EFFECT OF HYSTERECTOMY AT DIFFERENT STAGES IN THE LIFE CYCLE OF THE CORPUS LUTEUM IN THE GUINEA-PIG

I. W. ROWLANDS

*Agricultural Research Council Institute of Animal Physiology,
Babraham, Cambridge*

(Received 3rd February 1961)

Summary. Hysterectomy on the 5th day of the luteal phase inhibited the dioestrous cycle for at least 8 months. The corpora lutea persisted throughout this period and, at the end, contained an appreciable amount of progesterone.

Hysterectomy on the 10th day of the luteal phase caused the corpora lutea to enlarge as in pregnancy and to persist for a period about equal to that of gestation.

Hysterectomy on the 15th day of the luteal phase prevented the impending ovulation and oestrus in 70 % (24/35) of animals. In these, the major effect was the arrest of the regressive changes in the corpora lutea, which became only slightly enlarged. In the remaining animals, ovulation and oestrus occurred shortly after the operation. The regression of the corpora lutea occurred normally but the corpora lutea that formed post-operatively responded in the same way as those in animals operated on the 5th or 10th days of the luteal phase.

The nature of the stimulus of hysterectomy on the corpus luteum of the guinea-pig is discussed briefly.

INTRODUCTION

The effects of hysterectomy on the ovaries, mammary gland and oestrous behaviour of the unmated guinea-pig were extensively studied by Loeb more than 30 years ago (Loeb, 1923, 1927, 1932; Loeb & Kountz, 1928). The principal effect was a persistence of the corpora lutea in a functional condition which resulted in the inhibition of ovulation, the cessation of the dioestrous cycle and the retention of the vaginal epithelium in a secretory state for periods equivalent to or longer than that of pregnancy. Other workers including Desclin & Brouha (1932), Herlant (1933) and Klein (1939) have confirmed the main features of Loeb's work. Loeb also noted that the period over which the effects lasted was proportional to the amount of uterine tissue excised and that hysterectomy performed in immature animals evoked a similar response in the first set of corpora lutea formed some weeks later when the ovarian cycle is initiated. To account for these changes, he suggested that the uterus (endometrium) produced an internal secretion that controlled the life-span of the corpus

luteum, but his attempts to demonstrate this in hysterectomized guinea-pigs with transplanted uterine tissue were unsuccessful. The transplanted tissue was susceptible to the formation of placentomata which, as was suggested by Bradbury, Brown & Gray (1950), could have interfered with normal uterine function and prevented the tissue from exerting a replacement effect, i.e. causing regression of the corpora lutea. Such an effect has been demonstrated in the rat (Hechter, Fraenkel, Lev & Soskin, 1940) and rabbit (Chu, Lee & You, 1946).

A similar effect of hysterectomy on the corpus luteum during pregnancy has been shown by Desclin (1932) and Klein (1939). The demonstration by the latter worker that the removal of only the foetuses and placentae resulted in a prompt collapse of the corpora lutea and the termination of the secretory activity of the vaginal epithelium, with the return to the oestrous condition, clearly signifies the importance of the uterus in the control of luteal function. Apart from the observation of Rowlands & Short (1959) that the persistent corpora lutea of the hysterectomized guinea-pig contain a high concentration of progesterone, no other work seems to have been done on this problem in this species. Many experiments have been performed in several other species (see Perry & Rowlands, 1961) and although the persistence of the corpora lutea is not invariable there are some, e.g. the pig (Du Mesnil du Buisson & Dauzier, 1959; Spies, Zimmerman, Self & Casida, 1960) and the sheep and cow (Wiltbank & Casida, 1958), in which it is comparable to that occurring in the guinea-pig.

In the present paper, the effects of hysterectomy in the guinea-pig have been re-examined, particularly in relation to the time in the ovarian cycle when the operation is performed, in the hope that they would throw some light on the nature of the mechanism involved in the control of luteal function by the uterus.

METHODS

Nulliparous guinea-pigs of the M.R.C. strain, bred in this Institute, were observed daily for vaginal opening to estimate the time of occurrence of ovulation. The latter was considered to have occurred on the day following that of maximal vaginal opening (see Rowlands, 1957). All animals were observed through at least two dioestrous cycles before performing hysterectomy on the 5th, 10th or 15th day after ovulation.

After a preliminary subcutaneous injection of 0.5 ml of 0.5% atropine, anaesthesia was induced by the intraperitoneal injection of a 2.5% solution of tribromoethanol ('Avertin') in 0.9% sodium-chloride solution at the rate of 1 ml/100g body weight followed by ether. Operative details have been described previously (Rowlands & Short, 1959).

Control measures consisted of (a) anaesthesia of comparable duration to that necessary for hysterectomy (25 min), (b) sham operations in which nylon ligatures were tied tightly in the positions needed for hysterectomy, and in which loops of the same material were tied so as not to restrict the blood supply to the uterus.

All the animals were killed with chloroform and the ovaries and vagina fixed

in Bouin's fluid if required for histological examination. Ovaries were cut serially at $5\ \mu$ and every tenth section mounted and stained in Ehrlich's haematoxylin and eosin. The volume of every corpus luteum was calculated from the product of three diametric measurements. One diameter was obtained from the product of the number of sections and section thickness and the others by measurements made at right angles through the centre of the largest section.

The corpora lutea from one group, hysterectomized on the 5th day after ovulation, were dissected and immersed in cold ethanol for extraction of progesterone. The extract was assayed by Dr R. V. Short by the method described by Rowlands & Short (1959).

RESULTS

HYSTERECTOMY ON THE 5TH DAY AFTER OVULATION

At this early stage of the normal luteal phase the corpus luteum was found by Rowlands & Short (1959) to have an average weight of 3.1 mg and to contain $0.050\ \mu\text{g}$ of progesterone. It was also found that following hysterectomy at this stage oestrus and ovulation were suppressed for at least 8 weeks, that at the end of this period the average weight of the corpus luteum was 4.8 mg, and that this amount of fresh luteal tissue contained $0.180\ \mu\text{g}$ of progesterone. The result of a further experiment is given below.

Another five guinea-pigs, hysterectomized on the 5th day after ovulation, were observed daily for 8 months. The vagina of four remained completely closed throughout this period and that of the remaining animal opened once only for a period of 3 days about 10 weeks after operation; oestrus and ovulation probably occurred during these 3 days. Many of the ovaries contained numerous rete cysts and consequently were greatly enlarged. All the corpora lutea (seventeen) were dissected, weighed individually and immersed in ice-cold ethanol for progesterone assay. The average weight of the corpus luteum was 3.7 mg, and the pooled material had a progesterone concentration of $16.5\ \mu\text{g/g}$ of fresh luteal tissue; that of the ovarian residues was about $2\ \mu\text{g/g}$. The progesterone content of a corpus luteum of average weight was $0.061\ \mu\text{g}$. The total weight of the corpora lutea in each of these five animals varied from 19.2 to 4.8 mg; progesterone content therefore varied from $0.32\ \mu\text{g}$ to $0.08\ \mu\text{g}$. The only evidence obtainable that indicated the functional activity of these corpora lutea 8 months after their formation was the occurrence of a highly mucified vagina in all five animals.

HYSTERECTOMY ON THE 10TH DAY AFTER OVULATION

Table 1 shows that the regression of the corpus luteum which normally begins at about the 10th day after ovulation in the unmated guinea-pig can be prevented by hysterectomy at this time and that the corpora lutea become significantly enlarged within 10 days. The extent and rate of this enlargement is compared with that occurring in the corpora lutea of pregnancy in Text-fig. 1; they are closely similar in both conditions. The corpora lutea are maintained at maximum size for about 50 days after hysterectomy before regression occurs. The difference in the rate of luteal regression in the hysterectomized and pregnant animal is very striking.

E*

The oestrous cycle was inhibited completely in all forty-one animals killed at intervals up to the 80th day after operation. Another three hysterectomized animals were set aside for observations over 120 days; oestrus occurred in two of these during the last week of this period and their ovaries were later found to contain recently formed corpora lutea. The vagina of the third animal remained closed until death on the 120th day after operation and the size of the corpora

TABLE 1
EFFECT ON THE CORPORA LUTEA OF UNMATED GUINEA-PIGS OF
HYSTERECTOMY AND SOME CONTROL PROCEDURES ON THE 10TH
DAY AFTER OVULATION

Treatment	Duration of experiment (days)	No. animals	Corpora lutea	
			Total No.	Mean volume (cu. mm $\pm$ S.E.)
Nil	5th (N.C.)	6	32	1.52 $\pm$ 0.05
	10th (N.C.)	6	27	2.00 $\pm$ 0.05
	15th (N.C.)	6	29	1.06 $\pm$ 0.04
Hysterectomy	5	6	33	2.19 $\pm$ 0.11
	10	6	32	2.88 $\pm$ 0.08
	20	6	33	2.83 $\pm$ 0.08
	40	6	28	2.77 $\pm$ 0.08
	53	5	22	2.57 $\pm$ 0.16
	67	6	28	1.57 $\pm$ 0.11
	80	6	29	0.90 $\pm$ 0.09
	120	1†	4	0.51
Anaesthesia	5	3	16	0.86 $\pm$ 0.05
	11	3	13	0.31 $\pm$ 0.04
			13*	1.12 $\pm$ 0.06
Anaesthesia + ligation of vascular and nervous connexions	5	3	14	2.13 $\pm$ 0.07
Anaesthesia + nylon loops without restriction of blood and nerve supplies	5	3	14	0.66 $\pm$ 0.06

N.C. = 5th, 10th or 15th day after ovulation in normal cycle.

* New corpora lutea, 3 to 4 days old.

† Two other guinea-pigs of this group came into oestrus and ovulated within 7 days before killing on the 112th day after hysterectomy.

lutea is given in Table 1. The growth and maturation of Graafian follicles in these animals was not impeded by the operation and in general the ovaries were indistinguishable from those found during pregnancy.

Table 1 also gives the results of experimental procedures (see p. 342) to test the specific nature of the response to the operation. Anaesthesia alone did not maintain the corpora lutea or prevent the recurrence of oestrus and ovulation at the normal time, 6 to 7 days later. The results of the sham operations clearly

indicate that the effects produced by hysterectomy are due to the interference with the nerve supply and/or interruption of the blood supply to the uterus.

HYSTERECTOMY ON THE 15TH DAY AFTER OVULATION

At this stage of the luteal phase, the regression of the corpora lutea is normally well advanced and the rupture of another set of follicles is imminent. In all, thirty-five guinea-pigs were hysterectomized and another fourteen were used to test the effect of anaesthesia alone on the impending ovulation. The latter tests showed that, on average, the ovarian cycle in which this treatment was given was 2.5 days longer ($P = 0.01$) than that which occurred before or after treatment, and ovulation was delayed to a similar extent.

TEXT-FIG. 1. Mean volume of corpora lutea in relation to age. ●—● In the normal unmated guinea-pig; ○—○ following hysterectomy in the unmated animal 10 days after ovulation; x—x during pregnancy. P = parturition.

Recurrence of oestrus after operation

Oestrus occurred post-operatively in eleven of the thirty-five animals; in six of them it occurred within 2 days after operation and in five it was delayed from 5 to 16 days as shown in Table 2. Four of these animals were killed to confirm that oestrus was accompanied by ovulation and the remaining seven were observed for periods varying from 33 to 152 days for the recurrence of further cyclical activity. The vagina of five of them failed to open again and when they were killed the ovaries contained very large corpora lutea dating presumably from ovulation in the early post-operative period. In only one animal (No. 12L) was there unequivocal evidence of further cyclical vaginal activity and in the remaining animal (No. 30L) the vagina, after being completely sealed for 11 weeks, began to show signs of opening intermittently during the next 4 weeks before it was killed. The ovaries contained several recently formed corpora lutea which clearly indicated that ovulation occurred during this period of slight vaginal activity.

TABLE 2
FATE OF THE NEW CORPORA LUTEA IN ELEVEN OF THIRTY-FIVE GUINEA-PIGS IN WHICH OVULATION OCCURRED
AFTER HYSTERECTOMY PERFORMED ON THE 15TH DAY OF THE CYCLE

Guinea-pig No.	Time ovulation occurred after hysterectomy (days)	Time guinea-pigs killed after operation (days)	Corpora lutea formed post-operatively			Notes
			No.	Age (days)	Mean volume (cu. mm)	
13	2	2	5	0	0.59	Five tubal eggs recovered
20	1	6	4	5	1.91	Tubal eggs not found
112	6	8	5	2	0.73	Four fragmenting eggs recovered
11	1	8	4	7	0.93	" "
160	8	33	5	25	3.65	" "
10	5	42	3	37	3.42	No further recurrence of oestrus or ovulation
164	16	52	1	36	3.35	" "
2L	1	63	5	62	2.27	" "
30L	2	105	5	?	1.61	" "
44L	10	120	5	110	1.99	Slight tearing of vaginal closure membrane
12L	2	152	6	?	1.34	No further recurrence of oestrus or ovulation
						Oestrus on 30th and 46th days after operation

Fate of the regressing corpus luteum

Material for this study was obtained from the twenty-four guinea-pigs in which the vagina failed to open after hysterectomy. Four animals were killed on the 4th, 8th, 12th, 16th and 20th days after operation and the remaining four at variable periods afterwards (see Table 3).

TABLE 3

FATE OF THE OLD CORPORA LUTEA IN TWENTY-FOUR GUINEA-PIGS IN WHICH OVULATION WAS INHIBITED BY HYSTERECTOMY ON THE 15TH DAY OF THE CYCLE

Time guinea-pigs killed after hysterectomy (days)	Guinea-pig No.	No. corpora lutea	Volume of corpora lutea (cu. mm)		
			Mean	Range	Group mean ($\pm$ S.E.)
4	98	5	1.40	0.98 to 2.20	0.65 $\pm$ 0.11
	8	5	0.54	0.48 to 0.59	
	4L	6	0.29	0.19 to 0.46	
	39L	4	0.40	0.36 to 0.46	
8	16L	2	1.00	0.67 to 1.34	1.16 $\pm$ 0.27
	23L	3	2.43	2.05 to 2.70	
	33L	3	0.24	0.14 to 0.30	
	101	4	0.63	0.20 to 1.71	
12	13L	3	3.30	3.15 to 3.44	1.60 $\pm$ 0.31
	166	3	0.51	0.38 to 0.67	
	35L	5	1.04	0.29 to 2.29	
	14L	4	1.87	0.60 to 3.29	
16	19L	1	1.82	—	1.49 $\pm$ 0.32
	169	6	2.86	2.46 to 3.02	
	5L	4	0.63	0.49 to 0.78	
	34L	4	0.22	0.11 to 0.51	
20	170	3	0.27	0.16 to 0.44	1.61 $\pm$ 0.31
	1L	4	1.42	0.94 to 1.97	
	24L	5	3.19	2.49 to 4.17	
	171	4	0.82	0.38 to 1.81	
42	24	3	0.30	0.14 to 0.60	
96	26L	4	0.31	0.18 to 0.46	
96	29L	4	1.32	0.78 to 1.67	
120	32L	4	0.18	0.14 to 0.23	

The mean volume of the corpora lutea in individual animals throughout the five groups mentioned is extremely variable but with few exceptions (e.g. Nos. 14L and 35L) the size differences of the corpora lutea within individual animals were quite small. Only in three of these twenty animals are the corpora lutea equivalent in size to those found regularly in guinea-pigs that were hysterectomized on the 5th and 10th days after ovulation or in pregnant guinea-pigs. The group means suggest the occurrence of a slight but significant increase in size of the corpora lutea by the 12th day after operation, which is maintained until at least the 20th day. It is clear, however, that they are not restored to the size they normally reach by the 10th day after ovulation.

In three of the four guinea-pigs surviving for much longer periods than 20 days, the corpora lutea were extremely small and from their histological appearance would seem to be inactive. Nevertheless, the vaginal epithelium of these and all other animals described in this section was in a mucified condition, which suggests the retention by the corpora lutea of sufficient hormonal activity to suppress ovulation and oestrus.

DISCUSSION

The results of these investigations confirm the observations of the earlier workers that hysterectomy inhibits the dioestrous cycle of the guinea-pig and that this effect is produced by the persistence of the corpora lutea in a functional condition for very long periods. Loeb's (1923) statement that the duration of this effect is equivalent to or slightly longer than that of gestation is, however, not substantiated, for it has been shown that oestrus recurs only sporadically and in only an occasional animal. It would seem reasonable to conclude, therefore, that the effect is virtually permanent, and, as far as can be determined from other work involving attempts to restore the cycle with exogenous hormones, is irreversible.

The quantitative effects on the corpora lutea differ according to the physiological condition of the latter at the time of operation and would seem, therefore, to depend on the varying sensitivity of the lutein cells to stimulation. It may be assumed that the enlargement of the corpora lutea following hysterectomy on either the 5th or 10th day after ovulation is caused by the hypertrophy of the lutein cells during the time the latter are undergoing their normal development. But when hysterectomy is performed later (15th day after ovulation), when the corpora lutea are regressing, it would seem that these cells have only a very limited capacity to grow again. The variability in the size of the corpora lutea, between animals hysterectomized at this very late stage in the cycle, is probably related to the differences in the extent to which regression had proceeded when the operation was performed. The effect of the operation at this time would seem to be predominantly one of preventing, or at least greatly decreasing, the rate of the regression so that the corpora lutea retain a sufficient degree of secretory activity to inhibit oestrus for long periods. The retention of small, aged, corpora lutea for very long periods of time contrasts very sharply with their rapid involution and disappearance in the normal animal, especially after ovulation has recurred.

The rate and extent of growth, and the histological appearance of the corpus luteum following hysterectomy on the 10th day after ovulation, are remarkably similar to those in the pregnant animal. The finding by Desclin & Brouha (1932) and Herlant (1933) of similar cytological changes in the anterior pituitary gland of the pregnant and hysterectomized guinea-pigs provides some evidence that the changes in the corpora lutea are brought about in a similar manner but there is no information on the nature of the stimulus effecting the pituitary changes.

When hysterectomy was performed during the early part of the luteal phase, the corpus luteum was shown to persist in an apparently functional state for a

period at least four times that of gestation. The extension of luteal activity beyond the physiological limit by these means is comparable with the results obtained in rats in which the pituitary gland was removed from its normal situation and transplanted beneath the renal capsule (Everett, 1956). In these circumstances, the corpora lutea of the dioestrous cycle acquired the characteristics of those of the pseudopregnant rat, but only after the administration of oestrogen did they become enlarged to the size of those found in the pregnant rat. Since the active corpus luteum of the dioestrous cycle in the normal guinea-pig may be regarded as being equivalent in the physiological sense to the corpus luteum of pseudopregnancy in the rat, it would seem that the change induced by hysterectomy in the former species is similar to that produced by exogenous oestrogen in the rats in Everett's experiments. The luteotrophic activity of oestrogen in some species is well known, e.g. the rabbit (Robson, 1937), and the possibility of such a mechanism being involved in the maintenance of the corpus luteum in the hysterectomized guinea-pig cannot be ignored. A supply of oestrogenic hormone could be provided by the follicles which continue to mature throughout the post-operative period. The presence of similar follicular activity during the luteal phase in the intact animal would at first sight appear to refute this explanation, but, in the light of Heckel's (1942) work on the sparing effect of hysterectomy on endogenous oestrogen in the rabbit, it may well be that much larger amounts of this hormone become available to accelerate and maintain the secretion of a pituitary luteotrophin for prolonged periods after the uterus of the guinea-pig is removed.

ACKNOWLEDGEMENTS

I am grateful to Dr R. V. Short for the determination of the progesterone content of the corpora lutea in the hysterectomized guinea-pigs described on p. 343, and to Miss G. Needham for skilled technical assistance.

REFERENCES

- BRADBURY, J. T., BROWN, W. E. & GRAY, L. A. (1950) Maintenance of the corpus luteum and physiologic actions of progesterone. *Recent Progr. Hormone Res.* **5**, 151.
- CHU, J. P., LEE, C. C. & YOU, S. S. (1946) Functional relation between the uterus and corpus luteum. *J. Endocrin.* **4**, 392.
- DESCLIN, L. (1932) À propos des interactions entre l'utérus et le corps jaune au cours de la grossesse chez le cobaye. *C. R. Soc. Biol., Paris*, **109**, 972.
- DESCLIN, L. & BROUHA, L. (1932) Étude expérimentale des modifications gravidiques de l'hypophyse chez le cobaye. *Arch. Biol., Paris*, **42**, 167.
- DU MESNIL du BUISSON, F. & DAUZIER, L. (1959) Contrôle mutuel de l'utérus et de l'ovaire chez la truie. *Ann. Zootech. Suppl.*, p. 147.
- EVERETT, J. W. (1956) Functional corpora lutea maintained for months by autografts of rat hypophyses. *Endocrinology*, **58**, 786.
- HECHTER, O., FRAENKEL, M., LEV, M. & SOSKIN, S. (1940) Influence of the uterus on the corpus luteum. *Endocrinology*, **26**, 680.
- HECKEL, G. P. (1942) The estrogen sparing effect of hysterectomy. *Surg. Gynec. Obstet.* **74**, 379.
- HERLANT, M. (1933) Recherches préliminaires sur les modifications provoquées par l'hystérectomie chez le cobaye. *C. R. Soc. Biol., Paris*, **114**, 273.
- KLEIN, M. (1939) Action du placenta sur le corps jaune gravidique et sur le cycle vaginal chez le cobaye. *C. R. Soc. Biol., Paris*, **130**, 1393.
- LOEB, L. (1923) Effects of extirpation of the uterus on the life and function of the corpus luteum in the guinea pig. *Proc. Soc. exp. Biol., N.Y.* **20**, 441.

- LOEB, L. (1927) Effects of hysterectomy on system of sex organs and on periodicity of sexual cycle in guinea pig. *Amer. J. Physiol.* **83**, 202.
- LOEB, L. (1932) Some mechanisms in the sexual cycle of the guinea pig. *Aust. J. exp. Biol.* **9**, 141.
- LOEB, L. & KOUNTZ, W. B. (1928) The effect of injection of follicular extract on the sex organs in the guinea pig and the interaction between the follicular substances and substances given off by the corpus luteum. *Amer. J. Physiol.* **84**, 283.
- PERRY, J. S. & ROWLANDS, I. W. (1961) Effect of hysterectomy on the ovarian cycle of the rat. *J. Reprod. Fertil.* **2**, 332.
- ROBSON, J. M. (1937) Maintenance by oestrin of the luteal function in hypophysectomized rabbits. *J. Physiol.* **90**, 435.
- ROWLANDS, I. W. (1957) Insemination of the guinea-pig by intraperitoneal injection. *J. Endocrin.* **16**, 98.
- ROWLANDS, I. W. & SHORT, R. V. (1959) The progesterone content of the guinea-pig corpus luteum during the reproductive cycle and after hysterectomy. *J. Endocrin.* **19**, 81.
- SPIES, H. G., ZIMMERMAN, D. R., SELF, H. L. & CASIDA, L. E. (1960) Effect of exogenous progesterone on the corpora lutea of hysterectomized gilts. *J. Anim. Sci.* **19**, 101.
- WILTBank, J. N. & CASIDA, L. E. (1956) Alteration of ovarian activity by hysterectomy. *J. Anim. Sci.* **15**, 134.