INDUCTION OF DECIDUOMATA IN NORMAL AND HISTAMINE-DEPLETED RATS

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[*Manuscript received August* 23, 1967]

Summary

In the rat large deciduomata were obtained by crushing and by intra-uterine injections of saline, peanut oil, or compound 48/80. Effects of injections of saline made with a 28-gauge needle were largely confined to the site of insertion, but injections made with a 26-gauge needle induced very large, full-length deciduomata almost as large as those produced by crushing. Three histamine-releasing agents were given intraperitoneally: pyrathiazine which induced large deciduomata and damaged the abdominal organs, and compound 48/80 and polymyxin B which were non-irritant and did not induce deciduomata.

The induction of deciduomata was not prevented by prior depletion of tissue histamine by compound 48/80 or polymyxin B although the growth of decidual tissue produced by local compound 48/80 was slightly less in rats depleted with polymyxin B.

From these results it is concluded that histamine is not a key factor in the induction of decidualization.

I. Introduction

Since the discovery of the artificial deciduoma by Loeb (1907), many workers have used this reaction to study the maternal aspect of implantation. The usual stimulus for the induction of deciduomata involves tissue injury (Blandau 1961), and Shelesnyak (1952) has proposed that the tissue injury factor is histamine, since local antihistaminic drugs prevent decidualization in the rat. Further evidence is the finding that intraluminal histamine induces deciduomata in the intact rat (Shelesnyak 1952), in the ovariectomized rat (Zarrow, Peters, and Caldwell 1958; Nakao and Aizawa 1963), and in the rabbit (Chambon and Lefrein 1952). However, other workers have been unable to demonstrate any significant difference between injections of histamine and of saline in the rabbit (Elton 1966), the rat (Finn and Keen 1962*a*; De Feo 1963; Orsini 1963; Banik and Ketchel 1964; Wrenn *et al.* 1964), and the mouse (Miyake, Kakushi, and Hara 1964; Humphrey and Martin 1968).

The antihistamine pyrathiazine, when given systematically, has been stated to induce deciduomata in the pseudopregnant rat, presumably by releasing mast cell histamine (Kraicer and Shelesnyak 1958; Kraicer 1960; Psychoyos 1961; Shelesnyak and Kraicer 1961; De Feo 1963). However, pyrathiazine was reported to be inactive in the rat, hamster, and mouse by Orsini (1963), Finn (1965), and Humphrey and Martin (1968).

Repeated treatment with histamine-liberating compounds such as compound 48/80, polymyxin B, or pyrathiazine depletes the tissues of histamine and abolishes some histamine-dependent reactions (Feldberg and Talesnik 1953). Kraicer, Marcus,

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and Shelesnyak (1963) found that decidualization in the rat was reduced or abolished by such histamine depletion, but Banik, Kobayashi, and Ketchel (1963) reported that nidation in the rat was unaffected by alterations in the tissue histamine levels.

This paper describes the induction of deciduomata in both normal and histamine-depleted rats.

II. Materials and Methods

(*a*) *Animals*

Randomly bred female rats of the Wistar strain, weighing 190–230 g, were caged in an enclosed animal house kept at 72°F, with artificial lighting from 0600 to 1800 hr. Daily vaginal smears were taken with cotton-wool swabs throughout the experiments. After each rat had displayed two normal oestrous cycles, pseudopregnancy was induced by electrical stimulation of the cervix on the day(s) of cornified vaginal smears. In this colony, 50% of the rats had oestrous smears for 1 day and the remainder for 2 days. The first day of predominantly leucocytic smears after cervical stimulation was termed day 1 of pseudopregnancy. Rats with cornified smears after day 1 were excluded from the experiments.

(*b*) *Induction of Deciduomata*

On the day of induction of deciduomata, the rats were assigned to a treatment group and were anaesthetized with ether. For local application the right ovary and uterine horn were exposed through a dorsal body wall incision, and an Agla micrometer syringe used to inject 50 μl of medium through the ovarian tip. A 28-gauge needle was used unless stated otherwise. In some rats the right horn was traumatized by crushing with artery forceps. Parenteral administration of histamine-releasing agents was by intraperitoneal injection, given under light ether anaesthesia.

(*c*) *Deciduoma Weights and Scores*

Rats were killed 96 hr after induction by cervical dislocation under Nembutal anaesthesia. The reproductive tract was dissected and the deciduomata scored and weighed. The deciduoma induction score (DIS) was based on the length of the treated horn which developed deciduomata, scores of 0–4 being allotted as follows:

4, for a full-length reaction of the treated horn;

3, when three-quarters of the horn contained deciduomata;

2, when more than one-quarter but less than three-quarters of horn contained deciduomata;

1, when one-quarter of horn or less contained deciduomata;

0, for absence of deciduomata.

After intra-uterine injection or crushing, the DIS was based on the treated (right) horn only. The uterine horns were separated at the cervix, washed in saline, blotted dry, and weighed to the nearest milligram on a torsion balance. After intra-uterine injection, the deciduoma weight in each rat (w) was taken as the difference in weight between right (treated) and left (untreated) horns. In a few rats deciduomata were found in the left horn and in these animals the mean weight of the left horn in the remainder of the group was substituted. When decidualization was attempted with intraperitoneal injections, the deciduoma scores and weights were based on both horns, using total uterine weight (W) in the latter instance.

(*d*) *Schedules of Experiments*

Schedule 1

To determine the time of maximum uterine sensitivity, deciduomata were induced by crushing or by intraluminal peanut oil (50 μl with a 28-gauge needle) on days 3, 4, or 5 of leucocytic smears.

Schedule 2

A series of experiments was then performed to investigate the deciduomagenic properties of intraluminal peanut oil, saline, or compound 48/80. Rats received 50 μl saline using either a 26- or 28-gauge needle. Other animals had the right horn crushed or received 50 μl peanut oil or varying doses of compound 48/80, using a 28-gauge needle.

Schedule 3

In these experiments the activity of systemically administered histamine-releasing agents was studied. Firstly the incidence of spontaneous deciduomata was investigated in 38 untreated pseudopregnant animals. Other rats received 150 μg compound 48/80, 750 μg polymyxin B, or 2·0 mg pyrathiazine by intraperitoneal injections in 0·5 ml saline. Control rats received saline only. Other animals received 50 μl peanut oil to the right horn, or the right horn was crushed. These doses of polymyxin B and compound 48/80 have been shown to release most of the tissue histamine (Parratt and West 1957; Riley 1959).

Schedule 4

Decidualization was then attempted in histamine-depleted animals. Depletion was achieved by repeated intraperitoneal injections of compound 48/80 or polymyxin B, on days 1–4 of pseudopregnancy as follows:

	Compound 48/80 (μg)		Polymyxin B (mg)	
	0900 hr	1700 hr	0900 hr	1700 hr
Day 1	100	150	0·8	0·8
Day 2	200	250	1·2	1·2
Day 3	300	350	2·0	2·0
Day 4	400	—	2·5	—

Control (non-depleted) rats received corresponding injections of saline. At 1200 hr on day 4 depleted and non-depleted rats were randomized into groups and deciduomata induced by crushing, by intraluminal injections of peanut oil or compound 48/80, or by intraperitoneal injections of pyrathiazine. Uterine sections and pieces of omentum were taken from groups of five control and depleted rats killed on day 4 and from all rats killed on day 8. These were fixed in Carnoy's solution and stained with 0·5% toluidine blue to demonstrate mast cells.

(*e*) *Analyses and Solutions*

Analyses of variance of the DIS and the deciduoma weights were performed but to conserve space they are not included in the tables. Statistically significant comparisons are referred to in the text. The standard errors of all deciduoma weights are given in the tables. In all experiments groups of control rats received intraluminal or intraperitoneal injections of saline. A double-blind technique was used when injecting and killing the rats and scoring the deciduomata. All solutions were freshly prepared and were stored at 5°C for the duration of the experiment.

III. Results

The results in Table 1 show that maximum responses with both stimuli were obtained by treatment on day 4 of pseudopregnancy ($P<0·001$). Reactions to oil on day 3 or 5 were commonly confined to the site of injection and the differences between responses to peanut oil and crushing indicate that peanut oil is the more specific and effective stimulus of decidualization. In this and all other experiments an occasional rat developed bilateral reactions.

In the experiments of schedule 2, it was found (Table 2) that peanut oil given with a 28-gauge needle was much more effective than saline ($P<0\cdot001$ for scores and weights) but saline injections made with a 26-gauge needle were almost as effective

TABLE 1

INDUCTION OF DECIDUOMATA ON DAYS 3, 4, AND 5 OF PSEUDOPREGNANCY IN THE RAT

Each result is expressed as a mean for eight rats, killed 4 days after induction of deciduomata by crushing one horn or by intraluminal injection of 50 μl peanut oil

Treatment	D(%)*	DIS	w†±S.E. (mg)
Day 3			
Crushing	75·0	1·8	229·5± 39·0
Peanut oil	62·5	0·6	66·5± 29·0
Day 4			
Crushing	100·0	3·6	911·0±182·0
Peanut oil	100·0	3·6	864·0±124·0
Day 5			
Crushing	100·0	2·3	282·0± 66·5
Peanut oil	87·5	1·6	226·0± 97·5

* Percentage rats with deciduomata.

† Mean weight difference between right (treated) and left (untreated) horns.

TABLE 2

INDUCTION OF DECIDUOMATA IN RATS BY INTRA-UTERINE INJECTIONS OF VARIOUS SUBSTANCES

Treatment	No. of Rats	D(%)*	Mean DIS	w†±S.E. (mg)
Crushing	9	100·0	2·8	704±132·0
Saline, 50 μl‡	9	100·0	2·0	416±102·0
Peanut oil, 50 μl	21	100·0	3·5	836± 52·0
0·9% saline, 50 μl	21	76·2	0·9	182± 34·0
Compound 48/80				
1 μg	12	91·7	1·0	264± 53·5
10 μg	12	100·0	3·9	986± 85·5
100 μg	12	100·0	4·0	1·052± 78·5
1000 μg§	7	42·9	0·5	176±109·5

* Percentage of rats with deciduomata.

† Mean weight difference between right (treated) and left (untreated) horns.

‡ These injections given with a 26-gauge needle, all other injections with a 28-gauge needle.

§ 16 of 24 rats died, one developed cornified smears. Deciduomata present in only 3 of the 7 survivors.

as crushing ($P<0\cdot05$ for DIS). Compound 48/80 was most effective ($P<0\cdot001$); doses of 10 and 100 μg produced massive, full-length deciduomata (DIS of 3 or 4) in all rats, with an occasional bilateral response, but 1·0 μg was no more effective than saline.

The results in Table 3, line 1, show that on day 8, six of 38 untreated rats (15·8%) had "spontaneous" deciduomata with a mean DIS of 0·18. These were at the cervices in all animals and one rat had a second large reaction in the centre of the right horn.

Intraperitoneal injections of saline, polymyxin B, or compound 48/80 induced deciduomata in 2, 2, and 4 respectively of 13 rats, with a mean DIS of 0·15, 0·15, and 0·38 respectively; these differences were not statistically significant.

TABLE 3

INDUCTION OF DECIDUOMATA IN THE RAT BY INTRAPERITONEAL INJECTIONS OF VARIOUS SUBSTANCES

Results are expressed as means

Treatment on Day 4	No. of Rats	D(%)*	Mean DIS	W†±S.E. (mg)
No injection	38	15·8	0·18	257± 8·0
Saline, 0·5 ml	32	18·8	0·12	257± 14·0
Polymyxin B, 750 μg	13	15·4	0·15	276± 22·0
Compound 48/80, 150 μg	13	30·8	0·38	280± 24·0
Pyrathiazine, 20 mg	19	78·9	2·2	930±160·0
Crushed right horn	13	100·0	2·00	1180±264·0
Peanut oil, 50 μl‡	19	100·0	2·0	1071±212·0

* Percentage of rats with deciduomata.

† Total uterine weight.

‡ Intra-uterine injection.

Of 25 pseudopregnant rats given 20 mg pyrathiazine intraperitoneally, six animals died, and large deciduomata were found in 15 of the 19 survivors (see Table 3). Analysis of variance of uterine weights and the DIS showed that systemic pyrathiazine was as effective as local peanut oil in the induction of deciduomata (treatments *v.* saline, $P<0·001$ for all parameters). There were marked pathological changes in the abdominal organs of 16 of the survivors. These animals had peritonitis with much fluid exudate and there were gross adhesions between the abdominal organs. The livers usually showed fatty degeneration and the edges were fibrosed and thickened. Occasionally haemorrhages in the intestinal walls were found. Three of the saline-treated rats had such intestinal haemorrhages but this was the only pathological change found.

The results obtained in histamine-depleted rats are presented in Table 4. Prior depletion of tissue histamine did not reduce the induction of deciduomata and the only significant difference in deciduoma weights was with local induction by compound 48/80 in rats depleted with polymyxin B ($P<0·05$). In the groups of rats not traumatized on day 4, all four depleted animals were negative while one control rat had a deciduoma at the cervix. After each injection of the histamine-releasing agents the rats collapsed and showed signs of histamine release. On day 8 depleted rats were debilitated and all weighed 25–50 g less than control rats. On day 4 stained omental spreads from control rats were packed with mast cells, and uterine sections showed isolated mast cells in the mesentery, serosa, and myometrium. In depleted

animals the peritoneal spreads were devoid of mast cells, as were the uterine sections. It appears that both compound 48/80 and polymyxin B, given on days 1–4, caused disruption of the tissue histamine levels.

TABLE 4

EFFECTS OF DECIDUAL INDUCERS IN HISTAMINE-DEPLETED RATS

Rats were first depleted of histamine by twice daily intraperitoneal injections of either compound 48/80 or polymyxin B [see schedule 4, Section II(*d*)] on days 1–4 of pseudopregnancy. Control (non-depleted) rats received corresponding injections of saline. Deciduomata were induced either locally or systemically

Treatment on Day 4	No. of Rats	Prior Treatment	*D*(%)*	Mean DIS	*w*†±S.E. (mg)
Local induction: rats depleted with compound 48/80					
Crush	5	Control	100·0	4·0	753·0± 69·5
	5	Depleted	100·0	4·0	555·0±124·0
Peanut oil, 50 μl	5	Control	100·0	3·6	849·0±160·0
	5	Depleted	100·0	3·8	784·0±181·0
Compound 48/80, 10 μg	5	Control	100·0	3·4	570·0± 83·5
	5	Depleted	100·0	3·2	742·0±121·5
No treatment	4	Control	25·0	0·1	49·0± 50·0
	4	Depleted	0·0	0·0	2·0± 2·5
Local induction: rats depleted with polymyxin B					
Crush	6	Control	100·0	3·7	806·0±162·0
	6	Depleted	100·0	3·8	778·0±110·5
Peanut oil, 50 μl	6	Control	100·0	3·7	1014·0±195·0
	6	Depleted	100·0	2·8	743·0±172·5
Compound 48/80, 10 μg	6	Control	100·0	3·7	902·0± 66·5
	6	Depleted	100·0	2·8	434·0±142·0
Systemic induction: rats depleted with compound 48/80					
					W±S.E. (mg)
Pyrathiazine, 20 mg	8	Control	62·5	1·5	647·0±151·0
	8	Depleted	50·0	1·4	830·0± 33·5
Systemic induction: rats depleted with polymyxin B					
Pyrathiazine, 20 mg	6	Control	33·3	0·8	721·0±103·0
	6	Depleted	50·0	0·5	768·0± 59·5
Pyrathiazine, 20 mg	6	Control	33·3	0·8	721·0±103·0
	6	Depleted	50·0	0·5	768·0± 59·5

* Percentage of rats with deciduomata.

† Mean weight differences between right (treated) and left (untreated) horns. For systemic induction experiments, total uterine weights (*W*) are given.

On day 8 the peritoneal spreads from control rats were packed with large mast cells as on day 4, whilst those from depleted animals had only a scattering of small rounded cells. This agrees with the findings of Riley (1959) who showed that the mast cells reappear in the tissues about 3 or 4 days after the cessation of depletion treatment.

IV. Discussion

The decidual cell reaction in the rat uterus is apparently readily induced. Intraluminal injections of saline made with a large needle were almost as effective as crushing, while "spontaneous" deciduomata developed in many untreated animals (see also Evans 1929; Innes and Bellerby 1929; Hain 1932; Chambon 1960; Kraicer 1960; Finn and Keen 1962*a*; De Feo 1963; Wrenn *et al.* 1963; Coppola, Ball, and Brown 1966). The frequent occurrence of these non-specific reactions shows the ambiguous results possible when testing for deciduomagenic activity in the rat, unless the experimental design incorporates random groups given saline or sham injections and unless the data are statistically analysed.

This is illustrated by the results obtained with local compound 48/80 by different workers. In the present work at a dose of 1·0 μg this substance is no more effective than saline, but 10 or 100 μg induce massive full-length reactions in virtually all rats. Kraicer and Shelesnyak (1958) have reported that local compound 48/80 induces deciduomata in the rat at a dose of 0·03 μg, although higher doses were no more effective and some rats given saline also developed deciduomata (see also Kraicer 1960). Apparently these data were not analysed. From the data presented by Kraicer and Shelesnyak (1958) it is impossible to determine the size and incidence of the reactions. In contrast, De Feo (1963) found that both saline and compound 48/80 induced massive deciduomata in rats, and the differences between the treatments were not statistically significant. In the present work reactions to injections of saline were almost always confined to the site of needle insertion, so that the deciduomagenic activity of the higher doses of compound 48/80 could be demonstrated. The low activity of 1000 μg of local compound 48/80 may perhaps be due to a direct inhibitory or toxic action of the substance (Paton 1957).

Intraluminal compound 48/80 also induces deciduomata in pseudopregnant and ovariectomized mice (Humphrey and Martin 1967). The effective dose of 10 μg corresponds to concentrations of 1·0 and 0·2 mg/ml in the mouse and rat respectively. Compound 48/80 releases histamine at very low concentrations but has direct effects at such high levels (Paton 1957), including a direct effect upon capillary permeability (Brocklehurst, Humphrey, and Perry 1957; Gözsy and Kato 1957) and causing apparent thrombosis of blood vessels in the skin (Miles and Miles 1952). The induction of deciduomata by local compound 48/80 in mice and rats was not prevented by prior depletion of tissue histamine (Humphrey and Martin 1967; the present results). These findings indicate that the decidualization following compound 48/80 is probably a direct action and not associated with release of histamine.

Intraluminal peanut oil is a most effective deciduomagenic stimulus. In experiments in this laboratory over the past five years it has proved to be a non-traumatic, highly specific stimulus for decidualization in mice and rats. Finn and Keen (1963) and Finn (1965) have described its activity in these species.

Systemic pyrathiazine induced many large deciduomata but also produced marked inflammatory changes and some necrosis in the abdominal organs and subcutaneous tissue. The corrosive action of pyrathiazine has also been described by Kraicer (1960) and Orsini (1963). Kraicer (1960) showed that pyrathiazine is

deciduomagenic only at high, near-toxic dose levels, and believed this action was due solely to histamine release. However, systemic compound 48/80 and polymyxin B release most of the tissue histamine (Paton 1957) but do not produce pathological changes in the tissues, or induce deciduomata (see also Kraicer and Shelesnyak 1958; De Feo 1963). It is clear that at the concentrations needed for decidualization, pyrathiazine has corrosive, traumatic properties not associated with histamine release and can hardly be called a physiological stimulus.

The induction of deciduomata is not impaired by drastic depletion of tissue histamine and it would appear that decidualization in the rat is not histamine-dependent, although the results given here and those obtained by Banik, Kobayashi, and Ketchel (1963) differ from those of Kraicer, Marcus, and Shelesnyak (1963). The latter workers depleted rats of histamine by repeated injections of compound 48/80 or pyrathiazine on days 1–3 and found that the induction of deciduomata by pyrathiazine was almost completely prevented, while the decidual responses to crushing or to implanting blastocysts were markedly reduced. The repeated injections of pyrathiazine used by these authors produced great inflammatory changes in the abdominal organs (Kraicer 1960) and this may have interfered with decidualization. Also, the antihistaminic properties of pyrathiazine may inhibit the growth of decidual tissue as occurs with mepyramine in the rat (Finn and Keen 1962*b*).

It is concluded that mast cell factors, including histamine (Shelesnyak 1963) and heparin (Finn and Keen 1963), are probably not important in the induction of decidualization in the rat.

V. Acknowledgments

The author wishes to thank Professor C. W. Emmens and Dr. L. Martin for their interest and criticism. This work has been supported in part by grants from the Population Council (U.S.A.) and the Australian Wool Board.

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