ENDOMETRIAL RESPONSE TO POLY-OESTRADIOL PHOSPHATE (P. E. P.) IN AMENORRHEIC WOMEN

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In reports by Diczfalusy (1954), Diczfalusy & Westman (1956) and Diczfalusy et al. (1956) a long-acting, water soluble, high molecular weight polyester of phosphoric acid and oestradiol-17\beta, poly-oestradiol phosphate (hereafter called P. E. P.) has been described. It was shown that P. E. P., injected in a single dose to spayed mice, had an oestrogenic effect – measured by the duration of vaginal cornification – which persisted much longer than that of oestradiol benzoate or ethinyl oestradiol when the duration of vaginal cornification was used as an index of response. It was also found that a single intramuscular injection of P. E. P. to oophorectomized women resulted in an increased and greatly prolonged excretion of oestrone, oestradiol-17\beta, and oestriol. Finally Diczfalusy et al. studied the distribution of administered P. E. P. tagged with radioactive phosphorus in spayed mice.

The present report deals with the findings obtained in the course of a study of the endometrial response of amenorrheic women to P. E. P.

MATERIAL AND METHODS

Three groups of patients are included in this investigation; surgically castrated women, patients with primary amenorrhea, and those with secondary amenorrhea. They were all between 20 and 40 years old. If the patients had been treated previously with oestrogen the experiment was not commenced until the oestrogen had been withdrawn for at least 1½ months.

A single intramuscular injection of P. E. P. in a dose of 10, 20, 40, 80, or 160 mg, was given on the first day of the experiment. Endometrial biopsies were taken on this day as well as on two or three subsequent occasions. The time-interval between the biopsies is indicated in the figures. The endometrial strips were taken from different
regions of the endometrium in order to avoid damage caused by previous biopsies. On some occasions no material could be obtained from the inner surface of the uterus in spite of two or three attempts with the biopsy instrument. Such a result of a curettage was interpreted as indicating marked hypoplasia of the endometrium. In several of the patients examined, a previous curettage had given the same result.

The endometrial specimens were fixed immediately in 10 per cent formol saline. Paraffin embedding as well as preparation of the slides were made according to routine methods.

The presence of mitoses was found to be the only satisfactory index of a proliferative activity present at the time of biopsy. Thus the number of mitoses per glandular cross-sections was determined. Three grades of proliferative activity were established; low activity with less than one mitosis per eight glandular cross-sections; intense activity showing more than one mitosis per three cross-sections; and moderate activity showing mitoses in a number ranging between the two grades just mentioned. If no mitoses were found in the glands or in the stroma the endometrium was considered to be inactive, even if it had an otherwise normal appearance. This grading of the proliferative activity is roughly based on experience derived from a study of the frequency of mitoses in normal endometrial glands during early, mid, and late proliferative phases.

\[ \text{SECONDARY AMENORRHEA} \]

Graphical presentation of the endometrial response to 10 and 20 mg. P. E. P. administered to patients with secondary amenorrhea. Proliferative activity (P.A.) indicated along the ordinate and graded from 0 to 3 according to the description in the text. The time-interval between the injection and the biopsy is indicated along the abscissa. Open circles indicate that no endometrial material could be obtained.

Fig. 1.
In order to make it possible to evaluate the duration of oestrogenic effect of a single injection of P. E. P., the investigation also included a study of the endometrial response after a single intramuscular injection of 1, 2, or 4 mg. oestradiol benzoate in oily solution, performed along the same lines as those described above.

**RESULTS**

The results are shown graphically in Figs. 1 to 4. All subjects injected with P. E. P. showed signs of proliferative activity in their endometrial glands. This proliferation could already be observed in biopsies taken a week after the injection.

The duration of the proliferative activity was not definitely determined in this investigation. However, intense proliferative activity (grade 3) was observed in an oophorectomized woman, injected with 160 mg. P. E. P., as long as 15 weeks after the injection (Fig. 4).

**SECONDARY AMENORRHEA**

Endometrial response in secondary amenorrhea after the injection of 40, 80, and 160 mg. P. E. P. -- Black rectangles indicate uterine bleeding. Biopsies showing cystic glandular hyperplasia are marked by the letters »m. h. c.«.
PRIMARY AMENORREA

Fig. 3.
Endometrial response in primary amenorrhea treated with different doses of P. E. P., given in a single injection.

Comparing the results with those obtained after injection of oestradiol benzoate the oestrogenic effect of P. E. P. was found to be more prolonged. When oestradiol benzoate was injected in a single dose proliferative activity generally ceased after 2 to 3 weeks.

Two of the patients treated with 80 mg. P. E. P. as well as three of the four
women treated with 160 mg. P. E. P. showed a slight to moderate cystic glandular hyperplasia, developed as a result of the P. E. P. administration (Fig. 5). The other biopsies all showed a normal proliferative endometrium (Fig. 6). Secretory activity was not observed.

Uterine bleeding was not reported by the patients treated with 10 or 20 mg. P. E. P. When higher doses were used uterine bleeding was frequently observed, but the bleeding was never more marked than that appearing during a normal menstruation.

**DISCUSSION**

The data of the present investigation indicating that P. E. P. has a markedly prolonged oestrogenic effect on the human endometrium agree very well with the observations made by Diczfalusy et al. who showed that a single dose of P. E. P. causes a prolonged urinary excretion of oestrogens.

The present study is not sufficiently extensive to allow of a calculation of
Fig. 5. Microphotograph showing the endometrium obtained from an oophorectomized patient 5 weeks after the injection of 160 mg. P. E. P. Moderate cystic glandular hyperplasia. Moderate proliferative activity according to the number of glandular mitoses. × 60.

Fig. 6. Microphotograph showing a normal endometrium observed in a patient with secondary amenorrhea 4 weeks after the administration of 80 mg. P. E. P. Moderate proliferative activity. × 60.

the optimal dose of P. E. P. but it seems probable that a dose of 20 to 40 mg. P. E. P. is sufficient to produce a proliferative activity corresponding to that found in a normal mid-proliferative phase. If higher doses are used cystic glandular hyperplasia may develop.

There were no side effects following the administration of P. E. P. The injection caused no pain and no local irritation. The castrated patients all suffered from sweating, nervousness, etc. prior to the commencement of the treatment. These symptoms disappeared shortly after the injection of P. E. P. and did not reappear until at least 1½ months had elapsed.

SUMMARY

A study of the endometrial changes following a single intramuscular injection of P. E. P., a water soluble, high molecular weight polyester of phosphoric acid and oestradiol-17ß, indicates that P. E. P. has a much more prolonged oestrogenic effect than oestradiol benzoate. A proliferative endometrium was found in a surgically castrated woman as late as 15 weeks after the injection of P. E. P. Furthermore, P. E. P. was found to abolish symptoms following castration, such as sweating and nervousness, for at least 1½ months.
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REFERENCES