Influence of Progesterone, Estradiol Benzoate, and Relaxin upon Placentomata Formation in Mice.* (28448)

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There are several papers showing that relaxin is not a simple substance(1,2,3). It has been reported that relatively crude preparations of relaxin contain two factors. One factor produces an inhibitory effect on extensive modification of stromal connective tissue which accompanies decidual growth the second factor has been reported to have a potentiating effect on placentomata or decidual development in synergism with progesterone. When relaxin loses the latter activity as a result of purification or heating, an inhibitory effect on placentomata development was observed(1). The exact function of relaxin in placentomata production in the uterus is of interest because such information should indicate if it plays a role in the normal implantation of fertilized eggs.

This paper is concerned with the interaction of relaxin and ovarian steroid hormones on the placentomata reaction in the mouse.

Methods and materials. Adult Swiss-Webster mice, kept in a room artificially illuminated during daylight hours at a uniform temperature of 78 ± 1°F were ovariectomized 7 to 10 days previous to the study. They were then given 0.1 μg estradiol benzoate (EB) for 3 days, followed by graded levels (0.35 to 10 mg) of progesterone (P) alone for 9 days.

On day 5, the right uterine horn was traumatized throughout its entire length with a sharp needle point. On day 4 post-traumatization, the uterus was removed, right and left horns separated, measured and weighed. Results are expressed in terms of percent increased weight of right horn over left horn. A relaxin preparation W1164A-66,‡ assayed 150 GPU per milligram, was suspended in sesame oil containing 5% beeswax, and injected subcutaneously. Various levels of EB and relaxin were administered during the pre-trauma period followed by 1 mg P plus relaxin during the post-trauma period (Table II).

Results. It is well known that estrogenized, castrated mice and rats given progesterone and traumatized produce a placentomata or decidual reaction in the uterus(4). The reaction increased as the level of P was increased from 0.35, 0.50, 0.75, and 1.0 mg per day (Table I). A marked but reduced response resulted with P levels of 2, 3, 5 and 10 mg/day. When EB was reduced to 0.01 μg/day, 1 mg of progesterone produced a limited response. The addition of 5 GPU of relaxin to 0.01 μg of estradiol benzoate during pretreatment increased response slightly which was further increased slightly with 0.001, 0.002 and 0.005 μg of estradiol benzoate per day.

Without EB as pretreatment, P at 1.0 mg level produced limited development, and relaxin (5 GPU) failed to improve reaction. However, when this level of relaxin was added to P (1.0 mg) during pre- and post-trauma periods, the reaction was increased. In subsequent trials 0.1 μg of EB was used during pretreatment. When 1 mg of P was supplemented with 5 GPU of relaxin during the post-trauma period, the largest decidual reaction was produced but relaxin had no effect in absence of P at this time as a complete substitute for progesterone even at a level of 30 GPU/day.

The introduction of low levels of EB alone (0.5 and 0.01 μg) or concurrently with relaxin (5 GPU) during the post-trauma period reduced the size of the placentomata reaction.

Discussion. The hormonal requirement involved in placentomata production in the uterus should represent also the hormones

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‡ The relaxin preparation kindly supplied by Drs. R. L. Kroc and B. G. Steinetz, Chilcott Laboratories, Morris Plains, N. J.

TABLE I. Effect of Graded Levels of Progesterone on Placentomata Reaction in Mice.

No. of mice	EB* (μg/day)	Pre- and post-trauma progesterone (mg/day)	Placentomata reaction on day 9: Max diameter of uterus Control (mm)	Max diameter of uterus Traumatized (mm)	Wt of uterus Control (mg)	Wt of uterus Traumatized (mg)	Uterine wt increase (%)
7	.1	.35	1.0	3.2	27.8	89.3	235
9	.1	.50	1.2	3.4	28.7	101.3	247
5	.1	.75	1.3	4.4	33.0	184.9	429
6	.1	1.0	1.0	4.1	26.7	178.7	613
6	.1	2.0	1.3	4.3	31.4	166.4	346
6	.1	3.0	1.4	4.2	32.9	160.7	467
6	.1	5.0	1.1	3.5	27.0	141.7	436
7	.1	10.0	1.3	3.6	36.9	146.9	293

* EB = Estradiol benzoate.

involved in the normal implantation or nidation of the fertilized egg. In ovariectomized mice, it was shown that 0.1 μg/day of estradiol benzoate for 3 days followed by 1 mg per day of progesterone alone for 9 days with traumatization on day 5 induced marked placentomata reactions. EB pretreatment at 0.01 μg/day for 3 days prevented the reaction. P levels below or above 1 mg were less effective.

In the rat, graded levels of P (1 to 3 mg/day) induced increased response but 6 mg did not increase the reaction further(4). When P 3 mg and EB 1 μg were administered, slight but not significant increases in various indices of placentomata growth were observed(5).

Whether relaxin plays a role in placentomata production at any point was studied at levels of estrogen below optimum during pretreatment. Relaxin appeared to have a slight beneficial effect on placentomata production. The greatest effect of relaxin was noted when 5 GPU was administered concurrently with progesterone during the post-trauma period. However, relaxin was without value as a substitute for progesterone at this time or during the entire period. These results suggest that relaxin is not involved in placentomata production nor in

TABLE II. Effect of Estrogen and Relaxin on Placentomata Reaction in Mice.

	Pretreatment		Pre-trauma		Post-trauma		Decidual reaction on 9th day: Diam of uterus		Wt of uterus		
No. of mice	EB (μg)	R GPU	P (mg)	R GPU	P (mg)	R GPU	Cont (mm)	Trauma (mm)	Cont (mg)	Trauma (mg)	Increase (%)
7	.01		1.0		1.0		1.4	2.0	24.0	32.8	36.0
7	.01	5	1.0		1.0		1.3	1.7	30.6	55.6	103
4	.001		1.0		1.0		.8	2.4	9.7	30.0	209
5	.001	5	1.0		1.0		.9	2.2	11.0	33.8	237
5	.002		1.0		1.0		1.0	1.7	13.2	25.8	71
6	.002	5	1.0		1.0		1.2	3.2	31.4	93.7	251
4	.005		1.0		1.0		1.1	2.0	15.7	27.5	77
7	.005	5	1.0		1.0		1.2	2.5	31.3	72.8	161
15			1.0		1.0		1.0	2.1	21.0	58.5	144
6		5	1.0		1.0		1.0	2.1	14.3	35.0	142
8			1.0	5	1.0	5	.8	2.5	19.4	55.5	197
10	.1		1.0		1.0	5	1.2	4.2	14.1	171.3	755
13	.1		1.0			5	1.5	2.7	38.5	75.1	124
6	.1			5		5	1.0	1.3	14.6	21.5	36
6	.1			30		30	.9	1.0	20.6	24.4	27
5	.1		1.0		1.0	*	1.5	3.3	48.0	148.0	218
5	.1		1.0		1.0	†	1.7	2.6	35.0	76.9	112
4	.1		1.0		1.0	‡	1.2	2.5	35.4	98.5	161

R: Relaxin
EB: Estradiol benzoate
P: Progesterone

* EB: .01 μg
† EB: .01 μg + R 5 GPU
‡ EB: .5 μg

normal implantation of the fertilized egg in the mouse. It is possible that endogenous relaxin production might synergize with P toward the end of the period. The fact that estrogen depresses decidual reaction after traumatization in the mouse suggests that early pregnancy is a period when estrogen is undesirable. The secretion of estrogen at this time might interfere with normal implantation.

In a study of the hormonal requirements for mammary gland growth in ovariectomized mice, it was shown that 1 μg EB plus 3 mg P administered for 19 days induced the greatest lobule-alveolar development as measured by DNA. However, the level of DNA attained experimentally by this treatment was only 57.6% of the DNA observed in normal mice pregnant 18 days(6). The present observations suggest that EB during the first 9 or 10 days might be a depressing factor in lobule-alveolar growth just as it depresses the placentomata reaction. In contrast, in the rat, daily administration of 1 μg EB plus 2 or 3 mg P for 19 days resulted in mammary gland growth as measured by DNA comparable to that of rats pregnant 18-20 days(7). These differences in the mouse and rat in regard to the role of estrogen in placentomata production and mammary gland growth suggest that mice secrete little or no estrogen during the first half of pregnancy.

Summary. Mature mice, ovariectomized for 7 to 10 days, were administered 0.1 μg EB for 3 days as a pretreatment followed by graded levels of P alone for 9 days. On day 5, the right uterine horn was traumatized by a sharp needle throughout its length. On day 4 post-trauma, the right and left horns were separated and compared in weight. The placentomata reaction increased as P was increased to 1.0 mg/day, then decreased with levels of 2 to 10 mg/day. Relaxin alone as a substitute for P had no effect. During pretreatment on reduced levels of EB, relaxin showed a slight response and during the post-trauma period with P a marked response was observed. When EB was administered with P during post-trauma period, the reaction was reduced. This observation suggests that mice secrete little or no estrogen during the first half of pregnancy.

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Anti-Androgenic Activity of a Series of 17-Iminosteroids. (28449)

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In recent years there has been considerable interest in compounds which inhibit the action of testosterone. Dorfman and Nes(1) reported that dihydroisosteviol inhibited the action of testosterone on the chick comb but not on the seminal vesicles or prostate of the rat. Dorfman and Stevens(2) also reported that a perhydrophenanthrene derivative inhibited the action of testosterone on both the chick comb and the accessory glands of the castrate rat. This latter compound was not estrogenic but was slightly androgenic and produced inhibition of pituitary gonadotrophin secretion in parabiotic rats. Byrnes *et al.*(3) reported that 11α-hydroxy-progesterone partially inhibited the stimulatory ef-