
<td>5.90 ± 1.13</td>
<td>3.02 ± 0.42</td>
<td>-48.8</td>
</tr>
<tr>
<td>mean ± S.E.M.</td>
<td>6.71 ± 1.76</td>
<td>4.04 ± 0.66</td>
<td>-(33.3 ± 8.8)</td>
</tr>
</tbody>
</table>

|       | LH     |       |                     |
| ♂ 01  | 4.08 ± 0.68 | 3.48 ± 1.84 | -14.7 |
| 02    | 8.62 ± 0.94 | 5.78 ± 0.98 | -32.9 |
| 03    | 12.42 ± 3.36 | 4.94 ± 0.78 | -60.2 |
| 04    | 5.70 ± 1.02 | 3.00 ± 0.70 | -47.3 |
| 05    | 5.32 ± 0.98 | 2.78 ± 0.64 | -47.7 |
| 06    | 10.56 ± 1.46 | 6.16 ± 1.20 | -41.7 |
| mean ± S.E.M. | 7.78 ± 1.34 | 4.36 ± 0.60 | -(40.8 ± 6.4) |

* Calculated from the formula: \[
\text{Percentage change} = \left( \frac{\text{mean value day 14} - \text{mean value day 6}}{\text{mean value day 6}} \right) \times 100
\]
**TABLE 6.3:** Characteristics of pulsatile gonadotrophin release in six normal women sampled at 15 minute intervals for 3 hours before (day 4) and during (day 6) ethinyl-oestradiol administration

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
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<td>FSH</td>
<td>LH</td>
<td>FSH</td>
<td>LH</td>
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<tr>
<td>Number of peaks</td>
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<td>0.83</td>
<td>0.16</td>
<td>0.33</td>
</tr>
<tr>
<td>(per 3 hours)</td>
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<tr>
<td>Absolute peak</td>
<td>1.70 ± 0.68</td>
<td>2.26 ± 0.56</td>
<td>1.40</td>
<td>4.36 ± 1.34</td>
</tr>
<tr>
<td>magnitude (mU/ml)</td>
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<td></td>
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<tr>
<td>(mean ± S.E.M.)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Relative peak</td>
<td>39.3 ± 13.0</td>
<td>37.4 ± 3.0</td>
<td>32.6</td>
<td>185.7 ± 1.8</td>
</tr>
<tr>
<td>magnitude (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(mean ± S.E.M.)</td>
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</table>
the within-assay variation for FSH is 11.8% and for LH 15.4%; Hunter, Edmond, Watson and McLean, 1974).

(2) for each peak the absolute amplitude was calculated from the difference between the highest peak-level and the baseline, and the relative peak amplitude was found by expressing the absolute amplitude as a percentage of the baseline which was taken as 100%.

Before exogenous oestrogen treatment pulsatile release of either FSH or LH was present in 5 out of 6 subjects (not in 0.04) but in only one instance did a FSH-peak coincide with a LH-peak (Figure 6.2). FSH-release was less frequent and could be detected in only 2 of the women (02 and 06). Neither the presence (or absence) of episodic FSH-release or the magnitude of the FSH-peaks appeared to be related to the transverse mean FSH-level or the endogenous 17β-oestradiol level. Pulsatile LH-release on the other hand occurred more regularly (in 4 out of 6 subjects) and, although in absolute terms the amplitude of the peaks varied between 1.10 and 4.00 mU/ml (coefficient of variation 55.5%), the relative magnitude was remarkably constant (range 31.4-48.8%, coefficient of variation 18.1%).

Oestrogen administration completely abolished all episodic gonadotrophin release except in those women in whom gonadotrophin secretion was not adequately suppressed. Thus FSH-release was still present in 0.05 and LH release in 0.01 (compare with Table 6.2). It is noteworthy that in this latter subject the magnitude of the LH-peaks, in absolute as well as in relative terms, was significantly greater during the oestrogen treatment than before.
FIGURE 6.2: Plasma FSH and LH concentrations in samples collected at 15 minute intervals for a period of 3 hours from a normal woman (♀ 06) before (broken line) and during (solid line) ethinyloestradiol treatment. Arrows indicate episodic gonadotrophin release.
(b) Normal men (200 µg ethinyloestradiol a day for 3 days)

Daily hormonal changes

Mean (± S.E.M.) peripheral gonadotrophin levels and their relative changes from pretreatment values are shown in Figure 6.3 and gonadotrophin patterns in individual subjects are illustrated in Figure 6.4.

Basal FSH as well as LH concentrations were within the normal range in all individuals studied. Oestrogen administration resulted in a prompt suppression of both gonadotrophins, although LH levels were more markedly suppressed than FSH. The profile of changes in FSH during oestrogen treatment of men was very similar to that seen in women. The pattern of LH on the other hand was qualitatively quite different between the two sexes and there was no indication of a biphasic effect of the administered oestrogen on LH secretion in men. Indeed, mean LH levels remained suppressed to approximately 50% of their initial value until the end of treatment after which they gradually increased again to reach a value of 158·0 ± 28·2% of the basal level at the end of the observation period. The decrease in LH levels during treatment as well as their recovery following treatment varied markedly between individuals (Figure 6.4) but was always related to circulating 17α-ethinyloestradiol concentrations as illustrated by the highly significant correlation between these two parameters (y = 90·2 - 69·5 log x where y = percentage change in LH from basal values and x = plasma 17α-ethinyloestradiol level; r = -0·6158, n = 25, p < 0·001). A similar relation did not exist for FSH, nor was there any correlation between the degree of suppression of FSH and that of LH. The recovery of FSH levels after stopping the oestrogen was also slower than that of LH, and peripheral FSH remained suppressed for at least 24 hours longer than LH.
FIGURE 6.3: Mean (± S.E.M.) gonadotrophin-levels and their relative change from the mean basal level in 6 normal men during oestrogen provocation test (4 x 50 µg ethinyloestradiol per day was given on days 5-7). Values significantly (p<0.05) different from mean pretreatment level are indicated by an asterisk.
FIGURE 6.4: Circulating FSH, LH and 17α-ethinylestradiol concentrations during an oestrogen provocation test in 6 normal men.
Peripheral testosterone and 17β-oestradiol levels during the oestrogen provocation test as well as their relative change from the mean pretreatment level are illustrated in Figure 6.5.

The pattern of plasma testosterone during ethinyloestradiol administration was qualitatively very similar to that described for LH although in quantitative terms testosterone was more markedly suppressed than LH. This was further illustrated by regression analysis of the observed changes in plasma testosterone and LH which revealed that although both variables were positively correlated \((y = -32.5 + 0.6x)\) where \(y\) and \(x\) represent percentage change from mean pre-treatment level in testosterone and LH respectively; \(r = 0.6656, n = 25, p < 0.001\), the regression line did not pass through the origin. The fact that the intercept with the y-axis (-32.5%, 95% confidence limits -45.6 and -19.3%) was significantly different from zero at the 0.001 level, suggests that the decrease in plasma testosterone during oestrogen could not completely be accounted for by the concomitant decrease in LH. Following clearance of ethinyloestradiol from the circulation, the rise in LH led to a coincident increase in plasma testosterone but the recovery of this latter hormone was slower and peripheral testosterone levels were still significantly below pre-treatment values at the end of the test.

The profile of plasma 17β-oestradiol was basically similar to that of testosterone and changes in the level of these 2 hormones, both during and following oestrogen-suppression, were positively correlated \((y = -3.2 + 0.4x)\) where \(y\) and \(x\) represent percentage change from mean pre-treatment level in 17β-oestradiol and testosterone respectively; \(r = 0.4438, n = 36, p < 0.01\).
FIGURE 6.5: Mean (± S.E.M.) testosterone and 17β-oestradiol levels and their relative changes from the mean pre-treatment level in 6 normal men during oestrogen provocation test (1 x 50 µg ethinyloestradiol per day on days 5-7). Values significantly (p<0.05) different from mean pre-treatment level are indicated by an asterisk.
During treatment, mean plasma $17\alpha$-ethinylestradiol concentrations (Figure 6.6) were only about half those seen in women, and in only one instance (\textit{\textsuperscript{c}} 0\textdegree; Figure 6.4) were levels comparable to those of women achieved. Since a sex-related difference in gastro-intestinal absorption of $17\alpha$-ethinylestradiol seemed unlikely, the lower plasma levels in men suggested a more rapid clearance of this compound from the circulation and this was further illustrated by the rapid decrease in peripheral levels of the hormone after stopping treatment.

\textbf{Episodic gonadotrophin release}

The data with respect to episodic gonadotrophin release before and during oestrogen administration are presented in Tables 6.4 and 6.5.

Mean FSH and LH levels during the second sampling period (i.e. 24 hours after start of treatment) were significantly lower in all but one subject (Table 6.4) although the degree of suppression was on average smaller than that seen in women (Table 6.2).

Episodic gonadotrophin release before oestrogen (Table 6.5) was both in terms of frequency and magnitude of the secretory episodes, comparable to that of women (Table 6.3). In contrast to women however, ethinylestradiol treatment had very little effect on pulsatile gonadotrophin secretion. Peak frequency remained essentially unchanged, nor was there any change in the amplitude of the FSH peaks. The relative magnitude of LH peaks on the other hand tended to increase but the difference was not significant.

\textbf{(c) Normal men (500 \textmu g a day for 3 days)}

The patterns of plasma FSH and LH in men taking 500 \textmu g ethinylestradiol per day (Figures 6.7 and 6.8 a,b) were very similar to those
FIGURE 6.6: Mean (± S.E.M.) plasma FSH, LH, testosterone, 17β-oestradiol and 17α-ethinyloestradiol in six normal men during an oestrogen provocation test (4 x 50 μg ethinyl-oestradiol per day was given on days 5-7).
TABLE 6.4: Transverse means (± S.D.) in mU/ml and percentage change of peripheral gonadotrophin levels in 6 normal men sampled at 15 minute intervals for 3 hours before (day 4) and during (day 6) ethinyloestradiol administration.

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 6</th>
<th>Percentage change*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>σ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>2.27 ± 0.34</td>
<td>2.04 ± 0.26</td>
<td>-10.1</td>
</tr>
<tr>
<td>02</td>
<td>2.60 ± 0.32</td>
<td>1.88 ± 0.26</td>
<td>-27.7</td>
</tr>
<tr>
<td>03</td>
<td>8.44 ± 0.99</td>
<td>6.48 ± 0.93</td>
<td>-23.2</td>
</tr>
<tr>
<td>04</td>
<td>5.35 ± 0.35</td>
<td>4.88 ± 0.52</td>
<td>-8.8</td>
</tr>
<tr>
<td>05</td>
<td>6.68 ± 1.04</td>
<td>3.77 ± 0.34</td>
<td>-43.6</td>
</tr>
<tr>
<td>06</td>
<td>3.32 ± 0.41</td>
<td>3.45 ± 0.41</td>
<td>+3.9</td>
</tr>
<tr>
<td>mean ± S.E.M.</td>
<td>4.78 ± 1.01</td>
<td>3.75 ± 0.71</td>
<td>-(18.3 ± 6.8)</td>
</tr>
</tbody>
</table>

| LH  |        |        |                     |
|     |        |        |                     |
| σ   |        |        |                     |
| 01  | 3.06 ± 0.52 | 1.88 ± 0.32 | -38.6               |
| 02  | 2.71 ± 0.42 | 1.10 ± 0.23 | -59.4               |
| 03  | 4.13 ± 0.53 | 3.40 ± 0.77 | -17.7               |
| 04  | 4.08 ± 0.65 | 2.58 ± 1.15 | -36.8               |
| 05  | 3.82 ± 0.75 | 1.95 ± 0.21 | -49.0               |
| 06  | 4.76 ± 0.88 | 6.08 ± 2.54 | +27.7               |
| mean ± S.E.M. | 3.76 ± 0.31 | 2.83 ± 0.72 | -(29.0 ± 12.7) |

* Calculated from the formula: \[ \frac{\text{mean value day 4}}{\text{mean value day 6}} \times 100 - 100 \]
# TABLE 6.5: Characteristics of pulsatile gonadotrophin release in six normal men sampled at 15 minute intervals for 3 hours before (day 4) and during (day 6) ethinyloestradiol administration

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th></th>
<th>Day 6</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSH</td>
<td>LH</td>
<td>FSH</td>
<td>LH</td>
</tr>
<tr>
<td>Number of peaks</td>
<td>0.67</td>
<td>0.83</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>(per 3 hours)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute peak</td>
<td>1.19 ± 0.56</td>
<td>1.44 ± 0.20</td>
<td>1.27 ± 0.41</td>
<td>1.98 ± 0.66</td>
</tr>
<tr>
<td>magnitude (mU/ml)</td>
<td>(mean ± S.E.M.)</td>
<td>(mean ± S.E.M.)</td>
<td>(mean ± S.E.M.)</td>
<td>(mean ± S.E.M.)</td>
</tr>
<tr>
<td>Relative peak</td>
<td>31.9 ± 3.1</td>
<td>46.5 ± 5.7</td>
<td>30.9 ± 1.0</td>
<td>96.7 ± 26.7</td>
</tr>
<tr>
<td>magnitude (%)</td>
<td>(mean ± S.E.M.)</td>
<td>(mean ± S.E.M.)</td>
<td>(mean ± S.E.M.)</td>
<td>(mean ± S.E.M.)</td>
</tr>
</tbody>
</table>
seen with the lower dose except for a few minor differences.

With the higher dose, inhibition of pituitary gonadotrophin secretion appeared to be more effective and both FSH and LH were already maximally suppressed 24 hours after starting the ethinyloestradiol. Neither of the gonadotrophins showed any significant further decrease thereafter despite a further rise in circulating 17α-ethinyloestradiol (see below). It is of interest to note that even though peripheral 17α-ethinyloestradiol levels were 2 to 3 times higher with the 500 µg dose, peripheral gonadotrophin levels were on average not significantly more suppressed than with the lower dose. Thus, FSH declined to 64.7 ± 5.5% (mean ± S.D.) of the initial level on 500 µg and to 70.5 ± 17.0% on the 200 µg dose (p > 0.05), while the corresponding values for LH were 48.3 ± 13.5% and 46.2 ± 19.1% (p > 0.05).

Although mean circulating 17α-ethinyloestradiol levels were higher than those in women, ethinyloestradiol did not have a biphasic effect on gonadotrophin release, and LH levels were always inversely correlated with plasma 17α-ethinyloestradiol concentrations (\( y = 55.3 -43.9 \log x \) where \( y \) = percentage change in LH from pre-treatment level and \( x \) = 17α-ethinyloestradiol concentration; \( r = -0.666; n = 33; p < 0.001 \)). Statistical comparison of this regression line and that of the previous experiment indicated that neither the slopes or the intercepts of both lines were significantly different. FSH levels on the other hand were not correlated with plasma 17α-ethinyloestradiol, and, as with the lower dose, recovered much more slowly than LH after stopping the oestrogen.

But for a quantitatively more pronounced suppression, the changes in plasma testosterone and 17β-oestradiol in men taking the 500 µg dose of ethinyloestradiol (Figure 6.9) were essentially similar to those seen with the lower dose.
FIGURE 6.7: Mean (± S.E.M.) plasma FSH and LH and their relative changes from pre-treatment levels in 7 normal men treated with 500 μg ethinyloestradiol per day on days 5-7. Values which are significantly (p<0.05) different from mean pre-treatment level are indicated by an asterisk.
FIGURE 6.8 a: Plasma FSH, LH and 17α-ethynylestradiol in normal men during an oestrogen provocation test (500 μg ethynylestradiol per day on days 5-7).
**Figure 6.8 b:** Plasma FSH, LH and 17α-ethinylestradiol in normal men during an oestrogen provocation test (500 μg ethinylestradiol per day on days 5-7).
During oestrogen-suppression, fluctuations in circulating testosterone were correlated with changes in FSH and LH, but the correlation with the latter was better \( y = -4.02 + 0.7 x_1 \) and \( y = -4.5 + 0.5x_2 \) where \( y \) = percentage change in testosterone from mean pretreatment level and \( x_1 \) and \( x_2 \) = percentage change in FSH and LH respectively; \( r_1 = 0.4010; r_2 = 0.7792; n_1 = n_2 = 33; 0.01 < p < 0.05 \) and \( p_2 < 0.001 \). Intercept and slope of this latter regression line were not significantly different from those in the previous study and, as in the previous study, the regression line did not pass through the origin (intercept \(-4.5\), 95% confidence limits \(-5.7\) and \(-3.3\)).

Twenty-four hours after the start of oestrogen administration, plasma 17α-ethinylestradiol levels (Figure 6.10) in men on the 500 µg dose were comparable to those seen in women on 200 µg but, in contrast to women, levels in men were not stable but showed a further significant (\( p < 0.01\), paired t-test) rise over the next 24 hours. After stopping the oestrogen, levels rapidly declined during the first 12 hours but from then onwards the rate of decrease and hence also the half-life of the hormone (mean \( ±\) S.E.M.: \( 927.6 \pm 91.4\) minutes or \( 15h 27 \) min \( ±\) 1h 31 min) were similar to those seen in women.

6.1.4 Discussion
(a) Feedback effects of 17α-ethinylestradiol

The present studies on gonadotrophin secretion in women treated with exogenous oestrogen confirm the now numerous reports that oestrogens, when given in a sufficiently high dose for a minimum period of time, have a biphasic effect on pituitary gonadotrophin release: following an initial period of suppression, pituitary gonadotrophin secretion is stimulated resulting in an acute surge of LH (for references see Chapter 1).
FIGURE 6.2: Mean (± S.E.M.) plasma testosterone and 17β-oestradiol and their relative changes from the mean pre-treatment values in 7 normal men during an oestrogen provocation test (500 μg ethinyloestradiol per day on days 5-7). Asterisks indicate values which are significantly (p < 0.05) different from mean pre-treatment value.
FIGURE 6.10: Mean (± S.E.M.) plasma FSH, LH, testosterone, 17β-oestradiol and 17α-ethinyloestradiol in 7 normal men during an oestrogen provocation test (500 μg ethinyloestradiol per day was given on days 5-7).
The initial decrease of peripheral gonadotrophin levels is likely to be the result of a direct inhibitory action of the administered oestrogen on hypothalamic LRF-secretion as well as on pituitary gonadotrophin secretion. Administration of oestrogens has been shown to lower hypothalamic LRF content in rats (McCann, 1973), to interrupt episodic gonadotrophin release and to diminish pituitary responsiveness to LRF when given acutely to women (Yen, Tsai, Vandenberg and Rebar, 1972; Keye and Jaffe, 1974). Most of the studies on the effects of exogenous oestrogen on pulsatile gonadotrophin secretion have been performed on agonadal (e.g. Yen et al., 1972) or ovariectomized (e.g. Yamaji, Dierschke, Bhattacharya and Knobil, 1972) females since in these conditions pulsatile gonadotrophin secretion is most pronounced. The present study however indicates that a similar effect of oestrogens can be demonstrated in intact women in the early to mid-follicular phase of the cycle (Table 6.3).

This initial period of gonadotrophin suppression was brief and the negative feedback effect was soon offset by the positive feedback action of the administered oestrogen, resulting in a significant rise in peripheral LH which started between 48 and 72 hours after initiation of treatment. Since no significant decrease in plasma \(^{17\alpha}\)-ethinyl-oestradiol occurred over that period, it seems unlikely that oestrogen-induced LH surges are triggered off by an acute fall rather than a rise in circulating oestrogen as has been suggested by some authors (e.g. Nillius and Wide, 1971b). A similar conclusion was reached by Karsch, Weick, Butler, Dierschke, Krey, Weiss, Hotchkiss, Yamaji and Knobil (1973) following their studies on the strength-duration characteristics of the oestrogen stimulus required to induce LH-surges in the rhesus monkey. Evidently, this does not exclude that the decline in
circulating $17\beta$-oestradiol during the peri-ovulatory period of the normal menstrual cycle might not enhance the magnitude and/or duration of the midcycle LH peak. For $17\beta$-oestradiol to decline however, an initial rise in LH seems to be essential. An increase in LH concentrations has been shown to inhibit oestrogen secretion from sheep Graafian follicles both in-vivo and in-vitro (Moor, 1974). Conversely, when this initial rise in LH is absent as in anovulatory dysfunctional bleeding, follicular oestrogen secretion is often enhanced and extended beyond the time of expected ovulation (see further).

Under our experimental conditions, the magnitude of the induced LH-surge was comparable to that of the spontaneous midcycle LH-peak. Similar results were obtained by Yen and Tsai (1972) who treated 3 women with ethinyloestradiol (200 µg a day) between days 7 and 10 of the menstrual cycle. Tsai and Yen (1971) on the other hand reported that, using the same treatment-regime during the early follicular phase, LH surges were much smaller than those seen at midcycle. This difference in magnitude of the LH response at different stages of the cycle probably reflects differences in hypothalamic-pituitary sensitivity for the positive feedback effect of oestrogen, and may be related to the degree of previous oestrogen-exposure. Fink and Jamieson (1976) have shown that hypothalamic sensitivity for electrical stimulation of LH release in the rat, increases between di-oestrus and pro-oestrus, when ovarian oestradiol secretion is maximal. Since oestrogens also promote pituitary gonadotrophin synthesis (Apfelbaum and Taleisnik, 1976) and enhance pituitary sensitivity to LRF (Jaffe and Keye, 1974), it seems not unreasonable to assume that the magnitude of the induced LH-surge may be related, at least within certain limits, to the extent of previous oestrogen-priming of the hypothalamic-pituitary unit. This latter
effect may possibly also account for the variability in time-lag between the start of oestrogen treatment and the initiation of the LH-surge. In the present study, peak LH levels were invariably observed 96 hours after initiation of treatment in all but one subject. In this latter woman pre-treatment 17\(\beta\)-oestradiol levels were significantly higher than in the remaining five, and the LH-surge occurred 24 hours earlier. A similar phenomenon was encountered in patients with polycystic ovarian disease who have elevated circulating oestrogen levels (see further). The earlier activation of the positive feedback mechanism in this woman may also explain why peripheral LH levels were less markedly suppressed and episodic LH release preserved on the second day of oestrogen treatment. The fact that the amplitude of her LH pulses increased during oestrogen is consistent with the observations of Yen, Vandenberg, Tsai and Parker (1974) who reported a similar finding during the peri-ovulatory period of the normal menstrual cycle.

Although in most instances the spontaneous midcycle LH-peak is accompanied by an increase in FSH (see e.g. Figure 1.2), the induction of FSH-surges with exogenous oestrogen has generally been less successful (Nillius and Wide, 1971; Monroe, Jaffe and Midgley, 1972) and this was also the case in our study (Figure 6.1). The absence of a stimulatory effect of ethinyloestradiol on FSH secretion was illustrated by the significant inverse correlation between peripheral FSH and 17\(\alpha\)-ethinyloestradiol levels which suggests that, under our experimental conditions, FSH secretion was regulated by a negative feedback mechanism only. It would however be hazardous to infer from this conclusion that the naturally occurring oestrogen, 17\(\beta\)-oestradiol, does not have a positive feedback effect on FSH release, particularly in view of the observation that ethinyloestradiol, in contrast to 17\(\beta\)-oestradiol, inhibits the release of FSH in response to LRF (Schally,

Unlike in women, the profile of peripheral LH levels during and following oestrogen administration to normal men, was qualitatively completely different and there was no indication of a stimulatory effect of ethinyloestradiol on gonadotrophin release (Figure 6.3). Yamaji, Dierschke, Hotchkiss, Bhattacharya, Surve and Knobil (1971) also failed to detect a positive feedback action of oestradiol in intact male rhesus monkeys, but recent reports have tended to suggest that, in the human intact male, oestrogen may be capable of inducing LH release (Kulin and Reiter, 1976; Dorner, Rohde, Stahl, Krell and Masius, 1975). The evidence on which this conclusion has been based however, is rather unconvincing. Dorner et al. (1975) reported that LH levels in homosexual men rose significantly above pre-treatment levels after intravenous administration of conjugated equine oestrogens. Neither peripheral oestrogen or testosterone concentrations were measured in that study and it cannot be excluded therefore that the observed rise in LH was not due to a release from the negative feedback effect of the administered oestrogen at a time when testosterone levels were markedly suppressed. This conclusion is all the more tempting since the reported profile of peripheral LH in these men was very similar to that observed in our volunteers on the low oestrogen dose (Figure 6.3) (The alternative explanation i.e. that all our male volunteers were homosexuals, seems unlikely!)

The rise in LH observed by Kulin and Reiter (1976) after intramuscular administration of oestradiol-benzoate to normal men can be explained on the same basis. These latter authors did measure peripheral $17\beta$-oestradiol and testosterone and could thus show that LH
levels increased at a time when oestradiol levels were still above the normal male range. In every instance however, increases in LH became only detectable after oestradiol levels had markedly decreased. Since at the same time testosterone concentrations were grossly suppressed, it seems reasonable to assume that the increase in LH was due to a stimulatory effect of the low testosterone levels which could no longer be offset by the inhibitory action of the declining oestrogen levels. It is well known that LH secretion in the human male is not sensitive to the suppressive effect of low doses of oestrogen (Kulin and Reiter, 1972), and our observation that episodic gonadotrophin secretion was still present in the male volunteers on the 200 µg dose of ethinyloestradiol (Table 6.5) would seem to support this.

Apart from the obvious qualitative difference in the profile of peripheral gonadotrophin levels, the absence of a positive feedback effect of ethinyloestradiol in men was also illustrated by the highly significant inverse relationship between peripheral LH, expressed as a percentage of the mean pre-treatment level, and the logarithm of peripheral 17α-ethinyloestradiol levels. This inverse correlation could be demonstrated with both the low and high dose of ethinyloestradiol treatment, and since in both instances the regression lines did not differ statistically, the data have been pooled in Figure 6.11.

Correlation analysis of the combined results gave the equation

\[ y = 51.04 - 4.41 \log x \]  

where \( y \) = percentage change in LH and \( x = 17\alpha\text{-ethinyloestradiol concentration; } r = -0.5959; n = 58; p < 0.001. \]

It is evident from Figure 6.11 that on several occasions low 17α-ethinyloestradiol levels were associated with elevated LH levels. Such combinations were only encountered during the post-treatment period when circulating oestrogen levels were declining and testosterone levels
\[ y = 51.04 - 44.41 \log x \]

\[ r = -0.5959 \]

\[ p < 0.001 \]

**FIGURE 6.11:** The regression line and its 95% confidence limits for LH suppression by 17α-ethinyloestradiol in normal men.
markedly depressed, and may therefore not be interpreted as evidence for a positive feedback effect of the administered oestrogen. The upper 95% confidence limit of the regression line intercepts the x-axis at 25 pg/ml. Assuming a Gaussian distribution about the regression line, this would indicate that only when plasma 17α-ethinyloestradiol levels are higher than 25 pg/ml, one may expect with reasonable (95%) confidence to find a suppression of the mean LH level. Judging from the 17α-ethinyloestradiol concentrations in men on 200 and 500 μg of this hormone, it would appear that, in order to achieve such a level, a daily dose of 50 μg or more would be required. This would be in agreement with the data of Kulin and Reiter (1972) who showed that plasma LH levels in men could be suppressed with 50 μg of ethinyloestradiol but not with lower doses.

In contrast to LH, changes in FSH were quantitatively much smaller and not related to plasma 17α-ethinyloestradiol concentrations. Swerdloff and Odell (1968) gave 200 μg of ethinyloestradiol per day to 14 normal men and reported that FSH levels were unaffected in 6 of the subjects after 2 days of treatment although they were suppressed after 4 days. Using much smaller doses of ethinyloestradiol, Kulin and Reiter (1972) observed a significant FSH suppression at the end of a 7-day treatment regime with 30-40 μg per day. LH levels on the other hand were not suppressed by this dose. It is likely therefore, that the relative ineffectiveness of ethinyloestradiol with respect to FSH suppression in the present study, is due to an intrinsic property of the feedback mechanisms involved in FSH regulation, which appear to be more sluggish in adapting to changes in the hormonal environment than those involved in LH secretion.
(b) Gonadal effects of ethinyloestradiol

In women, the decrease in plasma 17β-oestradiol during oestrogen administration suggests that follicular development was arrested. It seems unlikely that this inhibition of follicle maturation was due to a direct antigonadal effect of the administered ethinyloestradiol since oestrogens promote rather than inhibit follicular development (Bradbury, 1961; Goldenberg, Vaitukaitis and Ross, 1972). The decrease in ovarian oestrogen secretion may therefore be attributed to the decrease in circulating gonadotrophins. The dependency of the growing follicle on continuous gonadotrophic support at this stage of the cycle is further illustrated by the observation that in 5 out of the 6 subjects, 17β-oestradiol levels remained low after discontinuation of treatment, suggesting that 48-72 hours of relative gonadotrophin-deprivation were sufficient to make the developing follicles atretic. As a result, the onset of the next menstrual period in these women was delayed for 12.4 ± 3.7 days (mean ± S.E.M.) (p < 0.05), and serial measurements of urinary total oestrogen and pregnanediol excretion indicated that this delay was due to a prolongation of the follicular phase and the development of a new crop of follicles. Using the same treatment regime in 3 women between days 7 and 10 of the cycle, Yen and Tsai (1972) reported a mean increase in cycle length of 11.0 days.

In one subject (♀01) plasma 17β-oestradiol levels, after having been suppressed during oestrogen administration, rapidly rose as soon as treatment was discontinued. The failure of the developing follicle(s) to become atretic in this woman may have been the result of a variety of factors. Since pre-treatment levels of 17β-oestradiol were higher, it may be that follicular maturation in this woman had further progressed.
than in the remaining five and hence that the follicle was less
dependent on gonadotrophic support, although the decrease in 17β-
oestradiol which occurred during treatment would seem to argue against
this. Alternatively, the follicle may have been saved from atresia
by the earlier activation of the positive feedback mechanism. It is
noteworthy that although plasma 17β-oestradiol levels were well within
the normal midcycle range on day 10 of the test, ovulation did not
occur in this woman and urinary pregnanediol excretion remained below
1mg/24h. The reason for her failure to ovulate is uncertain but could
be related to temporary refractoriness of the positive feedback
mechanism. In the intact female rhesus monkey, Weick, Dierschke, Karsch,
Yamaji and Knobil (1972) have shown that a second injection of oestradiol-
benzoate consistently induces LH release only when given 8 days after
the first.

In men, administration of ethinyloestradiol resulted in a con-
comitant suppression of LH and testosterone but, in relative terms, the
decrease in testosterone was greater than that of LH. Changes in
testosterone were always correlated with changes in LH however, and
since the regression lines in both experiments (200 and 500 µg per day
of ethinyloestradiol) did not differ statistically, the data have been
pooled in Figure 6.12. Correlation analysis of the combined data gave
the equation \( y = -39.83 + 0.55x \) where \( y \) and \( x \) are percentage change in
testosterone and LH respectively; \( r = 0.6963; n = 58; p < 0.001 \). The
negative intercept of this regression line (-39.83%, 95% confidence
limits - 32.83 and -46.79%) with the y-axis suggests that ethinyloestradiol
has a direct antigonadal effect on testicular testosterone secretion,
resulting in a decrease in peripheral testosterone levels of approx-
imately 40% even in the absence of a decrease in plasma LH. In the rat,
FIGURE 6.12: The regression line and its 95% confidence limits for changes in plasma LH and testosterone during ethinyl-oestradiol treatment of normal men.
Tcholakian, Chowdhury and Steinberger (1974) have shown that oestradiol-<br>benzoate significantly suppresses plasma testosterone within 1 hour,<br>and testicular testosterone content within 2 hours after injection<br>without depressing LH. In men suffering from prostatic cancer, intra¬<br>venous administration of diethylstilboestrol for 20 days resulted in a<br>decrease of the serum LH level to about 50% and of testosterone levels<br>to less than 5% of the initial values (Dorner, Stahl, Rohde and<br>Schnorr, 1975). The observation that chlorotrianisene, a synthetic<br>oestrogen which is structurally related to clomiphene and stilboestrol,<br>suppresses testosterone without affecting gonadotrophin release (Baker,<br>Burger, De Kretser, Hudson and Straffon, 1973) adds further support to<br>the view that oestrogens may inhibit testicular androgen production<br>directly. The mechanism by which this effect is produced is not known,<br>although the demonstration of deficient in-vitro conversion of C21-<br>steroids to androgens in testes from oestrogen-treated animals (Samuels,<br>Short and Huseby, 1964) and the observation that specific oestrogen<br>receptors are present in the interstitial cells of the rat (Brinkmann,<br>Mulder, Lamers-Stahlhofen, Mechielsen and van der Molen, 1972) suggest<br>that oestrogens may be acting directly on the steroidogenic enzymatic<br>apparatus within these cells.

It is of interest to note in Figure 6.12 that extrapolation of<br>the regression line to x = -100% gives y = -94.83% (95% confidence limits,<br>-105.58% and -84.01%). Thus in the absence of LH (and hence of<br>testicular testosterone secretion), mean peripheral testosterone levels<br>would decrease to approximately 5% of their initial value (i.e. to 0.25 -<br>0.50 ng/ml). This is in accordance with constant isotope infusion data<br>which indicate that, but for 3-4%, the entire blood production rate of<br>testosterone is derived from direct testicular secretion of the hormone<br>(Baird, Horton, Longcope and Tait, 1969).
Plasma $17\beta$-oestradiol in men on the other hand originates for nearly equal amounts from direct testicular secretion and from peripheral conversion of testosterone (Longcope, Kato and Horton, 1969). Changes in $17\beta$-oestradiol may therefore be expected to reflect changes in testosterone. That this was so is illustrated in Figure 6.13. The slope of this regression line ($0.14; 95\%$ confidence limits $0.36$ and $0.52$) indicates that the decrease in circulating $17\beta$-oestradiol could completely be accounted for by the decrease in peripheral conversion of the precursor, testosterone, which evidently would imply that the other source of circulating oestradiol, i.e. direct testicular secretion, was virtually unaffected during exogenous oestrogen treatment. The lack of correlation between $17\beta$-oestradiol and LH appears to support this.

(c) Profile of plasma $17\alpha$-ethinyloestradiol

Despite the widespread use of ethinyloestradiol in contraceptive medication, data on plasma-levels of this hormone during treatment are virtually non-existent and any comparison of the plasma levels found in the present study with previously published work is therefore not possible. Several lines of indirect evidence however indicate that the reported plasma concentrations are within the range one might have expected on the basis of previous work on the metabolism of radioactive ethinyl-oestradiol. Thus, Reed, Fotherby and Steele (1972) reported that 4 hours after oral administration of $^{14}C$-ethinyloestradiol to normal men peripheral plasma contained 2.5% of the administered dose per litre. More than 70% of the radioactivity was associated with sulphate conjugates and only 3.5% (or approximately 0.08% of the administered dose) behaved chromatographically in the same way as unconjugated $17\alpha$-ethinyloestradiol. Since after oral administration, absorption of ethinyloestradiol is
% CHANGE IN
17\(\beta\)-OESTRADIOL

FIGURE 6.13: The regression line and its 95% confidence limits for changes in plasma testosterone and 17\(\beta\)-oestradiol in normal men during ethinyloestradiol treatment.
virtually complete (Reed et al., 1972), doses of 200 or 500 µg would result in peripheral plasma levels of approximately 160 and 400 pg per ml respectively which is well within the range observed in our male volunteers (Figures 6.4 and 6.8 a, b).

Using the constant infusion technique, Bird and Clark (1973) have shown that the metabolic clearance rate of ethinyloestradiol in normal young women is $1345 \pm 221 \mu l/\text{min}$ hour (mean ± S.D.; n = 4). Since under steady-state conditions

$$P.R. = MCR \times i$$

where P.R. is the blood production rate, MCR the metabolic clearance rate and i the plasma concentration, it can be calculated that a daily dose of 200 µg ethinyloestradiol corresponds to a plasma concentration of $149 \pm 24 \text{ pg/ml}$, a value which is not significantly different from the $206 \pm 54 \text{ pg/ml}$ observed in our female volunteers during treatment.

In a preliminary communication Warren and Fotherby (1971) recently reported that 8 hours after ingestion of 50 µg ethinyloestradiol, plasma levels of the hormone were 38 pg/ml, which is very similar to our results (range 21-35 pg/ml) obtained in patients treated with this dose and from whom bloodsamples were taken 10-12 hours later.

6.1.5 Summary

The effects of exogenous oestrogen administration on pituitary gonadotrophin secretion and gonadal function were examined in normal women during the early-mid follicular phase of the cycle and in men.

In women, ethinyloestradiol (200 µg a day for 3 days) had a biphasic effect on LH release. Following an initial period of suppression, LH levels rapidly increased to reach peak values which were 3 to 4 times higher than pre-treatment values. The oestrogen-induced LH peak was
in terms of magnitude and duration comparable to the spontaneous midcycle LH-surge. A similar positive feedback effect of ethyline-
oestradiol on FSH secretion could not be demonstrated. Follicular
development, as reflected by peripheral 17β-oestradiol levels, was
arrested during oestrogen treatment and, in most instances, this
arrest was irreversible and follicles became atretic.

In men, no evidence for a stimulatory effect of ethinyloestra-
diol (in doses of 200 µg a day for 3 days and 500 µg a day for 3 days)
on gonadotrophin release could be obtained even though with the latter
treatment regime circulating 17α-ethinyloestradiol levels were higher
than those seen in women. Changes in peripheral LH, but not FSH, were
inversely related to changes in plasma 17α-ethinyloestradiol. During
oestrogen administration, the decrease in plasma testosterone levels
was correlated with the decrease in LH, but the results suggest that
ethinyloestradiol may also inhibit testicular androgen secretion
directly. Changes in plasma 17β-oestradiol on the other hand reflected
those of testosterone.
6.2 Studies on positive feedback in male pseudohermaphroditism

6.2.1 Background

The studies described in the previous section indicate that feedback regulation of gonadotrophin secretion in adult, intact humans is a sexually dimorphic characteristic. In the female, oestrogens can inhibit as well as stimulate the release of gonadotrophins while in the male, gonadotrophin secretion appears to be regulated by negative feedback only.

In rodents, it is now well established that this sexual dimorphism in hypothalamic responsiveness to oestrogen is due to intrauterine or neonatal exposure to androgens (and/or oestrogens) which are held responsible for the permanent suppression of the centre(s) involved in cyclical gonadotrophin release.

The situation in primates however appears to be different. Clinical and experimental evidence tends to suggest that pre- or neonatal androgen exposure of female monkeys and humans does not necessarily result in permanent anovulatory sterility. Moreover, since under special conditions, male primates can be made to release LH in response to exogenous oestrogens, the existence of a sex-related difference in hypothalamic control of gonadotrophin secretion has been questioned. In particular, Karsch, Dierschke and Knobil (1973) have suggested that the difference between the sexes in their responsiveness to oestrogen may be quantitative rather than qualitative, and that the failure to demonstrate positive feedback in intact males may be due to a blocking action of testosterone. If this hypothesis is correct it should be possible to induce gonadotrophin release in genetic males in whom this blocking effect of testosterone is not present. Castrated men could
theoretically be used for this purpose, but it seemed to us that the syndrome of testicular feminization (Morris, 1953) would actually represent a better test model. Indeed, although patients with this condition have a female phenotype, from a genetic, gonadal and endocrine point of view they are comparable to normal men (for review see e.g. Naftolin and Judd, 1973), and as such are probably more representative of the intact male situation than oestrogen-suppressed castrated males. The syndrome of testicular feminization however is not a very common condition (estimated prevalence 1 per 62,400 males, Jagiello and Atwell, 1962), and we are therefore very grateful to Dr. J. Scrimgeour for allowing us access to the two siblings under his care. The reported studies were carried out in collaboration with Dr. D. T. Baird. For comparison, an additional patient with pure gonadal dysgenesis and XY-karyotype has also been studied.

6.2.2 Subjects and design of study

The proposita, Miss J. H., aged 26 years, presented at the gynaecological clinic with primary amenorrhoea. The medical history dated back to 9 years previously when she was first seen at another hospital for the same problem and, at laparotomy, was reported to have bilateral ovaries but undeveloped internal genitalia. Her sister, Miss A. H., aged 18 years, also suffered from primary amenorrhoea and so did 2 of her great-aunts and 2 of her aunts, one of whom (aged 56 years) was still alive but could not be investigated.

At examination, Miss J. H. weighed 50 kg for a height of 160 cm. Span and pubis height were 160 and 84 cm respectively. She had normal female secondary sex characteristics but axillary and pubic hair were absent. No internal genitalia were felt on pelvic examination and the
vagina was blind-ending. Her buccal smear was negative for Barr bodies and the karyotype of her peripheral leucocytes was 46, XY. A clinical diagnosis of testicular feminization was made and subsequently confirmed when at laparoscopy, intra-abdominal testes were visualised. Following this, an oestrogen provocation and IRF-test were performed as described in Chapter 4. After completion of the oestrogen provocation test, the patient underwent laparotomy. Paired peripheral and testicular vein blood collections were made, and the gonads removed and sent for histological examination.

Miss A. H., the younger sister of J.H., was 172 cm tall and weighed 70 kg. But for the absence of axillary and pubic hair, her secondary sex characteristics were those of a normal female. The vagina was about 5 cm long and blind-ended and uterus could not be felt. No Barr bodies could be detected in the buccal smear and the karyotype was 46,XY. The protocol of endocrine investigations performed in this patient was similar to that described for her sister.

The third patient, Miss L. I., aged 14\(\frac{1}{2}\) years, was referred to the gynaecological endocrine clinic for investigation of delayed puberty. At birth, the patient had had marked oedema of both feet which however had disappeared following treatment. During childhood she had suffered from an asthmatic condition and had also received conservative orthopaedic treatment for a slight genu-varum deformity. There was no family history of primary amenorrhoea and the patient had no sisters or female cousins. Her height was 147 cm and her weight 49 kg. Breast development was minimal and pubic and axillary hair absent (Plate 6.1). There was some degree of cubitus valgus and genu varum, as well as ptosis of the eyelids, but webbing of the neck and lymphoedema of the extremities were not conspicuous. The chest had a shield-like appearance.
Blood pressure and heart auscultation were normal. The external genitalia were female and no gonadal tissue could be felt in either the inguinal canals or labia. No Barr bodies were seen in the buccal smear and chromosome studies of the peripheral blood leucocytes showed her to have 46 chromosomes with a XY sex-chromosome complement. X-rays of the hands were normal. Urinary total oestrogen excretion was consistently less than 2 μg/24 hours but urinary gonadotrophin excretion was markedly elevated (FSH: 32·5 iu/24 hours, LH 109-371 iu/24 hours). At diagnostic laparoscopy she was found to have bilateral streak gonads but otherwise normally developed female internal genitalia. Miss L. I. was therefore considered to represent a case of pure gonadal dysgenesis. The hormonal studies performed in this patient were similar to those described for the previous two patients. At laparotomy, eight months later, both streak gonads were excised and sent for histological examination. A portion of the right gonad as well as pieces of rectus muscle and abdominal skin were kept for culture and complementary chromosome studies.

6.2.3 Results
(a) Histological and cytogenetical studies

Testicular feminization

In both patients the histological appearance of the testes was similar and characteristic of this condition, although each testis showed within itself a certain variation in structure between different fields. The larger, central part of each gonad was made up by closely packed small seminiferous tubules lined with immature Sertoli-cells and devoid of germinative elements. The tubules were interspersed with numerous Leydig cells showing heavy brown pigmentation. In the
peripheral portions of the gonad (Plate 6.2), the Leydig cells often tended to form cords or small clusters. Groups of spindle-shaped cells, morphologically indistinguishable from ovarian stroma, were also present here but no ovarian follicles were seen. In each testis, remnants of the Mullerian duct system were easily identifiable. They were usually attached to the poles of the testis and consisted of bundles of smooth-muscle fibres resembling myometrium, and immature Fallopian tube-like structures. Malignant changes were not detected in any of the 4 testes.

**Pure gonadal dysgenesis**

Microscopically the streak gonads of this patient consisted of a thick layer of ovarian stroma-like tissue without any suggestion of follicular or seminiferous tubule development. In the adjacent vascular hilum, numerous substantial clumps of hilus cells with abundant eosinophilic cytoplasm were identified (Plate 6.3). No Barr bodies could be seen in these cells nor in the ovarian stroma, and there was no evidence of malignancy.

In all tissues examined, chromosome-analysis confirmed the presence of a Y chromosome which on fluorescence-staining, was indistinguishable from that of a normal male.

**(b) Endocrine studies**

**Oestrogen provocation test**

Results for the two siblings with testicular feminization are illustrated in Figures 6.14 and 6.15.

Basal gonadotrophin levels were elevated in both patients but the increase in LH was more marked than that of FSH, and the FSH/LH ratio was less than 1. Both FSH and LH were higher in the younger of the two siblings (A.H.) which was probably related to the fact that
PLATE 6.2: Histological appearance of the testis in testicular feminization (Miss A.H.; magnification x 40). Note the seminiferous tubules (T), the clusters of Leydig cells (L) and the spindle-shaped cells resembling ovarian stroma (S).
PLATE 6.3: Cluster of hilus cells in the streak gonad of a patient with XY pure gonadal dysgenesis.
FIGURE 6.14: Plasma gonadotrophins, testosterone, 17β-oestradiol and 17α-ethinylestradiol levels during oestrogen provocation test in a patient with testicular feminization (J.H.). Values on day 1 represent mean ± 1 SD of 5 consecutive daily samples.
MISS A.H., TESTICULAR FEMINIZATION

FIGURE 6.15: Plasma gonadotrophins, testosterone, 17/β-oestradiol and 17α-ethynylestradiol levels during oestrogen provocation test in a patient with testicular feminization (A.H.). No samples were collected on the first two days.
$17\beta$-oestradiol levels were only half those of her sister (J.H.). Ethinyloestradiol administration rapidly and effectively suppressed both gonadotrophins to values which at the end of the treatment period (day 8) were well within the normal male range. After stopping the oestrogen, gonadotrophins started to rise although they had not completely reached pre-treatment levels on day 10. The post-treatment rise of FSH was much slower than that of LH. As in normal men, changes in LH, but not in FSH, were inversely related to circulating $17\alpha$-ethinyloestradiol levels ($y = 18\cdot9 - 42\cdot5 \log x$ where $y =$ percentage change in LH from pretreatment value and $x =$ plasma $17\alpha$-ethinyloestradiol concentration; $r = -0\cdot7056$; $n = 10$; $p < 0\cdot05$).

The profile of peripheral testosterone during and following ethinyloestradiol appeared qualitatively similar to that of LH, and this was further substantiated by correlation analysis of these two parameters ($y = -31\cdot43 + 0\cdot43x$ where $y$ and $x$ are percentage change in testosterone and LH respectively; $r = 0\cdot6930$; $n = 10$; $p < 0\cdot05$). Neither the intercept or slope of this regression line were significantly different from those found in normal men, and, as in the group of normal men, the intercept ($-31\cdot43\%$, 95% confidence limits $-55\cdot87$ and $-6\cdot99\%$) was significantly different from zero.

Changes in plasma $17\beta$-oestradiol on the other hand paralleled those of testosterone ($y = 0\cdot39 + 0\cdot82x$ where $y$ and $x$ represent the percentage change in $17\beta$-oestradiol and testosterone respectively; $r = 0\cdot7789$; $n = 10$; $p < 0\cdot01$) but, in contrast to normal men, were also related to LH ($y = -18\cdot30 + 49x$ where $y =$ percentage change in $17\beta$-oestradiol and $x =$ percentage change in testosterone; $r = 0\cdot7132$; $n = 10$; $p < 0\cdot05$).
Plasma 17α-ethinylestradiol levels were on average about 50% higher than those found in men on the same dose and were not much different from those seen in women. The rate of clearance of the hormone after stopping treatment was much slower than in men and levels were still detectable at the end of the test. The half-life of the hormone, calculated from the difference in concentration between days 8 and 9 as described earlier, was 1280 min (or 21 h 20 min), a value comparable to that found in women (945 ± 259 min, mean ± SD).

Figure 6.16 illustrates the pattern of gonadotrophin and 17α-ethinylestradiol levels in the patient with pure gonadal dysgenesis.

Pre-treatment values of FSH (51.7 ± 10.0, mean ± SD) and LH (16.0 ± 5.3) were markedly elevated in this patient but, in contrast to the siblings with testicular feminization, the FSH/LH ratio was greater than 1. The presence of undetectable (< 10 pg/ml) 17β-oestradiol levels and of testosterone concentrations in the low female range indicated that the increase in pituitary gonadotrophin secretion was due to primary gonadal failure.

As in women, ethinylestradiol had a biphasic effect. Following an initial suppression, LH levels increased to a peak value of 36.4 mU/ml on day 9 after which they declined again. The oestrogen-induced LH peak was qua timing and absolute magnitude similar to that of women although in relative terms, peak-height was smaller (200% of pretreatment value as compared to 350%).

Episodic gonadotrophin release

Although in all three patients oscillations in peripheral FSH and LH levels were present, the number of genuine secretory episodes
FIGURE 6.16: Plasma gonadotrophins, testosterone, 17β-oestradiol and 17α-ethinyloestradiol during an oestrogen provocation test in a patient with XY pure gonadal dysgenesis.
was small (one FSH and one LH peak occurring asynchronously in L.I before oestrogen) and a comparison between the two sampling periods could therefore not be made.

**ERF-test**

On all three occasions, intravenous injection of 50 μg of ERF caused a dramatic rise in peripheral gonadotrophin concentrations, the magnitude of which was related to basal values (Figure 6.17). Thus, the elevation in LH was larger in the two siblings with testicular feminization while the FSH response was greater in the patient with pure gonadal dysgenesis. In relative terms however, pituitary gonadotrophin responses to ERF were similar in the two conditions: peak FSH levels were about 200% of basal levels and LH 500 to 600%. In the patients with testicular feminization, the rise in plasma gonadotrophins was followed by a 25% increase in plasma testosterone 60 minutes after injection of ERF.

**Testicular vein steroid concentrations**

Concentrations of both testosterone and 17β-oestradiol in testicular vein plasma, obtained at laparotomy from the patients with testicular feminization, were much higher than those in peripheral plasma indicating direct testicular secretion of both steroids (Figure 6.18). The mean ratios testicular vein/peripheral vein concentration were 16.3 and 7.5 for testosterone in J.H. and A.H. respectively while the corresponding values for 17β-oestradiol were 26.6 and 17.7.

**6.2.4 Discussion**

Although the first description of bilateral, intra-abdominally located testes in an otherwise apparently normal woman dates back to the early 19th century (Naftolin and Judd, 1973), it was not until
FIGURE 6.17: Plasma FSH and LH responses to intravenous injection of 50 μg of LRH in testicular feminization (broken line) and pure gonadal dysgenesis (solid line.). Values for testicular feminization represent the mean of two patients.
FIGURE 6.18: Peripheral (black bars) and testicular (open bars; R = right, L = left) vein concentrations of testosterone and $17\beta$-oestradiol in two patients with testicular feminization.
Morris' classic paper (1953) that the syndrome of testicular feminization received the attention it truly deserved. Since then, the literature on this condition has swollen considerably and the number of articles devoted to it, probably exceeds by far that of identified cases. To the pragmatist this may seem ludicrous, yet there are a variety of reasons which may explain the widespread interest in this unusual syndrome. Firstly, testicular feminization is the only well-documented example of a naturally occurring insensitivity to a steroid hormone, in casu testosterone. As such, it is of major importance in the study of the effects of this steroid at tissue or organ-level. Secondly, since the end-organ unresponsiveness in this syndrome arises from defective action of testosterone at the cellular level, the elucidation of this primary defect could considerably advance our knowledge and understanding of the mechanism of action of steroid hormones on their target-cells. Finally, the recognition that a similar condition may be found in several other animal species including such common laboratory species as the mouse (Iyon and Hawkes, 1970), has considerably widened the scientific interest in this syndrome not only from a comparative endocrinological point of view but also because it expanded the range of experimental investigative techniques which henceforth could be used in the study of this condition.

There is no doubt that the two siblings described in this study are genuine cases of the syndrome of testicular feminization. The clinical features, laboratory findings and gonadal histology of both patients were characteristic of this condition. The presence of ovarian stroma-like tissue and Müllerian remnants in their testes may appear rather unusual, although it should be pointed out that these anomalies
are relatively frequent in this syndrome, a fact already appreciated by Morris (1953).

The correct diagnosis in the third patient of this study on the other hand, is less certain. Some of the clinical features of this girl (e.g. the short stature, shield-like thorax, bone malformations of the extremities, ptosis of the eyelids) were suggestive of Turner's syndrome, yet the karyotype of peripheral leucocytes was 46, XY. XY/X0 sex-chromosome mosaicism as well as structural abnormalities of the Y chromosome have been encountered in patients with the Turner phenotype (Grumbach and Van Wyk, 1974), but all tissues of our patient had a 46, XY karyotype, the Y chromosome was indistinguishable from that of a normal male on fluorescence staining and the remaining X chromosome as well as the autosomes were structurally normal (Dr. K. Buckton, personal communication).

In 1955, Swyer described two chromatin-negative girls who were of tall stature and lacked the anomalies and malformations usually encountered in Turner's syndrome. Harnden and Stewart (1959) subsequently demonstrated the presence of a normal male chromosome complement in these patients and classified the condition as "pure gonadal dysgenesis". The use of this term was later expanded to accommodate patients with other karyotypes provided they did not have the somatic stigmata associated with Turner's syndrome. About two-thirds of patients with pure gonadal dysgenesis have a XY-karyotype (Grumbach and Van Wyk, 1974). Their clinical features include: female phenotype, primary amenorrhoea, streak gonads with normal female internal genitalia, normal or tall stature and eunuchoid habitus. Microscopically, the gonadal streaks consist of fibrous stroma without ova or follicles. Clumps of hilus cells, morphologically indistinguishable from Leydig cells, may
be present (Brunsteau, Sipahioglu, Byrd and Greenblatt, 1976) and are thought to account for the slightly elevated testosterone levels and the minor degree of virilization found in some of these patients. It is believed that this syndrome is a result of intra-uterine degeneration of the testes which occurred early enough in foetal life to allow development of the internal and external genitalia along female lines.

Our patient, Miss L.I., does not really fit into any of the two described syndromes. However, since the various forms of male pseudohermaphroditism are usually classified on the basis of their chromosomal constitution and the clinical picture within any given karyotype can be extremely variable, we consider L.I. to represent a case of XY pure gonadal dysgenesis unless otherwise proven.

The profile of peripheral gonadotrophin levels (Figure 6.16), as well as the pituitary FSH and LH response to LRF (Figure 6.17) in the patient with pure gonadal dysgenesis were characteristic of the castrate situation. The complete absence of gonadal steroid secretion in this patient was illustrated by the low levels of testosterone and $17\beta$-oestradiol. In addition, neither of these two gonadotrophins showed any significant change following gonadectomy (Figure 6.19), indicating that any gonadal steroid secretion which might have been present, was not operational in feedback control of gonadotrophin release. Consequently, it must be assumed that the hyperplastic hilus cells identified in the gonads, were not functional or, if they were, that the secreted steroids did not have any biological activity.
FIGURE 6.19: Plasma FSH and LH following gonadectomy in a patient with testicular feminization (Miss A.H.) and in a patient with XY pure gonadal dysgenesis (Miss L.I.). Vertical bars represent mean (± SD) of pre-gonadectomy values.
Basal gonadotrophin levels (Figures 6.14 and 6.15) and LRF-responses (Figure 6.17) in the siblings with testicular feminization on the other hand were quite different. The marked increase in basal LH in both patients is in accordance with previous studies on gonadotrophin secretion in this condition (e.g. Judd, Hamilton, Barlow, Yen and Kliman, 1972; Faiman and Winter, 1974) and is probably a result of hypothalamic-pituitary unresponsiveness to the negative feedback effect of testosterone. However, the elevation in LH seen in our patients and particularly in A.H., appeared to be greater than that reported by these authors and, unlike in their studies, LH levels tended to decrease rather than increase following gonadectomy (Figure 6.19). Furthermore, in contrast to most other workers (e.g. Judd et al., 1972; Faiman and Winter, 1974; Naftolin and Judd, 1973, but not Zarate, Canales, Soria and Carballo, 1974) who found normal or near normal FSH, the peripheral concentrations of this hormone in our patients and (again) particularly in A.H., were well above the normal male range although a further rise occurred after gonadectomy. The factor(s) which may account for these discrepancies is (are) uncertain. A possible explanation however may be derived from the testicular vein testosterone and 17β-oestradiol concentrations found in our subjects (Figure 6.18).

There is every reason to believe (and evidence to support it) that the endocrine function of the gonads in testicular feminization is normal and that the plasma concentrations and blood production rates of testosterone and 17β-oestradiol in this condition are similar or higher than those found in normal men (Judd et al., 1972; Wilson, Harrod, Goldstein, Hemsell and MacDonald, 1974; Naftolin and Judd, 1973). Although patients with testicular feminization are unresponsive to
testosterone, they are sensitive to the negative feedback effect of oestrogen and it is likely therefore that the pattern of peripheral gonadotrophin levels will depend on the plasma concentration and production rate of this latter steroid. Data on 17β-oestradiol concentrations in testicular vein blood are scarce although it would appear from the studies which have been reported that the results obtained in our patients may definitely be considered subnormal. Saez, Morera, de Peretti and Bertrand (1972) measured peripheral and testicular vein concentrations in one patient and found values of 64 and 6,300 pg/ml respectively. Spermatic vein 17β-oestradiol levels in two subjects studied by Kelch, Jenner, Weinstein, Kaplan and Grumbach (1972) were 4,600 and 2,210 (post-hCG) pg/ml. In five additional subjects, investigated by Morris and Mahesh (1963) and Pion, Dignam, Lamb, Moore, Frankland and Simmer (1965) concentrations ranged from 1,500 to 18,000 pg/ml. These results are in marked contrast to our estimates of 564 and 742 pg/ml for right and left testicular vein in J.H., and of 333 and 338 pg/ml in A.H. Since a 5 to 50-fold difference in testicular blood flow seems extremely unlikely it would appear that the testicular 17β-oestradiol secretion rate in our subjects was markedly less than that usually encountered in patients with testicular feminization. Moreover, extraglandular production of 17β-oestradiol from circulating testosterone was probably also impaired in our patients since testicular vein testosterone concentrations were markedly lower than those previously reported, and peripheral testosterone levels were near or below the limit of the normal male range.

It should be pointed out that the gonadectomy in our patients was performed either one day after (J.H.) or on the last day (A.H.) of the oestrogen provocation test. Since on the day of laparotomy
peripheral oestradiol levels were only 70% of the pre-treatment value in J.H. and 86% in A.H., spermatic vein 17\(\beta\)-oestradiol concentrations under basal conditions would probably have been somewhat higher although still well below the values found in other studies.

Thus, it would appear that gonadal steroid secretion in both siblings was impaired and it may therefore not be surprising that FSH and LH levels were elevated and that the increase was more marked in A.H. who had the lowest peripheral and testicular 17\(\beta\)-oestradiol levels.
The changes in plasma FSH and LH following gonadectomy of this latter patient (Figure 6.19) suggest that although this subnormal oestrogen production was completely ineffective in suppressing LH secretion, it did have some inhibitory effect on FSH. This is in agreement with the observation of Kulin and Reiter (1972) that, in males, the secretion of FSH is more sensitive to the negative feedback action of oestrogen than that of LH. The tendency of plasma LH levels to decrease after gonadectomy is probably a reflection of the fact that in men (Wang, Lasley and Yen, 1975) as in women (Jaffe and Keye, 1974), 17\(\beta\)-oestradiol enhances pituitary sensitivity to LRF.

Although it may be hazardous to draw definite conclusions from such a small number of observations, the changes in gonadotrophin secretion during and following exogenous oestrogen treatment of these three patients (Figure 6.20) raise some intriguing questions. In the girl with XY pure gonadal dysgenesis, ethinyloestradiol administration induced a LH discharge which qua timing and absolute magnitude was similar to that seen in women. It seems unlikely that this LH peak was due to release from negative feedback suppression rather than to a positive feedback effect since the plasma 17\(\alpha\)-ethinyloestradiol
FIGURE 6.20: Relative changes in FSH and LH during oestrogen provocation test in normal men (mean ± S.E.M., n = 7), testicular feminization (mean of 2), XY pure gonadal dysgenesis (n = 1) and normal women (mean ± S.E.M., n = 6). Horizontal black bars indicate ethinyloestradiol treatment (500 µg a day in normal men, others 200 µg a day).
level on the day of the LH surge was 30 pg/ml. Following gonadectomy this patient has been treated with 50 µg ethinyloestradiol per day. Plasma levels of the hormone on this dose ranged from 21 to 35 pg/ml and LH concentrations were near the limit of sensitivity of the assay except for the odd isolated LH peak. The presence of such LH peaks has previously been described in women during the oestrogenic phase of sequential contraceptive treatment (Swerdloff and Odell, 1968).

The ability of oestrogen to stimulate LH release in patients with X0 gonadal dysgenesis has previously been demonstrated (Yen, Tsai, Vandenberg and Rebar, 1972; Reiter, Kulin and Hamwood, 1974) and from this it has been inferred that the maturation of the positive feedback mechanism in girls is independent of gonadal steroid secretion. The present study tends to suggest that this maturational process is not restricted to females but does also occur in males with pure gonadal dysgenesis. Since it is very likely that Miss L.I.'s brain cells have a male karyotype in view of the 46,XY chromosomal constitution of her skin, this would indicate that the ability to respond to oestrogen with a LH surge is an intrinsic property of the hypothalamic-pituitary unit which is not related to the genetic make-up of its cells. The failure to demonstrate positive feedback in the siblings with testicular feminization (and in normal men) on the other hand, suggests that the presence of functionally active gonads may suppress this intrinsic hypothalamic "cyclicity" although it cannot be concluded from the present studies whether this suppression is permanent and due to intra-uterine, neonatal or postnatal steroid "imprinting" or whether it is a reversible phenomenon. Our results however do permit us to conclude that the absence of positive feedback in intact adult men cannot be attributed to a blocking action of testosterone as suggested by Karsch, Dierschke and Knobil (1973) for the rhesus monkey.
It is noteworthy that in the siblings with testicular feminization, like in normal men, the regression line relating changes in plasma testosterone to those of LH did not pass through the origin, which adds further support to the earlier expressed view that ethinylestradiol may affect testicular androgen secretion directly. Changes in plasma 17β-oestradiol on the other hand were related not only to the decrease in testosterone but also to LH, which is in accordance with the observation that in testicular feminization, the testes are the main source of circulating 17β-oestradiol, extraglandular production being relatively less important than in normal men due to the increase in sex hormone binding globulin (Mauvais-Jarvis, Bercovici, Crepy and Gauthier, 1970) and hence lower conversion rate of testosterone to 17β-oestradiol (Naftolin and Judd, 1973).

6.2.5 Summary

The effects of exogenous oestrogen and LRF administration on pituitary gonadotrophin and gonadal steroid secretion were studied in two intact siblings with testicular feminization and in a patient with XY pure gonadal dysgenesis. Spermatic vein testosterone and 17β-oestradiol concentrations were also measured in the patients with testicular feminization.

In the subject with XY pure gonadal dysgenesis basal gonadotrophin and steroid levels as well as the pituitary response to LRF were in the castrate range. No further increase in FSH or LH occurred following gonadectomy and the FSH/LH ratio remained greater than 1.

In the siblings with testicular feminization both FSH and LH levels were elevated but the increase in FSH was smaller and that of LH larger (FSH/LH ratio less than 1) when compared to the patient with
XY pure gonadal dysgenesis. A similar quantitative and qualitative difference in the pattern of gonadotrophin release was also demonstrated following LRH. Spermatic vein steroid concentrations in both patients were lower than those previously reported. The implications of this finding with respect to the feedback regulation of gonadotrophin secretion are discussed.

An oestrogen provocation test was performed in all three patients before gonadectomy but a LH surge was elicited in the patient with XY pure gonadal dysgenesis only. In the subjects with testicular feminization, the profile of peripheral gonadotrophins during and following oestrogen administration was qualitatively similar to that of normal men. It is concluded from these results that the absence of positive feedback in the intact male cannot be attributed to a blocking effect of testosterone on oestrogen-induced LH release.
6.3 Studies on positive feedback in adolescent dysfunctional uterine bleeding

6.3.1 Background

Although anovulatory cycles may occur in normal women at any time during reproductive life, they are most common immediately after the menarche and before the menopause (Doring, 1969). In most adolescents, this period of anovulatory sterility is usually short and a regular pattern of ovulatory cycles soon becomes established. The temporary failure to ovulate in this age-group is probably due to the fact that maturation of the hypothalamic-pituitary mechanisms involved in pre-ovulatory gonadotrophin release is not complete until mid to late puberty (Reiter, Kulin and Hamwood, 1974).

There exists however a small minority of adolescents in whom anovulatory cycles tend to persist for several years after menarche. Frequently these girls present at gynaecological clinics with dysfunctional uterine bleeding (DUB), a condition characterised clinically by "irregular, often heavy menstrual bleeding not due to a recognisable local or systemic disease" (Baird and Fraser, 1973).

Few studies have been carried out on the endocrine features of adolescent patients with persistent menstrual irregularities. The association between DUB and anovulation, suspected from the earlier work on endometrial and ovarian histology (Shaw, 1929), has been confirmed by serial measurements of the urinary excretion of total oestrogen and/or pregnanediol (Wilson, Randall and Osterberg, 1938; Brown and Matthew, 1962). However, although these studies provided data on the variation in the pattern of ovarian activity and indicated that regular coordinated follicular growth was present in most of the patients, they
did not give any direct information on the pathophysiological mechanisms underlying the ovulatory failure. In a long-term serial study of plasma gonadotrophins and urinary total oestrogen and pregnanediol excretion in adolescents with a history of DUB, Fraser, Michie, Wide and Baird (1973) were able to demonstrate an absence of the normal midcycle LH peak in three patients with persistent anovulatory cycles. The present study was designed to test the hypothesis of these authors that the failure to ovulate in this condition may be due to a failure of the positive feedback mechanism by which oestrogen evokes a LH discharge from the pituitary at midcycle. Preliminary results of this study have been published in abstract form (Van Look, Fraser, Hunter, Michie and Baird, 1975).

6.3.2 Design of the study

Clinical details of the patients are given in Table 6.6

A total of nine girls (aDUB 01-09), with ages ranging from $16^{2}/12$ to $27^{9}/12$ years, were studied. They were selected from patients attending the gynaecological endocrine clinic with a history of irregular menstrual bleeding since adolescence (adolescence being defined according to Southam and Richart, 1966 as the first 10 years after menarche). During selection, preference was given to patients who had shown cystic glandular hyperplasia on endometrial curettage. In all nine patients irregular menstruation had been present for at least 2 years (range 2-8 years) prior to the date of entry to the study and in most instances irregular bleeding had started soon after menarche. A typical case history is given below.

The control group consisted of 6 women of proven fertility and with a history of regular menstrual cycles. Selection-criteria and
<table>
<thead>
<tr>
<th>PATIENT'S NUMBER</th>
<th>AGE AT START OF STUDY (years, months)</th>
<th>AGE AT MENARCHE (years)</th>
<th>MENSTRUAL HISTORY</th>
<th>ENDOMETRIAL HISTOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>aDUB 01</td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>Proliferative</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
<tr>
<td></td>
<td>21/12</td>
<td>1</td>
<td>Irregular since menarche</td>
<td>CGH*</td>
</tr>
</tbody>
</table>

*CGH* = cystic glandular hyperplasia

**TABLE 6.6:** Clinical details of adolescents with dysfunctional uterine bleeding (aDUB).
clinical details of these women who served also as controls for the positive feedback studies in normal men, have been given in section 6.1 and Chapter 4.

In both the patients' and control group, ovarian activity was monitored throughout the entire duration of the study by serial measurements of total oestrogen and pregnanediol excretion in 24-hour urine samples collected three times a week. An oestrogen provocation test, the details of which have been described in Chapter 4, was started between the first and second study cycle when urinary total oestrogen excretion was less than 12 ug/24 hours. The latter criterion was also used when deciding on the most appropriate timing of the LRF-test (Chapter 4), which in most instances was performed between the second and third study cycle. All samples were assayed for FSH and LH and, where indicated, for 17β-oestradiol and 17α-ethinylestradiol. Menstrual records were kept by all participants throughout the period of study.

Case history (patient no. 02)

After menarche at the age of 10, menstrual bleeding in this patient had been fairly regular until she was admitted at 13 years of age because of persistent vaginal bleeding of 4 months' duration. Haemoglobin was 6.4 g/ml and a blood transfusion was given. Curettage showed the typical changes of cystic glandular hyperplasia. She was treated with cyclical oestrogen-progesterone medication for approximately 18 months. After stopping hormone therapy, bleeding became heavy and irregular but, at 17 years of age, she became pregnant after 6 months of marriage. The pregnancy however ended at 24 weeks of gestation when she aborted triplets. Following this, her periods became heavy and irregular again and required a second curettage which showed proliferative
changes. After the birth of her child at the age of 20, she had several episodes of irregular, prolonged bleeding and 2 further curetages, one of them during the course of this study. On both occasions the endometrium showed the classical changes of cystic glandular hyperplasia (Plate 6.4).

6.3.3 Results

(a) Urinary measurements

The pattern of urinary total oestrogen and pregnanediol excretion in all six control women was consistent with normal follicular development and ovulation. Mean cycle length during the first and third study cycle was 29.0 ± 0.4 (SE) and 32.0 ± 3.0 days respectively and these were not significantly different. However, the second study cycle (i.e. the cycle following the oestrogen provocation test) was significantly longer (39.5 ± 3.7 days). As discussed in section 6.1, the profile of urinary total oestrogen and pregnanediol excretion during this cycle indicated that the delay in the onset of menstruation was due to a prolongation of the follicular phase (from 16.3 ± 1.5 to 27.0 ± 3.5 days, p<0.05) and the development of a new crop of follicles. There were no significant differences between the three cycles in either the height of the urinary total oestrogen peak at midcycle, the length of the luteal phase or luteal pregnanediol excretion.

Of the 9 adolescents with DUB, 7 girls consistently failed to ovulate as evidenced by the pregnanediol excretion which remained below 1.5 mg/24 hours. Two typical examples are illustrated in Figure 6.21. In the two remaining patients (aDUB 08 and 09) the mid-cycle oestrogen peak was followed by an increase in pregnanediol excretion but the rise was small and short-lived and menstrual bleeding in these girls started
Endometrial histology in an adolescent girl with DUB showing the typical changes of cystic glandular hyperplasia (magnification x 25).
respectively 9 and 10 days later. Both patients were therefore considered to represent cases of defective corpus luteum function and their results were excluded when the data was analysed.

Since in the patients' group anovulatory cycles were not always followed by vaginal bleeding (Figure 6.21) and, conversely, menstrual bleeding could be present despite rising oestrogen levels (Figure 6.21) cycle length as judged from the menstrual records was highly variable and no valid comparison with the control group could therefore be made on this basis. The profiles of urinary total oestrogen excretion however suggested that in the patients' group menstrual cycle length as calculated from one oestrogen nadir to the next, was on average about 5-6 days longer due to a prolongation of the follicular phase (see e.g. Figure 6.21). The amount of oestrogen excreted during the initial stages of follicular development was comparable in patients and controls and reached a level of about 30 μg/24 hours after approximately 2 weeks (Figure 6.22). In control women, this concentration appeared to represent the critical stimulus required for inducing LH release and ovulation as evidenced by the subsequent rise in urinary pregnanediol excretion. In the patients' group however ovulation did not occur and follicular oestrogen secretion continued at an exponentially increasing rate for an additional 5 to 6 days. As a result, both the total amount of oestrogen excretion during the proliferative phase and the height of the "midcycle" oestrogen peak were significantly greater in patients as compared to controls. Within the patients' group, increased oestrogen excretion was not always related to the severity of bleeding or the endometrial histology at previous curettage, although the two highest "midcycle" peaks (125.8 and 80.0 μg/24 hours) were found in aDUB 02 and 03 both of whom had a history of cystic glandular hyperplasia.
FIGURE 6.21: Profile of urinary total oestrogen and pregnanediol excretion in two adolescent girls with anovulatory dysfunctional uterine bleeding. Each open vertical bar represents one 24-hour urine-collection. Black bars indicate days of ethinyloestradiol and LRF administration and the hatched bars the days of menstrual bleeding.
Mean (± S.E.M.) urinary total oestrogen and pregnanediol excretion in normal women (open bars) and adolescents with anovulatory dysfunctional uterine bleeding (black bars). Each bar represents a 24-hour urine-collection. Collections were made three times a week and results in each group were centred round the midcycle oestrogen peak.
Urinary pregnanediol remained low in all seven anovulatory girls throughout the study. On several occasions, a short-lived rise in pregnanediol excretion, coincident with the "midcycle" oestrogen peak, could be observed particularly in those patients who had high urinary oestrogen levels (Figure 6.21), but normal luteal function never developed.

(b) Oestrogen provocation test

Daily samples

Results obtained in the control group have been discussed in section 6.1 (Figure 6.1 and Table 6.1). In summary, administration of ethinyloestradiol had a biphasic effect on LH secretion characterised by a brief, initial period of suppression followed by a dramatic rise in plasma LH to a peak value on day 9 of 35.0 ± 5.5 (mean ± S.E.M.) mU/ml (Table 6.7). This oestrogen-induced LH surge was not accompanied by an increase in FSH. In 5 out of the 6 women, the decrease in peripheral gonadotrophin levels during the initial 2½ - 4½ hours of treatment resulted in follicular atresia.

Mean (± S.E.M.) plasma gonadotrophins, 17β-oestradiol and 17α-ethinyloestradiol levels in the patients with anovulatory cycles are illustrated in Figure 6.23 and individual values for FSH and LH are summarised in Table 6.7.

Pre-treatment FSH (overall mean ± S.E.M. of days 1-4, 5.51 ± 0.43 mU/ml) and LH (6.58 ± 0.80 mU/ml) were significantly (p < 0.05) lower in the patients' group than in the control group (FSH: 8.40 ± 1.02 mU/ml; LH: 10.26 ± 0.80 mU/ml) but 17β-oestradiol levels were on average about 50% higher in the patients (83.2 ± 8.7 pg/ml as compared to 55.0 ± 5.1 pg/ml for normal women; n = 49; p < 0.01).
FIGURE 6.23: Mean (± S.E.M.) FSH, LH, 17β-oestradiol and 17α-ethinylestradiol levels during an oestrogen provocation test in 7 adolescents with anovulatory dysfunctional uterine bleeding. Ethinylestradiol (h x 50 µg a day) was given on days 5-7.)
TABLE 6.7: Daily changes in plasma FSH and LH (in mIU/ml) during oestrogen provocation test in 7 adolescents with anovulatory dysfunctional uterine bleeding and in normal women (mean ± S.E.M. taken from Table 6.1)

<table>
<thead>
<tr>
<th></th>
<th>DAY OF THE OESTROGEN PROVOCATION TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>FSH</td>
<td></td>
</tr>
<tr>
<td>aDUB</td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>4.8</td>
</tr>
<tr>
<td>02</td>
<td>4.5</td>
</tr>
<tr>
<td>03</td>
<td>7.2</td>
</tr>
<tr>
<td>04</td>
<td>5.2</td>
</tr>
<tr>
<td>05</td>
<td>4.2</td>
</tr>
<tr>
<td>06</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>1.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>mean (±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6 5.1 5.6 6.6 6.8 4.6 3.9 4.7 6.3 6.8</td>
</tr>
<tr>
<td>±0.7 ±0.6 ±0.8 ±1.1 ±0.8 ±0.6 ±1.0 ±0.9 ±1.0 ±0.5</td>
</tr>
</tbody>
</table>

| LH |    |    |    |    |    |    |    |    |    |    |
|    |    |    |    |    |    |    |    |    |    |    |
| aDUB |    |    |    |    |    |    |    |    |    |    |
| 01 | 4.8| 4.4| 12.2| 10.6| 11.8| 4.0| 4.0| 17.0| 20.2| 11.2|
| 02 | 3.8| 6.2| 6.4| 5.8| 5.8| 8.4| 6.4| 15.0| 12.2| 12.8|
| 03 | 4.6| 2.8| 2.8| 3.6| 3.6| 2.4| 2.4| 2.0| 4.8| 5.0| 2.0|
| 04 | 10.6| 17.4| 13.8| 12.6| 19.4| 10.4| 10.6| 23.0| 27.6| 25.4|
| 05 | 4.4| 5.6| 3.8| 5.6| 7.2| 5.0| 5.4| 4.8| 7.4| 6.0|
| 06 |    |    |    |    |    |    |    |    | 11.4| 6.2|
| 07 | 1.2| 4.0| 7.4| 4.2| 5.0| 3.4| 4.8| 5.6| 29.4| 28.4|

<table>
<thead>
<tr>
<th>mean (±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9 6.7 7.7 6.9 7.7 5.0 4.9 10.5 16.2 13.1</td>
</tr>
<tr>
<td>±1.3 ±2.2 ±1.8 ±1.3 ±1.2 ±1.2 ±2.6 ±1.2 ±2.5 ±3.8</td>
</tr>
</tbody>
</table>

| 01-06 | 10.4| 10.6| 10.0| 10.0| 9.9| 5.0| 7.1| 19.5| 35.0| 17.8 |
|       | ±1.8| ±1.2| ±1.8| ±1.9| ±1.6| ±0.9| ±0.9| ±3.7| ±5.5| ±2.9 |
The changes in FSH and LH during and following oestrogen administration were qualitatively similar to those seen in normal women (Fig. 6.24). Following an initial decrease, LH levels started to rise 48 to 72 hours after initiation of treatment to reach a peak of $16.2 \pm 3.7$ mIU/ml on day 9. The magnitude of the oestrogen-induced LH surge was significantly ($p < 0.005$) smaller than that seen in controls (Table 6.7) and in only 2 out of the 7 patients (aDUB 04 and 07) fell the value within the normal range. Peak LH levels in the four patients with a history of cystic glandular hyperplasia (aDUB 02, 03, 05 and 06) were significantly lower than those of the remaining three patients in whom the endometrium showed proliferative changes only. The difference in peak magnitude between patients as well as between patients and controls could not be attributed to differences in the plasma $17\alpha$-ethinyloestradiol concentration.

The oestrogen-induced LH release was not accompanied by an increase in FSH, hence changes in the plasma level of this latter hormone were inversely related to plasma $17\alpha$-ethinyloestradiol concentrations ($y = 1.21 - 70.1 \log x$ where $y$ = percentage change in FSH from basal value and $x$ = plasma $17\alpha$-ethinyloestradiol concentration; $n = 35$; $r = -0.6678; p < 0.001$). Neither slope or intercept of this regression line was significantly different from those in controls.

Plasma $17\alpha$-ethinyloestradiol levels during treatment ($253.0 \pm 23.2$ pg/ml, mean $\pm$ S.E.M.) were on average somewhat higher than those in control women ($197.7 \pm 16.6$ pg/ml) but the difference was not significant. The half-life of the hormone calculated from the difference in plasma concentration between day 8 and 9, was $812 \pm 87$ minutes ($13h 32$ min $\pm$ 1h 27 min; mean $\pm$ S.E.M.), a value similar to that of the control group ($9h5 \pm 116$ minutes).
FIGURE 6.21: FSH and LH levels (mean ± S.E.M.) in normal women (broken line) and adolescents with anovulatory DUB (solid line) during an oestrogen provocation test. Asterisks indicate values which were significantly different between the two groups.
Episodic gonadotrophin release

Results are summarised in Tables 6.8 and 6.9.

During oestrogen treatment mean FSH and LH were significantly suppressed in all subjects but one (aDUB 02) in whom LH, but not FSH, remained virtually unchanged. Mean FSH level as well as the percentage change in FSH during treatment were similar to those seen in controls (Table 6.2). Pretreatment LH levels on the other hand were somewhat lower (though not significantly so) and suppressed less readily than in control women. The difference could not be accounted for by differences in the hormonal environment since 17β-oestradiol levels were similar in both groups during both sampling periods and 17α-ethinyloestradiol concentrations were, on average, higher in the patients' group. The less marked suppression of LH during treatment was also reflected by the fact that episodic LH release persisted, albeit with a reduced frequency, in 1 out of the 7 girls (as compared to 1 out of 6 controls). There were no significant differences in either the relative or absolute magnitude of the pulsatile release between the two groups. As in normal women, LH pulses tended to become larger and FSH pulses smaller during ethinyloestradiol administration (Table 6.9).

(c) LRF-test

The pituitary FSH and LH responses to the intravenous injection of 50 μg LRF are shown in Figure 6.25.

In the control group, LRF administration resulted in a rapid increase in the levels of both FSH and LH within 5 minutes of injection. The magnitude of the response was greater for LH (23.9 ± 4.7 mU/ml; mean ± S.E.M.) than for FSH (11.0 ± 2.7 mU/ml), but the increase of the latter lasted longer. The subsequent decrease in peripheral gonadotrophin
<table>
<thead>
<tr>
<th></th>
<th>Day h</th>
<th>Day 6</th>
<th>Percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aDUB 01</td>
<td>5.55 ± 0.48</td>
<td>3.68 ± 0.73</td>
<td>-33.7</td>
</tr>
<tr>
<td>02</td>
<td>5.30 ± 0.51</td>
<td>3.39 ± 0.61</td>
<td>-34.0</td>
</tr>
<tr>
<td>03</td>
<td>6.50 ± 0.65</td>
<td>5.76 ± 0.53</td>
<td>-11.4</td>
</tr>
<tr>
<td>04</td>
<td>5.62 ± 2.15</td>
<td>4.46 ± 0.49</td>
<td>-20.6</td>
</tr>
<tr>
<td>05</td>
<td>3.72 ± 0.37</td>
<td>2.68 ± 0.32</td>
<td>-28.0</td>
</tr>
<tr>
<td>06</td>
<td>13.02 ± 0.68</td>
<td>4.94 ± 0.52</td>
<td>-62.1</td>
</tr>
<tr>
<td>07</td>
<td>3.69 ± 0.54</td>
<td>2.11 ± 0.23</td>
<td>-42.8</td>
</tr>
<tr>
<td>mean ± S.E.M.</td>
<td>6.20 ± 1.20</td>
<td>3.86 ± 0.48</td>
<td>- (33.2 ± 6.2)</td>
</tr>
<tr>
<td>LH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aDUB 01</td>
<td>6.32 ± 1.64</td>
<td>4.10 ± 1.22</td>
<td>-35.1</td>
</tr>
<tr>
<td>02</td>
<td>5.10 ± 0.44</td>
<td>5.48 ± 1.48</td>
<td>+ 7.5</td>
</tr>
<tr>
<td>03</td>
<td>2.24 ± 0.54</td>
<td>1.88 ± 0.44</td>
<td>-16.1</td>
</tr>
<tr>
<td>04</td>
<td>11.30 ± 1.44</td>
<td>8.64 ± 1.04</td>
<td>-23.5</td>
</tr>
<tr>
<td>05</td>
<td>4.80 ± 0.58</td>
<td>4.10 ± 0.56</td>
<td>-14.6</td>
</tr>
<tr>
<td>06</td>
<td>3.72 ± 0.86</td>
<td>1.76 ± 1.16</td>
<td>-52.7</td>
</tr>
<tr>
<td>07</td>
<td>3.64 ± 1.78</td>
<td>2.60 ± 0.46</td>
<td>-28.6</td>
</tr>
<tr>
<td>mean ± S.E.M.</td>
<td>5.30 ± 1.12</td>
<td>4.08 ± 0.92</td>
<td>- (23.3 ± 7.1)</td>
</tr>
</tbody>
</table>
TABLE 6.2: Characteristics of pulsatile gonadotrophin release in seven adolescents with anovulatory DUB sampled at 15 minute intervals for 3 hours before (day 4) and during (day 6) ethinyloestradiol administration.

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSH</td>
<td>LH</td>
</tr>
<tr>
<td>Number of peaks (per 3 hours)</td>
<td>0.43</td>
<td>1.14</td>
</tr>
<tr>
<td>Absolute peak magnitude (mU/ml) (mean ± S.E.M.)</td>
<td>2.72 ± 1.22</td>
<td>1.82 ± 0.44</td>
</tr>
<tr>
<td>Relative peak magnitude (%) (mean ± S.E.M.)</td>
<td>56.9 ± 25.8</td>
<td>43.9 ± 4.3</td>
</tr>
</tbody>
</table>
FIGURE 6.25: Pituitary FSH and LH responses to the intravenous injection of 50 µg of LRF at time 0. Illustrated are the means of normal women (broken line, n = 6) and adolescents with anovulatory dysfunctional uterine bleeding (solid line, n = 7)
levels was slower than might have been expected from the half-lives of FSH and LH. The disappearance curves of both FSH and LH appeared to consist of two components. Following an initial period of relatively rapid clearance, the rate of decrease abruptly diminished 90 minutes after LRF injection. From then onwards FSH and LH declined much more slowly and plasma levels of both hormones were still significantly elevated above basal values six hours after LRF injection.

The FSH response in the patients' group was on average smaller than that of the control group but the difference was not significant. LH responses were similar to those of controls.

In both groups, the rise in gonadotrophins was followed by an increase in peripheral 17β-oestradiol which became first detectable 4 hours after LRF injection and reached maximal values 2 hours later (Fig. 6.26). The magnitude of this increase in peripheral 17β-oestradiol was similar in the two groups and varied between 111 and 387% of basal level. There was no correlation between the increase in plasma 17β-oestradiol and that of plasma LH or between the increase in 17β-oestradiol and the pre-injection 17β-oestradiol level.

6.3.4 Discussion

The occurrence of periodical bleeding without previous ovulation and without formation of a corpus luteum constitutes an anovulatory cycle. In these instances menstrual bleeding originates from a mucosa which lacks the characteristic secretory changes (Novak, 1930). Such oestrogen-withdrawal bleedings are frequently less controlled and tend to be heavier than those which follow a normal ovulatory cycle but the exact mechanisms involved are unknown. Anovulatory cycles are not
FIGURE 6.26: Increase in plasma 17β-oestradiol (mean ± S.E.M.), expressed as a percentage of the mean basal level (= 100%) following LRF injection into normal women (open bars) and adolescents with anovulatory dysfunctional uterine bleeding (black bars). Asterisks indicate values significantly different from basal value.
always followed by withdrawal bleeding however (see e.g. Figure 6.21) and the menstrual flow, apart from its tendency to increase, is therefore often of irregular nature. Clinically, this type of bleeding-pattern is usually referred to as "dysfunctional uterine bleeding", provided no underlying local pelvic or systemic disease can be identified.

In women, anovulatory cycles may occur at any time during reproductive life but they are most common following menarche and before the menopause. Between the ages of 12 to 17 years nearly 50% of cycles are anovulatory as compared to less than 10% in the age group from 18-40 years (Doring, 1969). In a minority of adolescent girls anovulatory cycles tend to persist for several years after menarche. The profile of urinary total oestrogen excretion in these girls is consistent with regular follicular development (Brown, Kellar and Matthew, 1959) but there is an absence of the normal midcycle LH peak (Fraser, Michie, Wide and Baird, 1973). This latter finding, together with the observation that in the rhesus monkey (Dierschke, Weiss and Knobil, 1974) as well as in the human (Reiter, Kulin and Hamwood, 1974) exogenous oestrogen is not capable of inducing LH release until several months after menarche, suggested that anovulatory DUB in adolescents might be due to an "immaturity" of the positive feedback mechanism. To test this hypothesis the present study was undertaken.

Before considering the results, it should be emphasized that the subjects selected for this project, do not represent an unbiased sample of the group of adolescents with menstrual irregularities. Indeed, although irregular menstruation is fairly common amongst post-menarchial girls, specialist treatment is seldom required and any study based on patients attending a gynaecological clinic is therefore unavoidably
biased from the start. The predominance of "bad" cases was further increased purposely in the present study by selecting girls with a prolonged history of DUB and/or with recent evidence of endometrial cystic glandular hyperplasia. By doing so, it was hoped to gather a group of patients who in terms of endocrine features could be expected to be as uniform as possible. The extent of bias introduced by using these selection criteria can be assessed from the clinical histories of the participating subjects summarised in Table 6.6. Of the adolescent girls seeking gynaecological advice for DUB, approximately 50% establish a pattern of regular menstrual cycles within 1 year after the onset of their symptoms (Southam and Richart, 1966). In this study, irregular bleeding had on average been present for nearly five years (range 2-9 years). Four of the seven adolescents (i.e. 57%) with persistent anovulation had a recent curettage showing cystic glandular hyperplasia of the endometrium. In the adolescent population of the South-Eastern region of Scotland from which the present patients' material was recruited, cystic glandular hyperplasia of the endometrium is rare and accounts for only 4.0% of all cases of abnormal uterine bleeding requiring curettage in adolescents (Fraser and Baird, 1972).

Thus there can be little doubt that from the spectre of DUB patterns in adolescence, only the more severe forms are represented in this study. The results seem nevertheless worth reporting since they relate to a group of women which albeit small in quantitative terms, is important from an epidemiological point of view because of the increased risk of gynaecological morbidity for its members (Southam and Richart, 1966; Fraser and Baird, 1972). Apart from persistent menstrual disturbance and the need for recurrent curettages, infertility, ovarian cysts, endometrial carcinoma and variants of the polycystic ovary syndrome are
all more frequently encountered in these patients than in the population at large. All our seven patients with persistent anovulation had had at least one curettage, three of them had required blood transfusions, one (aDUB 04) underwent a diagnostic laparoscopy for primary infertility and another (aDUB 05) a laparotomy and unilateral salpingo-oopherectomy for removal of a fimbrial cyst. Since completion of the study (in June 1975) 3 additional curettages have been carried out.

The profiles of total oestrogen excretion illustrated in Figure 6.22, confirm the earlier work of e.g. Brown, Kellar and Matthew (1959) who showed that the amount of oestrogen excreted in the urine is often increased in anovulatory DUB particularly if associated with cystic glandular hyperplasia. Since it is unlikely that there are differences in oestrogen metabolism between women with DUB and normal women, it seems reasonable to assume that this enhanced oestrogen excretion is a direct reflection of an increase in ovarian 17β-oestradiol secretion. The data shown in Figure 6.22 suggest that this increase becomes manifest only during the final stages of the (prolonged) follicular phase, urinary oestrogen excretion during the first two weeks of follicular development being similar to that of normal women. This is in accordance with results obtained by Fraser and Baird (1974) who, using isotope dilution techniques combined with direct sampling of ovarian vein blood, could demonstrate that blood production rates of 17β-oestradiol in 10 perimenopausal women with DUB were within the same range as those found in normal women, except for one patient studied on day 16 of an anovulatory cycle and who had the highest 17β-oestradiol production rate (497 μg/24 hours) ever recorded by these authors. The same workers also reported that some of their patients in the early to mid follicular phase of the cycle appeared to have oestrogen production rates which
were inappropriately high. Our findings do not support this. The discrepancy is probably due to the fact that Fraser and Baird (1974) estimated the day of the cycle in their patients from the menstrual history which, as illustrated in Figure 6.21, is not always a very reliable index of ovarian activity in this condition.

From our data it is not possible to decide whether the elevated oestrogen secretion observed during the latter part of the extended follicular phase originates from one single pre-ovulatory follicle or from a crop of follicles which failed to become atretic prior to the expected time of ovulation. Work by several other investigators however favours the latter possibility. Dysfunctional uterine bleeding in the perimenopausal age group is frequently associated with multiple bilateral follicular cysts (Shaw, 1929; Schroder, 1954). Direct measurement of ovarian vein 17β-oestradiol concentrations has indicated that these cysts are functionally active (Fraser and Baird, 1974). This is in marked contrast to normal women where only one pre-ovulatory follicle usually develops and in whom more than 95% of circulating 17β-oestradiol is secreted by one ovary (Baird and Fraser, 1974). Follicular cysts were present in our patients aDUB 04 and aDUB 05 at laparoscopy, respectively laparotomy and direct evidence for multiple follicular development was also obtained when subject aDUB 02 aborted triplets, four years prior to this study. The development of more than one follicle may however not be a constant feature of this condition as illustrated by the fact that the same patient was subsequently delivered of a single child.

The factor(s) responsible for the simultaneous growth of more than one pre-ovulatory follicle in these patients is (are) uncertain. It seems unlikely that this phenomenon is a result of an increased
ovarian sensitivity for the stimulatory effect of gonadotrophins since the rise in plasma $17\beta$-oestradiol levels following LRF-induced gonadotrophin release (Figure 6.26) was not significantly greater than that seen in normal women. Moreover, the dynamics of the H.P.O. axis are such that any increase in ovarian sensitivity would automatically have been compensated for by a decrease in pituitary gonadotrophin secretion. Hence the number of growing follicles and the oestrogen production rate in women with "sensitive" ovaries would have been similar to those of women with a "normal" ovarian sensitivity and not higher as in our patients. It seems more likely therefore that the abnormality of follicular development in DUB is due to a relative decrease in hypothalamic-pituitary sensitivity to the negative feedback effect of oestrogen. A similar mechanism has been put forward to explain differences in ovulation rate between different breeds of sheep (Land, Wheeler and Carr, 1976). Our observation that LH levels were less readily suppressed during ethinyloestradiol administration to patients (Table 6.8) as compared to normal women (Table 6.2) appears to support this view. The difference between the two groups was not significant however which may have been due to the fact that the dose of ethinyloestradiol employed in this study was too large to allow detection of minor differences in negative feedback sensitivity. Further work using smaller doses of exogenous oestrogens will obviously be required.

Despite the presence of apparently adequate, if not exaggerated, follicular development, seven out of nine patients consistently failed to ovulate as indicated by the absence of a significant rise in urinary pregnanediol excretion. When challenged with an oestrogen provocation test, four of these girls failed to release LH, while in the remaining three the response was either below or just above the lower limit of the
range seen in normal women (Table 6.7). The present study thus confirms the suggestion made by Fraser et al (1973) that the failure to ovulate in this condition is associated with an inability to discharge an adequate amount of LH in response to oestrogen. In addition, our results indicate that this failure is probably of hypothalamic origin since the pituitary LH-response to LRF in patients with DUB is comparable to that seen in women with regular ovulatory cycles (Figure 6.25). It should be emphasized however that this latter conclusion requires additional proof. Indeed, the amount of LH released following the acute injection of 50 µg LRF is small compared to that secreted at midcycle and it can therefore not be excluded that the abortive oestrogen-induced LH peak of the patients' group is due to a relative failure of the pituitary to increase the synthesis and/or secretion of LH during prolonged, intense hypothalamic stimulation. Studies involving continuous infusion of LRF may solve this question.

Although the results of this study leave no doubt that oestrogen-induced LH release in adolescents with anovulatory DUB is deficient when compared to normal women, they also clearly indicate that this positive feedback failure is not absolute. From clinical experience it is well known that these patients may ovulate occasionally either spontaneously or following Clomiphene administration. The two pregnancies in patient aDUB are direct proof of this. It seems likely therefore that the hypothalamic disorder in this condition is of intermittent nature or, alternatively, that the basic defect is a decrease rather than a complete loss of hypothalamic positive feedback sensitivity. The results seem to favour the latter possibility. Indeed, in terms of magnitude and duration, the abortive LH peaks observed in the patients are very similar to those described by Karsch, Weick, Butler, Dierschke,
Krey, Weiss, Hotchkiss, Yamaji and Knobil (1973) in female rhesus monkeys given a subthreshold dose of oestrogen. This would suggest that these patients in order to release an adequate amount of LH may require a more potent oestrogen stimulus than normal women do. It is of interest to note that the LH peaks in all four patients with a history of cystic glandular hyperplasia were significantly smaller than those in the remaining three who did not have such a history. This might indicate that hypothalamic positive feedback sensitivity in these four girls was more markedly impaired and hence that spontaneous ovulations are less likely to occur. Two of these girls had the highest urinary total oestrogen peaks encountered, suggesting that negative feedback sensitivity also may have been more profoundly affected. Evidently, these two factors i.e. the presence of markedly elevated oestrogen levels for prolonged periods of time and the absence of ovulation and hence of progesterone secretion, constitute the ideal endocrine environment for the development of cystic glandular hyperplasia of the endometrium (Schroder, 1954).

6.3.5 Summary

Of nine adolescent girls with a history of dysfunctional uterine bleeding of at least 2 years duration, seven failed to ovulate during at least 3 consecutive cycles. The profiles of urinary total oestrogen excretion were consistent with the presence of regular follicular development but the follicular phase was prolonged and the amount of oestrogen excretion increased as compared to normal women. Oestrogen administration led to an increase in peripheral LH levels but the induced LH surge was significantly smaller than that of normal women. Pituitary
gonadotrophin release following acute injection of IRF was normal. It is concluded that the failure to ovulate in this condition is due to a failure to release adequate amounts of LH in response to a physiological oestrogen stimulus and that this defect is likely to be hypothalamic in origin. The suggestion is made that the basic abnormality in adolescent anovulatory DUB is a decrease in hypothalamic sensitivity for the negative and positive feedback effects of oestrogen.
6.4 Studies on positive feedback in premenopausal dysfunctional uterine bleeding

6.4.1 Background

It is well known that menstrual irregularities become increasingly common towards the menopause. In women after the age of 40, there is a large variation in menstrual cycle length (Treloar, Boynton, Behn and Brown, 1967) which is probably a reflection of the dramatic rise in the incidence of anovulation and defective corpus luteum function in this age-group (Doring, 1969). At no other time during reproductive life is cystic glandular hyperplasia of the endometrium so often encountered at curettage than between the ages of 40 - 50 years (Schroder, 1951; Fraser and Baird, 1972). Yet, despite the fact that dysfunctional uterine bleeding is a common problem in perimenopausal patients, very few studies have been performed with a view to elucidating the underlying endocrine defects. Urinary measurements have indicated that basal gonadotrophin levels may be raised several years before the menopause (Adamopoulos, Loraine and Dove, 1971) and that urinary oestrogen levels may be markedly elevated particularly in patients with a history of cystic glandular hyperplasia (Brown, Kellar and Matthew, 1959).

In the present study it was intended to study H-P-O relationships in a group of premenopausal women with DUB in order to gain some insight into the pathophysiological mechanisms underlying this condition.

6.4.2 Design of the study

Clinical details are given in Table 6.10.

A total of 10 women (pDUB 01-10) with ages ranging from 37 to 52 years participated in this study. They were recruited from the
<table>
<thead>
<tr>
<th>PATIENT'S NUMBER</th>
<th>AGE AT START OF STUDY (years, months)</th>
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<th>DURATION OF DUB</th>
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<td>13</td>
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<td>6 mo</td>
</tr>
<tr>
<td>pDUB 02</td>
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<td>13</td>
<td>3+3</td>
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</tr>
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</tr>
<tr>
<td>pDUB 05</td>
<td>51 3/12</td>
<td>16</td>
<td>3+0</td>
<td>10 y</td>
</tr>
<tr>
<td>pDUB 06</td>
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<td>13</td>
<td>2+0</td>
<td>1 y</td>
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</tr>
<tr>
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</tr>
<tr>
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**ENDOMETRIAL HISTOLOGY**

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<th>pDUB 03</th>
<th>pDUB 04</th>
<th>pDUB 05</th>
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</table>

*CGH = cystic glandular hyperplasia*
gynaecological clinic which they attended because of irregular menstruation except for one subject (pDUB 10) whose cycles had been regular prior to insertion of an intra-uterine device. All women were of proven fertility and had experienced regular menstrual cycles until 5 months – 10 years before the start of the study. They were otherwise healthy. All nine women with idiopathic DUB underwent curettage either before or during the study and four were shown to have cystic glandular hyperplasia of the endometrium. A few illustrative case histories are given below.

The experimental design of the study was similar to that used in the adolescents with DUB except that from 6 of the 10 women (not in pDUB 03-06) blood and urine samples were collected daily for periods ranging from 5-12 weeks. In addition, clomiphene (100 mg a day for 5 days) was administered to three of the patients (pDUB 01, 02 and 10) but no LRF-test was done in pDUB 01. Menstrual records were kept by all participants throughout the study period.

Results were compared to those obtained in the group of 6 regularly menstruating women who also served as controls for the previous study (section 6.3).

Case histories

Case pDUB 02

This lady, aged 39 years, was admitted as an emergency to the gynaecological ward because of continuous, heavy vaginal bleeding of seven days' duration. For the last five years menstruation had become progressively heavier but remained regular until 1 month previously when she suddenly missed her period. A pregnancy test was negative. The next period started off as usual but the menstrual flow became gradually
more profuse and severe. Haemoglobin on admission was 8g% and the endometrium showed the typical changes of cystic glandular hyperplasia. She was discharged from hospital on iron therapy but re-admitted 7 weeks later because of recurrence of her symptoms. Bleeding was controlled with oral gestagen treatment which was discontinued 6 days later. Following the gestagen withdrawal bleeding, daily blood and urine samples were collected for 5 weeks and, after a 14 weeks' interval, for another 6 weeks. Three months after completion of the study the patient underwent hysterectomy.

Case pDUB 07

Irregular bleeding in this woman of 48 years had started at the age of 40 but had been fairly well controlled with the combined contraceptive pill. She stopped the pill at the age of 45 and had only 4 episodes of vaginal blood loss over the next two years until she was admitted for continuous vaginal spotting which had been present for 10 weeks. At curettage the endometrium showed signs of early cystic hyperplasia. Her periods remained irregular over the next 12 months and required a second curettage which gave a similar histological picture. Following this and before the start of the study 5 months later, the patient had one scanty episode of bleeding. Blood and urine samples were collected daily for a period of 8 weeks during which time she menstruated twice. Towards the end of the sampling period the patient experienced the onset of hot flushes and in the 6 months since the end of the study has not had any further bleeding.

Case pDUB 09

Menorrhagia in this patient began at the age of 49 years. Menses had always been very regular but she then missed a period and had
subsequently 2 episodes of heavy vaginal bleeding, each lasting for about a week, within the space of a month. No menstruation occurred in the following two months but bleeding restarted thereafter. The endometrium was proliferative. Urine samples from this patient were collected three times a week and blood samples daily for a period of 5 weeks. Since completion of the study this woman's menses have remained irregular although bleeding has become scantier.

6.4.3 Results

In contrast to the adolescents, the profiles of urinary total oestrogen and pregnanediol excretion in the group of perimenopausal women with DUB were far less uniform and at least 5 different patterns could be distinguished. Each pattern is discussed separately below.

(a) Anovulatory cycles with regular follicular development

Two patients (pDUB 01 and 02) both of whom were studied on a daily basis, belonged to this group. They had several characteristics in common (see also case history pDUB 02). Both women were in their mid thirties when menorrhagia started. Bleeding was extremely severe, much more so than in any of the other patients, and the endometrium had shown the classical changes of cystic glandular hyperplasia on at least two occasions. Repeated curettages were required, often within the space of a few months. Their periods were fairly well controlled on cyclical gestagen therapy but became irregular and heavy again as soon as treatment was stopped. Both women eventually underwent hysterectomy.

Hormone measurements are illustrated in Figures 6.27 and 6.28.

Following the onset of menstrual bleeding (at the beginning of week 2 in pDUB 01 and at the start of the study in pDUB 02), urinary
FIGURE 6.27: Daily changes of urinary total oestrogen, pregnanediol and gonadotrophin excretion, and of plasma FSH, LH, 17β- oestradiol and androstenedione in a perimenopausal woman with DUB. Black bars indicate menstrual bleeding.
FIGURE 6.28: Daily changes of urinary total oestrogen and pregnanediol excretion, and of plasma FSH, LH, 17β-oestradiol and progesterone in a perimenopausal woman with DUB. Hatched bars indicate menstrual bleeding.
total oestrogen and plasma $17\beta$-oestradiol gradually started to rise in both patients (somewhat slower in pDUB 01) over the first 14-18 days. At the end of this period, $17\beta$-oestradiol levels were similar to those seen at the time of the pre-ovulatory $17\beta$-oestradiol peak during the normal menstrual cycle (approx 300 pg/ml). LH levels also rose at this stage but the magnitude of the induced LH surge was less than half that observed during ovulatory cycles (see Figure 1.2). The slight increase in urinary pregnanediol and plasma progesterone which occurred concomitantly with the rise in LH, suggests that some luteinization of the growing follicle(s) may have taken place but ovulation and normal corpus luteum formation failed to occur. The abortive LH peak did not appear to have any significant effect on follicular oestrogen secretion since plasma $17\beta$-oestradiol levels continued to rise exponentially to reach a peak of about 500 pg/ml at the end of the third week. Urinary total oestrogen excretion at this stage was comparable to that seen in adolescents with DUB at "midcycle" (Figure 6.22). Following this, plasma $17\beta$-oestradiol concentrations declined rapidly and fell below 100 pg/ml on the fifth day after the peak. In patient pDUB 02, menstrual bleeding started on the same day. Thereafter and until the start of ethinyloestradiol treatment, plasma $17\beta$-oestradiol remained fairly stable although at an elevated level as compared to early follicular phase values during the normal menstrual cycle. But for the absence of a normal midcycle LH peak, peripheral gonadotrophin levels were always within the range seen in normal women during the follicular phase of the cycle.

In contrast to normal women, ethinyloestradiol did not have any consistent suppressive effect on gonadotrophin secretion. Thus, FSH was lower but LH higher in pDUB 01 24 hours after the start of treatment
while the reverse was true for pDUB 02. In both patients however, LH levels subsequently showed a significant increase throughout the remainder of the treatment period. The magnitude of this rise was similar to that seen in normal women but, unlike in normal women, LH levels did not increase any further after stopping treatment. The height of the LH surge induced by the exogenous oestrogen was similar (pDUB 01) to or somewhat lower (pDUB 02) than that of the spontaneous abortive midcycle LH-peak.

After completion of the oestrogen provocation test no further samples were collected from patient pDUB 02 during the first 3 weeks of the subsequent cycle but collections were restarted at the end of that cycle. No vaginal bleeding had occurred during this period and measurements of urinary pregnanediol and plasma progesterone indicated that the cycle had been anovulatory. LRF was then given to this patient in order to assess the pituitary gonadotrophin response to this hormone. Peak FSH (10.8 mU/ml) and LH (27.4 mU/ml) levels were within the range found in controls (Figure 6.25). There was however a significant (p<0.05) difference in timing of the response. Peripheral FSH was highest at 120 min and LH at 150 min following LRF injection (controls: mean ± SD, FSH:24 ± 6 min; LH:28 ± 9 min). The delayed response could be attributed to the higher circulating 17β-oestradiol level which was 188 pg/ml in pDUB 02 at the time of LRF testing as compared to 62.4 ± 19.7 (SD) pg/ml in controls (p<0.05).

The changes in steroid and gonadotrophin concentrations following Clomiphene administration (100 mg/day for 5 days) to patient pDUB 01 indicated that ovulation had been induced with this compound. During the treatment cycle peripheral 17β-oestradiol and urinary total oestrogen excretion rose to peak values which were more than double those seen
during the spontaneous cycle. The plasma $17\beta$-oestradiol peak was followed, 24 hours later, by a LH surge of normal magnitude and duration and the subsequent luteal phase was of normal length. However, despite the presence of adequate luteal $17\beta$-oestradiol and progesterone secretion, LH levels remained markedly elevated and showed large fluctuations throughout the remainder of the study period.

In subject pDUB 02 on the other hand, ovulation did not occur following Clomiphene. Plasma $17\beta$-oestradiol levels during the treatment cycle were not much higher than those observed during the spontaneous cycle of this patient, and the abortive oestrogen-induced LH peak was also of similar magnitude. The $17\beta$-oestradiol peak was recorded on day 21 of the cycle but bleeding did not start until 14 days later. Peripheral $17\beta$-oestradiol levels during this period remained elevated between 100-150 pg/ml but, unlike LH, did not show any significant oscillation.

(b) Irregular follicular development with anovulation

Probably the most important common characteristic of the 5 women (pDUB 03-07) who fell into this group was the fact that all of them became menopausal either during (pDUB 03, 06 and 07) or very soon after completion of the study. All 5 patients were of comparable age (late forties to early fifties) and each of them had been subjected to 1-3 curettages but on none of these occasions did the endometrium show the typical changes of cystic glandular hyperplasia. Menorrhagia had been present for various lengths of time (5 months - 10 years) but was never as severe as in the previous two patients.

Follicular development in these women was extremely erratic (Figure 6.29). Ovarian activity, as reflected by the urinary total
FIGURE 6.29: Patterns of urinary total oestrogen and pregnanediol excretion in two perimenopausal women with dysfunctional uterine bleeding. Each vertical bar represents one 24-hour urine specimen. Collections were made three times a week.
oestrogen excretion, appeared to be minimal for periods of up to 4 weeks until eventually follicular growth was stimulated and oestrogen levels started to rise. Ovulation however never occurred.

**Oestrogen provocation test**

**Daily hormonal changes**

Means of plasma gonadotrophins and 17α-ethinylestradiol are shown in Figure 6.30 and FSH and LH concentrations in individual patients are summarised in Table 6.11.

Basal gonadotrophin levels were markedly elevated in these 5 patients despite the fact that circulating 17β-oestradiol (mean ± S.E.M. on days 1-4: 89.9 ± 9.8 pg/ml) was significantly higher than in controls (55.0 ± 5.1 pg/ml; p < 0.005). Mean (± S.E.M.) pre-treatment FSH was 30.8 ± 4.5 mU/ml (controls 8.4 ± 5.0 mU/ml, p < 0.001) and LH 35.8 ± 1.7 mU/ml (controls 10.2 ± 0.8 mU/ml; p < 0.001).

Changes in FSH during and following ethinylestradiol administration were qualitatively similar to those seen in control women. FSH levels were maximally suppressed 48 hours after the start of treatment to 55.2 ± 8.7% of their initial value (control women 61.8 ± 16.5%; p > 0.05). They subsequently increased slightly, concomitant with the increase in LH, but were still well below pretreatment levels at the end of the test (66.0 ± 15.0% of initial value).

LH levels on the other hand decreased initially during the first 24-48 hours of treatment but then started to rise from a value of 25.8 ± 5.2 mU/ml (mean ± S.E.M.) on day 7 to 59.0 ± 16.4 mU/ml on day 8. This increase in peripheral LH occurred in the absence of any significant decrease in circulating 17α-ethinylestradiol and was of similar magnitude as that found in control women when expressed as a percentage.
FIGURE 6.30: Plasma gonadotrophins and 17α-ethinylestradiol levels (mean - S.E.M.) in normal women (broken line) and perimenopausal women with DUB (solid line) during oestrogen provocation test. Asterisks indicate a statistically significant difference between the values of the two groups.
TABLE 6.11: Daily changes in plasma FSH and LH (in mU/ml) during oestrogen provocation test in 5 perimenopausal women with dysfunctional uterine bleeding and in normal women (mean ± S.E.M. taken from Table 6.1)

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of basal values. However, three of the 5 women did not show any significant further increase in LH over the next 24 hours (Table 6.11). Peripheral gonadotrophin levels in these women remained either stable or declined again. Unequivocal LH surges were found only in the two remaining patients and, on both occasions, were accompanied by a marked coincident rise in FSH.

Plasma 17α-ethinyloestradiol concentrations in the patients were not significantly different from those found in controls, nor was there any difference between the two groups in the half-life of the hormone (pDUB 1112 ± 193 min; controls 945 ± 116 min).

**Episodic gonadotrophin release**

Results are summarised in Tables 6.12 and 6.13.

Twenty-four hours after the start of treatment, mean FSH and LH levels were significantly suppressed in all but one subject. The degree of suppression expressed as a percentage of pre-treatment values, was on average virtually identical for both gonadotrophins and comparable to that found in controls (Table 6.2).

Analysis of the gonadotrophin changes during the frequent sampling period before oestrogen administration indicated that the elevated FSH concentrations in the patients' group were associated with an increase in amplitude, but not infrequency of episodic FSH release (Tables 6.13 and 6.3). FSH peaks were more often encountered than LH peaks which was in marked contrast to the findings in men, women or adolescents with DUB. The fact that LH pulses were relatively rare in the patients' group suggests that the raised circulating gonadotrophin level was probably maintained by an increase in the tonic secretion of this hormone.
### TABLE 6.12: Transverse means (± SD) in mIU/ml and percentage change of peripheral gonadotrophin levels in 5 perimenopausal women with DUB sampled at 15 minute intervals for 3 hours before (Day 4) and during (Day 6) ethinylestradiol administration

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 6</th>
<th>Percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FSH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pDUB 03</td>
<td>12.89 ± 2.94</td>
<td>7.98 ± 1.98</td>
<td>-38.1</td>
</tr>
<tr>
<td>04</td>
<td>63.15 ± 4.34</td>
<td>47.87 ± 4.93</td>
<td>-24.2</td>
</tr>
<tr>
<td>05</td>
<td>29.78 ± 6.44</td>
<td>13.28 ± 1.37</td>
<td>-55.4</td>
</tr>
<tr>
<td>06</td>
<td>20.82 ± 4.42</td>
<td>10.85 ± 2.63</td>
<td>-47.9</td>
</tr>
<tr>
<td>07</td>
<td>8.67 ± 1.25</td>
<td>13.94 ± 2.40</td>
<td>+60.8*</td>
</tr>
<tr>
<td>mean ± S.E.M.</td>
<td>27.06 ± 9.72</td>
<td>18.78 ± 7.35</td>
<td>-(41.4 ± 6.7)</td>
</tr>
</tbody>
</table>

| **LH** |             |             |                   |
| pDUB 03 | 20.22 ± 6.06 | 8.68 ± 1.54 | -58.0             |
| 04     | 38.28 ± 2.72 | 23.72 ± 3.40 | -39.0             |
| 05     | 40.94 ± 2.88 | 21.70 ± 8.32 | -47.0             |
| 06     | 25.54 ± 2.96 | 19.60 ± 2.14 | -23.3             |
| 07     | 27.46 ± 1.94 | 35.44 ± 5.98 | +29.1*            |
| mean ± S.E.M. | 30.49 ± 3.93 | 21.83 ± 4.28 | -(41.8 ± 7.3)     |

*not included in mean
**TABLE 6.13:** Characteristics of pulsatile gonadotrophin release in 5 perimenopausal women with DUB sampled at 15 minute intervals for 3 hours before (day 4) and during (day 6) ethinyloestradiol administration

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th></th>
<th>Day 6</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSH</td>
<td>LH</td>
<td>FSH</td>
<td>LH</td>
</tr>
<tr>
<td>Number of peaks (per 3 hours)</td>
<td>0.60</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Absolute peak magnitude (mU/ml) (mean ± S.E.M.)</td>
<td>6.00 ± 1.73</td>
<td>16.20</td>
<td>3.05</td>
<td>9.70</td>
</tr>
<tr>
<td>Relative peak magnitude (%) (mean ± S.E.M.)</td>
<td>39.9 ± 4.4</td>
<td>118.2</td>
<td>38.9</td>
<td>60.2</td>
</tr>
</tbody>
</table>
After the oestrogen provocation test, two women (pdUB 04 and 05; Figure 6.29) had an anovulatory cycle followed by withdrawal bleeding, respectively 9 and 6 weeks after the test, while in the remaining three patients urinary total oestrogen excretion decreased to values within the menopausal range. The hormonal changes during the menopausal transition in one of these women are shown in Figure 6.31.

The cycle prior to the oestrogen provocation test in this patient was anovulatory as indicated by the absence of a significant rise in either urinary pregnanediol or plasma progesterone levels. Gonadotrophins were markedly elevated and did not show any suppression despite the presence of mid-follicular phase 17β-oestradiol levels. The absence of a mid-cycle LH discharge, and hence of ovulation, during this cycle was probably due to an insufficient strength of the endogenous 17β-oestradiol stimulus rather than to a positive feedback defect, since exogenous oestrogen was capable of inducing LH release. Shortly after the oestrogen provocation test, endogenous 17β-oestradiol levels became undetectable. The decrease in circulating 17β-oestradiol coincided with a rise in FSH and LH although castrate levels were not reached until 4 days after 17β-oestradiol levels became undetectable. It is of interest to note that the patient did not experience any hot flushes before circulating gonadotrophin levels had reached their plateau.

**LRF-test**

Mean plasma FSH and LH on days 1-4 of the LRF-test are compared to the corresponding basal levels on days 1-4 of the oestrogen provocation test in Figure 6.32.
**FIGURE 6.31:** Daily changes of urinary total oestrogen and pregnanediol excretion and of plasma FSH, LH, 17β-oestradiol and progesterone during the menopausal transition in a woman with a history of DUB. Hatched bars indicate menstrual bleeding.
FIGURE 6.32: Mean FSH and LH levels on days 1-4 of the oestrogen provocation and LRF-test respectively in 5 perimenopausal women with DUB
Both FSH and LH had significantly risen in all three patients whose urinary total oestrogen excretion during the interval between the two tests had been consistently below 5 µg/24 hours and in whom plasma 17β-oestradiol was in the menopausal range. However, FSH levels in pDUB 03 were much lower than those in the other two women. Three weeks after the LRF-test, urinary oestrogen levels in this patient rose temporarily to a peak of 10·9 µg/24 hours and although she did not experience any bleeding and continued to have hot flushes, she was re-admitted 14 months after the end of the study because of another episode of slight vaginal spotting. No pathology could be found and the endometrium contained only a few inactive glands.

In patient pDUB 01, FSH levels were in the castrate range at the time of the oestrogen provocation test, but, following the anovulatory cycle with prolonged elevated urinary oestrogen excretion in the interval between the two tests (Figure 6.29), gonadotrophins had returned to normal. In the remaining woman, no significant change in either FSH or LH had occurred. Urinary oestrogen levels of this patient had never been higher than 17·4 µg/24 hours between the two tests.

Pituitary gonadotrophin responses to LRF (Figure 6.33) expressed either as the absolute or relative magnitude of the peak or as the absolute or percentage increment above baseline, were always related to the basal gonadotrophin level before LRF-injection except for the FSH response in pDUB 03. The apparent discrepancy between the basal FSH level and the pituitary FSH response to LRF in this patient might indicate that FSH secretion under basal conditions was, at least partially, suppressed by some factor(s) acting at the pituitary level. As might be expected, peripheral 17β-oestradiol concentrations following LRF did not show any rise in the 3 women with menopausal oestrogen levels (pDUB 03, 06 and
FIGURE 6.33: Basal FSH and LH (mean of 16 measurements) and peak FSH and LH responses to LRF in 5 perimenopausal women with DUB.
07). In the other 2 women circulating $17\beta$-oestradiol concentrations increased from 108 pg/ml to 140 pg/ml in pDUB 04 and from 124 pg/ml to 168 pg/ml in pDUB 05 in the samples collected 6 hours after LRF-injection. Expressed as a percentage of the basal level, the increase (129% and 135% respectively) was somewhat smaller than that found in controls (range 139-191%).

(c) Regular cycles with short follicular phase

The pattern of urinary total oestrogen and pregnanediol excretion in subject pDUB 08 (Figure 6.3u) was consistent with regular follicular development and ovulation, but plasma $17\beta$-oestradiol levels were somewhat lower and the follicular phase much shorter than normally seen in women of younger age. In both the control and LRF-test cycle, urinary levels of total oestrogen excretion reached their maximum on day 9 of the cycle and were followed by a luteal pregnanediol rise which was of normal 14 days' duration. The most striking hormonal feature of this patient was the presence of markedly elevated basal FSH levels which remained high throughout the luteal phase and rose dramatically at the beginning of the next cycle. But for one exception, FSH concentrations were always greater than LH, even at midcycle. Levels of this latter hormone were within the normal range during the follicular phase and at mid-cycle and in the high normal range during the luteal phase.

Gonadotrophin responses to ethinyloestradiol were similar to those seen in controls except that the oestrogen-induced LH peak occurred 24 hours earlier and was associated with a concomitant rise in FSH.

No samples could be obtained during the larger part of the subsequent cycle but the hormone measurements at the end of that cycle indicated that ovulation had occurred.
**FIGURE 6.34:** Daily changes in urinary total oestrogen and pregnanediol excretion and of plasma gonadotrophin and steroid levels in a perimenopausal woman with a history of DUB. Hatched bars indicate menstrual bleeding.
Pituitary FSH and LH responses to LRF were within the normal range.

(d) Irregular cycles with occasional anovulation

Results are shown in Figure 6.35.

The pattern of pituitary-ovarian activity in this patient, although somewhat similar to that of the previous patient, was much more variable from one cycle to the next and this was also reflected by the menstrual bleeding record. Following the first cycle, during which she neither ovulated nor menstruated, urinary total oestrogen excretion and plasma 17β-oestradiol rapidly rose again to a pre-ovulatory peak which coincided with the LH peak. Since urine-samples from this patient were not collected on a daily basis, the length of this short follicular phase cannot be determined exactly but must have been between 7 and 9 days.

Due to unfortunate timing, ethinyloestradiol was administered immediately after the spontaneous midcycle LH-surge when circulating progesterone levels were rising, hence the apparent positive feedback failure and the short luteal phase. It is well known that progesterone blocks the stimulatory effect of oestrogen on gonadotrophin release (Dierschke, Yamaji, Karsch, Weick, Weiss and Knobil, 1973) and that oestrogens have a luteolytic effect (Karsch, Krey, Weick, Dierschke and Knobil, 1973).

The hormonal changes during the initial part of the subsequent cycle were similar to those encountered in the previous patient and characterised by a rapid increase in peripheral FSH to values which were well above the upper limit of the normal range. Follicular development however was not accelerated and the follicular phase was of normal length (15 days). As in the previous spontaneous cycle, pre-ovulatory oestrogen secretion appeared to be much higher than that normally seen in younger
FIGURE 6.35: Urinary oestrogen and pregnanediol excretion and plasma FSH, LH, 17β-oestradiol and progesterone in a perimenopausal woman with DUB. Menstrual bleeding is indicated by the hatched bars.
women. During the ensuing luteal phase, which was of normal length, FSH and LH levels remained adequately suppressed until the onset of bleeding after which FSH rapidly rose again.

The pituitary LH response to LRF was similar to that found in controls but FSH secretion fell outside the normal range (peak FSH level 31.1 mU/ml; normal range: 6.3-23.7 mU/ml).

(e) Ovulatory cycles

Results obtained in the remaining patient (pDUB 10) who had a history of regular menstrual cycles but became a "dysfunctional bleeder" after insertion of an intra-uterine device, are shown in Figure 6.36.

Circulating levels of pituitary gonadotrophins and ovarian steroids during the two spontaneous ovulatory cycles of this patient were similar to those seen in women of younger age. FSH levels were highest during the early follicular phase but then progressively declined as follicular maturation progressed and peripheral 17β-oestradiol levels rose. LH levels on the other hand were lowest at the beginning of the cycle and increased gradually towards midcycle. During the luteal phase, gonadotrophins were lower than during the proliferative phase.

The hormonal changes observed following Clomiphene administration indicated that this compound when used in the dosage employed, induced ovulation in perimenopausal women with regular ovarian cycles.

Ethinyloestradiol given during the luteal phase of the cycle failed to induce LH release, thus confirming that the absence of a LH surge in the previous patient was a physiological rather than a pathophysiological positive feedback failure.

Pituitary gonadotrophin responses to LRF were within the range seen in controls of younger age.
FIGURE 6.36: Daily changes in urinary steroid excretion and plasma gonadotrophin and steroid hormone levels in a perimenopausal woman with regular ovarian cycles but irregular menstruation caused by an intra-uterine device. Hatched bars indicate menstrual bleeding.
Discussion

Considering the fact that nearly half the world's entire human population experiences at one stage or another the physiological changes associated with the menopause, our factual knowledge of this stage in reproductive life is surprisingly small. A variety of factors may account for this, but two are clearly outstanding.

Firstly, the type of menopause which occurs in the human female, appears to have no counterpart in any of the other mammalian species, except perhaps for certain strains of mice (Aschheim, 1976). Admittedly, fertility may decrease and ovarian activity may become irregular with advancing age in females of other species, but evidence (and this is based mainly on work in the rat, see e.g. Aschheim, 1976) tends to suggest that, unlike in man, reproductive failure in these instances is not of primary ovarian origin. In effect this means that the only experimental model which is suitable for the study of the physiology of the menopause is man himself which evidently puts a severe restriction on the range of experimental approaches which could be applied.

The second and probably even more important cause for our relative ignorance is of practical rather than scientific nature. Though peri-menopausal symptoms may be troublesome, the knowledge that they are of a temporary character and herald the end of reproductive life, and hence of the risk of further pregnancies, would tend to make these women less motivated towards participation in investigative procedures than e.g. younger patients with menstrual irregularity or infertility. It may therefore not be surprising that most of the information on the characteristics of the menopausal transition, particularly where it relates to pituitary-ovarian function, is based on indirect measurements such as basal body temperature or menstrual bleeding recordings. These studies
left little doubt that abnormalities in ovarian function (Doring, 1969) and hence also in menstrual function (Treloar, Boynton, Behn and Brown, 1967) become increasingly common in women approaching the menopause, but they did not yield any information as to the pathophysiological mechanisms underlying these changes. In order to obtain such information the present study was undertaken.

The reported results relate to a group of women recruited from a gynaecological clinic which they attended because of irregular menstrual bleeding. They do not therefore represent an unbiased sample of the perimenopausal age-group, and our observations and conclusions may hence not necessarily pertain to the common or garden-type perimenopausal woman. However, in view of the high prevalence of irregular menstruation, anovulation and deficient luteal function amongst these women (Doring, 1969; Treloar et al., 1967) it seems not unlikely that the results of the present study may be relevant for a much larger section of the perimenopausal population than that represented in our patient material.

The variability in menstrual cycle length observed in our patients (9 days-11½ months) is in agreement with the monumental prospective study by Treloar et al., (1967) on the changes in menstruation interval throughout reproductive life. As pointed out by these authors, the variation in cycle length found in the perimenopausal age group results not only from a tendency to bleed at irregular, less frequent intervals but also from the presence, in a proportion of women, of unusually short cycles. These may alternate with cycles of normal or increased duration, but they may also occur on a regular basis. The former observation seems easily explainable from what we already know on the hormonal changes and the bleeding pattern associated with disorders such as occasional or persistent anovulation (section 6.3; Figure 6.27) or deficient luteal
function (Sherman and Korenman, 1974), but the finding of regular cycles of short duration is rather surprising.

It is well known that the number of primordial follicles present in the ovaries declines exponentially from birth onwards so that by the age of 40-50 years the oocyte stock is drastically reduced (Block, 1952). Other factors being equal, one might therefore theoretically expect that, as the number of follicles sensitive to gonadotrophins diminishes, cycle length would, if anything, increase rather than decrease. This is apparently not necessarily true which brings us automatically to the conclusion that "other factors might not always be equal", a view supported by the results illustrated in Figure 6.34. From these data, it becomes evident that short cycles in regularly menstruating women are due to a marked decrease in the length of the follicular phase from the normal 14 days to 9 days in this particular patient. This apparent acceleration of follicle growth was associated with a remarkable increase of basal FSH levels which rose dramatically at the beginning of the cycle and did not show any significant suppression prior to the midcycle gonadotrophin surge. FSH levels during the luteal phase were also higher than those normally seen in young women but LH levels were normal.

It seems reasonable to assume that both phenomena, i.e. the elevated FSH concentrations on the one hand and the accelerated follicular maturation on the other hand are causally related in view of the well-known physiological effects of FSH on ovarian follicular development (for references see Chapter 1). The question however then arises which mechanism(s) might be responsible for this elevation in peripheral FSH. The answer to this question is at present uncertain but a few suggestions are made below. It is of interest to note at this point however, that
even in regularly menstruating women between the ages of 20-40 years, mean cycle length is not constant but shows a gradual decrease of between 2 and 3 days (Treloar et al., 1967).

The hormonal findings in subject pDUB 09 (Figure 6.35) resembled somewhat those of the previous patient in that FSH levels during the early follicular phase were also markedly elevated although they did decline when peripheral 17β-oestradiol rose and the follicular phase was of normal length. Menstrual bleeding in this patient was more irregular and the profiles of urinary steroid excretion indicated that apart from having ovulatory cycles with normal or short follicular phases, she occasionally also failed to ovulate. Unfortunately the oestrogen provocation test in this patient, having been performed during the luteal phase of the cycle, gave a false negative result and it is therefore not possible to know whether her failure to ovulate was due to a decrease in positive feedback sensitivity. However, the fact that circulating levels of 17β-oestradiol rose to unusually high pre-ovulatory maxima suggests that such a decrease might have been present.

The five patients (pDUB 03-07) with irregular, infrequent follicular development (Figures 6.29 and 6.31) had several features in common. All of them were at the very extreme of their reproductive lives. Two subjects (pDUB 06 and 07) became menopausal during the study, one patient (pDUB 03) appeared to have become menopausal but had another episode of vaginal bleeding 1½ months later, and the remaining two women (pDUB 04 and 05) stopped menstruating within one year after completion of the study. All five patients had elevated gonadotrophins (Figure 6.30 and 6.31) despite the presence of peripheral 17β-oestradiol levels in the early to mid-follicular range (43-187 pg/ml on days 1-4 of the oestrogen provocation test). Elevations in circulating 17β-oestradiol to values
between 100-150 pg/ml did not appear to have any inhibitory effect on either FSH or LH secretion, even when maintained for 2 to 3 weeks (Figure 6.31). Prolonged periods of follicular activity which were associated with high urinary oestrogen levels however eventually suppressed gonadotrophins to within the normal range (patient pDUB 04 in Figures 6.29 and 6.32). Responses to oestrogen provocation were variable (Table 6.11). The patient from whom daily blood samples were collected (Figure 6.31) was capable of releasing LH in response to ethinyloestradiol which suggests that her failure to ovulate during the study-cycle was probably due to an insufficient strength of the endogenous oestrogen stimulus. Patient pDUB 04 on the other hand appeared to have adequate follicular oestrogen secretion (Figure 6.29) which suppressed but did not stimulate gonadotrophin release. Ethinyloestradiol administration to this patient induced an abortive LH peak suggestive of a central rather than ovarian origin of her ovulatory failure. The pituitary response to LRF was augmented in these patients who had elevated gonadotrophin levels at the time of testing. Since pituitary sensitivity to LRF is dependent, apart from other possible but as yet still unidentified factors, upon the steroid hormone environment (Lasley, Wang and Yen, 1975) as well as upon previous exposure of the gland to the "priming" effect of endogenous LRF (Aiyar, Shiappa and Fink, 1974) it seems likely that the enhanced responsiveness in these patients was a reflection of intense hypothalamic LRF secretion since 17β-oestradiol levels in 3 of the 4 women were in the menopausal range.

Finally, the two patients with persistent anovulation (pDUB 01 and 02, Figures 6.27 and 6.28) appeared to be in a category of their own. They were much younger than the other women who were studied and, in fact, could not truly be called "perimenopausal". In terms of endocrine
characteristics, both patients resembled the adolescent girls with anovulatory dysfunctional uterine bleeding. Due to the absence of a normal midcycle LH peak, follicular oestrogen secretion was extended beyond the usual 14-day period and urinary oestrogen levels were markedly elevated probably as a result of multiple follicular development and the presence of functionally active follicular cysts (Schroder, 1954; Fraser and Baird, 1974). The results of the oestrogen provocation test were compatible with decreased positive feedback sensitivity which was probably of hypothalamic origin in view of the normal pituitary response to LRH. An increase in the strength of the endogenous oestrogen stimulus following Clomiphene treatment induced ovulation in pDUB 01.

Although the number of patients in the present study is small and conclusions must therefore remain speculative, the observations reported here permit the development of a few hypotheses which seem worth testing in future work.

On the basis of the clinical histories it appears reasonable to divide the nine patients with idiopathic DUB into two groups. In seven of the patients (pDUB 03-09) the pattern of menstrual bleeding, although irregular, may to a certain extent be considered as "physiological" in that it is common among women of this particular age-group. In the remaining two patients (pDUB 01-02) the menstrual irregularity is not characteristic of their age-group and is therefore abnormal or pathological although, as will be seen below, it could maybe also be labelled as being physiological but with premature onset.

There is no doubt that reproductive failure in the human female is due to primary ovarian failure. The number of oocytes declines exponentially with advancing age (Block, 1952) and few, if any, follicles are present in postmenopausal ovaries. The few oocytes which may be
found after the menopause (T.G. Baker, personal communication) are probably abnormal or at least resistant to the high circulating level of gonadotrophins.

There are however a number of observations which appear difficult to reconcile with the view that the one and only factor which determines the pattern of ovarian activity in women of advancing age is the progressive loss of ovarian oocytes. Indeed, it seems rather unusual that although the total number of ovarian follicles decreases with age, the relative proportion of growing follicles increases with age (Block, 1952). In the absence of any change in H-P-O relationships, this latter phenomenon should theoretically not influence the number of follicles which eventually develop into mature Graafian follicles since the negative feedback effect of the secreted oestrogen on FSH release would ensure that all but one of these follicles become atretic prior to midcycle (Speroff and Vande Wiele, 1971). This however does not appear to occur. Apart from the fact that multiple follicular cysts and bilateral ovulations are common in perimenopausal women (Schroder 1954; Fraser and Baird, 1974), the observations that cystic glandular hyperplasia (which is a reflection of intense oestrogen stimulation) is most frequently found after the age of 35 (Schroder, 1954) and that the dizygotic twinning rate increases with advancing age (Parkes, 1969) suggest that the incidence of multiple follicular development may be higher in older women. It seems not unreasonable to assume that this is due to a disturbance in the mechanism(s) concerned with the regulation of follicular atresia.

A number of factors could account for this failure of developing follicles to become atretic but, on the basis of the present results it is tempting to suggest that it is due to a change in the feedback
regulation of FSH, and, more specifically, to an increase in basal FSH secretion which appears to become less suppressible towards the menopause. The age at which this change occurs is uncertain, although we are inclined to think that it may be a gradual process which begins as soon as the organogenesis of the ovary is completed and the first crop of follicles starts growing during intra-uterine life.

It should be pointed out that a decrease in feedback inhibition of FSH release must not necessarily result in a gradual elevation of basal FSH levels with advancing age. Indeed, provided the stock of gonadotrophin-sensitive follicles is not drastically reduced, the tendency of FSH levels to increase during the early follicular phase of the cycle can be easily offset by more rapid follicular maturation. There are no published data on the length of the follicular phase in women of different age, but the observation that mean menstrual cycle length between the ages of 20-40 years shows a gradual decrease of between 2-3 days (Treloar et al., 1967) strongly suggests that the follicular phase may indeed become shorter with increasing age. Elevated peripheral FSH levels will therefore only be encountered in women in whom this change in feedback regulation of the hormone reaches a certain threshold level. The age at which this occurs is unknown although recent work by Sherman and Korenman (1975) appears to indicate that it may be between the ages of 40 and 45. These latter authors studies the hormonal changes during the menstrual cycle in women aged 40-41 (mean cycle length 25.4 days) and in premenopausal women aged 46-51 (mean cycle length 23.3 days). Hormone values in the first group did not differ from those of younger women (cycle length 30.0 days) but FSH levels were elevated and 17β-oestradiol levels lower than normal in the premenopausal women with short follicular phases. Similar findings were obtained in our patient pDUB 08.
It thus would appear that the first hormonal evidence of approaching menopause is the presence of a monotropic elevation in FSH, the levels of which start rising as soon as luteal steroid secretion declines. The subsequent course of events is then likely to be determined by the size of the ovarian oocyte pool, which determines the number of follicles which, within a given period of time, start growing and hence become sensitive to gonadotrophin stimulation (Krarup, Pedersen and Faber, 1969). If the oocyte stock is still relatively large (i.e. in women who are not on the verge of the menopausal transition), the interval between the onset of the rise in FSH and the moment one or more follicles become gonadotrophin-sensitive, is not likely to be very long. The follicular phase in these instances will therefore be short or of normal length and oestrogen secretion may be decreased, normal or increased depending on the number of follicles which start growing. Whether FSH levels will subsequently return to the normal range (pDUB 09) or remain elevated (pDUB 08) once follicular growth is initiated is probably dependent on the initial FSH level and on the number of growing follicles. On average however FSH concentrations will increase progressively with each cycle and this increase will be inversely related to the reduction of the oocyte pool. If this pool becomes small, follicular development occurs at infrequent intervals during which time both FSH and LH rise (pDUB 03-07).

The elevation in LH may appear surprising in view of the fact that peripheral 17β-oestradiol levels in our patients were within the early to mid-follicular phase range. It is well known however that LH secretion is not very sensitive to the suppressive effect of oestrogens, given in low or moderate doses. In the castrated female rhesus monkey, early to mid-follicular phase concentrations of circulating 17β-oestradiol
do not suppress the elevated LH level even when maintained for several weeks (Karsch, Weick, Hotchkiss, Dierschke and Knobil, 1973).

Following such prolonged periods of relative ovarian inactivity, another follicle(s) may eventually be stimulated, but the amount of oestrogen excretion often appears to be small which may be due to premature exposure of the growing follicle(s) to the elevated gonadotrophin levels. It has been shown that doses of LH comparable to the amount released at midcycle inhibit oestrogen secretion from sheep Graafian follicles both in-vivo and in-vitro (Moor, 1974). The inadequate oestrogen secretion by the growing follicle may account for the increased incidence of anovulatory cycles in perimenopausal women.

The speculations made so far allow us to explain some of the hormonal changes observed in perimenopausal women. The main question however remains to be answered, i.e. what factor(s) is (are) responsible for the proposed change in feedback regulation of FSH? There are without any doubt a number of possible answers to this question but two are presently being considered in particular.

It seems not impossible that the metabolic processes associated with ageing or the chronic exposure to steroid hormones, may affect the sensitivity of the hypothalamic centres involved in the control of gonadotrophin secretion. In particular, a decrease in sensitivity for both the negative and positive feedback effects of oestrogen could explain a large proportion of the changes described above. Since LH is more sensitive than FSH to the negative feedback effect of 17β-oestradiol
(Speroff and Vande Wiele, 1971) (see footnote), a decrease in negative feedback sensitivity will lead initially to an increase in the secretion of FSH only but, as suggested earlier, this may remain undetected until the oocyte stock is drastically reduced or until the change in sensitivity reaches a certain threshold level. Similarly, the initial changes in positive feedback sensitivity may be compensated for by the enhanced follicular oestrogen secretion.

For reasons discussed above, it seems likely that this change in hypothalamic sensitivity is a gradually occurring process which starts early in life and continues, uninterruptedly, into old age. It is of interest to note in this respect that the onset of puberty is attributed to a decrease in feedback sensitivity (for references, see Chapter 1). The presence (or absence) of perimenopausal DUB and the duration and severity of the symptoms would then depend on the rate of decrease in feedback sensitivity in relation to that of the number of oocytes. Few symptoms would be expected if the oocyte stock is exhausted before feedback sensitivity is markedly impaired, but cycle abnormalities would be severe if the sequence should be reversed, such as in our patients pDUB

Footnote: This finding is not inconsistent with the numerous studies showing that chronic treatment with low or moderate doses of exogenous oestrogens suppresses FSH more readily than LH. In these latter instances, circulating LH levels are the algebraic sum of negative and positive feedback effects. Since negative feedback suppression of LH is virtually maximal at low oestrogen levels, LH secretion during such treatment is primarily regulated by the positive feedback effect of the administered oestrogen (Speroff and Vande Wiele, 1971). A simultaneous slight decrease in negative and positive feedback sensitivity will therefore tend to reduce rather than increase peripheral LH.
01 and 02. The present study failed to detect any consistent difference in negative feedback sensitivity between perimenopausal women and controls. The administered dose of ethinyloestradiol however was large and any difference which may have been present would have been obscured by the activation of the positive feedback mechanism. Further studies using smaller amounts of oestrogen will obviously be required.

A second, very attractive suggestion was recently made by Sherman and Korenman (1975). In order to explain the monotropic elevation of FSH in perimenopausal women, these authors hypothesized the existence of a specific FSH-inhibiting substance of follicular origin, similar to the as yet still unidentified "inhibin" of the male. Assuming that the amount of follicular "inhibin" secretion is proportional to the number of residual oocytes, this hypothesis could explain most of the changes encountered in perimenopausal women except for the changes in positive feedback sensitivity.

Apart from Sherman and Korenman's work (1975) and the present observations, there are to our knowledge no other published studies which contain indirect evidence for the existence of an "inhibin" in the female. It is noteworthy in this respect that in the mouse, the only known species with a "human-type" menopause, the ratio growing follicles/total number of oocytes increases with age like in the human female. If however the oocyte population is experimentally reduced in young females, it becomes apparent that this ratio is a function of the number of oocytes present and not of the age of the animal (Krarup, Pederson and Faber, 1969). Assuming that the relative increase in the number of growing follicles is due to FSH, this would add further support to the existence of a FSH-inhibiting substance of follicular origin, for which we propose the name "FSH-release inhibiting substance" (FRIS). The
presence of FRIS which is likely to act at the pituitary level, could explain the differential pituitary release of FSH and LH in response to a single hypothalamic releasing hormone. The increase in pulsatile secretion of FSH but not of LH in our patients pDUB 03-07 would then become easier to explain.

6.4.5 Summary

In a group of 10 perimenopausal women with irregular menstrual bleeding serial measurements were made of urinary total oestrogen and pregnanediol excretion and of plasma gonadotrophin and steroid levels under basal conditions and using dynamic tests (oestrogen provocation and LRF).

Five different patterns of ovarian activity were identified. One patient in whom bleeding was due to an intra-uterine device had regular ovulatory cycles. In two patients with a history of persistent anovulation and cystic glandular hyperplasia, basal gonadotrophins were normal but there was a failure to release LH in response to endogenous and exogenous oestrogen stimulation. One woman had regular ovulatory cycles but the follicular phase was much shorter than normal and characterised by a marked elevation of basal FSH secretion. A similar monotropic FSH increase was also present in a further patient who had irregular cycles including an ovulatory cycle with short follicular phase, an ovulatory cycle of normal length and an anovulatory cycle. In the remaining five patients, two of whom became menopausal during the study, follicular development was infrequent and anovulation the rule. FSH and LH levels in these women were elevated despite the presence of circulating 17β-oestradiol levels in the early-mid follicular phase range. Following a prolonged period of follicular development with
elevated urinary total oestrogen excretion in one of these women, basal gonadotrophins as well as their response to LRF were within the normal range. LRF responses in the other four patients were augmented. Oestrogen administration failed to induce a normal LH surge in 3 out of the 5 patients.

The implications of these findings are discussed and it is suggested that the increase in pituitary secretion of FSH and subsequently in that of LH in perimenopausal women is due either to a change in hypothalamic sensitivity for the feedback effects of oestrogen or to a decrease in the ovarian secretion of a hypothetical substance produced by the growing follicle and for which the name "FSH-release inhibiting substance" (FRIS) is proposed.
CHAPTER SEVEN

CLINICAL STUDIES ON NEGATIVE FEEDBACK
INTRODUCTION

In women of reproductive age, the occurrence of regular follicular development is dependent upon the delicate interplay between the three components of the negative feedback loop, i.e. ovaries, pituitary and hypothalamus, and their respective "messengers" i.e. steroid hormones, gonadotrophins and releasing factor(s). In the absence of intervening factors (e.g. pregnancy), the presence of menstrual bleeding, occurring at regular monthly intervals is usually the obvious clinical manifestation of functional integrity of this system.

A variety of factors, functional or structural, may disturb the normal activity of the negative feedback loop (Baird, Van Look and Hunter, 1976). In such instances, the absence of regular co-ordinated follicular growth, and hence the alteration in the pattern of ovarian steroid secretion, often presents clinically as a disturbance of menstrual function, in casu amenorrhoea. Apart from the uterus, other target organs for pituitary and/or ovarian steroid hormones may also be affected such as for example the breast in the galactorrhoea-amenorrhoea syndromes, or the hair follicles in the polycystic ovary syndrome.

It is well recognised that women with amenorrhoea, although presenting with a similar clinical symptom, may vary considerably with respect to endocrine characteristics, responsiveness to therapy and prognosis of their disorder. These differences are probably a reflection of varying degrees of H.P.O. dysfunction, but the mechanisms underlying them are poorly understood. In an attempt to gain some information on this point, H.P.O. relationships were studied in a group of women with proven polycystic ovary syndrome and in patients with secondary amenorrhoea due to hypothalamic-pituitary failure.
7.1 The polycystic ovary syndrome

7.1.1 Background

The term polycystic ovary syndrome is applied to a group of cases of unknown or uncertain aetiology in which characteristic polycystic ovaries are found.

The first, albeit very incomplete, clinical description of this condition was probably given by Aristotle (384-322 B.C.) when he reported (De Generatione Animalium, p 215-216): "Others become sterile ... because they have put on too much flesh: in women who are too fat ... no menstrual discharge is formed ..." This observation evidently received little attention until 1935, when Stein and Leventhal discovered seven examples of a syndrome characterised by oligo- or amenorrhoea, infertility, hirsutism, obesity and the presence of bilateral, enlarged polycystic ovaries.

From subsequent work however, it has become abundantly clear that the clinical picture allegedly associated with polycystic ovaries is seldom complete. In their extensive literature review on the subject, Goldzieher and Green (1962) reported that obesity, hirsutism and amenorrhoea were present in respectively 33, 56 and 47% of cases and that even the most common complaint, infertility, was present in only 75%. Many patients with polycystic ovaries may have dysfunctional bleeding or regular menses (21 and 16% respectively) and in about 19% corpora lutea have been found at the time of operation (Goldzieher and Green, 1962). This variability in symptomatology may be partly due to differences in the diagnostic criteria used or in the sources from which patients were obtained by individual observers, although it also suggests that the underlying aetiology may not always be identical in women presenting with this condition.
The factor(s) responsible for the development of polycystic ovaries is (are) unknown. Our complete ignorance on this aspect of polycystic ovarian disease (P.C.O.) is probably most convincingly illustrated by the wide range of hypotheses which have been put forward to explain one or other symptom of this disorder (for reviews see e.g. Goldzieher and Green, 1962; Jeffcoate, 1964 or Ferriman, 1969). Of these, studies attributing the clinical and endocrine abnormalities in P.C.O. to changes in ovarian and/or adrenal steroid metabolism or to alterations in the patterns of pituitary gonadotrophin secretion have undoubtedly attracted most of the attention.

The hormonal features of patients with P.C.O. have been studied on numerous occasions. Several investigators have reported elevated and fluctuating levels of LH both in urine (McArthur, Ingersoll and Worcester, 1958) and in plasma (Yen, Vela and Rankin, 1970). FSH levels were usually found to be normal or lower than normal. Urinary total oestrogen excretion is within the normal range but there is an absence of the biphasic pattern characteristic of the normal cycle (Brown and Matthew, 1962). Blood production rates of androstenedione and testosterone are elevated (Lipsett, Migeon, Kirschner and Bardin, 1968), possibly as a result of increased ovarian androgen secretion under the influence of the raised LH level (Kirschner and Jacobs, 1971). Oestrogen production rates as measured by urinary isotope dilution techniques are within the normal range (Jeffcoate, Brooks, London, Smith, Spathis and Prunty, 1968), but the concentration of oestrone in peripheral plasma exceeds that of 17β-oestradiol (Baird, 1973).

Very few studies have been performed on the dynamics of the HPG-axis in patients with P.C.O. Work by Yen, Vela and Rankin (1970) has indicated that continuous infusion of 17β-oestradiol suppresses the
elevated basal LH levels, but at the time the present study was started, no comparable data were available with respect to the functional status of the positive feedback mechanism in this condition. In collaboration with Dr. D. T. Baird acting as principal investigator, it was therefore decided to undertake an investigation in patients with P.C.O. of gonadotrophin and steroid levels under basal conditions as well as of their response to specific dynamic tests, including oestrogen provocation and LRF-tests and Clomiphene stimulation. Preliminary results of this study have been published in abstract form (Baird, Corker, Fraser, Hunter, Michie and Van Look, 1975).

7.1.2 Design of study

Clinical details of the twelve women (P.C.O. 01-12) who participated in this study are summarized in Table 7.1 and a typical case history is given below.

In ten out of the 12 patients the clinical diagnosis of P.C.O. was confirmed by laparoscopy or laparotomy, while in the remaining two patients (P.C.O. 03 and 11) the diagnosis was made on clinical grounds and confirmed by pelvic examination and hormone measurements. All women had a history of oligo- or amenorrhoea of several years' duration and three of them (P.C.O. 01, 10 and 12) had been admitted 2-3 years previously for ovarian wedge resection. Hirsutism was present in 8 patients and 4 of the 6 married women complained of infertility. Two patients (P.C.O. 05 and 09) were grossly overweight.

The design of the study was similar to that used in the investigation of patients with dysfunctional uterine bleeding except that in two subjects (P.C.O. 01 and 02) daily blood and urine-samples were collected for a period of about 9 weeks. In addition to an oestrogen
<table>
<thead>
<tr>
<th>PATIENT'S NO.</th>
<th>AGE (yrs)</th>
<th>MENARCHE (yrs)</th>
<th>PARITY</th>
<th>HEIGHT (cms)</th>
<th>WEIGHT (kgs)</th>
<th>MENSTRUAL HISTORY</th>
<th>HIRSUTISM</th>
<th>CYCLE PRE-OSTROGEN TEST</th>
<th>PRE-LRF TEST</th>
<th>COMMENTS</th>
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<tr>
<td>01</td>
<td>26</td>
<td>14</td>
<td>1+1</td>
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<td></td>
<td>0</td>
<td>-</td>
<td>An</td>
<td></td>
<td>Wedge resection (3 yrs)</td>
</tr>
<tr>
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<td>27</td>
<td>12</td>
<td>1+1</td>
<td>160</td>
<td>69</td>
<td>0</td>
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<td>Infertility (3 yrs)</td>
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<td>23</td>
<td>15</td>
<td>0+0</td>
<td>169</td>
<td>52</td>
<td>0</td>
<td>+</td>
<td>An</td>
<td></td>
<td></td>
</tr>
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<td>04</td>
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<td>12</td>
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<td>-</td>
<td>An</td>
<td></td>
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<td>86</td>
<td>0</td>
<td>+</td>
<td>An</td>
<td></td>
<td>An</td>
</tr>
<tr>
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<td>2+0</td>
<td>155</td>
<td>51</td>
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<td>+</td>
<td>An</td>
<td></td>
<td>An</td>
</tr>
<tr>
<td>07</td>
<td>19</td>
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<td>75</td>
<td>0</td>
<td>+</td>
<td>An</td>
<td></td>
<td>An</td>
</tr>
<tr>
<td>08</td>
<td>19</td>
<td>11</td>
<td>0+0</td>
<td>162</td>
<td>60</td>
<td>0</td>
<td>+</td>
<td>An</td>
<td></td>
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</tr>
<tr>
<td>09</td>
<td>28</td>
<td>12</td>
<td>0+0</td>
<td>169</td>
<td>135</td>
<td>0</td>
<td>+</td>
<td>Ov</td>
<td></td>
<td>Infertility</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>15</td>
<td>0+0</td>
<td>156</td>
<td>62</td>
<td>0</td>
<td>-</td>
<td>Ov</td>
<td></td>
<td>Wedge resection (2 yrs).Infertility</td>
</tr>
<tr>
<td>11</td>
<td>21</td>
<td>16</td>
<td>0+0</td>
<td>164</td>
<td>54</td>
<td>A</td>
<td>+</td>
<td>An</td>
<td></td>
<td>Ov</td>
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<tr>
<td>12</td>
<td>25</td>
<td>11</td>
<td>0+0</td>
<td>162</td>
<td>55</td>
<td>0</td>
<td>+</td>
<td>Ov</td>
<td></td>
<td>Wedge resection (2 yrs)</td>
</tr>
</tbody>
</table>

0 = oligomenorrhoea  
A = amenorrhoea (> 6 months)  
An = anovulatory  
Ov = ovulatory
provocation test, both women also received Clomiphene but no LRF was given to subject P.C.O. 01.

Results were compared to those obtained in a group of 6 regularly menstruating women who served also as controls for the studies in patients with dysfunctional uterine bleeding (Chapter 6, sections 6.1, 6.3 and 6.4).

Case history (patient P.C.O. 02)

Menstrual bleeding in this 26 year-old patient started at the age of 12 years and was regular until, at the age of 22, menstruation became progressively scantier and irregular. One year later she was admitted for investigation of secondary amenorrhoea and primary infertility. At diagnostic laparoscopy both ovaries were noted to be enlarged and their surfaces had a sclerocystic appearance. Although no other pelvic abnormalities were found, ovulation induction was not attempted since the husband's semen was reported to be oligospermic on two consecutive examinations. However, one year later and following two episodes of heavy vaginal bleeding she was started on Clomiphene therapy and subsequently became pregnant after the fifth treatment course. During her pregnancy Mrs. P.C.O. 02 underwent fasciotomy for progressively aggravating bilateral carpal tunnel syndrome. After the birth of her child and until the beginning of this study, 10 months later, menstrual bleeding occurred at 5 to 8 weeks' intervals and was usually fairly heavy. There was no hirsutism present and the patient weighed 69 kg for a height of 160 cm. Blood and urine samples from this patient were collected on a daily basis for a period of 9 weeks.

One year after completion of the study, Mrs. P.C.O. 02 was admitted for investigation of chronic pain in the right iliac fossa.
An exploratory laparotomy was carried out and the presence of bilateral, markedly enlarged polycystic ovaries confirmed (Plate 7.1).

7.1.3 Results

(a) Urinary measurements

As discussed in section 6.3, the pattern of urinary total oestrogen and pregnanediol excretion in all six control women was consistent with normal follicular development and ovulation. Mean cycle-length of the first study-cycle was $29.0 \pm 0.4$ (SEM) days but this increased to $39.5 \pm 3.7$ days following the oestrogen provocation test. Serial measurements of urinary steroid levels during this cycle indicated that the delay in the onset of menstruation was due to a significant prolongation of the follicular phase from $16.3 \pm 1.5$ to $27.0 \pm 3.5$ days ($p < 0.05$) and the development of a new crop of follicles.

Nine out of the 12 women with P.C.O. did not show any evidence of co-ordinated follicular growth or ovulation during the 4 to 6 weeks' tracking period prior to ethinyloestradiol administration (Table 7.1). Mean urinary oestrogen excretion in these patients ranged from 10.2 to 19.1 µg/24 hours, which was comparable to the levels found during the mid-follicular phase in controls (Figure 7.1).

Following the oestrogen provocation test Clomiphene was administered to subjects P.C.O. 01 and 02, both of whom responded with an ovulatory cycle (see below). Of the remaining ten women, seven failed to ovulate during the 4-6 weeks prior to IRF injection, thus giving a total of 6 spontaneous ovulations over a study period covering at least 22 months (i.e. an ovulatory incidence of 27%). Three of these ovulatory cycles were encountered in the two patients (P.C.O. 10 and 12) who had ovarian wedge resection two years prior to the start of the present
PLATE 7.1: Macroscopic appearance of the ovarian wedge biopsy taken from patient P.C.0. 02.
FIGURE 7.1: Profiles of urinary total oestrogen and pregnanediol excretion (means ± S.E.M.) in normal women (open bars) and patients with anovulatory polycystic ovarian disease (closed bars) during the four weeks prior to the oestrogen provocation test. Results in the normal women were centred round the midcycle oestrogen-peak.
study. Both these subjects ovulated before and P.C.O. 12 also after oestrogen administration. The three remaining ovulations were encountered in P.C.O. 09 (before oestrogen provocation) and in P.C.O. 08 and 11 (before LRF). There were no significant differences in either the length of the luteal phase (13.5 ± 0.4 days; mean ± S.E.M.), or the urinary total oestrogen excretion at midcycle (41.1 ± 5.1 μg/24 hours) between ovulating patients with P.C.O. and control women (length of luteal phase: 13.0 ± 1.1 days; midcycle oestrogen peak: 30.7 ± 3.0 μg/24 hours).

(b) Oestrogen provocation test

Daily hormonal changes

Mean (± S.E.M.) levels of FSH, LH, 17α-ethinylestradiol, 17β-estradiol, oestrone and androstenedione in patients with P.C.O. and normal women are illustrated in Figures 7.2 and 7.3.

In both these figures, results obtained in the 3 patients who had an ovulatory cycle prior to ethinylestradiol administration have not been included in the calculation of the mean since it was assumed that the endocrine events associated with ovulation and corpus luteum formation might have altered the characteristic hormonal environment normally found in anovulatory P.C.O. That this assumption was correct is illustrated in Table 7.2 which summarizes basal gonadotrophin and steroid levels (means ± S.E.M. of samples taken on days 1-4 of oestrogen provocation test or LRF-test) in patients who ovulated in the 4-6 weeks prior to one test but not prior to the other ("mixed P.C.O."). For comparison, basal gonadotrophin and steroid levels in normal women (overall means of days 1-4 of oestrogen provocation and LRF-test) have also been included in Table 7.2.
OESTROGEN PROVOCATION TEST IN POLYCYSTIC OVARY SYNDROME

*Significantly different from Basal levels (p<0.05)

FIGURE 7.2: Mean (± S.E.M.) levels of plasma FSH, LH and 17α-ethinylestradiol during oestrogen provocation test in 6 normal women (broken lines) and 9 patients with polycystic ovary syndrome, who had been anovulatory for at least 4 weeks prior to the test.
OESTROGEN PROVOCATION TEST IN POLYCYSTIC OVARY SYNDROME

Figure 7.3: Mean (± S.E.M.) plasma levels of androstenedione, oestrone and 17β-oestradiol during oestrogen provocation test in 6 normal women and in 9 patients with polycystic ovary syndrome who had been anovulatory for at least 4 weeks prior to the test.

* Significantly different from Basal levels (p<0.05)
Table 7.2: Mean (± S.E.M.) plasma steroid and gonadotrophin levels in 4 patients with polycystic ovary syndrome and in normal women (for explanation see text).

<table>
<thead>
<tr>
<th></th>
<th>Normal Women</th>
<th>Anovulatory Cycle</th>
<th>Ovulatory Cycle</th>
<th>P&lt;sub&gt;*&lt;/sub&gt;1</th>
<th>P&lt;sub&gt;*&lt;/sub&gt;2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mU/ml)</td>
<td>7.55 ± 0.60</td>
<td>6.51 ± 0.61</td>
<td>5.19 ± 0.66</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LH (mU/ml)</td>
<td>9.83 ± 0.55</td>
<td>18.83 ± 3.03</td>
<td>10.56 ± 2.21</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>17α-Oestradiol (pg/ml)</td>
<td>55.6 ± 3.1</td>
<td>152.7 ± 11.7</td>
<td>152.7 ± 11.7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Oestrone (pg/ml)</td>
<td>80.4 ± 3.3</td>
<td>55.2 ± 10.3</td>
<td>55.2 ± 10.3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>0.96 ± 0.08</td>
<td>2.95 ± 0.19</td>
<td>2.35 ± 0.19</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*<sub>1</sub> Student's t-test comparing anovulatory cycle and ovulatory cycle in P.C.O. patients.
*<sub>2</sub> Student's t-test comparing normal women and ovulatory P.C.O. patients.
It is evident from this data that peripheral LH levels in P.C.O. patients following an ovulatory cycle are on average about 50% lower than those found after a 4-6 week period of anovulation. Circulating levels of oestrone and androstenedione, although somewhat lower after the ovulatory cycle, were still markedly elevated when compared to regularly menstruating women. Since peripheral gonadotrophin levels following an ovulatory cycle were similar to those seen in controls, this might indicate that the abnormalities in steroid secretion found in patients with P.C.O. are unlikely to be caused by a primary disturbance in pituitary gonadotrophin release, although, as will be discussed below, such a disturbance might be implementary in maintaining the abnormal pattern of steroid hormone secretion once it has been initiated.

In the nine patients who did not ovulate prior to ethinyl-oestradiol administration basal concentrations of LH, 17β-oestradiol, oestrone and androstenedione were all significantly elevated as compared to normal women (Figures 7.2 and 7.3 and Table 7.3). Basal FSH levels on the other hand were significantly lower than those in controls (P.C.O. 6.33 ± 1.87 mU/ml; controls 8.40 ± 1.02 mU/ml; p < 0.05).

The changes in plasma FSH during and following ethinyl-oestradiol treatment in anovulatory P.C.O. patients were basically similar to those seen in normal women. FSH levels were maximally suppressed on days 6 and 7 of the test after which they returned to basal values.

In contrast to controls, LH concentrations in P.C.O. patients 24 hours after the start of oestrogen administration (mean ± S.E.M.: 13.18 ± 2.46 mU/ml) were not significantly lower than pre-treatment values (15.90 ± 1.18 mU/ml; p > 0.05) which may have been due to the earlier activation of positive feedback in the patient's group. Indeed,
TABLE 7.3: Basal (means ± S.E.M.) steroid and gonadotrophin levels in normal women and in P.C.O. patients who did not ovulate during a 4-6 week period prior to collection of the samples.

<table>
<thead>
<tr>
<th></th>
<th>NORMAL WOMEN</th>
<th>ANOVULATORY P.C.O.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mU/ml)</td>
<td>8·40 ± 1·02 (n=24)</td>
<td>6·33 ± 1·87 (n=36)</td>
<td>&lt; 0·05</td>
</tr>
<tr>
<td>LH (mU/ml)</td>
<td>10·26 ± 0·80 (n=24)</td>
<td>15·90 ± 1·18 (n=36)</td>
<td>&lt; 0·001</td>
</tr>
<tr>
<td>17β-OESTRADIOL (pg/ml)</td>
<td>55·0 ± 5·1 (n=24)</td>
<td>74·2 ± 6·5 (n=35)</td>
<td>&lt; 0·05</td>
</tr>
<tr>
<td>OESTRONE (pg/ml)</td>
<td>84·5 ± 7·4 (n=15)</td>
<td>142·0 ± 16·5 (n=35)</td>
<td>&lt; 0·05</td>
</tr>
<tr>
<td>ANDROSTENEDIONE (ng/ml)</td>
<td>1·13 ± 0·13 (n=20)</td>
<td>3·60 ± 0·23 (n=35)</td>
<td>&lt; 0·001</td>
</tr>
</tbody>
</table>
in 5 out of the 9 patients (Table 7.4), peripheral LH levels were maximal on day 8 of the test i.e. 24 hours earlier than in controls. One patient (P.C.O. 07) did not show any significant increase in LH in response to exogenous oestrogen while in the remaining three women LH surges occurred on day 9. The magnitude of the oestrogen-induced LH peaks (mean ± S.E.M.: 35.04 ± 5.65 mU/ml) was not significantly different from that seen in control women (mean ± S.E.M.: 36.10 ± 4.84 mU/ml). Peripheral 17α-ethinylestradiol concentrations were also similar in both groups (Figure 7.2).

Plasma androstenedione levels were significantly suppressed during oestrogen treatment in both control women and anovulatory P.C.O. patients, but 17β-oestradiol concentrations did not show any significant suppression in the patient's group. Changes in peripheral oestrone levels paralleled those of androstenedione.

**Episodic gonadotrophin release**

Results with respect to episodic gonadotrophin release in anovulatory P.C.O. patients are summarised in Tables 7.5 and 7.6. The corresponding data for control women have been presented in Tables 6.2 and 6.3 (section 6.1).

In all but one patient (P.C.O. 02) transverse mean FSH and LH concentrations were significantly lower during oestrogen treatment (day 6) than the corresponding basal values (day 4). For the group as a whole, mean FSH levels decreased from 5.89 ± 0.43 mU/ml (mean ± S.E.M.) to 4.03 ± 0.37 mU/ml (p < 0.001, paired t-test) or 68.7% ± 4.66% of the initial level, a value comparable to that found in control women (66.73 ± 8.76%). Mean peripheral LH concentration on day 6 was 10.30 ± 2.10 mU/ml or 73.76 ± 8.05% of the pretreatment level as compared to 4.36 ± 0.60 mU/ml (p < 0.05) or 59.23 ± 6.36% (p > 0.05) for the control group.
TABLE 7.4: Peripheral FSH and LH levels (mIU/ml) during oestrogen provocation test in 9 patients with P.C.O.

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<tr>
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</tr>
<tr>
<td>FSH</td>
<td></td>
</tr>
<tr>
<td>PGO 01</td>
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</tr>
<tr>
<td></td>
<td>5.8</td>
</tr>
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<td>8.8</td>
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<tr>
<td></td>
<td>6.2</td>
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<td></td>
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<td>4.3</td>
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<tr>
<td>mean (±SEM)</td>
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</tr>
<tr>
<td>±0.8</td>
<td>±0.7</td>
</tr>
<tr>
<td>LH</td>
<td></td>
</tr>
<tr>
<td>PGO 01</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>11.4</td>
</tr>
<tr>
<td>mean (±SEM)</td>
<td>15.4</td>
</tr>
<tr>
<td>±1.8</td>
<td>±3.8</td>
</tr>
</tbody>
</table>
TABLE 7.5: Transverse means (± S.D.) in mU/ml and percentage change of peripheral gonadotrophin levels in anovulatory P.C.O. patients sampled at 15 minute intervals for 3 hours before (Day 1) and during (Day 6) ethinyloestradiol administration.

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 6</th>
<th>Percentage change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FSH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCO 01</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>5.39 ± 0.57</td>
<td>5.09 ± 0.86</td>
<td>-5.6</td>
</tr>
<tr>
<td>03</td>
<td>6.88 ± 0.87</td>
<td>3.61 ± 0.37</td>
<td>-47.5</td>
</tr>
<tr>
<td>04</td>
<td>5.33 ± 0.17</td>
<td>4.12 ± 0.27</td>
<td>-22.7</td>
</tr>
<tr>
<td>05</td>
<td>6.90 ± 0.63</td>
<td>4.30 ± 0.42</td>
<td>-37.7</td>
</tr>
<tr>
<td>06</td>
<td>5.63 ± 0.70</td>
<td>3.70 ± 0.60</td>
<td>-34.3</td>
</tr>
<tr>
<td>07</td>
<td>3.63 ± 0.70</td>
<td>2.24 ± 0.40</td>
<td>-38.3</td>
</tr>
<tr>
<td>08</td>
<td>7.55 ± 0.42</td>
<td>5.71 ± 0.39</td>
<td>-24.4</td>
</tr>
<tr>
<td>11</td>
<td>5.77 ± 1.50</td>
<td>3.48 ± 0.60</td>
<td>-39.7</td>
</tr>
<tr>
<td>mean ± SEM</td>
<td>5.89 ± 0.43</td>
<td>4.03 ± 0.37</td>
<td>-(31.28 ± 4.66)</td>
</tr>
</tbody>
</table>

| **LH**  |       |       |                   |
| PCO 01  | ND    | ND    |                   |
| 02     | 18.30 ± 2.38 | 21.92 ± 4.86 | +19.8         |
| 03     | 14.52 ± 5.42 | 5.58 ± 1.86 | -61.6         |
| 04     | 17.60 ± 1.40 | 13.40 ± 1.60 | -23.9         |
| 05     | 10.40 ± 1.20 | 8.10 ± 0.60 | -22.1         |
| 06     | 11.00 ± 1.40 | 7.40 ± 0.70 | -32.7         |
| 07     | 5.20 ± 1.10  | 4.10 ± 1.60 | -21.1         |
| 08     | 2.40 ± 5.18  | 14.70 ± 3.34 | -38.9         |
| 11     | 10.20 ± 1.40 | 7.20 ± 1.10 | -29.4         |
| mean ± SEM | 13.90 ± 2.10 | 10.30 ± 2.10 | -(26.24 ± 8.05) |

ND = not done
TABLE 7.6: Characteristics of pulsatile gonadotrophin release in anovulatory polycystic ovary syndrome.

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th></th>
<th>Day 6</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSH</td>
<td>LH</td>
<td>FSH</td>
<td>LH</td>
</tr>
<tr>
<td>Number of peaks</td>
<td>0.25</td>
<td>0.88</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>(per 3 hours)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute peak</td>
<td>3.13</td>
<td>5.90 ± 1.40</td>
<td>1.78</td>
<td>1.65</td>
</tr>
<tr>
<td>magnitude (mU/ml)</td>
<td></td>
<td>(mean ± S.E.M.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative peak</td>
<td>66.9</td>
<td>49.8 ± 7.7</td>
<td>43.3</td>
<td>37.3</td>
</tr>
<tr>
<td>magnitude (%)</td>
<td></td>
<td>(mean ± S.E.M.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analysis of gonadotrophin changes during the 15 minute interval sampling period before oestrogen treatment (Table 7.6) indicated that the low FSH levels in the patient’s group were associated with a reduction in frequency, but not in amplitude, of episodic FSH release. In women with anovulatory P.C.O. FSH peaks occurred on average at 12-hourly intervals as compared to 6-hourly intervals in controls. LH-peak frequency on the other hand was similar to that seen in control women (approx. 1 peak every 3 hours), but there was a striking increase in the absolute magnitude of the pulses from a value of 2.26 ± 0.56 mU/ml (mean ± S.E.M.) in controls to 5.90 ± 1.40 mU/ml (p < 0.05, one-tailed t-test) in P.C.O. patients. Frequency and amplitude of pulsatile release during ethinyloestradiol treatment were comparable in both groups.

(c) LRF-test

Results are illustrated in Figure 7.4.

For reasons discussed earlier, P.C.O. patients were divided into two groups depending on whether they ovulated ("ovulatory P.C.O." in Figure 7.4) or not ("anovulatory P.C.O." in Figure 7.4) during the 4-6 weeks' period prior to the test.

Mean (± S.E.M.) basal FSH (5.42 ± 0.83 mU/ml), LH (6.83 ± 1.72 mU/ml) and 17β-oestradiol (4.7 ± 18 pg/ml) in ovulatory P.C.O. patients were similar to the corresponding values in control women (5.68 ± 0.90 mU/ml; 7.62 ± 1.36 mU/ml and 62 ± 8 pg/ml), but basal levels of oestrone (168 ± 26 pg/ml) and androstenedione (2.31 ± 0.58 ng/ml) were significantly higher in ovulatory P.C.O. patients than in controls (71 ± 4 pg/ml, p < 0.001 and 1.05 ± 0.19 ng/ml, p < 0.05 for oestrone and androstenedione respectively). In the anovulatory P.C.O. group, basal levels of FSH (mean ± S.E.M.: 5.75 ± 0.77 mU/ml), 17β-oestradiol (5.4 ± 10 pg/ml), oestrone (117 ± 27 pg/ml) and androstenedione (3.26 ± 0.74 ng/ml) were similar to those of ovulatory P.C.O. patients but LH levels (13.14 ± 2.69 mU/ml) were twice as high.
RESPONSE TO LRF IN POLYCYSTIC OVARY SYNDROME (PCO)

FIGURE 7.1: Plasma FSH and LH levels (means) during LRF-test in normal women and patients with polycystic ovary syndrome. The terms ovulatory and anovulatory P.C.O. are explained in the text. Vertical bars represent 1 S.E.M.
There was no significant difference between the three groups in either timing, absolute or relative magnitude of the FSH-peak following LRF-injection (Figure 7.4). Peak concentrations of LH were also virtually similar in controls and ovulatory P.C.O. patients. However, the LRF-induced LH peak in the anovulatory P.C.O. group was significantly greater whether expressed in absolute terms (60·37 ± 14·14 mU/ml vs 24·76 ± 4·66 mU/ml in controls, p<0·05) or as a percentage of the pre-treatment value (457 ± 48% vs 325 ± 25% in controls, p<0·05). This significant increase in LH secretion following LRF in the anovulatory P.C.O. group is unlikely to be a result of the higher circulating levels of androstenedione or oestrone since the ovulatory P.C.O. patients had similarly elevated concentrations of these hormones but a normal gonadotrophin response.

(d) Clomiphene-test

In two subjects (PCO 01 and 02) daily measurements were made of urinary total oestrogen and pregnanediol excretion and of plasma gonadotrophin and steroid levels for 6 weeks prior to and following Clomiphene administration (Figures 7.5 and 7.6).

Neither of these patients ovulated during the initial 6 week study period and their peripheral steroid and gonadotrophin levels were characteristic of "anovulatory PCO". Basal androstenedione and LH levels were markedly higher but FSH lower than those found in regularly menstruating women during the early follicular phase of the cycle.

Urinary and plasma oestrogen levels were similar to those found in the mid-follicular phase but, in contrast to normal women, the plasma concentration of oestrone exceeded that of 17β-oestradiol. Levels of this latter hormone were steady and did not show any evidence of regular follicular growth.
FIGURE 7.5: Daily changes in plasma $17\beta$-oestradiol, oestrone, androstenedione, FSH and LH and in the urinary excretion of total oestrogens and pregnanediol in a patient with polycystic ovary syndrome (subject P.C.O. 01). Black bars indicate menstrual bleeding.
FIGURE 7.6: Daily changes in plasma 17β-estradiol, oestrone, androstenedione, FSH and LH and in the urinary excretion of total oestrogens and pregnanediol in a patient with polycystic ovary syndrome (subject P.C.O. 02). Black bars indicate menstrual bleeding.
Both patients responded positively to ethinyloestradiol provocation with LH release.

Clomiphene administration resulted in an increase in peripheral gonadotrophin levels which induced follicular development as indicated by the rising levels of urinary total oestrogen excretion and of plasma 17β-oestradiol. The subsequent increase in urinary pregnanediol excretion confirmed the ovulatory nature of their cycle (see Footnote). It is noteworthy that in both women LH levels at the end of the luteal phase and during menstruation were only half those seen during the initial anovulatory 6-week period. Oestrone and androstenedione levels on the other hand were virtually unchanged (Figure 7.6) or slightly lower (Figure 7.5), which is in agreement with the results obtained in the other four patients who had both an "anovulatory" and an ovulatory cycle during the study period (Table 7.2).

7.1.4 Discussion

Despite the overwhelming body of clinical, histological and endocrine data, the pathophysiology of the polycystic ovary syndrome remains obscure. In fact, it might be argued if there exists such a thing as "the" polycystic ovary syndrome. Indeed, apart from the fact that polycystic ovaries might be encountered in a number of other conditions such as e.g. Cushing's disease, adrenal hyperplasia etc. (Ferriman, 1969), the variation in frequency and severity of symptoms even within the so-called "idiopathic" group of P.C.O. patients is so large that it often may be difficult to accept that one is dealing with

Footnote: Due to technical difficulties no LH estimate of the sample taken at mid-cycle in patient P.C.O. 01 (Figure 7.5) could be obtained; hence the apparent absence of a pre-ovulatory LH peak.
a single, associated syndrome (Goldzieher and Green, 1962). If variability of the clinical picture is one of the main features of this condition, the present patient population may be considered as being highly representative.

The changes in basal hormone levels observed in this study are similar to those previously described. It has been known for many years that urinary excretion of LH as measured by bioassay, is elevated in patients with this disorder (McArthur, Ingersoll and Worcester, 1958) and that radioimmunoassayable concentrations of plasma LH are about twice as high as those found in normal women during the follicular phase of the cycle (Yen, Vela and Rankin, 1970). A number of authors (e.g. Duignan, Shaw, Rudd, Holder, Williams, Butt, Logan-Edwards and London, 1975) however have failed to detect elevated plasma LH levels in patients with laparoscopic evidence of P.C.O. which has led to the suggestion (Berger, Taymor and Patton, 1975) that increased LH concentrations might only be present in patients with enlarged polycystic ovaries but not in those with ovaries of normal size.

The present study indicates that these discordant findings might result from the pattern of ovarian activity during the period which preceded the collection of samples. In patients with evidence of co-ordinated follicular activity and ovulation in the month prior to investigation, plasma LH levels were significantly lower than those measured in the same patient following a period of anovulation, even though peripheral steroid levels were similar on both occasions and characteristic of P.C.O. (Table 7.2). This observation evidently stresses the need for careful monitoring of the patterns of spontaneous ovarian activity in P.C.O. patients participating in studies on the endocrine characteristics of this condition. In the present study 6 spontaneous
ovulations were recorded during an observation period covering 22 months. In terms of ovulatory incidence (27%), this value is not much different from the average frequency with which corpora lutea are found at operation in patients with P.C.O. (19% according to Goldzieher and Green, 1962).

The factor(s) responsible for this increase in circulating LH is (are) uncertain. Our observations indicate that the raised plasma LH levels are maintained by an increase in amplitude, but not in frequency, of pulsatile LH discharge (Table 7.6), and that this may be attributed to an enhanced pituitary sensitivity to LRF (Figure 7.4). The results do not however offer any clue as to the cause of this increase in pituitary responsiveness. Since P.C.O. patients with recent evidence of ovulation did not show an enhanced LH response to LRF (Figure 7.4) even though their circulating levels of oestrone and androstenedione were similar to the anovulatory P.C.O. group, it seems unlikely that either of these hormones is responsible for the observed increase in pituitary sensitivity in the anovulatory P.C.O. group.

It has been shown that pituitary responsiveness to LRF is determined not only by the hormonal environment in terms of 17β-oestradiol and progesterone levels (Lasley, Wang and Yen, 1975), but also by the extent of previous exposure of the pituitary gland to endogenous LRF (Aiyer, Shiappa and Fink, 1974). It may be therefore that anovulatory P.C.O. patients have a higher secretion rate of hypothalamic LRF. Obviously, the question then arises which mechanism might be responsible to account for this. Since the majority of P.C.O. patients respond favourably to Clomiphene administration with a rise in peripheral gonadotrophin levels it seems unlikely that their increased hypothalamic LRF secretion is a result of a decreased sensitivity to the negative
feedback effect of 17β-oestradiol. The observations that circulating LH levels in P.C.O. patients are suppressed during the luteal phase of the cycle (Figures 7.5 and 7.6), following ethinyloestradiol treatment (Table 7.5) and following intravenous oestrogen administration (Yen, Vela and Rankin, 1970) would appear to support this view.

It has been suggested that the peripheral level of LH is the algebraic sum of the negative and positive feedback effects of circulating 17β-oestradiol (Speroff and Vande Wiele, 1971). If this is correct, the elevated LH levels in P.C.O. patients might be a result of an increased sensitivity to the positive feedback effect of oestrogen. The finding of a "premature" LH discharge in 5 out of the 9 anovulatory P.C.O. patients (Table 7.4) is not inconsistent with this hypothesis. It is of interest to note in this respect that subthreshold doses of 17β-oestradiol which are ineffective in inducing LH release in normal ewes, will evoke a LH surge in animals chronically implanted with androstenedione or oestrone-containing Silastic capsules (Van Look, unpublished observations).

The present study has confirmed the observation that FSH levels in P.C.O. patients are significantly lower than those found during the early follicular phase of the cycle (Yen, Vela and Rankin, 1970). The explanation for this disparity in pituitary FSH and LH release in P.C.O. is not clear, but may be related to differential sensitivity for FSH and LH of the hypothalamus to the negative feedback effect of oestrogen and of the pituitary to the stimulatory effect of LRF (Rebar, Judd, Yen, Rakoff, Vandenberg and Naftolin, 1976). The possibility of a specific pituitary-FSH-release inhibiting substance (FRIS, see section 6.4) of follicular origin must also be considered, particularly in view of the fact that FSH secretion in response to LRF appears to be unimpaired in P.C.O. (Figure 7.4; Duignan et al, 1975).
Although there is little doubt that the production rates of androstenedione and testosterone are elevated in patients with P.C.O. (Lipsett et al, 1968), controversy exists as to the source of this increased androgen production. Prunty (1966) has suggested that in some cases of P.C.O. the primary pathology may be adrenal in origin, a view apparently substantiated by the observation that the elevated urinary 17-ketosteroid levels found in a proportion of women with P.C.O. can be suppressed with dexamethasone (Mahesh and Greenblatt, 1964). However, the normal adrenal secretes significant amounts of androstenedione which can be suppressed by dexamethasone (Rosenfield, Ehrlich and Cleary, 1972). It seems more likely therefore that in most patients with idiopathic P.C.O. the excess androgen arises from increased ovarian androgen secretion under the influence of the elevated level of LH (Kirschner and Jacobs, 1971). In the present study peripheral androstenedione concentrations were measured in 3 patients (one of whom is illustrated in Figure 7.7) following injection of LRF. In all three patients the increase in LH was followed by a rise in plasma androstenedione which in the patient shown in Figure 7.7 became detectable after 25 min. and preceded the rise in oestrone and that of 17β-oestradiol.

The increased plasma level of androstenedione (and hence of oestrone since the latter arises to a large extent from peripheral conversion of the former, Siiteri and MacDonald, 1973) would tend to create a vicious circle by suppressing FSH release (and hence normal follicular development) while at the same time, facilitating the release of LH (and hence ovarian androgen production). The question however remains what factor(s) might conceivably trigger off this cascade-effect. This problem is all the more important in view of the fact that permanent therapeutic success in this condition can be expected only when the
LRF TEST IN POLYCYSTIC OVARY SYNDROME

FIGURE 7.7: FSH, LH, 17β-oestradiol, oestrone and androstenedione levels during LRF-test in a patient with polycystic ovary syndrome (subject P.C.O. 02).
primary cause has been identified. Therapeutic measures such as dexamethasone treatment or ovarian wedge resection may offer temporary improvement by reducing adrenal, respectively ovarian androstenedione production, but this effect cannot be permanent if the primary cause is left untreated. What this primary cause may be, remains to be elucidated. In overweight women with P.C.O., it seems likely that the obesity promotes the development of polycystic ovaries since the percentage conversion of androstenedione to oestrone increases with increasing body-weight (Siiteri and MacDonald, 1973). It may not be surprising therefore that in a number of P.C.O. patients dieting appears to be far more effective than ovarian wedge resection (Jeffcoate, 1964). As for the remaining, not obese women with polycystic ovaries, it is tempting to suggest that a temporary increase in adrenal androgen production may be the trigger which initiates the cascade of endocrine events leading to the formation of the "polycystic, enlarged ovaries with the pearl-white, smooth thick capsule" (Jeffcoate, 1964).

7.1.5 Summary
Hypothalamic-pituitary-ovarian relationships were studied in a group of 12 women with polycystic ovary syndrome (P.C.O.) under basal conditions and using dynamic tests (oestrogen provocation, LRF and Clomiphene test). Results were compared to a control group of 6 regularly menstruating women studied during the early-mid follicular phase of the cycle.
Serial measurements of urinary total oestrogen and pregnanediol excretion enabled us to detect 6 spontaneous ovulatory cycles in P.C.O. patients during a 22-months' observation period. Ovulatory cycles in P.C.O. patients ("ovulatory P.C.O.") were associated with a decrease in
LH, but not in oestrone or androstenedione, during the early part of the subsequent cycle. In patients who did not ovulate within 4-6 weeks prior to the collection of blood samples ("anovulatory P.C.O.") circulating basal levels of LH, oestrone and androstenedione were significantly higher and levels of FSH significantly lower than those seen in control women. Elevated LH levels in P.C.O. patients were associated with an increase in magnitude but not in frequency of pulsatile LH release.

Ethinyloestradiol administration suppressed and, in 8 out of 9 anovulatory P.C.O. patients, subsequently stimulated the release of LH, indicating that negative and positive feedback mechanisms are unimpaired in this disorder. The magnitude of the oestrogen-induced LH release was similar to that of controls but peak LH levels occurred 2½ hours earlier.

Pituitary FSH secretion in response to LRF was similar in ovulatory and anovulatory P.C.O. patients and in controls. LH responses in ovulatory P.C.O. patients were also identical to those of the control group but were significantly greater in the anovulatory P.C.O. group. Both patients to whom Clomiphene was given responded with an ovulatory cycle thus confirming the integrity of hypothalamic-pituitary feedback mechanisms.

The results are discussed and it is suggested that the abnormal pattern of gonadotrophin secretion in P.C.O. may be secondary to the abnormalities in steroid hormone secretion.
7.2 Secondary amenorrhoea

7.2.1 Background

Although the hypothalamus and/or pituitary may be destroyed by structural disease, by far the commonest cause of pathological secondary amenorrhoea in women of reproductive age is a functional failure of the hypothalamic-pituitary unit with secondary ovarian failure (for recent review see e.g. Jacobs, 1976). In these instances, the absence of regular follicular growth points to an abnormality in H.P.O. relationships and, more specifically, to a disturbance of the normal function of the negative feedback loop, the cause of which however is unknown.

Serial measurements of urinary oestrogen and pregnanediol excretion were amongst the first hormonal investigations which suggested that cessation of menstrual function was probably a manifestation of a functional disturbance of the H.P.O. axis. In women with secondary amenorrhoea, urinary oestrogen levels may be steady or fluctuating with a high or low baseline. Patients with higher oestrogen excretion tended to have a spontaneous recurrence of menstrual function, while those with persistently low oestrogen excretion (<10 mg/24 hours) usually remained amenorrhoeic (Brown and Matthew, 1962).

In general, basal gonadotrophin levels in plasma appear to mirror peripheral oestrogen levels (Akande, Bonnar, Carr, Corker, Dutton, MacKinnon and Robinson, 1972). Patients with FSH and LH concentrations in the normal range are more likely to ovulate in response to Clomiphene than those with low and unvarying gonadotrophin levels (Dignam, Parlow and Daane, 1969) suggesting that hypothalamic sensitivity for the negative feedback effect of oestrogen is higher in the latter group. The mechanism(s) underlying these differences in hypothalamic sensitivity and
hence in circulating hormone levels are uncertain, although they do not appear to be related to the factor(s) which apparently precipitated the hypothalamic-pituitary dysfunction.

Apart from the abnormalities in FSH and LH secretion, some patients with secondary amenorrhoea have elevated basal levels of prolactin with or without galactorrhoea. Recognition of this subgroup, which comprises between 15-20% of patients with idiopathic secondary amenorrhoea (Franks, Murray, Jequier, Steele, Nabarro and Jacobs, 1975; Bohnet, Dahlén, Wuttke and Schneider, 1976) is important since the hyperprolactinaemia may be a manifestation of organic pituitary disease (Friesen, Hwang, Guyda, Tolis, Tyson and Myers, 1972).

Numerous studies have been made on the pituitary gonadotrophin response to IRF in patients with hypothalamic-pituitary failure. In almost all women with secondary amenorrhoea, natural or synthetic IRF produces a rise in LH and usually in FSH also (Schally, Kastin and Arimura, 1972). "Non-responders" can be converted to "responders" by repeated administration of synthetic IRF suggesting that the impaired pituitary sensitivity to IRF in these conditions is due to defective endogenous IRF synthesis and/or release (Yoshimoto, Moridera and Imura, 1975).

In summary, it seems likely that the primary pathology in women with functional amenorrhoea is located in the hypothalamus, a view which is further substantiated by the observation that other hypothalamic functions such as the induction of a LH surge in response to oestrogen, are often impaired in women with secondary amenorrhoea (Shaw, Butt and London, 1975).

There is however very little information on the incidence, if any, of other possible manifestations of hypothalamic dysfunction apart
from the abnormalities in gonadotrophin secretion. In patients with anorexia nervosa, evidence has been presented which indicates that the regulation of thyroid and adrenal function may be disturbed (Reichlin, 1974a) but, when we reviewed the literature in 1973, we were unable to find any such information in a series of unselected patients with secondary amenorrhoea.

It was therefore decided to initiate a prospective study of all consenting patients presenting at the Gynaecological Endocrine Clinic with the complaint of secondary amenorrhoea of at least 6 months' duration. The results reported in this thesis relate to the first fifty patients, screened between January 1974 and January 1976.

7.2.2 Design of the study

All patients with a history of six months' amenorrhoea referred to the Gynaecological Endocrine clinic were screened for entry into the investigation.

Preliminary screening included general and pelvic examination, measurement of plasma-thyroxine (T₄), full blood count, lateral and frontal X-ray of the pituitary fossa and an initial assessment of pituitary-ovarian activity by the measurement once per week over a 4 week period of the urinary excretion of total oestrogen, pregnanediol, FSH and LH. Patients with evidence of cyclical ovarian activity or elevated urinary gonadotrophin levels were excluded from the series.

A combined hypothalamic-pituitary function test was performed at the completion of the initial tracking. On Day 1 of this test (Figure 7.8) the patient arrived at the clinic between 8.00-9.00 AM fasting and a butterfly needle was inserted into an antecubital vein as described in Chapter 4. After a rest period of one hour 7 peripheral blood samples
PITUITARY FUNCTION TEST

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>-180</td>
<td>INSERT CANNULA</td>
</tr>
<tr>
<td>-120</td>
<td>7 x 15 MINUTE SAMPLES FOR EPISODIC GONADOTROPHIN RELEASE</td>
</tr>
<tr>
<td>-30</td>
<td>SAMPLE</td>
</tr>
<tr>
<td>-5</td>
<td>SAMPLE</td>
</tr>
<tr>
<td>0</td>
<td>INJECT LRF 50μg TRH 200μg INSULIN 0.15 U/kg</td>
</tr>
<tr>
<td>+20</td>
<td>SAMPLE</td>
</tr>
<tr>
<td>+30</td>
<td>SAMPLE</td>
</tr>
<tr>
<td>+60</td>
<td>SAMPLE</td>
</tr>
<tr>
<td>+90</td>
<td>SAMPLE</td>
</tr>
</tbody>
</table>

Measure:- Glucose, Oestradiol, Cortisol, Prolactin, LH, FSH, GH, and TSH in each sample.

FIGURE 7.8: Protocol of the combined hypothalamic-pituitary function test performed in patients with secondary amenorrhoea. For explanation see text.
were collected at 15 minute intervals. FSH and LH were measured in each of these samples and 17β-oestradiol in every second sample. Glucose, 17-fluorogenic corticosteroids, TSH, hGH, prolactin and androstenedione were also measured in the last sample as well as in the subsequent sample which was taken 5 minutes before the intravenous injection of 50 µg IRF, 200 µg TRF and 0.15 U/kg body weight insulin. Further blood samples were then taken at +20, +30, +60 and +90 minutes and assayed for FSH, LH, TSH and prolactin. Glucose, 17-fluorogenic corticosteroids and hGH were measured in the +30, +60 and +90 minutes samples and androstenedione in the +60' or +90' sample.

Starting on Day 4 of the test, Clomiphene was given in a dose of 2 x 50 mg per day for 5 days. On the last day of Clomiphene administration (i.e. day 8 of the test) seven peripheral blood samples were taken from an indwelling catheter for measurement of FSH, LH and 17β-oestradiol. Patients were fasting during this procedure and had been allowed one hour of supine rest prior to the collection of samples. Ovarian responses to Clomiphene administration were monitored by measuring urinary total oestrogen and pregnanediol excretion in 24-hour urine specimens collected on days 0, 7, 14, 21 and 28 of the test (Figure 7.9). Ovarian responses were classified into (a) no response (N) i.e. no significant rise in urinary total oestrogen excretion; (b) abortive response (A) in which the total oestrogen excretion rose to a value below 25 µg/24 hours but there was no ovulation; (c) anovulatory response (A) in which the total oestrogen excretion rose above 25 µg/24 hours but no ovulation occurred as indicated by the persistently low excretion of pregnanediol and (d) an ovulatory response (O) in which a rise in urinary oestrogen levels was followed by an increase in pregnanediol excretion above 1.5 mg/24 hours.
FIGURE 7.9: Protocol of Clomiphene test performed in patients with secondary amenorrhoea. For explanation see text.
Results were compared to those obtained in a control group consisting of eight women with a history of regular menstrual cycles and who underwent the same investigative procedures except for the preliminary screening. The criteria used in the selection of these women have been discussed in Chapter 4. In controls, Day 1 of the test corresponded to Day 3-5 of the cycle, but Clomiphene administration was started on the next day (i.e. Day 2 of the test) to approximate as nearly as possible the endocrine environment of the patients' group at the start of therapy.

7.2.3 Results

(a) Epidemiological data

The age-distribution of the patient material is illustrated in Figure 7.10. Ages ranged from 16 to 35 years with a mean (± S.E.M.) of 23.9 ± 0.7 years (controls 32.3 ± 1.5 years; p < 0.05) and amenorrhoea had been present for periods of up to 13 years (mean ± S.E.M.: 30.7 ± 4.4 months).

In eleven patients (22%) (Table 7.7) the onset of amenorrhoea coincided with a considerable reduction (> 7kg) in body weight and 16 patients (32%) became amenorrhoeic after using the contraceptive pill for 33.9 ± 7.1 (S.E.M.) months. A combination of weight loss and pill-use was present in five patients (10%). In 3 women (6%) menstrual function had ceased following an easily identifiable psychological trauma (e.g. death of close relative, emigration) while in another 3 patients (6%) menses had not returned following childbirth. Of the remaining 12 women (24%), one patient had a markedly enlarged sella turcica and one patient had previously been treated with pituitary irradiation but had a normal sized pituitary fossa at the time of investigation. No precipitating cause could be identified in the other 10 women.
FIGURE 7.10: Age-distribution (2 year classes) of patients with secondary amenorrhoea.
**TABLE 7.7:** Etiology, previous menstrual history, duration of amenorrhoea and associated symptoms in 50 women with secondary amenorrhoea. Values represent the number of patients in each group and, between brackets, the incidence of a given symptom expressed as a percentage of the number of patients within each etiological group.

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Others</th>
<th>Weight Loss + Pill</th>
<th>Psychological</th>
<th>Pregnancy</th>
<th>Etiological History</th>
<th>MENSTRUAL HISTORY</th>
<th>Duration of AENOMORRHOEA (mean ± SD in months)</th>
<th>GALACTORRHOEA</th>
<th>Underweight</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>12</td>
<td>16</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>7 (64%)</td>
<td>4 (36%) 3.6 ± 1.9 9.3 17.1 ± 3.3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Weight Loss (&gt; 7 kg)</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>7 (64%)</td>
<td>2 (13%) 4 ± 2.2 7.2 ± 1.0 4.0 ± 2.4 13.0 ± 2.7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Weight Loss &amp; Pill</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4 (33%)</td>
<td>2 (67%) 4 ± 2.2 7.2 ± 1.0 4.0 ± 2.4 13.0 ± 2.7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Psychological</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1 (33%)</td>
<td>1 (33%) 1 ± 0.3 ± 2.2 22.7 ± 10.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MENSTRUAL HISTORY</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4 (33%)</td>
<td>2 (67%) 4 ± 2.2 7.2 ± 1.0 4.0 ± 2.4 13.0 ± 2.7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Irregular</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1 (33%)</td>
<td>1 (33%) 1 ± 0.3 ± 2.2 22.7 ± 10.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Regular</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4 (33%)</td>
<td>2 (67%) 4 ± 2.2 7.2 ± 1.0 4.0 ± 2.4 13.0 ± 2.7</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Values significantly (p < 0.01) higher than that of control population of student nurses.*
Twenty-seven patients (5.4\%) had a history of irregular menstruation. In a population of student nurses of comparable age (19.1 ± 0.4 years) the incidence of menstrual irregularities was 30\% (14/47) (J. Farboosingh, unpublished observation) which is significantly lower than that found in the groups "weight loss" ($64\%; \chi^2 = 7.38; p < 0.01$) and "others" ($67\%; \chi^2 = 7.68; p < 0.01$) but not significantly different from the incidence in the group of women who became amenorrhoeic after stopping the contraceptive pill and whose age at the start of pill-use (mean ± S.E.M.: 20.2 ± 0.5 years) was comparable to that of the control nurses' population.

In general, women with post-pill amenorrhoea were referred sooner for investigation than those in whom the onset of amenorrhoea was unrelated to the use of contraceptive medication. Galactorrhoea was present in all 3 women with postpartum amenorrhoea (Chiari-Frommel syndrome), in the patient with the irradiated pituitary tumour (Forbes-Albright syndrome) and in one further patient with amenorrhoea of unknown cause and a normal sized sella turcica (Argonz-del Castillo syndrome).

In seven women body weight was more than 15\% lower than the average weight of women of similar age and height (Scientific Tables, Geigy, p.711).

(b) Endocrine data

For analysis of endocrine data, the 50 patients with secondary amenorrhoea were divided into 4 groups on the assumption that in order for spontaneous follicular development to occur, basal gonadotrophin and prolactin levels had to approximate those found in regularly menstruating women during the early follicular phase of the cycle. In controls, basal prolactin levels were less than 20 ng/ml, FSH was greater than or equal to 3.7 mU/ml, LH was greater than or equal to 1.9 mU/ml and the FSH/LH
ratio was always greater than 1 (Figure 7.11, see also Figure 1.2). Accordingly, patients were divided into:

1. Group 1 (21 patients): $\text{FSH} < 3.7 \text{ mU/ml and/or LH} < 1.9 \text{ mU/ml}$
   - normal basal prolactin level

2. Group 2 (12 patients): $\text{FSH} > 3.7 \text{ mU/ml and LH} > 1.9 \text{ mU/ml}$
   - FSH/LH ratio $> 1$
   - normal basal prolactin level

3. Group 3 (9 patients): as group 2 but with FSH/LH ratio $< 1$


The estimates of basal gonadotrophin levels in patients and controls were based on the means of the seven samples collected at 15 minute intervals on day 1 of the test and basal prolactin levels on the mean of the 2 samples taken at -30 and -5 minutes on the same day (Figure 7.8).

As can be seen in Figure 7.11, most patients of Group 1 had low levels of both FSH and LH and all but one a FSH/LH ratio greater than 1. None of the amenorrhoeic women had a circulating FSH level which was higher than the upper limit of the range found in controls (10.6 mU/ml) but five patients had an elevated LH level ($\text{LH} > 7.8 \text{ mU/ml}$).

A comparison between the classification based on basal gonadotrophin levels and that based on the precipitating cause of the amenorrhoea (Table 7.7) is illustrated in Table 7.8.

Eleven of the 16 patients (i.e. 70%) in whom the cessation of menstrual function was related to weight loss with or without concomitant use of the pill, had low gonadotrophin levels (Group 1). Elevated prolactin levels were encountered in 2 of the 3 women with Chiari-Frommel syndrome, in 1 patient with post-pill amenorrhoea and in 5 of the women with amenorrhoea of unknown cause including both patients with previous or recent evidence of pituitary tumour.
Figure 7.11: Basal gonadotrophin levels in patient with secondary amenorrhoea and normal women during the early follicular phase of the cycle.
TABLE 7.8: Classification of women with secondary amenorrhoea. Values between brackets indicate the number of patients with galactorrhoea.

<table>
<thead>
<tr>
<th></th>
<th>GROUP</th>
<th></th>
<th></th>
<th></th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>WEIGHT LOSS</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>PILL</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>WEIGHT LOSS+ PILL</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>PSYCHOLOGICAL</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>PREGNANCY</td>
<td>1(1)</td>
<td>0</td>
<td>0</td>
<td>2(2)</td>
<td>3</td>
</tr>
<tr>
<td>OTHERS</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>5(2)</td>
<td>12</td>
</tr>
<tr>
<td>TOTAL</td>
<td>21</td>
<td>12</td>
<td>9</td>
<td>8</td>
<td>50</td>
</tr>
</tbody>
</table>
Basal measurements

Results are shown in Table 7.9.

Peripheral blood count and plasma thyroxine level were normal in all patients studied.

Mean height was similar in patients and controls. Patients with elevated prolactin levels (Group 1) and control women were of comparable age and body weight. Mean weight of Group 1 was significantly lower than that of Group 2 and 3 even though patients in these 3 groups were on average of similar age.

Urinary total oestrogen and pregnanediol excretion in the patients' groups during the 4 weeks prior to the hypothalamic-pituitary function test were compared to the values found in normal women on day 0 of the test (i.e. day 2-4 of the cycle). Oestrogen levels in Group 1 were significantly lower than those of the other groups and were also lower in Group 4 when compared to controls.

There were no significant differences between amenorrhoeic women in urinary gonadotrophin excretion except for the presence of higher LH levels in Group 3 as compared to Group 1.

Circulating basal concentrations of 17β-oestradiol on day 1 of the test mirrored the urinary oestrogen excretion during the preceding 4 weeks. Thus, 17β-oestradiol levels were lower in groups 1 and 4 than in groups 2 and 3. While the low plasma 17β-oestradiol levels in group 1 could be explained by the suppressed gonadotrophin concentrations in this group, a similar mechanism could not account for the reduced oestrogen levels in patients with elevated prolactin since basal gonadotrophins in this group were similar to those of control women. Basal levels of circulating gonadotrophins in the other amenorrhoeic group were as anticipated from the criteria used in composing these groups.
<table>
<thead>
<tr>
<th>AGE (yrs)</th>
<th>HEIGHT (cm)</th>
<th>WEIGHT (kg)</th>
<th>TOTAL OESTROGEN (µg/24h)</th>
<th>URINE PREGNADIOL (mg/24h)</th>
<th>FSH (IU/24h)</th>
<th>LH (IU/24h)</th>
<th>17β-OESTRADIOL (pg/ml)</th>
<th>PLASMA FSH (mU/ml)</th>
<th>PLASMA LH (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROLS</td>
<td>32·3±1·5</td>
<td>165·9±1·5</td>
<td>64·9±3·6</td>
<td>7·14±0·90</td>
<td>ND</td>
<td>ND</td>
<td>45±5</td>
<td>5·49±0·86</td>
<td>4·00±0·66</td>
</tr>
<tr>
<td>GROUP 1</td>
<td>22·7±1·0</td>
<td>162·3±1·2</td>
<td>49·9±1·3</td>
<td>2·11±0·22</td>
<td>2·66±0·28</td>
<td>33±0·3</td>
<td>28±2</td>
<td>2·96±0·34</td>
<td>1·36±0·20</td>
</tr>
<tr>
<td></td>
<td>(a,e)</td>
<td></td>
<td></td>
<td>(a,c,d,e)</td>
<td></td>
<td></td>
<td></td>
<td>(a,c,d,e)</td>
<td></td>
</tr>
<tr>
<td>GROUP 2</td>
<td>23·4±1·6</td>
<td>162·4±1·6</td>
<td>54·8±2·1</td>
<td>5·07±0·91</td>
<td>3·13±0·54</td>
<td>12·8±5·9</td>
<td>46±6</td>
<td>5·16±0·39</td>
<td>3·31±0·35</td>
</tr>
<tr>
<td></td>
<td>(a)</td>
<td></td>
<td></td>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
<td>(b)</td>
<td></td>
</tr>
<tr>
<td>GROUP 3</td>
<td>23·8±0·6</td>
<td>161·6±1·7</td>
<td>55·2±1·9</td>
<td>6·65±2·26</td>
<td>2·30±0·38</td>
<td>62·5±10·8</td>
<td>55±6</td>
<td>5·47±0·38</td>
<td>8·21±1·06</td>
</tr>
<tr>
<td></td>
<td>(a)</td>
<td></td>
<td></td>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
<td>(b,e)</td>
<td></td>
</tr>
<tr>
<td>GROUP 4</td>
<td>27·6±2·0</td>
<td>164·8±2·4</td>
<td>58·6±3·0</td>
<td>3·77±0·89</td>
<td>2·38±0·27</td>
<td>39·3±3·7</td>
<td>31±8</td>
<td>4·78±0·53</td>
<td>3·89±1·20</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
<td>(a,b)</td>
<td></td>
<td></td>
<td></td>
<td>(b)</td>
<td></td>
</tr>
</tbody>
</table>

a = significantly (p < 0·05) different from controls
b =           "       "       "       "       group 1
      "       "       "       "       group 2
c =           "       "       "       "       group 3
d =           "       "       "       "       group 4
e =           "       "       "       "       group 4
ND =          not done

TABLE 1.2: Means (± S.E.M.) of basal measurements in secondary amenorrhea and control women.
FSH and LH responses to LRF

Gonadotrophin responses to LRF were analysed after logarithmic transformation of the data and are shown in Figure 7.12.

In general, pituitary FSH and LH secretion following LRF was related to the basal level of these hormones. Thus, the magnitude of the FSH peak was similar to that of controls in patients with normal basal FSH, but significantly reduced in those with low FSH levels (Group 1). Peak FSH concentrations in this latter group were reached at 56.2 ± 4.9 min (mean ± S.E.M.) after LRF injection as compared to 23.8 ± 1.8 min in the control group (p < 0.001). LH responses on the other hand were significantly greater in Group 3 but reduced in Group 1 when compared to controls. In patients with hyperprolactinaemia, magnitude (30.1 ± 4.4 mU/ml) and timing (31.3 ± 4.4 min) of the LH peak were not significantly different from controls (22.1 ± 1.7 mU/ml and 28.8 ± 1.3 min respectively) but LH release appeared to be more sustained.

TSH responses to TRF

There was no significant difference in plasma TSH following TRF (Figure 7.13) between patients or between patients and controls. TSH responses tended to be lower in patients with low circulating levels of 17β-oestradiol (Groups 1 and 4) but no direct correlation between these two parameters could be demonstrated.

hGH response to Insulin

Results are shown in Figure 7.14.

In patients with low gonadotrophin levels (Group 1), basal concentrations of hGH (mean ± S.E.M.: 5.8 ± 1.0 µU/ml and 4.2 ± 0.6 µU/ml at -30 and -5 minutes respectively) were significantly elevated but hGH responses to Insulin (22.6 ± 3.8 µU/ml at +60 min; 22.0 ± 3.6 µU/ml
RESPONSE TO LRF IN SECONDARY AMENORRHOEA

FIGURE 7.12: FSH and LH response (means) to intravenous injection of 50 μg LRF in patients with secondary amenorrhoea. Shaded area represents range (mean ± 1 SD) in normal women during the early follicular phase of the cycle (Day 3-5). Values which are significantly different from those of controls are indicated by asterisks.
RESPONSE TO TRH IN SECONDARY AMENORRHOEA

![Graph showing TSH response to intravenous injection of 200 µg TRF in secondary amenorrhea. Shaded area represents the range (mean ± 1 SD) in normal women tested on Day 3-5 of the cycle.]

**FIGURE 7.13:** TSH response (means) to intravenous injection of 200 µg TRF in secondary amenorrhea. Shaded area represents the range (mean ± 1 SD) in normal women tested on Day 3-5 of the cycle.
FIGURE 7.14: Insulin-induced growth hormone secretion (means) in secondary amenorrhoea. Shaded area represents range (mean ± 1SD) in regularly menstruating women on Day 3-5 of the cycle. Values which are significantly different from control values are indicated by asterisks.
at + 90 min) significantly reduced when compared to controls (-30 min: 1.0 ± 0.9 μU/ml, p < 0.01; -5 min: 0.9 ± 0.8 μU/ml; p < 0.005; +60 min: 64.2 ± 35.6 μU/ml, p < 0.001 and +90 min: 82.7 ± 44.3 μU/ml, p < 0.001).

Circulating hGH levels in Group 4 were also significantly lower at +60 min (25.9 ± 18.8 μU/ml, p < 0.02) and at +90 min (32.9 ± 30.7 μU/ml, p < 0.025) but basal concentrations were in the normal range.

The impaired hGH elevations in Groups 1 and 4 are probably a reflection of the lower circulating 17β-oestradiol levels in these patients since both variables were positively correlated (y = 3.89 + 0.78 x where x = basal 17β-oestradiol level in pg/ml and y = hGH concentration in μU/ml at +90 minutes; r = 0.1.936; n = 97; p < 0.001).

Prolactin response to TRF and Insulin

The changes in peripheral prolactin concentrations following injection of TRF and Insulin are illustrated in Figure 7.15.

In controls and in women with normoprolactinaemic secondary amenorrhoea administration of TRF resulted in an immediate rise in peripheral prolactin, the magnitude of which was related to the basal 17β-oestradiol level (y = 49.98 + 0.76x where x = plasma 17β-oestradiol in pg/ml and y = plasma prolactin in ng/ml at +20 min; r = 0.3557; n = 47; p < 0.05). Following this, mean peripheral prolactin levels remained stable or increased further to a secondary peak at +60 minutes before declining to lower values at +90 min. The presence or absence of this secondary rise in plasma prolactin concentrations which was probably hypothalamic in origin since it coincided with significant elevations in hGH and cortisol, did not appear to be related to the degree of hypoglycaemia but was positively correlated with endogenous 17β-oestradiol levels (y = 64.00 + 0.91x where x = plasma 17β-oestradiol in pg/ml and y = plasma prolactin in ng/ml at +60 minutes).
**Figure 7.15:** Mean changes in peripheral prolactin concentrations in women with secondary amenorrhea following injection of TRF and Insulin. Shaded area represents the range (mean ± 1SD) in normal women during the early follicular phase of the cycle (Day 3-5). Values which were significantly different from those of the control group are indicated by asterisks.
Of the eight patients with hyperprolactinaemia, only four showed a further rise in plasma prolactin in response to the provocative stimuli. Basal prolactin levels (mean ± S.E.M.: 54.1 ± 9.6 ng/ml) in "responders" (i.e. the two patients with Chiari-Frommel syndrome, the patient with irradiated pituitary tumour and a girl with idiopathic amenorrhoea) tended to be lower than those in "non-responders" (mean ± S.E.M.: 77.1 ± 9.6 ng/ml) but the difference was not significant.

Cortisol response to Insulin

In all four amenorrhoeic groups, basal 17-fluorogenic steroid levels (Figure 7.16) were significantly higher than those found in the control group but their response to insulin-hypoglycaemia was similar to that of controls. The increase in basal corticosteroid secretion was associated with a significant elevation in plasma androstenedione, (Figure 7.17), the levels of which were highest in the amenorrhoeic patients with raised LH levels (Group 3). Following insulin injection, plasma androstenedione concentrations in control women increased from 0.93 ± 0.12 ng/ml (mean ± S.E.M.) to 1.94 ± 0.26 ng/ml, a value which was not significantly different from the mean levels found in amenorrhoeic women.

Clomiphene test

The data with respect to episodic gonadotrophin release before and on the fifth day of Clomiphene administration are presented in Table 7.10.

Before Clomiphene, the pattern of episodic gonadotrophin release, and particularly that of LH, in patients with secondary amenorrhoea was related to the degree of intrinsic spontaneous hypothalamic-pituitary activity as reflected by the peripheral basal gonadotrophin level. Thus,
FIGURE 7.16: Mean changes in plasma 17-fluorogenic corticosteroids in women with secondary amenorrhoea following insulin injection. Shaded area represents the range (mean ± 1SD) in regularly menstruating women tested on Day 3-5 of the cycle and asterisks indicate significant differences from the levels found in control women.
FIGURE 7.17: Mean peripheral androstenedione levels in women with secondary amenorrhoea before and after hypothalamic pituitary function test. The range (mean ± 1SD) found in normal women (Day 3-5 of the cycle) is indicated by the shaded area. Basal androstenedione levels in women with secondary amenorrhoea were significantly higher than those in controls as indicated by the asterisks.
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FSH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Peak frequency (per 3 hours)</strong></td>
<td>0.25</td>
<td>0.50</td>
<td>0</td>
<td>1.10</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>Absolute peak magnitude (mU/ml) (mean)</strong></td>
<td>1.25</td>
<td>0.85</td>
<td>0.77</td>
<td>2.25</td>
<td>0.38</td>
</tr>
<tr>
<td><strong>Relative peak magnitude (%)</strong></td>
<td>23.9</td>
<td>26.2</td>
<td>30.4</td>
<td>67.2</td>
<td>37.4</td>
</tr>
</tbody>
</table>

|               |          |         |         |         |         |
| **LH**        |          |         |         |         |         |
| **Peak frequency (per 3 hours)** | 1.00     | 0.10    | 0.67    | 2.25    | 3.25    |
| **Absolute peak magnitude (mU/ml) (mean)** | 2.50     | 1.80    | 1.90    | 2.62    | 2.34    |
| **Relative peak magnitude (%)** | 37.3     | 126.6   | 37.3    | 188.1   | 37.3    |
pulsatile LH release was virtually absent in Group 1 but detectable albeit with reduced frequency when compared to controls, in patients with normal or elevated basal LH level (Group 2 and 3). It is of interest to note that episodic gonadotrophin secretion appeared to be suppressed in patients with hyperprolactinaemia even though basal gonadotrophin levels of this group were on average within the normal range.

The characteristics of pulsatile LH release during Clomiphene treatment were similar in all three groups (i.e. controls, Groups 2 and 3) in which follicular development was induced with this compound (see further).

Mean peripheral concentrations of FSH, LH and 17β-oestradiol on the last day of Clomiphene administration and the corresponding pre-treatment values are illustrated in Figure 7.18.

In nine out of the 21 patients of Group 1, FSH and/or LH levels remained either unchanged or decreased during Clomiphene treatment. Mean gonadotrophin concentrations in this group during treatment remained significantly below those found in normal women and, with a few exceptions, normal follicular development was not induced as evidenced by the low levels of circulating 17β-oestradiol and of urinary total oestrogens during the follow-up period (Table 7.11). None of the patients of this group ovulated following Clomiphene although 5 women did have some menstrual blood loss.

Circulating levels of FSH, LH and 17β-oestradiol in the patients of Group 2 were similar to those of controls during Clomiphene therapy. In nine out of the twelve women, the profile of urinary total oestrogen excretion was consistent with normal follicular development but ovulation failed to occur in seven patients as indicated by the persistently low excretion of pregnanediol.
FIGURE 7.18: Mean plasma level of FSH, LH and 17β-oestradiol before and on the fifth day of Clomiphene treatment (5 x 100 mg) in patients with secondary amenorrhoea and normal women (shaded area; mean ± 1SD).
TABLE 7.11: Ovarian response to Clomiphene. Responses were classified as described in the section on the design of the study. The value between brackets refers to the number of patients in whom menstrual bleeding occurred.

<table>
<thead>
<tr>
<th>RESPONSE</th>
<th>CONTROLS</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>TOTAL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>0</td>
<td>7 (0)</td>
<td>1 (0)</td>
<td>0</td>
<td>2 (0)</td>
<td>10 (0)</td>
</tr>
<tr>
<td>ABORTIVE RESPONSE</td>
<td>0</td>
<td>11 (3)</td>
<td>2 (2)</td>
<td>0</td>
<td>2 (0)</td>
<td>15 (5)</td>
</tr>
<tr>
<td>ANOVULATORY RESPONSE</td>
<td>0</td>
<td>3 (2)</td>
<td>7 (6)</td>
<td>2 (2)</td>
<td>1 (0)</td>
<td>13 (10)</td>
</tr>
<tr>
<td>OVULATORY RESPONSE</td>
<td>8 (8)</td>
<td>0</td>
<td>2 (2)</td>
<td>7 (7)</td>
<td>1 (1)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>UNKNOWN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>8 (8)</td>
<td>21 (5)</td>
<td>12 (10)</td>
<td>9 (9)</td>
<td>8 (1)</td>
<td>50 (25)</td>
</tr>
</tbody>
</table>

*Not including controls
In Group 3, plasma LH during Clomiphene was significantly higher than in controls but similar to the levels observed in patients with polycystic ovary syndrome on this type of treatment (see e.g. Figures 7.5 and 7.6). All patients of this group showed a significant rise in urinary oestrogen excretion which was followed, in seven, by a rise in urinary pregnanediol levels above 1.5 mg/24 hours.

In women with hyperprolactinaemia, Clomiphene treatment resulted in a rise of both gonadotrophins to values which were similar to those of controls. However, the ovarian response to this compound, as reflected by peripheral 17β-oestradiol levels during therapy and by the urinary total oestrogen excretion following therapy was poor and only 1 patient of this group had pregnanediol levels in the luteal phase range (>1.5 mg/24 hours).

Six-month follow-up data

At the time of writing 6-month follow-up data on spontaneous return of menstrual function and the results of ovulation-induction therapy is available for 38 women (Table 7.12).

Of the 31 women not on ovulation induction therapy, 13 (42%) had experienced one or more spontaneous episodes of vaginal bleeding, and 5 (16%) menstruated on a regular basis. Sixty-four percent of patients (9 out of 14) with basal gonadotrophin and prolactin levels in the normal range (i.e. Groups 2 and 3) had evidence of spontaneous ovarian activity during the follow-up period as compared to 21% in Group 1 and 33% in Group 4.

Of the patients on ovulation-induction therapy for infertility, both women of Group 1 and the patient of Group 2 consistently failed to ovulate on 3 or more successive courses of Clomiphene and were therefore
TABLE 7.12: Spontaneous return of menstrual function within 6 months after testing hypothalamic-pituitary function in patients with secondary amenorrhea.

<table>
<thead>
<tr>
<th></th>
<th>GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Number of patients</td>
<td>16(76)*</td>
</tr>
<tr>
<td>Not menstruating</td>
<td>11</td>
</tr>
<tr>
<td>Menstruating</td>
<td></td>
</tr>
<tr>
<td>regular</td>
<td>3</td>
</tr>
<tr>
<td>irregular</td>
<td>1</td>
</tr>
<tr>
<td>Ovulation induction</td>
<td>2</td>
</tr>
</tbody>
</table>

* percentage of original group
treated with exogenous gonadotrophins (1 patient) or Clomiphene + hCG (2 patients). Ovulation was successfully induced with Clomiphene alone in the patient of Group 3 and with 2-Bromo-α-ergocryptine in two of the patients of Group 4. The remaining patient of this group was treated with exogenous gonadotrophins.

7.2.4 Discussion

In view of the different criteria used by individual investigators in classifying women with secondary amenorrhoea on an aetiological basis, it is virtually impossible to compare the composition of our patient population with that of recently published larger series (e.g. Franks, Murray, Jequier, Steele, Nabarro and Jacobs, 1975; Ingerslev, Jeppesen and Ramsing, 1976). However, the fact that the incidence of post-pill amenorrhoea in the present study was similar to that of other workers (e.g. Ingerslev et al, 1976) suggests that the patient material of the present study was probably not biased in any particular direction and hence may be considered as being fairly representative of the group of women with secondary amenorrhoea, or at least of those who seek medical advice. These two populations of amenorrhoeic women may indeed not necessarily coincide since our data (Table 7.7) tends to indicate that women with post-pill amenorrhoea (or their general practitioners) are more anxious about their amenorrhoeic state than e.g. patients in whom the cessation of menstrual function was precipitated by (usually self-imposed) weight loss. It seems not unreasonable to assume therefore that a certain number of these latter women may not be represented in study-material based on patients attending a gynaecological clinic.

Despite the widespread interest devoted to the syndrome of post-pill amenorrhoea since the original description by Shearman (1966), its cause remains obscure. Irregular menses prior to the institution of oral
contraceptive therapy, late onset of menarche, length of time on oral contraceptive agents and the use of combined oral contraceptives have all been suggested as predisposing factors (Evrard, Buxton and Erickson, 1976).

In their review of the literature on the etiology of post-pill amenorrhea, Buttram, Vanderheyden, Besch and Acosta (1974) reported that 37.3% out of 271 cases published, had a history of irregular menses prior to starting oral contraception. Since, according to these authors, the incidence of irregular menses and amenorrhea in reproductive women is about 17% (an estimate based on Israel, 1967), Buttram et al (1974) concluded that women with an irregular menstrual history are more susceptible to developing post-drug amenorrhea. However, the incidence of irregular cycles given by Israel (1967) relates to females of all reproductive age-groups and hence grossly underestimates that found in adolescents in whom menstrual irregularities are far more common as illustrated by the pronounced variation in cycle length of postmenarchial girls (Treloar, Boynton, Behn and Brown, 1967). In this study, 44% of girls with post-pill amenorrhea had a history of irregular cycles before the start of contraceptive medication at 20.2 ± 0.5 years of age. This incidence was not significantly different from that (30%) found in a control population of menstruating student nurses who were of comparable age (mean ± S.E.: 19.1 ± 0.4 years; p > 0.05).

Since it seemed reasonable to assume that the endocrine status of patients with secondary amenorrhea in association with stopping the combined contraceptive pill, weight loss, pregnancy, stress etc. might not necessarily be unique to the precipitating factor (an assumption which proved to be correct after preliminary analysis of the data), it was decided to classify the patients on the basis of an endocrine parameter rather than an etiological basis. For this purpose a variety of
endocrine measurements could theoretically have been used but since one of the aims of the present study was to try and identify the minimum of diagnostic investigations required for successful management of a patient presenting with amenorrhoea, it seemed desirable to select a simple hormonal criterion. Patients were therefore classified on the basis of their basal prolactin and gonadotrophin levels, the measurement of which seemed essential in routine gynaecological practice if only to exclude primary ovarian failure and to select patients for bromocriptine therapy.

Comparison of the patients' distribution according to this criterion with the classification based on the precipitating factor (Table 7.8) indicated that although none of the etiological causes was related uniquely to any of the endocrine subgroups, most of the patients with weight loss-associated amenorrhoea (with or without concomitant use of the pill) had circulating gonadotrophin levels below the normal range (Group 1). Although most of these patients as well as the remainder of this first group were not severely underweight at the time of testing, mean body weight of this group was significantly lower than that found in girls of comparable age but with normal gonadotrophin levels (Groups 2 and 3). This association between low pituitary gonadotrophin secretion, amenorrhoea and body weight is very reminiscent of the situation found in patients with anorexia nervosa (Jacobs, 1976). Most of the women in Group 1 had not undergone psychiatric evaluation and it is not possible therefore to know in how many of these patients the self-imposed weight loss was due to some form of psychiatric disturbance. However, it seems extremely unlikely that all women in this group suffered from anorexia nervosa. Consequently, it must be assumed that weight loss itself, even where present outside the context of anorexia nervosa, may affect basal gonadotrophin secretion.
The mechanism(s) underlying this causal relationship is (are) uncertain. Pituitary gonadotrophin secretion in response to LRF in these patients was significantly impaired which may have been due to the low circulating levels of 17β-oestradiol and/or to the lack of stimulation from endogenous LRF. The latter possibility seems the more likely since the reduced oestrogen levels are probably a result of the deficient pituitary gonadotrophin release rather than vice versa. This evidently implies that the primary defect in these patients is hypothalamic in origin, a conclusion supported by recent work of Warren, Jewelewicz, Dyrenfurth, Ans, Khalaf and Vande Wiele (1973). These authors also have demonstrated reduced basal pituitary gonadotrophin secretion and impaired LRF responsiveness in underweight patients. Pituitary responses to LRF could be restored to within the normal range however following prolonged LRF infusion which suggests that the pituitary deficiency in this condition is secondary to impaired hypothalamic LRF stimulation.

The present study has revealed several other abnormalities of hypothalamic-pituitary function in these patients. Episodic gonadotrophin release in these women was virtually absent and Clomiphene administration failed to induce a significant rise, indeed often caused a decline, in peripheral gonadotrophin levels. A similar paradoxical gonadotrophin response to Clomiphene has been described in prepubertal children and has been attributed to an increased hypothalamic negative feedback sensitivity for the intrinsic oestrogenic activity of this compound (Kulin, Grumbach and Kaplan, 1972).

The finding of significantly elevated basal growth hormone levels in patients of Group 1 was somewhat surprising. Although it is well recognised that severe forms of protein-calorie malnutrition (marasmus, kwashiorkor) and anorexia nervosa may lead to an increase
in basal growth hormone secretion (Pimstone, Becker and Hansen, 1972) and, conversely, that basal growth hormone levels are suppressed in obese people (Glick, 1969), there are to our knowledge no published data showing that minor degrees of weight loss may affect basal growth hormone levels. The mechanism(s) underlying this increase is (are) uncertain and may be a result of hypersecretion of the hormone, impaired peripheral degradation or a combination of both. Pimstone et al (1972) have suggested that the elevation in growth hormone might be an important compensatory factor for retaining protein in a person whose protein intake is meagre, but there is very little substantial evidence to support this view.

The impaired growth hormone response to insulin-induced hypoglycaemia of the patients in Group 1 is most likely a reflection of their hypo-oestrogenic state. It has been shown that administration of oestrogen to prepubertal children (Deller, 1970) or adult normal men (Merimee, Burgess and Rabinowitz, 1966) enhances growth hormone secretion following insulin or arginine and that in regularly menstruating women arginine-induced secretion is highest at midcycle (Merimee, Fineberg and Tyson, 1969). Our finding of a significant positive correlation between the circulating level of 17β-oestradiol and that of growth hormone after insulin is not inconsistent with the view that oestrogen may potentiate growth hormone responses to insulin but it cannot be concluded whether this is a result of a stimulatory effect at the pituitary or hypothalamic level or both.

Since both TRF (Bowers, Friesen, Hwang, Guyda and Folkers, 1971) and insulin-induced hypoglycaemia (Wilson, Singhal and Percy-Robb, 1972) are potent stimulators of prolactin secretion, it is not possible to know exactly the relative contribution of these 2 provocative stimuli to the observed changes in peripheral prolactin concentrations. However,
in view of the difference in timing between the prolactin rise following TRF and hypoglycaemia it seems reasonable to assume that the initial increase in plasma prolactin was predominantly a result of TRF-stimulated pituitary prolactin release while the secondary rise or plateau (after 30 minutes) was hypothalamic in origin. Evidently the magnitude of this second peak was probably also dependent to a large extent on the magnitude of the TRF-induced prolactin rise, unless marked differences should exist between individuals in the half-life of prolactin.

Although there was no statistically significant difference between patients of Group 1 and controls in the mean plasma prolactin level at 20 minutes after TRF, prolactin responses in the patients' group tended to be smaller. The significant positive correlation between circulating 17β-oestradiol and prolactin levels, suggests that this impaired pituitary responsiveness may be a manifestation of hypo-oestrogenism. It is well known that in several animal species, particularly in rodents, peripheral levels of prolactin mirror those of 17β-oestradiol but controversy exists as to whether oestrogen has any physiological role in the regulation of this hormone in the human species also (for references, see Chapter 1). Most authors have failed to detect any significant change in plasma prolactin during the human menstrual cycle which would seem to argue against any possible involvement of oestrogen in the control of prolactin release. On the other hand, the observations that circulating concentrations of prolactin are lower in men and postmenopausal women as compared to regularly menstruating women, that prolactin levels rise during pregnancy and following oestrogen treatment and that oestrogen potentiates prolactin release from pituitary transplants in-vivo and in-vitro (for references see Chapter 1) suggest that oestrogen may influence prolactin secretion through a direct effect at the pituitary
level, although the physiological significance, if any, of this action remains unclear. The present study indicates that pituitary sensitivity to TRF may also be related to the prevailing oestrogen environment. If this is correct, it should be possible to demonstrate changes in the magnitude of TRF-induced prolactin release in regularly menstruating women at different stages of the cycle. Clinical studies to test this hypothesis are presently being considered.

Prolactin responses to insulin-induced hypoglycaemia in patients of Group 1 were significantly lower than those observed in control women but related to basal 17β-oestradiol levels. In view of the positive correlation between TRF-induced prolactin release and circulating 17β-oestradiol, it is not possible to derive from this result any definite conclusions as to the role of oestrogen in insulin-mediated prolactin secretion. Further studies involving separate administration of insulin will be required to clarify this point.

A characteristic common to all patients with secondary amenorrhea was the presence of significantly elevated basal 17-fluorogenic steroids. Since basal androstenedione levels in these women were also raised, it seems unlikely that this rise in corticosteroid levels is a result of an increased plasma concentration of corticosteroid-binding globulin (BG) rather than a manifestation of increased adrenal steroid production. The aetiological factor(s) responsible for the rise in adrenal steroid secretion in our patients is (are) unknown. In view of the extensive precautions taken during collection of the blood samples (the first sample for corticol measurement was collected two and a half hours after inserting the intravenous cannula and the tests were performed in a quiet area of the hospital which is used exclusively for clinical research studies) it seems unlikely that the elevated cortico-
steroid levels are due to stress. Raised plasma cortisol and changes in diurnal cortisol secretion have previously been described in patients with anorexia nervosa (Reichlin, 1974a). Our observations suggest that these irregularities in hypothalamic-pituitary-adrenal function may be common among women with secondary amenorrhoea.

In summary, the present study has demonstrated a number of endocrine abnormalities in patients with secondary amenorrhoea, low basal gonadotrophin levels and normal basal prolactin levels (Group 1). In terms of control of gonadotrophin secretion these patients may be compared with prepubertal children, a comparison which is all the more tempting in view of the suggestion made by Frisch and Revelle (1971) of a critical body weight at which gonadal function is initiated at puberty. In the absence of circulating gonadotrophin levels in the normal range, follicular development in these patients fails to occur as indicated by the low 17β-oestradiol concentrations. The preferential catabolism of 17β-oestradiol to 2-methoxy-oestrone rather than to oestriol in these underweight women (Fishman, Boyar and Hellman, 1975) may further contribute to their profound hypo-oestrogenism since 2-methoxy-oestrone, unlike oestriol, has features of an anti-oestrogen (Jacobs, 1976). The impaired prolactin and growth hormone responses to TRH and/or insulin-induced hypoglycaemia in these patients are probably secondary to the oestrogen deficiency. The elevated basal corticosteroid and growth hormone levels however cannot be explained on this basis. Their cause is at present unknown but a possible mechanism to account for these changes is discussed below.

Apart from elevated basal corticosteroid and androstenedione levels and a failure to ovulate in response to Clomiphene-induced follicular growth, patients of Group 2 (normal basal prolactin and
gonadotrophin levels, FSH/LH ratio greater than 1) did not show any other evidence of hypothalamic-pituitary malfunctioning. The failure to ovulate in the presence of an apparently intact negative feedback mechanism in these patients is very reminiscent of the situation found in girls during the later stages of pubertal maturation (Reiter, Kulin and Hamwood, 1974). The fact that a large proportion of these women had a spontaneous resumption of ovarian activity during the follow-up period seems to add further support to the view that the re-establishment of ovarian cyclicity in amenorrhoeic women might proceed according to a strict maturational "program" which is similar to that of pubertal girls although probably completed in a shorter period of time. A similar view has been expressed by Boyar, Katz, Finkelstein, Kapen, Weiner, Weitzman and Hellman (1974) who demonstrated that, like in pubertal children, LH levels rise during nocturnal sleep in patients with anorexia nervosa when weight is regained.

The endocrine features of patients in Group 3 were similar to those of the previous group except for the presence of augmented pituitary LH secretion and a functionally intact positive feedback mechanism. In view of these latter two characteristics it is tempting to draw a parallel between these patients and women with the polycystic ovary syndrome (section 7.1). Diagnostic laparoscopies have not been performed on these women except for one patient of this group who was shown to have sclerocystic ovaries. Menstruation in this patient had always been regular until she became amenorrhoeic after stopping the combined contraceptive pill. The question as to whether the elevated basal androstenedione levels of patients with secondary amenorrhoea might be implementary in the development of sclerocystic ovaries in a proportion of these patients remains to be elucidated. The follow-up study of these patients might provide some indirect information on this point in the near future.
The incidence of hyperprolactinaemia (16%) in the present study was similar to that of recently reported larger series (e.g. Bohnet, Dahlén, Wuttke and Schneider, 1975; Glass, Williams, Butt, Logan-Edwards and London, 1976). In accordance with these workers, we agree that elevated levels of prolactin may occur in the absence of galactorrhoea (4 patients) and, conversely, that galactorrhoea may be found in women who have normal prolactin levels (one patient). As described in Chapter 1, the absence of milk secretion despite hyperprolactinaemia is probably a reflection of the fact that the initiation of lactogenesis requires, apart from prolactin, a number of other hormonal stimuli, including insulin, growth hormone, corticosteroids etc. The maintenance of established lactation on the other hand appears to become progressively less dependent on intense prolactin stimulation which may account for the finding of galactorrhoea with normal prolactin concentrations.

It has been suggested on several occasions (for references see Chapter 1) that the absence of ovarian activity in physiological and pathological hyperprolactinaemia may result from a direct antigonadotrophic effect of prolactin at the ovarian level. The present findings of markedly reduced 17β-oestradiol levels in spite of apparently normal gonadotrophin levels appears to support this view. In four of the six patients on whom data was available, Clomiphene administration failed to stimulate normal follicular development even though peripheral concentrations of FSH and LH on the last day of treatment were similar to those found in controls. Similar observations have recently been reported by Bohnet, Dahlén, Wuttke and Schneider (1976).

Although a direct ovarian action of prolactin could account for the absence of normal follicular growth in patients with hyperprolactinaemia, the possibility of concomitant hypothalamic dysfunction must also
be considered. Recent work by Glass, Shaw, Butt, Logan-Edwards and London (1975) has illustrated that women with hyperprolactinaemia virtually always fail to release LH in response to exogenous oestrogen administration indicating that, at least in this respect, hypothalamic function is abnormal.

A number of possible mechanisms could be hypothesized to explain the endocrine abnormalities observed in the present study. In view of the beneficial effects of L-Dopa (a dopamine precursor) and 2-Bromo-α-ergocryptine (a dopamine agonist) in a proportion of women with hyperprolactinaemia (Lutterbeck, Pryor, Varga and Wenner, 1971; Besser, Parke, Edwards, Forsyth and McNeilly, 1972) it could be assumed that the primary defect in these patients, or at least in those without evidence of pituitary tumour, is a selective dopamine deficiency. Such a deficiency is likely to be associated with decreased levels of other neurotransmitter catecholamines, particularly noradrenaline and adrenaline, both of which are synthetized from dopamine (Melmon, 1974). Since noradrenergic mechanisms have been implicated in the control of episodic gonadotrophin release (Bhattacharya, Diirschke, Yamaji and Knobil, 1972) as well as in the secretion of corticotrophin-release inhibiting factor (Yates and Maran, 1974), a decreased formation of noradrenaline could explain the increase in basal cortisol secretion and the impairment of pulsatile gonadotrophin secretion, observed in the patients of Group 4. The failure of these patients to release LH in response to oestrogen may have a similar basis since noradrenaline appears to be involved in progesterone-induced gonadotrophin release in the rat (Kalra, Kalra, Krulich, Fawcett and McCann, 1972) although apparently not in the rhesus monkey (Knobil, 1974).
In patients of Groups 2 and 3, a similar but more selective depletion in noradrenaline and adrenaline alone could account for the endocrine abnormalities of hypothalamic origin observed in this group (i.e. the elevated basal corticosteroid levels, the reduced pulsatile gonadotrophin secretion and, in a proportion of patients, the failure of positive feedback). Such selective noradrenalin and adrenalin deficiency appears not to be entirely inconceivable considering the fact that the enzymatic conversion of dopamine to noradrenaline is a rate-limiting step in catecholamine biosynthesis (Melmon, 1974).

Patients in Group 1 may suffer from a similar, albeit more profound defect in adrenaline and noradrenaline biosynthesis. Since growth hormone secretion appears to be regulated by a balance between α-adrenergic and β-adrenergic mechanisms and, independent from these, a serotonergic mechanism involved in diurnal, sleep-related increase in growth hormone secretion (Reichlin, 1974b), it could be postulated that a severe adrenaline-noradrenaline deficiency may result in a relative predominance of the stimulatory serotonergic neurotransmission, hence the elevated basal growth hormone levels in these patients. Since the diurnal rhythm in cortisol secretion is also mediated via serotonergic pathways (Yates and Maran, 1974), this mechanism could also account for the abnormalities in this rhythm observed in underweight patients with anorexia nervosa (Reichlin, 1974a).

7.2.5 Summary

A total of 50 patients with secondary amenorrhoea of at least 6 months' duration were screened for possible abnormalities in hypothalamic-pituitary function with respect to gonadotrophin, growth hormone, TSH, prolactin and ACTH secretion under basal conditions and following
appropriate stimulation with LRF, TRF, insulin and Clomiphene. ACTH secretion was assessed indirectly by measuring plasma 17-fluorogenic corticosteroids.

Preliminary screening included general and pelvic examination, measurement of plasma thyroxine, full blood count, lateral and frontal X-ray of the pituitary fossa and an initial assessment of pituitary-ovarian activity by measurement once per week over a 4 week period of the urinary excretion of total oestrogen, pregnanediol, FSH and LH. Patients with evidence of cyclical ovarian activity or primary ovarian failure (elevated urinary gonadotrophins) were not included in the series. Sellar enlargement was present in one patient.

A combined hypothalamic-pituitary function test was performed at the completion of the initial tracking. The test involved measurement of 17β-oestradiol, FSH, LH, prolactin, TSH, growth hormone and cortisol before and after administration of 50 µg LRF, 200 µg TRF and 0.15 U/kg insulin. Clomiphene (100 mg x 5) was then administered and the response assessed by measuring gonadotrophin and 17β-oestradiol concentrations in peripheral plasma on the last day of treatment and urinary oestrogen and pregnanediol excretion at weekly intervals over the subsequent 3 week period. Results were compared to those obtained in a control group of 8 regularly menstruating women who were studied during the early follicular phase of the cycle (Day 3-5).

For analysis of the endocrine data, patients were divided into 4 groups on the basis of their basal gonadotrophin and prolactin levels. Patients with low FSH and LH concentrations and normal prolactin levels were often underweight and had low urinary total oestrogen and plasma 17β-oestradiol levels. Pituitary sensitivity to LRF in these women was impaired and Clomiphene failed to induce follicular development,
suggestive of increased negative feedback sensitivity. Basal levels of growth hormone, 17-fluorogenic steroids and androstenedione were significantly elevated, but growth hormone responses to insulin-induced hypoglycaemia and prolactin responses to TRH and insulin were reduced as compared to normal women.

Patients with normal prolactin, FSH and LH levels and a FSH/LH ratio greater than 1 had elevated basal 17-fluorogenic steroid and androstenedione levels. Hormone responses to the injection of TRF, LRF and insulin were normal in these patients. Clomiphene induced follicular development but in the majority of these patients ovulation did not occur.

Patients with normal prolactin, FSH and LH levels but a FSH/LH ratio smaller than 1 had elevated basal 17-fluorogenic steroid and androstenedione levels. Pituitary LH secretion in response to LRF was enhanced in these patients but FSH, prolactin, growth hormone and TSH secretion were normal. All but two of the patients in this group had an ovulatory cycle following Clomiphene treatment.

Women with elevated prolactin levels had on average normal basal gonadotrophin concentrations but decreased urinary total oestrogen and plasma 17β-oestradiol levels. Pituitary responsiveness to LRF was normal and Clomiphene increased plasma gonadotrophins but follicular development did not occur. Basal prolactin, 17-fluorogenic steroid and androstenedione concentrations in peripheral plasma were raised. Growth hormone response to insulin-induced hypoglycaemia was reduced. Prolactin responses to TRH and insulin were variable.

Prognosis in terms of spontaneous return of menstrual function within 6 months after completion of the test was poor in women with elevated prolactin levels and in those with low gonadotrophin levels.

A possible mechanism based on selective neurotransmitter deficiencies which might account for the observed endocrine abnormalities in women with secondary amenorrhoea is discussed.
CHAPTER EIGHT

CONCLUSIONS
The studies reported in this thesis have posed more questions than they have provided answers. Results of the various projects have been discussed in the respective sections and will therefore not be considered further. It seems however worthwhile to review briefly some of the questions which have been encountered during the course of these studies and towards which future research might be directed.

Since the advent of radioimmunoassays for pituitary gonadotrophins and steroid hormones, it has become possible to investigate in more detail the various components of the hypothalamic-pituitary-ovarian axis. These investigations should eventually enable to classify disorders of reproductive function on the basis of their primary pathological defect, rather than on the basis of their dominant presenting symptom. Before any such classification can be made however, a better understanding of the dynamics of the H.P.O. axis seems essential.

Until now, H.P.O. relationships have been described in a qualitative manner: gonadotrophins stimulate the ovaries, androgens have negative feedback effects, oestrogens have positive and negative feedback effects etc. Very little information however is available on the quantitative characteristics (the "dose-response curves") of these relationships. Since all functional disorders of the H.P.O. axis are probably due to changes in sensitivity of one or other component of this axis, this information seems of utmost importance.

Obviously, in order to construct a "dose-response curve" from which the sensitivity of a particular component can be derived, a number of requirements have to be fulfilled. Firstly, one has to know what factors elicit a response from the target-organ under physiological conditions. We know about the existence of feedback effects of gonadal
steroids at the hypothalamic level, and of the stimulatory effects of
LRF and gonadotrophins at the pituitary respectively ovarian level but
a number of questions remain. Is there a non-steroidal substance which
regulates FSH secretion? Are there any other steroid hormones with
feedback effects apart from 17/β-oestradiol, progesterone and testosterone?
Does a specific FSH-releasing factor exist or is LRF the only hypo-
thalamic releasing hormone?

Secondly, before the response of a target-organ to a certain
stimulus can be assessed, it is essential to know whether there are
any substances which could interfere with the response and, if so, to
quantitate the extent of their interference. Our knowledge on this
aspect of H.P.O. relationships is virtually non-existent. Evidence
however is emerging that such interference may indeed exist. The demon-
stration that prolactin may have an antigonadotrophic action at the
hypothalamic and/or ovarian level and the naturally occurring 17/β-oestra-
diol metabolite, 2-methoxy-oestrone, an anti-oestrogenic effect are
but a few examples.

Thirdly, a dose-response curve can be constructed only when both
the dose and the response can be quantitated. Although self-evident,
this condition will probably form the most formidable obstacle in future
work on hypothalamic-pituitary function. Attempts to measure LRF in
peripheral plasma have been rather unsuccessful so far, but this problem
seems negligible in comparison to the difficulties which will have to be
envisaged when concentration estimates of LRF in portal venous blood
are to be obtained.
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