STUDIES ON THE INTERACTIONS OF OESTRIOL AND PROGESTERONE

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Summary. The interactions of oestriol and progesterone were studied in a series of assays for oestrogenic and progestational activities, and the responses were compared with data on progesterone-oestrone combinations in the same tests. The vaginal effects of these two oestrogens do not seem to differ, whereas the interactions of oestriol and progesterone are quite different from the interactions of oestrone and progesterone when uterine end-points are considered. Since oestriol is the dominant aromatic steroid excreted during pregnancy and the luteal phase of the menstrual cycle, we feel that explanations for many unsolved problems of luteal-phase and pregnancy physiology may reside in these interactions.

INTRODUCTION

Since oestriol was discovered in the urine of pregnant women over 30 years ago (Doisy, Mayer, Levinand & Curtis, 1930; Marrian, 1930), most workers have considered it simply as a metabolite of oestrone and oestradiol, an excretory product of no further physiological significance. More recently, interest in this material has increased. The studies of Hisaw, Velardo & Goolsby (1954) and Huggins & Jensen (1955) were stimulated by data that showed that the marked rise in oestrogens excreted during later pregnancy, and during the luteal phase of the menstrual cycle, is largely the result of changes in oestriol concentration (see Merrill, 1958, for review, and particularly the recent papers by Brown, 1955, 1956, 1959 a, b). Hisaw et al. found that oestriol would inhibit the uterine growth stimulated by either oestrone or oestradiol-17β in castrate adult rats, and Huggins & Jensen extended the study to the effects of oestriol and related agents on oestrone-induced uterine growth of young hypophysectomized rats. Systemically, oestradiol-17β, oestrone and oestriol, tested in pairs, had simple additive relationships when rat-vaginal-smear changes were employed as an index of effect (Claringbold, 1955; Edgren & Calhoun, 1957a), although they were mutually antagonistic by the intravaginal route. It has long been known that progesterone is a potent inhibitor of oestrogen-stimulated responses of various types (Roberts & Szego, 1953). However, when Huggins

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Interactions of oestriol and progesterone

(1956) studied the interactions of progesterone and oestriol, he found that while progesterone inhibited the uterine growth effects of large doses of oestriol, it potentiated the effects of small doses, a result similar to that obtained with mixtures of oestrone and oestriol by Edgren & Calhoun (1960a). Zarrow & Neher (1953) showed that, whereas oestradiol and oestrone blocked the action of progesterone on the stromal nuclei of mouse uteri (Hooker-Forbes test), oestriol was ineffective, even at very high dose levels.

Miyake & Pincus (1958) reported that oestriol is qualitatively similar to other oestrogens, both natural and synthetic, in its ability to antagonize the uterine carbonic anhydrase and glandular effects of progesterone in rabbits. They found oestriol to be about as potent as oestrone, oestradiol-17β or stilboestrol in this test, despite the fact that oestriol is usually considered to be a weak oestrogen when given systemically (Merrill, 1958).

Merrill (1958) has recently focused attention on certain of these considerations in a review of our present knowledge of oestriol that points up the probable physiological importance of the material, and the need for further research. We have been particularly impressed by the high urinary titres of oestriol during late pregnancy and the luteal phase of the cycle. These titres indicate that, in terms of actual quantity of material at least, these two periods may be dominated by oestriol and progesterone, rather than exclusively by progesterone, as appears to be assumed tacitly by many workers. Since it seemed probable that additional study of the interactions of oestriol and progesterone might throw light on the hormonal control of the luteal phase and pregnancy, we designed a series of experiments to test the effects of progesterone on certain well-known oestriol-stimulated responses and the effects of oestriol on some activities of progesterone. This report will describe these studies.

METHODS AND RESULTS

EFFECTS OF PROGESTERONE ON SOME ACTIVITIES OF OESTRIOL

Mouse uterine growth

The effects of progesterone upon oestriol-induced uterine growth have been evaluated by methods described previously (Edgren & Calhoun, 1957b). Briefly, the compounds to be studied were administered subcutaneously, either individually or in combination, in 0·3 ml of corn oil. One-tenth millilitre of solution was injected each day for 3 days into mice 22 to 24 days of age. Twenty-four hours after the final injection, the uteri were removed, cleaned, and weighed wet on a torsion balance. Groups of eight to ten mice were employed for each treatment, and interpretations were based upon the mean values from such treatment groups. In our mouse-uterine-growth studies, all doses indicated are total doses.

One hundred microgrammes of oestriol, which served as a standard stimulator, gave uterine weights ranging from 33 to 47·5 mg group averages, as compared with averages in oil-treated groups of about 10 mg. The standard oestriol groups varied around an overall mean of about 40 mg uterine weight. The simultaneous administration of progesterone at doses ranging from 1 to 1000 μg failed to produce any mensurable depression in this oestriol-stimulated
uterine growth (Text-fig. 1). In this test, the marked anti-oestriol effects of testosterone propionate (TP) were similar to those reported for both progesterone and TP when administered to oestrone-treated mice (Edgren & Calhoun, 1957b, 1959). Desoxycorticosterone, like progesterone, was ineffective as an oestriol antagonist (Edgren & Calhoun, 1960b).

Rat uterine growth

The interactions of progesterone and oestriol were studied in a replicated experiment. Oestriol was tested at doses extending from 1 to 100 µg/day for 3 days, giving a dose-response curve for this test. The same doses of oestriol were also tested when mixed with progesterone at 100, 1000 and 10,000 µg (Text-fig. 2). In order to allow study of the entire series within a single experiment, groups of four rats were used, and the entire experiment was replicated on three successive weeks, thus increasing each group to twelve rats. The animals employed had been spayed at approximately 30 days of age and then allowed to recover for about 10 days before use.

The combination of a high dose of progesterone with low doses of oestriol produced uterine growth responses that were considerably higher than those expected for either material alone, suggesting that the compounds were true potentiators. Lower levels of progesterone did not affect the responses to these doses of oestriol. At the higher doses of oestriol, the 1-mg dose of progesterone appeared to inhibit the response to oestriol. Thus, in these circumstances, progesterone was an antagonist of oestriol.

Confirmation of these potentiating and antagonistic effects was obtained in a series of studies involving low doses of oestriol with high doses of progesterone and high doses of oestriol with high or intermediate doses of progesterone (Table 1). A 10-mg dose of progesterone, although stimulating little uterine
Interactions of oestriol and progesterone

growth by itself, markedly potentiated the uterine-growth-stimulating activities of a 1-µg dose of oestriol. An intermediate dose of progesterone (1 mg) inhibited

![Graph of uterine weight vs oestriol dose](image)

**Text-fig. 2.** The effects of progesterone on the uterine growth produced by oestriol in young spayed rats. The horizontal lines represent the uterine weights of rats treated with oil or progesterone at the three doses employed. Dots: oestriol alone; circles: oestriol plus progesterone at 100 µg; half circles: oestriol plus progesterone at 1000 µg; crosses: oestriol plus progesterone at 10,000 µg. Each weight value represents the mean of twelve rats.

**Table 1**

<table>
<thead>
<tr>
<th>Test</th>
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<th>N</th>
<th>Uterine weight (mg)</th>
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<td>Oestriol</td>
<td>Progesterone</td>
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</tr>
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Means in bracketed groups differed significantly at * 1% and ** 5% levels. By Wilcoxon Rank Sum Test (Wilcoxon, 1949).

the response to the higher dose of oestriol (100 µg). A higher progesterone level (10 mg) caused a depression of the growth response in only one of the two experiments. It would appear that 10 mg of progesterone has less blocking
effect upon oestradiol-induced uterine growth than does 1 mg, and that this moderate effect is measurable, therefore, only in occasional experiments.

Vaginal smears

The interactions of progesterone and oestradiol have been considered in a series of earlier publications. Rat-vaginal-smear data were reviewed (Edgren, 1959); they suggested no significant inhibition of oestradiol-induced vaginal effects by progesterone when both steroids were administered simultaneously over a 2-day period. Progesterone was administered in doses up to 10 mg/rat, and oestradiol was employed at a dose of 100 µg. In this test, oestrone-stimulated changes were also unaffected by progesterone, whereas if the doses are injected over a 4-day period oestrone effects were blocked by progesterone (Edgren, 1960a).

Mouse-vaginal-smear studies have been pursued in greater detail. In a standard two-injection assay, in which oestradiol and progesterone were administered simultaneously, there was again no blockage (Edgren, 1960b). Where both materials were given simultaneously over a 4-day period, there was significant blockage (Edgren, 1960c). In the latter experiment, progesterone was injected in various patterns of 1 and 2 days. These studies suggested that progesterone best inhibits the oestradiol-induced response when it is administered midway in the 4-day oestrone-injection period. The oestrone-stimulated vagina showed the same pattern of responses (Edgren, 1960d).

EFFECTS OF OESTRIOL ON SOME ACTIVITIES OF PROGESTERONE

Epithelial arborization of rabbit uteri

The effect of oestradiol on progesterone-induced proliferation of the uterine epithelium of rabbit uteri was studied in Clauberg tests (Clauberg, 1930), by combining the oestrogen with standard doses of progesterone. The degree of antagonism was determined by the decreased glandular proliferation of uteri in rabbits that received oestradiol with the progesterone. Progesterone was administered subcutaneously at doses of 0·05, 0·1 or 0·2 mg/day, alone or in combination with doses of oestradiol that varied from 16 to 256 µg (Table 2). Oestradiol was administered either as a microcrystalline suspension or as a

<table>
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<th>Oestradiol dose (µg)</th>
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administered subcutaneously at doses of 0·05, 0·1 or 0·2 mg/day, alone or in combination with doses of oestradiol that varied from 16 to 256 µg (Table 2). Oestradiol was administered either as a microcrystalline suspension or as a
solution in corn oil along with the appropriate dose of progesterone. Progesterone at 0.05 mg daily for 5 days produced minimal proliferation of the endothelium; the average response was +2.5 on the McPhail scale. One-tenth milligramme produced an average response of +3.1 and 0.2 mg produced a near maximal response (+3.4). The end-point for antagonistic effect was depression of the average progestational response to less than +2.

In agreement with the observations with progesterone alone, the uterine response increased with increasing doses of progesterone also when this was combined with oestriol at 16, 32 or 64 µg (Table 2). In combination with 128 or 256 µg of oestriol, however, increasing doses of progesterone up to 0.2 mg did not lead to increases in uterine response. This is unlike the response pattern for a competitive inhibitor, and suggests oestriol may be a non-competitive antagonist of progesterone. Oestradiol and oestrone, on the other hand, seem to compete with progesterone, although effective amounts of the latter hormones vary widely in this type of study (unpublished).

**Rat deciduomata**

Progesterone causes decidual development in spayed, oestrogen-primed female rats (Elton & Edgren, 1958; Zarrow, Caldwell & Peter, 1958). Progesterone was administered at a dose of 8 mg daily for 9 days alone, or in combination with various doses of oestriol. Decidual response was estimated from the weight of the treated horn of the uterus. The progesterone-induced response was significantly reduced by 0.3 µg of oestriol (Text-fig. 3). Other

![Text-fig. 3. The effects of combinations of progesterone at 8 mg and oestriol at various doses (circles), and progesterone, oestrone (0.3 µg) and oestriol (dots) on the rat decidual reaction. The decidual reaction was evaluated from the weight of the treated horn of the uterus. The shaded area represents the 95% confidence band for rats treated with progesterone alone. Any point falling outside of the shaded area is significantly different from the progesterone-treated animals at the 5% level. All points represent groups of from six to eight animals.](image)

groups of animals received oestrone (0.3 µg) in addition to progesterone or to the oestriol-progesterone combination. This oestrone dose failed to inhibit the progesterone-induced response. When oestrone was added to the progesterone-oestriol mixture, an oestriol dose as low as 0.03 µg appeared to block the decidual response. Thus, oestriol seems to be a potent inhibitor of decidual development and to synergize with oestrone in this test.
DISCUSSION

The unique nature of oestriol and its close chemical relatives is now reasonably well known. In addition to potency differences and differences in slope of the dose-response curve, the interactions of progesterone and oestriol on the uterus are remarkably different from those between oestrone and progesterone. In our rat-uterine-growth test, in which progesterone largely fails to block oestrone effects, high doses of oestriol may be antagonized by progesterone. The uterine growth produced by low doses of oestriol was augmented by progesterone. This interaction would appear to be similar to the 'steroid buffering' phenomenon described elsewhere (Edgren, 1959; Edgren & Calhoun, 1960a; Edgren, Calhoun & Harris, 1960). However, no other pair of compounds studied has shown the potentiation observed with oestriol and progesterone.

In the mouse-uterine-growth test described here, oestriol-induced growth proved to be unaffected by progesterone. This fact, when considered in association with data on the effects of other steroids on oestrone- and oestriol-induced uterine growth in mice, suggested a model or mechanism consisting of two separate action sites for oestrone and oestriol (Edgren & Calhoun, 1959). This hypothesis is in the process of modification and extension at the present time.

The data at hand also suggest that in their anti-progesterone effects oestriol and oestrone are different. Oestriol appears to act as a non-competitive inhibitor of progesterone whereas oestrone and oestradiol appear to be competitive antagonists. These data may also fit the dual-receptor-site hypothesis when they are adequately explored.

Regardless of mechanism, or of qualitative as opposed to quantitative differences, the fact remains that oestrone and oestriol differ greatly in their interactions with progesterone. Although the implications of the individual tests remain unexplained, the differences are clear. Thus, one must conclude that the two dominant hormones of pregnancy, progesterone and oestriol, show patterns of interactions that differ from those of progesterone and oestrone, which may be considered as typical of the dominant oestrogens of the follicular phase of the oestrous-menstrual cycle.

REFERENCES


Interactions of oestriol and progesterone


