COMPARISON OF THE EFFECTS OF ETHINYL OESTRADIOL AND CONJUGATED EQUINE OESTROGENS IN OOPHORECTOMIZED WOMEN


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SUMMARY

Seventeen oophorectomized women were treated for 3 month periods, in random sequence, with ethinyl oestradiol 20 and 50 μg daily and conjugated equine oestrogens (Premarin) 0.625 and 1.25 mg daily. The serum cholesterol, clot lysis time, plasma fibrinogen, platelet adhesiveness and activated partial thromboplastin time remained unchanged throughout the different oestrogen regimes. There was a significant rise of serum triglyceride levels on both doses of ethinyl oestradiol but no significant change with Premarin. Serum luteinizing hormone levels were depressed most by ethinyl oestradiol 50 μg daily, although not down to the levels in premenopausal women.

An increased incidence of both arterial (Collaborative Study, 1973) and venous thrombosis (Inman & Vessey, 1968) in patients taking combined oestrogen–progestogen oral contraceptives has been reported, and the synthetic oestrogen component has, in particular, been implicated (Inman et al., 1970). In the present study we have tried to determine whether there are differences between the effects of administered synthetic and 'natural' oestrogens and, if so, whether they are due to qualitative differences between the two types of oestrogen preparation, or to quantitative differences in the oestrogenic potency of the doses commonly used. We have therefore compared the clinical and biochemical effects of ethinyl oestradiol and conjugated equine oestrogens (Premarin), given in doses considered to be of equivalent biological activity, in a group of oophorectomized women. Measurements have been made of serum cholesterol and triglycerides, the levels of which are thought to be of importance with regard to the risk of developing arterial disease (Kannel et al., 1971; Carlson & Bottiger, 1972), and of certain 'haemostasis-related parameters' thought to be relevant to the risk of venous thrombosis (Leading Article, 1972). As an index of the oestrogenic effect of the hormones, serum luteinizing hormone (LH) levels have also been measured.

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PATIENTS AND METHODS

Patients

Seventeen women agreed to take part in the study, the nature of which was fully explained to them. Their ages ranged from 32 to 46 (mean 40). All had been oophorectomized for non-malignant conditions, seven within 90 days of entering the study, and the remainder more than 90 days previously. Seven of the women were already taking an oestrogen preparation before the study started whilst the remainder were receiving no treatment.

Patients were prescribed in random sequence ethinyl oestradiol 20 μg or 50 μg daily or Premarin 0.625 mg or 1.25 mg daily. Each drug was given for approximately 3 months (mean 92 days, range 77–106 days) before the next oestrogen regime was instituted. At the end of each course, patients attended out-patients having fasted overnight. They were weighed and questioned as to symptoms, and after a 15 min rest, blood was withdrawn for the investigations detailed below. During the first part of the study the patients attended on two separate occasions at the end of each treatment regime for tests prior to changing therapy. Statistical comparison by a paired 't' test of the results obtained on these two visits showed no significant difference. Later in the study, therefore, the women were tested only once before going on to the next course. The untreated group was composed of patients who had been oophorectomized for more than 90 days and who were on no hormonal replacement therapy. It was made up of three of the original seventeen women and five others who fulfilled these conditions, but did not take further part in the study.

METHODS

Total serum cholesterol and serum triglycerides were measured on the Technicon AutoAnalyzer, cholesterol by a modification of the Liebermann Burchard method and triglycerides by the standard semi-automated Technicon method (Kessler & Lederer, 1965). Lipoprotein electrophoresis was carried out on agarose gel (Noble, 1968).

<table>
<thead>
<tr>
<th>Method</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Serum cholesterol (mg/dl)</td>
<td>1.3</td>
</tr>
<tr>
<td>2. Serum triglycerides (mg/dl)</td>
<td>3.4</td>
</tr>
<tr>
<td>3. Dilute clot lysis (h)</td>
<td>8.6</td>
</tr>
<tr>
<td>4. Plasma fibrinogen (mg/dl)</td>
<td>2.2</td>
</tr>
<tr>
<td>5. Adhesive platelet count (×10⁹/l)</td>
<td>21.5</td>
</tr>
<tr>
<td>6. Activated partial thromboplastin time (s)</td>
<td>3.6</td>
</tr>
<tr>
<td>7. Serum LH (mU/ml)</td>
<td>7.3% at 1.0 mU/ml</td>
</tr>
</tbody>
</table>

Fibrinolysis was determined by the dilute blood clot lysis time (Fearnley & Tweed, 1953). Plasma fibrinogen was assayed using a clot weight method (Fearnley & Chakrabarti, 1966). Platelet adhesiveness was measured in vivo from the difference between the venous platelet count and the platelet count of blood from a standardized scratch on the skin of
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Eflects of oestrogens in oophorectomized women (Borchgrevinck, 1960). Activated partial thromboplastin time was determined by the method of Eastham (1962).

Serum LH was measured by a double antibody radio-immunoassay method. Purified human pituitary LH (fraction IRC 2, kindly donated by Dr A. Stockell Hartree, Cambridge) was used for labelling with $^{131}$I. Anti-human pituitary LH (70/299) was supplied by the Medical Research Council. Results were expressed in terms of a human pituitary LH standard (MRC 68/40) ascribed an arbitrary value of 20 units per ampoule. The smallest amount of LH which could be measured was approximately 0.15 mU per ml serum. The mean serum LH in twenty-five premenopausal women (outside the mid-cycle) was $0.53 \pm 0.31$ (SD) mU per ml.

Serum cholesterol, triglycerides and LH on all sera from the same patient were each measured in the same assay. The accuracy of the methods used is shown in Table 1.

Statistical methods. The significance of changes was tested using Student's 't' test for comparison of mean values. The mean of the values obtained on the two attendances during the early part of the study was used for statistical purposes.

![Graph](image.png)

**Fig. 1.** Serum cholesterol levels on the different oestrogen regimes. EO 20 = ethinyl oestradiol 20 µg daily; EO 50 = ethinyl oestradiol 50 µg daily; P 0.625 = Premarin 0.625 mg daily; P 1.25 = Premarin 1.25 mg daily. No significant differences between results ($P > 0.05$).

RESULTS

**Clinical**

The different oestrogen preparations seemed to be similarly effective in relieving symptoms such as hot flushes. One woman developed severe nausea and tiredness on both doses of Premarin which she discontinued and another patient was similarly unable to tolerate ethinyl oestradiol 50 µg daily. Six patients preferred ethinyl oestradiol, seven Premarin and the remainder expressed no particular preference. There was no significant difference in body weight on the different oestrogen regimes.
Serum lipids

(a) Serum cholesterol (Fig. 1). The mean serum cholesterol level in the untreated group was 259 mg/100 ml. No significant change was observed on any treatment regime.

(b) Serum triglycerides (Fig. 2). The mean serum triglyceride level in the untreated group was 86.5 ± 11.1 (SEM) mg/100 ml. Significant elevations occurred with ethinyl oestradiol 20 μg (137.4 ± 13.0) and 50 μg (156.9 ± 14.3). Serum triglyceride levels during Premarin treatment with 0.625 mg (111.7 ± 12.2) and 1.25 mg (112.8 ± 10.1) were not significantly different from the levels in the untreated women. Lipoprotein electrophoresis reflected the changes, when present, in serum triglyceride levels. An intensified pre-β band was present when serum triglyceride levels were greater than approximately 160 mg/100 ml.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nil</th>
<th>EO 20</th>
<th>EO 50</th>
<th>P 0.625</th>
<th>P 1.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sign. vs. Nil</td>
<td>–</td>
<td>0.02</td>
<td>–</td>
<td>NS*</td>
<td>NS*</td>
</tr>
<tr>
<td>vs. EO 20</td>
<td>–</td>
<td>–</td>
<td>NS*</td>
<td>–</td>
<td>NS*</td>
</tr>
<tr>
<td>vs. EO 50</td>
<td>–</td>
<td>0.005</td>
<td>–</td>
<td>NS*</td>
<td>NS*</td>
</tr>
<tr>
<td>vs. P 0.625</td>
<td>–</td>
<td>–</td>
<td>0.025</td>
<td>–</td>
<td>NS*</td>
</tr>
<tr>
<td>vs. P 1.25</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 2. Serum triglyceride levels on the different oestrogen regimes (Abbreviations as in Fig. 1.) * N.S. = P > 0.05.

Haemostasis-related parameters. The results are shown in Table 2. There were no significant changes in any of the parameters during the four treatment regimes.

Serum LH (Fig. 3). The mean serum LH in the untreated patients was 6.68 ± 0.45 (SEM) mU/ml. Ethinyl oestradiol 20 μg (5.44 ± 0.32) and 50 μg (3.23 ± 0.46), and Premarin 1.25 mg (5.11 ± 0.46) caused significant depression of serum LH levels. Ethinyl oestradiol 50 μg daily caused significantly greater depression of serum LH levels than either ethinyl oestradiol 20 μg or Premarin 1.25 mg daily.

DISCUSSION

The rise in serum triglyceride levels observed during periods of ethinyl oestradiol administra-
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#### TABLE 2. 'Haemostasis related parameters' in oophorectomized subjects

<table>
<thead>
<tr>
<th>Regime</th>
<th>Nil</th>
<th>EO 20</th>
<th>EO 50</th>
<th>P 0.625</th>
<th>P 1.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCL* (h)</td>
<td>4.82 ± 2.31</td>
<td>5.51 ± 2.26</td>
<td>6.16 ± 1.81</td>
<td>5.38 ± 2.67</td>
<td>5.42 ± 1.75</td>
</tr>
<tr>
<td>(m ± SD)</td>
<td>n=9</td>
<td>15</td>
<td></td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Plasma fibrinogen (mg/dl)</td>
<td>302.4 ± 65.5</td>
<td>290.6 ± 47.7</td>
<td>288.1 ± 61.7</td>
<td>293.9 ± 67.2</td>
<td>291.4 ± 58.7</td>
</tr>
<tr>
<td>(m ± SD)</td>
<td>n=8</td>
<td>12</td>
<td></td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Adhesive platelet count (× 10⁹/dl)</td>
<td>64.66 ± 44.80</td>
<td>77.81 ± 33.78</td>
<td>87.86 ± 29.55</td>
<td>77.37 ± 32.62</td>
<td>81.73 ± 30.33</td>
</tr>
<tr>
<td>(m ± SD)</td>
<td>n=9</td>
<td>16</td>
<td></td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>APTT† (s)</td>
<td>37.44 ± 3.92</td>
<td>36.77 ± 4.01</td>
<td>36.57 ± 3.43</td>
<td>37.50 ± 4.84</td>
<td>37.75 ± 4.30</td>
</tr>
<tr>
<td>(m ± SD)</td>
<td>n=9</td>
<td>16</td>
<td></td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

All changes N.S. (*P* > 0.05).

* Dilute clot lysis time.
† Activated partial thromboplastin time.

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**Fig. 3.** Serum luteinizing hormone levels on the different oestrogen regimes. (Abbreviations as in Fig. 1.) * N.S. = *P* > 0.05.
tion is in keeping with the results of Stokes & Wynn (1971), who found that oral contraceptives with the highest synthetic oestrogen component resulted in the highest serum triglyceride levels, and Aitken et al. (1972) who reported elevated levels in oophorectomized women receiving mestranol 20–40 μg daily. Although Robinson & Le Beau (1965) found conjugated equine oestrogens in doses of 1·25 and 2·5 mg daily increased serum triglyceride levels in pre-menopausal women, the doses of Premarin used in the present study produced no such changes. It has been suggested that the increased levels in patients receiving oestrogens are due to an increased hepatic synthesis of triglycerides and lipoprotein (Wynn et al., 1969; Rossner et al., 1971), or a fall in lipoprotein lipase levels (Hazzard et al., 1969; Adams et al., 1970).

A depression of serum cholesterol levels was found by Boyd (1963) in patients receiving ethinyl oestradiol 100–200 μg daily, and by Robinson et al. (1960) in women during the first 3 months of therapy with Premarin 0·625 mg and 1·25 mg daily. We were unable to demonstrate any significant alteration of serum cholesterol levels on any of our four treatment regimes. We recognize that measurements of total serum cholesterol and serum triglycerides as undertaken during the present study do not take into account changes which may occur within individual lipoprotein groups. We have thus no definite information about changes of high density lipoproteins which have been reported to rise during oestrogen administration (Furman et al., 1967).

Alterations in haemostasis-related parameters in patients taking combined oestrogen–progestogen oral contraceptives have been reported (Leading Article, 1972). These include an increase in blood clotting factor levels, alterations in the fibrinolytic system and changes of properties of the platelets. Platelet behaviour in vitro had been reported to be affected by oral contraceptives (Bolton et al., 1968) and synthetic oestrogens (Elkeles et al., 1968) but not by natural oestrogens. In the present study, however, we were unable to show any alteration in the various haemostasis-related parameters studied.

The doses of ethinyl oestradiol and Premarin used in the study were those generally regarded as having equivalent oestrogenic potency (Israel, 1967; Yannone, 1967; Kistner, 1967). However, Rudel & Kincl (1966) found that ethinyl oestradiol in a dose of 20 μg daily from day 5 to 24 of the menstrual cycle produced more consistent suppression of ovulation than Premarin 1·25 mg daily. They concluded that Premarin 1·25 mg daily had more oestrogenic potency (as assessed by endometrial proliferation, vaginal cytology and ferning of cervical mucus in hypo-oestrogenic menopausal women) than ethinyl oestradiol 20 μg, but considerably less anti-ovulatory activity. Ethinyl oestradiol 50 μg and Premarin 3·75 mg daily were considered to have equivalent oestrogenic and anti-ovulatory potency. Schalch et al. (1968) treated three post-menopausal women with conjugated equine oestrogens 3·75 mg daily for 5 days and found marked suppression of serum LH levels in only one and modest suppression in another. In contrast, Wise et al. (1973) reported partial or complete suppression of serum LH levels in six out of eight post-menopausal women given ethinyl oestradiol 20 μg daily, and in all of four women given ethinyl oestradiol 50 μg daily, for periods of 21–60 days.

In our study, serum LH levels were measured to provide an index of oestrogenic potency of the different oestrogen preparations used. Ethinyl oestradiol 50 μg daily depressed the serum LH considerably more than did Premarin 1·25 mg daily. However, even with the synthetic oestrogen the levels remained higher than those of normal pre-menopausal women. Thus the elevation of serum triglycerides produced by ethinyl oestradiol cannot readily be
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attributed to the oestrogenic potency of the doses used, and suggests some qualitative difference between the synthetic oestrogen and endogenously secreted oestrogen.

ACKNOWLEDGMENTS

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