INTERACTIONS BETWEEN OESTRADIOL 3,17β AND PROGESTERONE ON THE INDUCTION AND GROWTH OF DECIDUOMATA IN OVARIECTOMIZED MICE

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Summary

The hormonal control of uterine sensitivity in progesterone-treated, ovariectomized mice was investigated by stimulating the deciduoma reaction by various means. Uterine sensitivity reached a peak on day 5 of progesterone treatment and then declined. Oestradiol before and after stimulation significantly increased the weight of the deciduomata induced by crushing, bradykinin, and compound 48/80 and increased uterine sensitivity so that intraluminal peanut oil could induce deciduomata. A single injection of 0·024 μg of oestradiol 8 hr before intraluminal peanut oil also greatly increased uterine receptivity to oil. The dose of oestradiol and the time of injection of oestradiol and progesterone were critical parameters. Surprisingly, the hormonal requirements for uterine sensitivity to intraluminal peanut oil are more stringent than for transferred blastocysts.

Uterine sensitivity on day 10 could be increased by multiple injections of oestradiol before stimulation, but responses were not as large as those obtained following oil on day 5.

From these data it is concluded that uterine sensitivity depends on the length of progestation, being maximal on day 5 of progesterone treatment, and on the amount of oestrogen before stimulation — uterine sensitivity may be increased by either a single or multiple doses of oestradiol.

I. INTRODUCTION

Uterine sensitivity has been defined as the capacity of the uterus to respond to a stimulus, either artificial or from the blastocyst, and transform to decidual tissue (Long and Evans 1922; De Feo 1967).

The hormonal requirements for implantation and for the artificially induced deciduoma reaction are not always identical. Deanesly (1963) showed that implantation occurs normally in untreated, ovariectomized, pregnant guinea pigs, but that deciduomata could be induced only if extra progesterone was administered. Conversely, Krehbiel (1941) could produce deciduomata in lactating rats while blastocysts remained in diapause in the other uterine horn. Deanesly (1963) suggested that the blastocysts may carry some factor essential for implantation — perhaps a hormone or enzyme. This may be true for the guinea pig (Blandau 1949). However, Krehbiel (1941) showed that in the lactating rat the electrical stimulus needed for deciduoma formation was stronger than normal, so that hormonal requirements for decidualization also seem to depend on the nature of the inducing stimulus.

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This paper describes the induction of deciduomata in ovariectomized mice with various stimuli of different intensities, and the interactions between progesterone and oestradiol in this induction. The data obtained from these experiments have been used to define the hormonal requirements of implantation and embryonic development following blastocyst transfer to ovariectomized mice (Humphrey 1967, 1968*a*).

II. Materials and Methods

Female mice of the QS strain were ovariectomized at 6–8 weeks of age, at a body weight of 22–25 g, leaving the greater part of the oviduct intact (Humphrey and Martin 1968).

The mice were randomly assigned to experimental groups 7–10 days later, and then received 0·8 mg/day of progesterone. The first day of progesterone treatment was termed day 1 of the experiment. Except in experiment 1, the mice were primed with 0·1 μg of oestradiol for 3 days immediately prior to treatment with progesterone. Deciduomata were induced either by crushing the right uterine horn with artery forceps, or by intraluminal injection to the right horn of 10 μl of peanut oil (given 8 hr after subcutaneous administration of 0·024 μg of oestradiol) or of 100 μg of the histamine releaser, compound 48/80 (Burroughs Wellcome), as described by Humphrey and Martin (1968).

The mice were killed by cervical dislocation 4 days after application of the stimulus unless stated otherwise. The deciduoma weight was taken as the difference in weight between the treated (right) horn and the untreated (left) horn (Humphrey 1968*b*; Humphrey and Martin 1968). The experimental schedules were as follows:

(1) *Importance of Oestradiol Priming.*—Mice were given 0, 0·01, 0·1, 1·0, or 10·0 μg of oestradiol for 1–4 days immediately preceding progesterone treatment, as indicated in Table 1. The stimuli used were crushing on day 4 or intraluminal injections of compound 48/80 or peanut oil on day 5.

(2) *Effects of Time of Stimulation.*—The time of maximal uterine sensitivity was investigated by crushing or by injections of compound 48/80 or peanut oil on days 2–6 of progesterone treatment.

(3) *Effects of Dose of Progesterone.*—Doses of progesterone ranging from 0·0 to 3·2 mg/day were given throughout the experiment. Deciduomata were stimulated by crushing on day 4 or compound 48/80 on day 5.

(4) *Growth of Deciduomata.*—Deciduomata were stimulated by crushing on day 5 and the mice were killed 2 to 9 days later.

(5) *Interactions between Progesterone and Oestradiol at Level I.*—Mice were given doses of oestradiol ranging from 0·0 to 0·064 μg/day on days 1–8 or 11. The stimulus used was compound 48/80 on day 5, and the mice were killed 4 or 7 days later.

(6) *Interactions between Progesterone and Oestradiol at Level II.*—Mice were given 0·0, 0·004, or 0·008 μg/day of oestradiol on days 1–11. On day 5, deciduomata were stimulated by crushing or injection of compound 48/80, peanut oil, or 0·1 μg of synthetic bradykinin (Humphrey and Martin 1968) intraluminally. In this experiment, 0·024 μg of oestradiol was not given before the peanut oil. In a further experiment, 12 mice received 0·008 μg/day of oestradiol from day 1 to 8, but were not stimulated, to see if oestradiol would induce the decidual cell reaction as claimed by Johnson and Shelesnyak (1958).

(7) *Effects of a Single Injection of Oestradiol on Uterine Sensitivity to Intraluminal Peanut Oil.*—A single injection of oestradiol or the vehicle only was given at various times on day 4 or 5, before peanut oil at 1200 hr on day 5. In these and subsequent experiments, the deciduomata were assigned a deciduoma induction score (DIS) of 0–4 (Shelesnyak and Kraicer 1961; Humphrey 1968*b*) since such scoring reflects subtle changes in uterine sensitivity which are not evident from measurements of deciduoma weights.

(8) *Importance of Progesterone on Day 5.*—In previous experiments, progesterone had been administered at 1200 hr on each day. In the next series of experiments, injections of progesterone or intraluminal peanut oil were made at 0600, 1200, or 1800 hr, following 0·024 μg of oestradiol at 0400 hr on day 5. Finally, 0·024 μg of oestradiol was given at 1000 hr, followed by progesterone and intraluminal peanut oil at 1800 hr on day 5 — some of these mice received an extra dose of progesterone at 1800 hr on day 4 in an attempt to overcome any deficiency of the hormone.

(9) *Effects of Oestradiol on Uterine Sensitivity on Day 10.*—Preliminary experiments had shown that crushing, and to a limited extent compound 48/80, on day 10 would induce deciduomata in many mice, but that peanut oil was completely ineffective. Varying doses of oestradiol were given on days 9–11 and peanut oil was given at 1200 hr on day 10.

All experiments were of factorial design with equal numbers of mice in each group, but the data from different experiments and replicates are pooled so that the final group sizes in the tables are often unequal. For simplicity, many of the results are presented as graphs or histograms. Analyses of variance of the deciduoma weights and scores were performed in each separate experiment and the levels of significance derived from these analyses are indicated in the text and tables. A double-blind technique was used throughout.

Progesterone and oestradiol were given by separate subcutaneous injections in 0·05 ml of peanut oil, usually at noon each day.

III. Results

(*a*) *Experiment* 1

From Table 1, it is seen that priming with oestradiol is essential for maximum decidualization (in all experiments, primed *v.* non-primed: $P<0·001$). The incidence of deciduomata in non-primed mice after crushing was not reduced, but the reactions were much smaller than in primed mice. The deciduomata in non-primed animals following compound 48/80 or peanut oil occurred only at the site of injection with the exception that compound 48/80 stimulated one full-length reaction, the deciduoma weighing 488 mg. In nearly all primed mice, the entire stimulated horn had decidualized. It seems that at least 0·1 μg of oestradiol for 2 days is required for adequate uterine sensitivity, but higher doses of oestradiol do not significantly increase the deciduoma weights. This oestrogen priming cannot be replaced by oestrogen given concurrently with progesterone before stimulation (Humphrey 1966).

(*b*) *Experiment* 2

Crushing on day 2 was only partly effective (Fig. 1) but large deciduomata developed when the uterus was crushed on days 3, 4, 5, or 6, with highly significant linear and quadratic differences between days ($P<0·001$ in each case). Intraluminal compound 48/80 and peanut oil were effective only on days 4 and 5 with significant quadratic effects between days ($P<0·001$ and $P<0·05$ respectively for each stimulus). Reactions after treatment on day 3 or 6 were confined to the site of injection. In other experiments, not presented here, it was found that in mice given lower doses of progesterone (0·2 or 0·4 mg/day) crushing on day 2, 3, or 6 was much less effective than crushing on day 4 or 5.

(*c*) *Experiment* 3

Figure 2 shows that progesterone doses of 0·4 mg/day or over were needed for consistent decidualization with significant linear effects of progesterone dosage

TABLE 1

EFFECTS OF OESTRADIOL PRIMING ON DECIDUOMA FORMATION IN OVARIECTOMIZED MICE

Deciduomata were induced on day 4 or 5 of progesterone treatment as indicated. Results are expressed as means

Oestradiol Priming Dose (μg/day)	No. of Days of Priming	No. of Mice	No. with Deciduomata (%)	Mean Deciduoma Weight (mg)
		Crushed on day 4		
0·00	3	20	90	47
0·01	1	20	100	45
	2	20	100	58
	3	20	100	62
0·1	1	20	100	63
	2	20	100	102
	3	20	100	101
1·0	1	20	100	62
	2	20	100	73
	3	20	100	107
		Compound 48/80 on day 5		
0·00	3	20	40	40
0·01	1	19	79	25
	2	19	90	32
	3	19	79	31
0·1	1	19	90	19
	2	47	92	117
	3	47	96	139
	4	28	100	128
1·0	1	19	90	53
	2	47	96	124
	3	47	100	146
	4	28	100	128
10·0	2	28	91	70
	3	28	100	89
	4	28	100	143
		Peanut oil on day 5*		
0·00	3	5	60	17
0·1	3	5	100	95

* Oestradiol (0·024 μg) given 8 hr before stimulation.

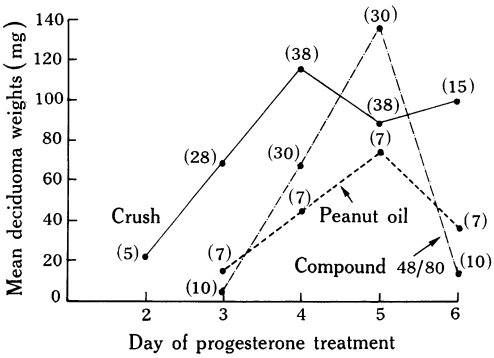

Fig. 1.—Effects of time of induction on the development of deciduomata. A dose of 0·024 μg of oestradiol was given 8 hr before induction with peanut oil. Numbers of mice per point given in parenthesis. A summary of the analyses of variance is as follows:

Source of Variation	Effect	D.F.	*P*
Crush	Linear	1	<0·001
	Quadratic	1	<0·002
Compound 48/80	Quadratic	1	<0·001
Peanut oil	Quadratic	1	<0·05

($P<0\cdot001$). In this and other experiments not presented, doses of 0·2 mg/day were of marginal activity when the deciduoma stimulus was crushing, but were ineffective when the stimulus was compound 48/80. There was virtually no increase in deciduoma weights when the progesterone dose was raised from 0·8 to 3·2 mg/day.

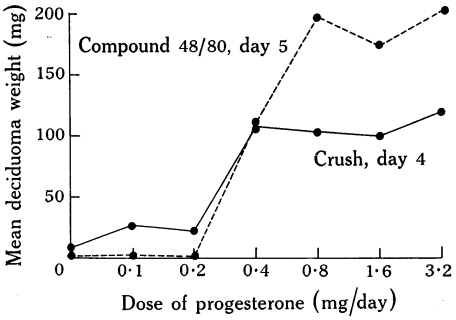

Fig. 2.—Effects of dose of progesterone on the development of deciduomata. There were 10 mice per group. A summary of the analyses of variance is as follows:

Source of Variation	D.F.	P
Progesterone *v.* control	1	$<0\cdot001$
Progesterone dose — linear effect	1	$<0\cdot001$

(*d*) *Experiment* 4

Deciduomata reached maximum size on day 12, 7 days after induction, and most deciduomata showed signs of resorption after day 13 (Fig. 3). The differences between weights were significant at the linear and quadratic levels ($P<0\cdot001$ for each).

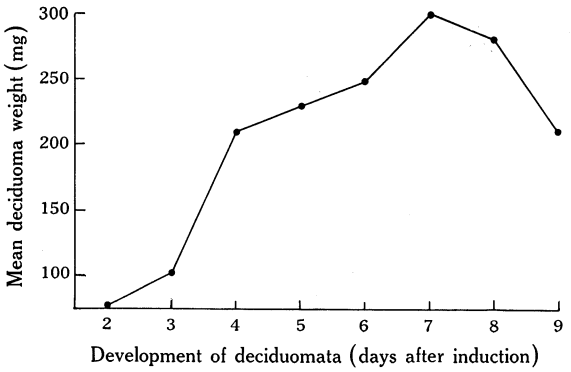

Fig. 3.—Growth of deciduomata which were induced by crushing on day 5, the mice being killed 2–9 days later. There were 10 mice per group.

(*e*) *Experiment* 5

From Figure 4, it is seen that daily doses of 0·032 μg or above of oestradiol partly inhibited deciduoma formation but slightly lower doses augmented deciduoma weights. The effects of oestradiol dosages were significant at the linear ($P<0\cdot001$) and quadratic levels ($P<0\cdot01$). Largest deciduomata were obtained in mice given 0·008 μg/day of oestradiol and killed 7 rather than 4 days after stimulation. These data confirmed earlier experiments in which deciduomata were induced by crushing on day 4.

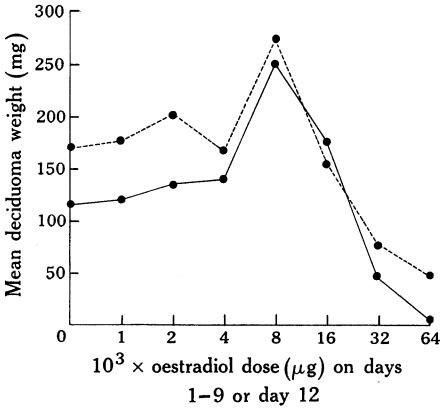

Fig. 4.—Effects of oestradiol at level I before and after induction on the development of deciduomata which were induced by compound 48/80 on day 5.

●——● 4 days development, 25 mice per point.

●---● 7 days development, 10 mice per point.

A summary of the analyses of variance is as follows:

Source of Variation	D.F.	P
Oestradiol, effect of dose		
Linear	1	$<0\cdot001$
Quadratic	1	$<0\cdot01$

(*f*) *Experiment* 6

Figure 5 shows that treatment with oestradiol on days 1–11 significantly increased the size of the deciduomata stimulated by crushing ($P<0\cdot05$), or intraluminal compound 48/80 ($P<0\cdot01$), peanut oil ($P<0\cdot01$), or bradykinin (for

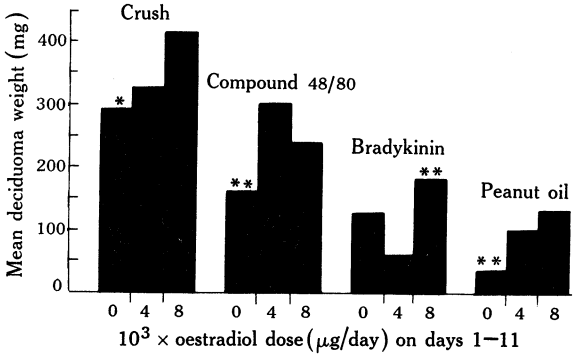

Fig. 5.—Effects of oestradiol at level II before and after induction at day 5 on the development of deciduomata after 7 days. Deciduomata were induced by crushing or by injection of compound 48/80, bradykinin, or peanut oil intraluminally. There were 8 mice per group in all treatments except peanut oil, when there were 16 mice per group. A summary of the analyses of variance is as follows:

Source of Variation	D.F.	P
Control *v.* oestradiol		
Crush	1	$<0\cdot05$
Compound 48/80	1	$<0\cdot01$
Peanut oil	1	$<0\cdot01$
Between doses of oestradiol		
Bradykinin	1	$<0\cdot01$

differences between doses of oestradiol, $P<0\cdot01$). Peanut oil was completely inactive in mice not given oestradiol — in these animals, deciduomata formed only at the injection site (DIS of 1, not shown). However, $0\cdot004$ or $0\cdot008\,\mu g$ of oestradiol on days 1–11 significantly increased uterine sensitivity so that peanut oil stimulated

massive, full-length deciduomata in nearly all mice given progesterone only. Of the 12 mice given oestradiol on days 1–8 and not stimulated, none had decidual reactions on day 9 (not shown).

(*g*) *Experiment* 7

Table 2 shows that peanut oil is ineffective in mice not given oestradiol, and that the largest reactions developed following 0·024 μg of oestradiol at 0400 hr on day 5, i.e. 8 hr before oil stimulation (see also Finn 1966). Oestradiol significantly increased deciduoma scores and weights ($P<0·001$). Higher doses of oestradiol inhibited deciduoma formation and oestradiol given less than 8 hr before stimulation or after stimulation was much less effective (see also Humphrey 1966).

TABLE 2

EFFECTS OF A SINGLE INJECTION OF OESTRADIOL UPON UTERINE SENSITIVITY

Deciduomata were induced with intraluminal peanut oil at 1200 hr on day 5. All results are expressed as means

Time		Oestradiol Dose (μg)	No. with Deciduomata (%)	Mean DIS	Mean Uterine Weight Difference (mg)
Day	Hour				
			7 mice per group		
5	0400	0·000	28·6	0·9	32·7
4	1200	0·016	100·0	1·6	78·7
	2000	0·016	85·7	1·9	83·7
5	0400	0·016	85·7	2·6	116·4
	1200	0·016	71·4	1·0	24·9
	2000	0·016	85·7	1·9	56·2
			10 mice per group		
5	0400	0·000	60·0	0·6	29·1
		0·016	100·0	3·4	214·3
		0·024	100·0	3·7	235·6
		0·036	90·0	2·3	98·3
		0·054	90·0	2·2	98·4
		0·108	30·0	0·6	35·3
			11 mice per group		
5	0400	0·000	55·5	0·6	13·6
5	0000	0·024	88·8	2·2	96·2
	0200	0·024	88·8	1·7	89·5
	0400	0·024	88·8	2·7	159·0
	0600	0·024	77·7	1·8	48·2
	0800	0·024	77·7	1·2	50·3
	1000	0·024	88·8	1·1	34·6

(*h*) *Experiment* 8

Table 3 shows that the time of injection of progesterone is an important factor controlling uterine sensitivity. Optimum conditions for decidualization are intraluminal peanut oil at 1200 hr with progesterone given at 0600 hr or at 1200 hr. If progesterone is delayed until 1800 hr then uterine sensitivity is reduced ($P<0·05$). Local injections of peanut oil at times other than 1200 hr are relatively ineffective

even if oestradiol is given 8 hr before induction at 1000 hr. An extra injection of progesterone at 1800 hr on day 4 before oil induction at 1800 hr on day 5 increased the deciduoma scores and weights but the increases were not statistically significant.

TABLE 3

EFFECTS OF TIME OF PROGESTERONE TREATMENT ON UTERINE SENSITIVITY

Deciduomata were induced with 10 μl of intraluminal peanut oil at various times on day 5, before or after treatment with 0·8 mg of progesterone and 0 or 0·024 μg of oestradiol. Results are expressed as means

Time of Treatment on Day 5			No. of Mice	No. with Deciduomata (%)	Mean DIS	Mean Deciduoma Weight (mg)
Oestradiol	Progesterone	Peanut Oil				
0400*	1200	1200	8	62·5	0·6	27
0400	0600	0600	8	100·0	1·9	51
	0600	1200	8	87·5	3·0	193
	1200	0600	8	75·0	1·6	25
	1200	1200	8	75·0	2·4	96
	1200	1800	8	87·5	1·4	45
	1800	1200	8	100·0	1·5	68
1000*	1800†	1800	9	78·0	0·8	34
1000	1800†	1800	9	78·0	1·8	82
	1800	1800	9	89·0	1·3	42

* Not given oestradiol. † Extra progesterone at 1800 hr on day 4.

TABLE 4

EFFECTS OF OESTRADIOL ON UTERINE SENSITIVITY ON DAY 10

Deciduomata were induced with peanut oil at 1200 hr on day 10. All injections were given at 1200 hr unless otherwise stated. Results are expressed as means

Oestradiol			No. of Mice	No. with Deciduomata (%)	Mean DIS	Mean Deciduoma Weight (mg)
Dose (μg)	Day(s)	Hour				
0	9–11	1200	35	43	0·4	22
0·016	9–10	1200	13	100	2·5	81
0·016	9–11	1200	7	100	2·6	78
0·024	9	1200	8	38	0·4	10
0·024	9	2000	6	83	1·7	62
0·024	10	0400	6	67	1·0	28
		1000	6	67	0·7	18
0·024	9–10	1200	23	83	1·6	56
0·024	9–11	1200	15	80	1·7	50

(*i*) *Experiment* 9

Intraluminal peanut oil on day 10 was completely inactive in mice given progesterone only, but oestradiol significantly increased the deciduoma weights and scores (Table 4). A single injection of 0·024 μg of oestradiol 8 hr before stimulation

was ineffective, and largest responses were obtained in mice given 0·016 μg on days 9–10 or 11, or 0·024 μg at 2000 hr on day 9 (DIS, $P<0·001$; weights, $P<0·05$). The higher dose of 0·024 μg on days 0–10 or 11 was less effective.

IV. Discussion

These experiments help to define the hormonal requirements for uterine sensitivity and the production of deciduomata in the ovariectomized mouse. The requirements appear to be:

1. Adequate oestrogen priming.
2. Adequate progesterone.
3. Correct time of application of the stimulus.
4. Presence of the correct amount of oestrogen before and after stimulation.

Different stimuli have different hormonal requirements. Crushing, a traumatic stimulus, is effective when uterine sensitivity is submaximal since crushing does not require oestrogen priming, and is effective at progesterone dose rates and at times when less traumatic stimuli, such as peanut oil or compound 48/80, are inactive (see also Shelesnyak 1962; De Feo 1963*a*, 1963*b* for rat; Elton 1966 for the rabbit; Finn 1966 for the mouse). Surprisingly, blastocysts have less stringent requirements than peanut oil, since oestrogen priming is not essential and transferred blastocysts will implant on day 6 or 8 of progesterone treatment (Humphrey 1968*a*).

The minimum effective dose of progesterone was 0·4 mg/day, and doses greater than 0·8 mg/day did not increase deciduoma weights (see also Zarrow, Peters, and Caldwell 1958; Finn 1966).

One of the major factors controlling uterine sensitivity is the duration of the preceding progestational stimulus. In ovariectomized rats and mice, uterine sensitivity to non-traumatic stimuli, such as compound 48/80 or peanut oil, is virtually restricted to day 4 or 6 of progesterone treatment and is very much less on succeeding days (Yochim and De Feo 1963; Brumley and De Feo 1964). This closely corresponds to the peak of uterine sensitivity for the induction of deciduomata in pseudopregnant mice (Humphrey and Martin 1968) and rats (De Feo 1963*a*, 1963*b*; Humphrey 1968*b*), and for the implantation of transferred ova in pseudopregnant rats and mice (Noyes and Dickmann 1960; Doyle, Gates, and Noyes 1963 respectively) and in ovariectomized mice (Humphrey 1968*a*).

This restricted period of uterine sensitivity in ovariectomized mice occurs even though a constant daily dose of oestradiol is given (Humphrey 1968*a*) and is thus not an artifact due to a deficiency of oestrogen, but is due to some progesterone-dependent differentiation and maturation of the endometrium (see Martin and Finn 1968). Uterine sensitivity takes at least 3 days to develop, and declines but does not completely disappear after 5 days of progesterone dominance, depending on the stimulus.

Oestrogen given during progestation has two effects on decidualization: (1) before stimulation it increases uterine sensitivity; (2) after stimulation it increases the growth of decidual tissue (see also Rothchild, Meyer, and Spielman 1940; and Yochim and De Feo 1963 for the rat).

Uterine sensitivity to oil can be greatly increased by treatment with oestradiol on either days 1–5, days 4 and 5, or day 5 only (Finn 1966; Humphrey 1966) — all treatments are equally effective. Repeated injections of oestradiol on the days

before peanut oil stimulation do not interfere with uterine sensitivity — the uterus does not become refractory or non-receptive as postulated by Psychoyos (1963) and Finn (1966). Any delay in injection of progesterone also prevents decidualization, perhaps because of rapid utilization of this hormone in the mouse.

On day 10, a single injection of 0·024 μg of oestradiol 8 or 24 hr before peanut oil did not significantly increase uterine sensitivity, but if the oestradiol was given 16 hr beforehand, large deciduomata developed, although these were smaller than those produced by peanut oil on day 5. Similar results have been reported by Cartoni and Bignami (1966) and De Feo (1967) in the ovariectomized rat.

At this stage, the pattern of oestrogen secretion before implantation in the pregnant mouse is unknown, but from the results of Finn and Martin (1967), Miller, Owen, and Emmens (1968) and Humphrey (1968*a*) it would seem not to be of the nature of a surge, as believed by Shelesnyak (1962).

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