INCREASED DURATION OF PSEUDOPREGNANCY ASSOCIATED WITH UTERINE DISTENSION IN THE RAT

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Summary. The duration of pseudopregnancy in rats was increased to 15.9 ± 3.4 days (control 12.5 ± 0.7) following surgical separation of the posterior parts of the uterine horns from their cervical attachments. Under these conditions, the uterine horns became distended with fluid. The uterine walls were stretched and thin, but appeared otherwise undamaged.

Deciduoma formation was readily induced in distended horns but the presence of deciduomata did not further prolong the duration of pseudopregnancy.

INTRODUCTION

The original observation by Loeb (1923) that hysterectomy prolongs the life-span of the corpus luteum in the guinea-pig has since been amply confirmed in many species of mammals (Bland & Donovan, 1966; Fischer, 1967; Ginther, 1967; Melampy & Anderson, 1968). This effect has been attributed to removal of a uterine luteolytic influence.

It has also been shown that the presence of certain foreign materials in the uterine lumen, in some cases associated with a variable degree of uterine distension, can influence the life-span of the corpus luteum in some species (Hawk, 1968). In most cases, the effect has been a shortening of the cycle (Moore & Nalbandov, 1953; Bland & Donovan, 1965; Yamauchi, Nakahara, Kaneda & Inui, 1967), but in certain circumstances, a prolongation may be produced (Moore, 1961).

In experiments involving partial hysterectomy in rats, Bradbury, Brown & Gray (1950) noted that when segments of the uterus were separated from their cervical attachments, they became extremely distended with fluid and the endometrium showed atrophic changes. Subsequently such rats "reacted like a hysterectomized animal" in that pseudopregnancy was prolonged. This prolongation of pseudopregnancy was later interpreted by Everett (1961) as being associated with destruction of the endometrium.

O'Shea (unpublished data) found that bilateral section of the uterine horns in the rat, leaving the greater part of the horns blind-ending posteriorly, did

not affect the duration of the oestrous cycle. Histological study of these animals, some of which were subsequently mated and showed abnormally long pseudopregnancies, indicated that, although distension of the blind-ending parts of the uterine horns had occurred, degenerative changes in the endometrium were not marked.

It appeared possible, therefore, that uterine distension without destruction or degeneration of the endometrium might lead to prolongation of the luteal phase in the pseudopregnant rat. This paper reports experiments designed to investigate this possibility.

MATERIALS AND METHODS

Adult, female, albino Wistar rats, weighing not less than 150 g, were used in these experiments. All operations were performed under ether anaesthesia, using a midline abdominal incision.

Tissues for histological examination were fixed in 10% buffered neutral formalin or in Bouin's fluid. Paraffin sections were stained routinely with haematoxylin and eosin. The periodic acid-Schiff (PAS) method, colloidal iron (CI), and azure A at pH 3.0 were used for selected sections.

Oestrous cycles were studied by daily vaginal smears. Rats for all experiments were selected after study of at least three complete oestrous cycles, and only those females showing cycles of between 4 and 6 days in length were included.

Duration of pseudopregnancy

Three groups of rats were used (see Table 1, Groups I to III).

Group I: Control. Laparotomy was performed and both horns of the uterus exposed and handled before repair of the abdominal wound.

Group II: Hysterectomy. Both uterine horns with their attendant vessels were removed, following section just anterior to the uterine bifurcation and at the utero-tubal junction (see Text-fig. 1). The ovaries, Fallopian tubes and posterior parts of the uterus were left *in situ*.

Group III: Posterior uterine section. Both uterine horns and their attendant vessels were sectioned at the posterior site only (Text-fig. 1). Before section by scalpel, the vessels were ligated and the uterine horns crushed with artery forceps immediately above and below the site of section. The broad ligament was divided anteriorly to a point close to the ovarian blood vessels (Greene, 1955) as shown in Text-fig. 1. The uterine blood supply was, thereafter, maintained by the ovarian vessels. The uterine horns were left *in situ*, attached only at their anterior ends.

All operations were performed on the 1st day of di-oestrus. Pseudopregnancy was induced by sterile mating with vasectomized male rats. Up to three pseudopregnancies were induced in each rat, the first being induced at the second oestrous period following operation. The end of pseudopregnancy was recognized as the 1st day of the return of vaginal oestrus.

Structure of the uterine wall

Rats from the above experiment were destroyed on the 1st day of di-oestrus

following completion of their first normal, short cycle after their third pseudopregnancy. All parts of the genital system were examined macroscopically, and sections of the uterus were prepared for histological examination.

Production of deciduomata

Four rats were subjected to posterior uterine section on the left side only. They were mated with vasectomized males at the second oestrous period following operation. A second laparotomy was performed 4 days later to induce deciduoma formation. Two 4/0 monofilament nylon sutures were placed transversely through both uterine horns at approximately one-third and two-thirds of the way from the uterine bifurcation to the utero-tubal junction. These

TEXT-FIG. 1. Diagram to show the position of the incisions in posterior uterine section (dotted line on the right) and hysterectomy (dotted line on the left).

sutures, which passed across the uterine lumen, were inserted with a minimum of handling of the uterus and were tied loosely. The resultant deciduomata were examined macroscopically and fixed for histological examination 5 days later.

Duration of pseudopregnancy prolonged by deciduoma formation

Two groups of rats were used (Table 1, Groups IV, V). At the first operation, performed on the 1st day of di-oestrus, control animals (Group IV) were subjected to laparotomy, while bilateral posterior uterine section was performed on the experimental group (V). The second operation, to induce deciduoma formation, was performed in both groups exactly as in the previous experiment. All rats were destroyed on the 1st day of di-oestrus following the oestrous period which marked the end of pseudopregnancy.

RESULTS

Duration of pseudopregnancy

Results are shown in Table 1. The mean duration of pseudopregnancy was increased in both experimental groups when compared with the controls. These increases, of approximately 5.5 days in Group II and 3.5 days in Group III, were highly significant ($P < 0.001$). The difference of 2 days between Groups II and III was not significant ($P > 0.05$). The mean duration of the first pseudopregnancy cycle in the seven rats in Group III was 16.9 ± 3.4 days, while that of the subsequent cycles was 15.4 ± 3.4 days. This difference was not significant.

TABLE 1

DURATION OF PSEUDOPREGNANCY FOLLOWING HYSTERECTOMY OR POSTERIOR UTERINE SECTION WITH OR WITHOUT DECIDUOMA FORMATION

Group	Treatment	No. of rats	No. of pseudopregnancy cycles	Duration of pseudopregnancy cycles in days (Mean $\pm$ S.D.)	P (Difference from Group I)
I	Control laparotomy	7	19	12.5 ± 0.7	
II	Hysterectomy	7	21	17.9 ± 3.3	< 0.001
III	Posterior uterine section	7	21	15.9 ± 3.4	< 0.001
IV	Control laparotomy with deciduomata	9	9	16.1 ± 2.5	< 0.001
V	Posterior uterine section with deciduomata	8	8	15.6 ± 2.1	< 0.001

Structure of the uterine wall

The uterine horns from control rats appeared normal at the time of destruction and measured 2 to 3 mm in diameter.

In the posterior uterine section group (III), the total period from operation to destruction was at least 49 days. The ends of the uterine horns had become completely sealed off at the points of crushing, and distension of their blind-ending anterior segments had occurred. The uterine horns in all but one of these rats were thin-walled, 4 to 5 mm in diameter, and regularly but not tensely distended with clear, colourless, watery fluid. One horn, which was unintentionally punctured during removal, contracted almost immediately to a normal diameter, squeezing out the contained fluid. A single horn in one rat was tensely distended with thicker, cloudy fluid and measured over 9 mm in diameter. The ovaries and Fallopian tubes appeared normal when compared with the controls although in two rats, there was unilateral, mild, flaccid distension of the ovarian bursa with watery fluid. The vagina and those parts of the uterus posterior to the point of section were of normal appearance in all rats.

On histological examination of the uterus, the walls of control horns (Pl. 1, Fig. 1) were generally between two and four times as thick as those of the sectioned horns (Pl. 1, Figs. 3, 5). The single horn which was punctured during removal had a wall, the thickness of which was within the normal range of the

PLATE 1

FIGS. 1 and 2. Wall of uterine horn from control rat. A mitotic figure is arrowed in Fig. 2. H & E, $\times 107$ and $\times 270$.
 FIGS. 3 and 4. Wall of uterine horn following posterior uterine section, showing a minimal degree of thinning of the wall. H & E, $\times 112$ and $\times 280$.
 FIGS. 5 and 6. As Figs. 3 and 4, showing a greater degree of thinning of the uterine wall due to distension. The integrity of the surface epithelium is retained, and a mitotic figure is present (arrowed). H & E, $\times 120$ and $\times 300$.

FIGS. 7 and 8. Wall of control uterine horn showing deciduoma formation. The greatly reduced uterine lumen is still recognizable (L). Fig. 8 shows details of the decidual cells. H & E, $\times 30$ and $\times 300$.

FIGS. 9 and 10. Deciduoma from uterine horn following posterior uterine section, for comparison with Figs. 7 and 8. H & E, $\times 30$ and $\times 300$.

(Facing p. 233)

controls. Both the endometrium and myometrium were greatly stretched and thinned in the sectioned horns but, apart from this, appeared almost normal. The luminal epithelium was retained as a complete layer of cuboidal or columnar cells (Pl. 1, Figs. 4, 6) which were, on the average, somewhat lower than in the control horns (Pl. 1, Fig. 2). Mitotic figures were frequently seen in this epithelium in both control and sectioned horns. Profiles of glands were present in the endometrium of sectioned horns (Pl. 1, Figs. 3, 4), but were less abundant, and often more flattened, than in the controls (Pl. 1, Figs. 1, 2).

The mucin stains employed produced similar reactions in the sectioned and control horns. In both cases, PAS-positive staining was observed inconsistently and faintly along the free borders of the luminal and glandular epithelial cells. Small quantities of PAS-positive secretion were present in the lumina of occasional glands. Positive staining with CI, indicative of acid mucopolysaccharides, was found in the same situations, and was both more intense and more widely distributed. At least one component of the glandular section, however, was PAS-positive but CI-negative. Azure A metachromasia, indicating the presence of sulphated acid mucopolysaccharides, was consistently absent from all epithelia. Mast cell granules were demonstrated by this method and mast cells were seen to be almost confined to the outer layers of the uterine wall. Though never abundant, they were slightly more numerous in the sectioned than in the control horns.

Production of decidualomata

At the time of the second operation, performed 11 to 15 days after the first, the blind-ending left uterine horns were mildly distended with watery fluid. Distension appeared slightly less than that observed at the end of the previous experiment.

At the time of destruction, decidualomata had formed at the sites of all sutures in both right (control) and left (sectioned) horns. In the control horns, three of the four rats showed continuous swellings 6 to 8 mm in diameter extending along 21 to 27 mm of the length of the horn. The decidualomata around the two sutures had become confluent. The affected parts of the uterine wall were greatly thickened, with the lumen almost obliterated. In the fourth rat, the swellings associated with the two sutures were distinct and the horn was swollen to a maximum diameter of 7.5 mm.

In the sectioned horns, confluent swellings were present in three rats, and two distinct swellings of 5 mm diameter in the fourth. In all rats with confluent swellings, decidualoma formation was more massive in the left than the right horn. In each case, the swelling was at least 8 mm in diameter and extended over 27 to 50 mm of the horn.

On histological examination (Pl. 2, Figs. 7, 8, 9, 10), decidualomata from both horns showed the characteristic features described by Velardo, Dawson, Olsen & Hisaw (1953).

Duration of pseudopregnancy prolonged by decidualoma formation

The mean duration of prolonged pseudopregnancy is shown in Table 1. In both Groups IV and V, pseudopregnancy was significantly longer than in the

control group (I) of the first experiment. However, there was no significant difference between Groups IV and V, nor between either of these groups and Group III.

At the time of destruction, the uterine horns in Group IV were of almost completely normal appearance and 2 to 3 mm in diameter. There was little if any swelling in the uterine wall at the sites of sutures. In Group V, the uterine horns were massively swollen to 5 to 10 mm in diameter. This swelling was due to distension of the lumen with dark brown fluid which was generally watery in consistency and the uterine wall was thin, even at suture sites.

DISCUSSION

The present findings confirm the observation of Bradbury *et al.* (1950) that when parts of the uterine horns of rats are separated from their cervical attachments, they become distended with fluid, and that pseudopregnancy is subsequently prolonged. The distension is presumably the result of retention of the fluid which fills the uterine lumen during oestrus and would normally escape through the cervix (Armstrong, 1968).

Contrary to the observations of Bradbury *et al.* (1950), endometrial atrophy was not demonstrated in these experiments. Such changes as were observed in the uterine wall were consistent with the effects of moderate stretching due to distension of the lumen.

If the changes leading to prolongation of pseudopregnancy had been fundamentally degenerative in nature, then a progressive increase in the lengths of pseudopregnancy cycles might have been expected. This was not the case and, in fact, the mean duration of the first pseudopregnancy cycles was slightly, though not significantly, longer than that of subsequent cycles. Furthermore, at least during the first pseudopregnancy cycle after operation, the endometrium was sufficiently viable to produce a well-marked deciduoma reaction.

It would appear that failure of uterine drainage and the resultant distension of the uterine lumen was probably the fundamental change leading to prolongation of pseudopregnancy. If so, the mechanism involved remains unknown. Even in the absence of recognized degenerative changes in the uterine wall, an interference with normal uterine luteolytic activity might still have occurred. Additional interference with the blood and nerve supply to the genital tract was undoubtedly caused by the operation, and the effects of these factors cannot be overlooked. In the case of the blood vessels, section of the uterine artery and vein at the site of operation would not be expected to produce more than a transient interference with uterine blood flow, in view of the very well developed anastomoses which exist with the ovarian vessels. The possible effects of nerve section cannot be assessed at present. It is, however, noteworthy that denervation was found to prevent alterations in the duration of the oestrous cycle which occurred following the intra-uterine insertion of beads in sheep (Moore & Nalbandov, 1953) and guinea-pigs (Moore, 1961). Denervation alone did not influence the length of the cycle (Moore & Nalbandov, 1953).

The influence of deciduomata in prolonging pseudopregnancy in the rat was first demonstrated by Erschoff & Duell (1943), who observed prolongation

to 22 days. Velardo, Olsen, Hisaw & Dawson (1953) showed that the extent of prolongation is related to the amount of decidual tissue present, and the relatively short mean duration of 16.1 days in the control animals in the present study was possibly related to the localized nature of the stimulus used to provoke decidualoma formation.

When decidualoma formation was induced following posterior section of the uterine horns, the duration of the prolonged pseudopregnancy was no longer than that of pseudopregnancy without decidualoma formation. The reason for this is not clear. Carlson & De Feo (1965) found that section of the pelvic nerves in rats prevented the lengthening of pseudopregnancy associated with decidualoma formation, suggesting the possibility that interruption of nerve pathways in the present experiments could have had a bearing on this result. There is little doubt that decidualomata were in fact formed in the rats in the present experiment. Well developed decidualomata were present in all of the rats destroyed 5 days after insertion of sutures, and the large amounts of brown-stained fluid present in the obstructed horns of all rats which went through a complete pseudopregnancy suggested that decidualoma formation had occurred.

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