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# Estrogen-Progestogen Interaction: Factors Influencing Progesterone-Induced Endometrial Changes of the Uterus of the Immature Rabbit (39266)

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The Clauberg-McPhail assay (1, 2) has been widely used to assess the potential progestational properties of a wide variety of compounds. However, the possible influence of estrogens other than estradiol-17$\beta$ and their route of administration on progesterone-induced endometrial changes have received little consideration (3, 4). This paper describes our observations on the title subject.

*Materials and Methods.* Progesterone and the estrogens were administered in sesame oil. The degree of progestational activity was assessed according to Clauberg-McPhail (1, 2). New Zealand immature female rabbits (900-1000 g body wt) were given estrogen once daily for 7 consecutive days. Estradiol-17$\beta$ (E) was administered subcutaneously, while 17$\alpha$-ethynyl-estradiol (EE) and its 3-cyclopentyl-ether (EECPE) were administered either subcutaneously or orally. Beginning on the morning of the seventh day, various doses of progesterone were administered subcutaneously once daily for 5 consecutive days along with one-tenth the initial amount of estrogen. Autopsy was performed on the 12th day. Midsections of each uterine horn were removed, sectioned, and stained. The degree of progestational activity was determined according to McPhail scale (2).

*Results.* Subcutaneous E was adequate for endometrial priming over a wide range of doses (0.312-5.0 $\mu$g/day). The 200 $\mu$g/day dose represented the lowest amount of progesterone capable of inducing adequate secretory changes with any priming dose of E. When the dose of progesterone was reduced to 100 $\mu$g/day, the degree of secretory changes increased parallel to any increase in the priming dose of E up to 1.25 $\mu$g/day, whereas further increases in the priming dose of E resulted in a marked decrease in the degree of secretory changes. In our experimental conditions the 400-$\mu$g/day dose was the amount of progesterone capable of inducing full secretory changes with any priming dose of E (Table I). Adequate secretory changes could be obtained when progesterone (400 $\mu$g/day) was administered to animals primed with subcutaneous doses of EE (2-4 $\mu$g/day) or EECPE (4 $\mu$g/day).

Higher doses of EE and lower as well as higher doses of EECPE resulted in poor progestational response (Table II). The subcutaneous dose of progesterone (400 $\mu$g/day) either failed to induce or produced poor secretory changes in animals orally primed with either EE or EECPE in doses ranging from 2 to 40 $\mu$g/day. Although a better progestational response could be obtained by raising the dose of progesterone to 800 $\mu$g/day, the degree of secretory changes was still inferior to that which could be obtained following the administration of a lower dose of progesterone (400 $\mu$g/day) in subcutaneously estrogen-primed animals. It was necessary to further increase the dose of progesterone to 1600 $\mu$g/day to bring about

TABLE I. ESTROGEN-PROGESTOGEN INTERACTION: INFLUENCE OF VARYING SUBCUTANEOUS DOSES OF ESTRADIOL-17$\beta$ AND PROGESTERONE ON THE ENDOMETRIAL RESPONSE OF THE UTERUS OF THE IMMATURE RABBIT.

| Number of animals | Priming daily dose ($\mu$g/animal) | Progesterone daily dose ($\mu$g/animal) | McPhail score | |
|---|---|---|---|---|
| | | | Average | Range |
| 6 | 0.312 | 100 | 2.0 | 1.5-2.5 |
| 5 | 0.312 | 200 | 3.2 | 2.5-3.5 |
| 4 | 0.312 | 400 | 4.0 | 4 |
| 5 | 0.625 | 100 | 2.7 | 0.5-4.0 |
| 6 | 0.625 | 200 | 3.3 | 2.5-4.0 |
| 4 | 0.625 | 400 | 3.9 | 3.5-4.0 |
| 10 | 1.250 | 100 | 3.0 | 1.0-4.0 |
| 10 | 1.250 | 200 | 3.3 | 3.0-4.0 |
| 9 | 1.250 | 400 | 3.9 | 3.5-4.0 |
| 5 | 2.500 | 100 | 1.3 | 0.0-2.0 |
| 5 | 2.500 | 200 | 1.3 | 2.5-3.0 |
| 4 | 2.500 | 400 | 3.6 | 3.0-4.0 |
| 4 | 5.000 | 100 | 1.0 | 0.0-2.5 |
| 16 | 5.000 | 200 | 3.2 | 2.5-4.0 |
| 9 | 5.000 | 400 | 3.7 | 3.0-4.0 |



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secretory changes comparable to those obtained following subcutaneous estrogen-priming and the 400-μg/day dose of progesterone (Table III).

*Discussion.* Our data clearly indicate that estradiol-17β (E) was adequate for endometrial-priming over a wide range of doses. When administered subcutaneously at relatively high doses, E markedly reduced the degree of secretory changes brought about by the lower dose of progesterone (100 μg/day). This confirms earlier observations, which similarly indicate that large priming doses of estrogens reduce progesterone-induced endometrial changes (2). This phenomenon could not be observed with larger doses of progesterone (200–400 μg/day).

Previous studies have shown 17α-ethynyl-estradiol-3-cyclopentyl-ether (EECPE) to be more effective than 17α-ethynyl-es-

TABLE II. ESTROGEN-PROGESTOGEN INTERACTION: INFLUENCE OF VARYING DOSES OF SUBCUTANEOUS 17α-ETHYNYLESTRADIOL (EE) AND 17α-ETHYNYLESTRADIOL-3-CYCLOPENTYL ETHER (EECPE) ON THE ENDOMETRIAL RESPONSE OF THE UTERUS OF IMMATURE RABBIT TO SUBCUTANEOUS PROGESTERONE.

| Treatment | Number of animals | Priming daily dose (μg/animal) | Progesterone daily dose | McPhail score | |
|---|---|---|---|---|---|
| | | | | Average | Range |
| EE | 9 | 2 | 400 | 2.8 | 2.0–4.0 |
| | 8 | 4 | 400 | 3.4 | 3.0–4.0 |
| | 8 | 10 | 400 | 1.5 | 0.5–3.5 |
| EECPE | 9 | 2 | 400 | 1.5 | 0–4.0 |
| | 6 | 4 | 400 | 3.1 | 1.5–4.0 |
| | 8 | 10 | 400 | 1.1 | 0–3.0 |

TABLE III. ESTROGEN-PROGESTOGEN INTERACTION: INFLUENCE OF VARYING DOSES OF ORAL 17α-ETHYNYLESTRADIOL (EE) AND 17α-ETHYNYLESTRADIOL-3-CYCLOPENTYL ETHER (EECPE) ON THE ENDOMETRIAL RESPONSE OF THE UTERUS OF THE IMMATURE RABBIT TO VARYING DOSES OF SUBCUTANEOUS PROGESTERONE.

| Treatment | Number of animals | Priming daily dose (μg/animal) | Progesterone daily dose (μg/animal) | McPhail score | |
|---|---|---|---|---|---|
| | | | | Average | Range |
| EE | 11 | 2 | 400 | 0.4 | 0–1.5 |
| | 9 | 4 | 400 | 0.3 | 0–1.0 |
| | 3 | 10 | 400 | 1.3 | 1.0–1.5 |
| | 3 | 20 | 400 | 1.5 | 1.5 |
| | 4 | 40 | 400 | 0.9 | 0–1.5 |
| | 6 | 2 | 800 | 1.4 | 0.5–3.0 |
| | 7 | 4 | 800 | 1.1 | 0–3.0 |
| | 4 | 10 | 800 | 2.2 | 1.5–3.0 |
| | 4 | 20 | 800 | 1.2 | 1.0–2.0 |
| | 4 | 40 | 800 | 1.5 | 1.0–2.0 |
| | 7 | 2 | 1600 | 2.0 | 1.5–4.0 |
| | 6 | 4 | 1600 | 3.3 | 2.0–4.0 |
| | 4 | 10 | 1600 | 3.9 | 3.5–4.0 |
| | 4 | 20 | 1600 | 3.2 | 1.5–4.0 |
| | 3 | 40 | 1600 | 2.8 | 2.0–3.5 |
| EECPE | 12 | 2 | 400 | 0.9 | 0–1.5 |
| | 12 | 4 | 400 | 0.9 | 0–1.5 |
| | 4 | 10 | 400 | 1.6 | 1.0–2.0 |
| | 4 | 20 | 400 | 0.2 | 0–0.5 |
| | 3 | 40 | 400 | 0.7 | 0–1.5 |
| | 8 | 2 | 800 | 1.6 | 0.5–2.5 |
| | 8 | 4 | 800 | 2.2 | 1.5–3.0 |
| | 4 | 10 | 800 | 2.5 | 1.0–4.0 |
| | 4 | 20 | 800 | 2.7 | 1.5–4.0 |
| | 4 | 40 | 800 | 2.1 | 1.5–3.0 |
| | 8 | 2 | 1600 | 3.0 | 2.0–4.0 |
| | 8 | 4 | 1600 | 3.6 | 3.0–4.0 |
| | 4 | 10 | 1600 | 1.5 | 1.0–2.0 |
| | 4 | 20 | 1600 | 2.2 | 0–4.0 |
| | 4 | 40 | 1600 | 2.3 | 2.0–2.5 |

tradiol (EE) in stimulating uterine growth (5, 6). Our results, however, have demonstrated that similar doses of either compound, whether administered subcutaneously or orally, were required to adequately prime the uterus of the immature rabbit.

With either EE or EECPE a marked difference was noticed in the characteristics of the endometrium, depending on route of administration. This was evidenced by the fact that in orally estrogen-primed animals four times as much progesterone was required to bring about secretory changes comparable to those obtained following subcutaneous priming.

It may be hypothesized that endometrial changes induced by oral estrogen were either different or inadequate as compared to those brought about following subcutaneous administration. This hypothesis is consistent with the observation that degree of progestational activity was largely independent, within a wide range, from the amount of oral estrogen.

It is possible that the larger doses of progesterone were able to modify the oral estrogen-induced endometrial changes to more normal or adequate proliferative ones so that transformation to a secretory pattern could be easily obtained.

The mechanism(s) through which progesterone restores the sequence of events leading to the transformation of the endometrium to a secretory pattern remains to be elucidated.

*Summary.* In orally estrogen-primed animals, four times as much progesterone is required to bring about secretory changes comparable to those obtained following subcutaneous priming.

These findings might be interpreted as evidence that: (a) oral estrogen-induced proliferative changes are either different or inadequate, and (b) excess progesterone is *conditio sine qua non* for the induction of those proliferative changes susceptible of being transformed into secretory ones.

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