

EFFECT OF BOVINE ENDOMETRIAL EXTRACTS, VASOPRESSIN AND OXYTOCIN ON THE DURATION OF PSEUDOPREGNANCY IN HYSTERECTOMIZED AND INTACT RATS

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(Received 4th August 1964)

Summary. Hysterectomy of pseudopregnant rats consistently increased the duration of vaginal dioestrus and the interval between ovulations. These observations suggested that the presence of the nongravid rat uterus limits the life span of pseudopregnancy corpora lutea.

Neither aqueous nor ether extracts of bovine endometrium collected at several stages of corpus luteum function significantly affected the life span of corpora lutea in hysterectomized pseudopregnant rats.

Vasopressin decreased the duration of vaginal dioestrus and hastened ovulation in hysterectomized, but not in intact, pseudopregnant rats, suggesting that it hastened luteal regression after hysterectomy.

Oxytocin failed to shorten the period of luteal function in the hysterectomized rat. Ovarian weight in intact and hysterectomized rats was increased by this means.

INTRODUCTION

The nongravid uterus appears to initiate or facilitate corpus luteum regression since its removal during the oestrous cycle markedly prolongs the duration of luteal function in guinea-pigs (Loeb, 1923), swine (Du Mesnil Du Buisson & Dazier, 1959) and in sheep and cattle (Wiltbank & Casida, 1956). Hysterectomy also prolongs the life span of pseudopregnancy corpora lutea in rats (Bradbury, Brown & Gray, 1950; Perry & Rowlands, 1961) and rabbits (Asdell & Hammond, 1933). Of the possible uterine factors which might terminate luteal function, a luteolytic uterine factor has been postulated most frequently.

Homotransplantation of uterine tissue into hysterectomized animals has provided both positive and negative evidence concerning a luteolytic function of the uterus. Hechter, Fraenkel, Lev & Soskin (1940) and Chu, Lee & You (1946) induced normal luteal regression with uterine homotransplants in rats and rabbits. Loeb (1927) and Spies, Zimmerman, Self & Casida (1960) obtained negative results from similar homotransplantation experiments in guinea-pigs and swine. Duncan, Bowerman, Anderson, Hearn & Melampy (1961) developed an in-vitro system of progesterone synthesis and provided further evidence in

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favour of a luteolytic substance. Endometrial filtrates prepared from swine uteri collected on the 16th and 18th days of the oestrous cycle decreased the *in vitro* progesterone synthesis.

The humoral nature of the luteolytic factor was further emphasized by uterine autotransplantation experiments in guinea-pigs (Butcher, Chu & Melampy, 1962) and swine (Anderson, Butcher & Melampy, 1963). Uteri were autografted to the abdominal oblique muscles in a two-stage operation designed to insure adequate vascularization. While the autografted uteri may have retained vasomotor nervous elements, the normal uterine afferent nerves were presumably excluded from the autografts. In both experiments the corpora lutea regressed and permitted a recurrence of oestrus in some of the autografted animals. This luteal regression was presumably due to a luteolytic substance secreted by the autograft or to a characteristic metabolism of the uterine tissue in the autograft.

Neurohypophysial hormones have recently been shown to affect corpus luteum function. Armstrong & Hansel (1959) observed precocious luteal regression in nonpregnant heifers injected with oxytocin. Faulkner & Hansel (1962) noted the regression of persistent corpora lutea in rats bearing hypophysial autografts by prolonged administration of either oxytocin or vasopressin. The luteolytic action of oxytocin and vasopressin in these two experiments and the reflex release of both hormones in response to neurogenic stimuli from the female reproductive tract (Debackere & Peeters, 1960) suggests that neurohypophysial hormones may participate in the regulation of luteal function by uterine factors.

The present experiments were therefore designed with two purposes in mind: (1) to test for the presence of a luteolytic substance in bovine endometrial tissue and (2) to investigate the possible luteolytic action of neurohypophysial hormones in hysterectomized rats.

MATERIALS AND METHODS

ENDOMETRIAL EXTRACTS

Bovine uteri were collected at slaughter and frozen immediately. After varying periods of storage, the uteri were thawed and split. The endometrial layer was stripped free with forceps and scalpel and placed in 100 to 150 ml saline. The tissue was chopped in a Servall Omni-Mixer and ground further in a Teflon tissue homogenizer. Both procedures were carried out in an ice water bath. The mixture was centrifuged for 30 min to concentrate the remaining tissue prior to vacuum filtration. The aqueous filtrate from each uterus was lyophilized and later made up to a volume of 12 to 16 ml and frozen until injection. Bladder mucosa was similarly treated, and the aqueous portion served as the control extract.

The residue from the vacuum filtration was extracted in ethyl ether vapours for 7 to 8 hr. The ether was evaporated, and the extracted material stored in a desiccator under vacuum until used. At the time of the first injection, the material was dissolved in corn oil and aliquots were used for each daily injection.

Uteri were also collected from normal dairy heifers at the 15th and 18th days of the oestrous cycle (oestrus = Day 0), and from seven heifers receiving daily injections of 7 U.S.P. units of oxytocin (Armours P.O.P.; 20 U.S.P. units/ml) per 100 lb body weight. Six of these seven heifers were from the experiments of Staples & Hansel (1961) and were slaughtered 4 to 8 days after a precocious ovulation induced by daily oxytocin injections. The other heifer was slaughtered on the fifth post-oestrous day while receiving daily oxytocin.

PSEUDOPREGNANCY

Pseudopregnancy was induced by electrical stimulation of the cervix on the night of a pro-oestrous vaginal smear. The day following stimulation was designated the first day of pseudopregnancy.

HYSTERECTOMY

The operation was performed under ether or pentobarbital anaesthesia on the second day of pseudopregnancy. The ovarian bursae were carefully dissected away to prevent the occurrence of cysts (Ranney, Peckham & Greene, 1947). The oviducts and associated broad ligament connexions to the ovary were severed, care being taken to avoid damage to the ovarian blood vessels. No attempt was made to preserve the utero-ovarian vascular supply to the ovaries. Sham operations, consisting of manipulation of the uterus and ovaries, were also performed on the second day of pseudopregnancy.

INJECTIONS

The endometrial extracts were injected subcutaneously in hysterectomized Sprague-Dawley rats beginning on the eighth day of pseudopregnancy. Other pseudopregnant rats were subcutaneously injected once daily with oxytocin (12 units) and vasopressin (8 units) beginning on the first or the eighth day. The vasopressin (Pitressin) was obtained from Parke, Davis & Co., and had a stated potency of 10 pressor units/ml. Daily injection of a 0.5% aqueous solution of chlorobutanol served as a control for the vasopressin and oxytocin injections. Both preparations contained this level of chlorobutanol and Duncan *et al.* (1961) reported that it inhibited in-vitro progesterone synthesis.

HISTOLOGY

Daily vaginal smears, obtained by lavage every afternoon, were examined immediately in the unstained condition. The occurrence of a full pro-oestrous smear was taken to represent the end of pseudopregnancy, but if the smear contained more than 25% epithelial cells, the animal was re-examined the same evening. Many animals displayed full pro-oestrus on this occasion. The animals were killed the following morning and the ovaries fixed for serial section in order to detect newly-formed corpora lutea.

RESULTS

ACCURACY OF THE VAGINAL SMEAR TECHNIQUE

The vaginal smear technique provided a reliable estimate of the length of pseudopregnancy but it could not accurately determine the time of the ovulation terminating pseudopregnancy. Ovaries obtained on the morning following

a pro-oestrous vaginal smear could be classified with regard to corpora lutea as follows: (1) no newly formed corpora lutea; (2) new corpora lutea, 5 to 15 hr postovulation; (3) new corpora lutea, 24 to 36 hr postovulation.

The first category contained approximately 20% of the animals, irrespective of treatment. The failure of these animals to possess any new corpora lutea may have represented a prolonged pro-oestrus prior to ovulation as Everett (1948) has reported. In the second group which comprised almost 70% of the animals, the vaginal smear results were completely verified by ovarian histology. These animals had apparently ovulated within the last 5 to 15 hr as the corpora lutea were composed exclusively of proliferating luteal cells which had not yet begun to hypertrophy. Animals of the third class usually displayed a dioestrous vaginal smear on the morning they were killed and had probably ovulated during the early morning hours of the previous day. The developing luteal cells of these corpora lutea had begun to hypertrophy, but connective tissue elements were still almost totally absent.

The duration of pseudopregnancy as estimated by the vaginal smear technique was later adjusted on the basis of the ovarian histology. All experimental animals were included in the calculation of 'Days to vaginal oestrus', but those lacking new corpora lutea when killed were excluded from the 'Days to ovulation' data. In both calculations the duration was reduced 1 day in those rats possessing the older type of recent corpora lutea. These adjustments of the data did not significantly alter them from the uncorrected data previously presented in abstract form (Malven & Hansel, 1962).

BOVINE ENDOMETRIAL EXTRACTS

Table 1 presents the adjusted duration of pseudopregnancy in hysterectomized rats treated with bovine endometrial extracts. None of the extracts significantly altered the length of pseudopregnancy as measured by 'Days to ovulation'. In the 'Days to vaginal oestrus' classification, the mean of 20.4 days for the Day 18 aqueous extract group was significantly ($P < 0.05$) greater than the control mean of 17.0 days by Duncan's New Multiple Range Test but not by Dunnett's Test (Steel & Torrie, 1960).

None of the mean durations of pseudopregnancy suggested the presence of a luteolytic factor in the endometrial extracts. However, in each of three different treatment groups one rat displayed a shortened pseudopregnancy (9 to 11 days). These three cases probably do not represent a true luteolytic action since injections of the same extracts into three other hysterectomized rats had no effect on the duration of their pseudopregnancies (17 to 19 days).

VASOPRESSIN AND OXYTOCIN

Table 2 reveals the luteolytic action of vasopressin in hysterectomized pseudopregnant rats. Vasopressin injections begun on either Day 1 or Day 8 markedly reduced the mean duration of pseudopregnancy so that it equalled that occurring in intact rats. Text-fig. 1 also illustrates this luteolytic action of vasopressin and also shows that the distributions of the hysterectomized vasopressin-treated group and the intact control group were somewhat dissimilar.

TABLE 1

EFFECT OF BOVINE ENDOMETRIAL EXTRACTS, PREPARED AT VARIOUS STAGES OF THE OESTROUS CYCLE, ON THE DURATION OF PSEUDOPREGNANCY IN HYSTERECTOMIZED RATS

	<i>No. rats</i>	<i>Days to vaginal oestrus</i>	<i>No. rats</i>	<i>Days to ovulation</i>
Bladder extracts	19	17.0	13	17.3
Endometrial extracts				
Aqueous extracts				
Day 15	12	16.8	10	16.4
Day 18	9	20.4†	8	20.2
Days 4 to 8*	7	16.3	7	16.3
Ether extracts				
Day 15	12	16.8	7	16.3
Day 18	12	18.8	7	19.7
Days 4 to 8*	7	17.1	5	17.2

* Oxytocin-treated heifers.

† Significantly ($P < 0.05$) greater than bladder extract group by Duncan's New Multiple Range Test but not by Dunnett's Test (Steel & Torrie, 1960).

TABLE 2

EFFECT OF VASOPRESSIN AND OXYTOCIN ON THE DURATION OF PSEUDO-PREGNANCY IN RATS

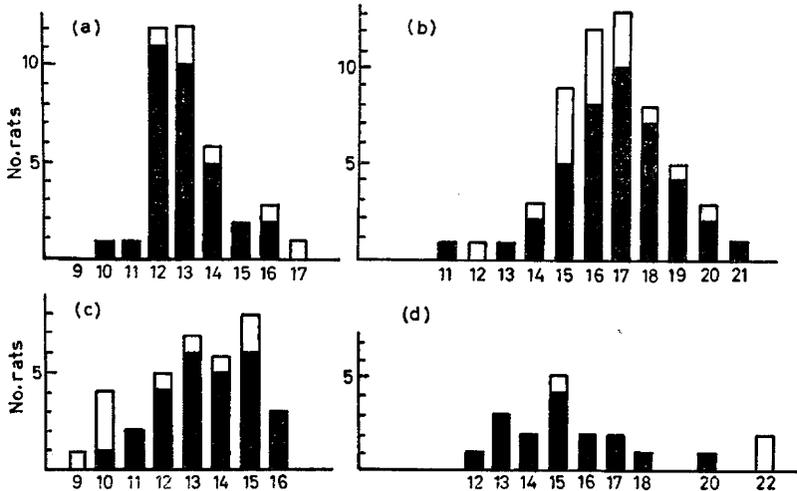
	<i>No. rats</i>	<i>Days to vaginal oestrus</i>	<i>No. rats</i>	<i>Days to ovulation</i>
Hysterectomized				
Controls	57	16.6	41	16.8
(non-injected)				
(bladder extracts)				
(chlorobutanol-treatment)				
Vasopressin-treated				
Injections begun Day 1	10	12.6*	6	12.8*
Injections begun Day 8	26	13.4*	21	13.8*
Oxytocin-treated	19	15.9	16	15.2†
(injections begun Day 8)				
Intact				
Controls				
Unoperated	29	13.2	25	13.1
Sham-hysterectomized	9	13.0	7	12.4
Vasopressin-treated	10	13.2	9	13.3
(injections begun Day 1)				
Oxytocin-treated	6	12.8	6	12.8
(injections begun Day 1)				

* Significantly ($P < 0.01$) less than appropriate control.

† Significantly ($P < 0.05$) less than the appropriate control by Duncan's New Multiple Range Test but not by Dunnett's Test (Steel & Torrie, 1960).

The hysterectomized controls in Table 2 and Text-fig. 1 represent a combination of several different types of controls. These groups were combined for easier presentation, but all statistical comparisons of the effects of vasopressin and oxytocin were made with appropriate contemporary controls. Chlorobutanol injection begun on either Day 1 or 8 had no effect on the duration of pseudopregnancy in hysterectomized rats.

The action of oxytocin was less clear-cut than vasopressin. Statistical analysis of the 'Days to vaginal oestrus' classification in Table 2 indicated no effect on the duration of pseudopregnancy. However, the 'Days to ovulation' classification was more difficult to interpret. The mean of 15.2 days was significantly



TEXT-FIG. 1. Proportions of intact and hysterectomized pseudopregnant rats displaying vaginal oestrus and ovulating after oxytocin or vasopressin treatment. (a) Intact control, mean = 13.2 days; (b) hysterectomized control, mean = 16.6 days; (c) hysterectomized vasopressin-treated, mean 13.2 days; (d) hysterectomized oxytocin-treated, mean 15.9 days. Open columns, rats displaying vaginal oestrus; black columns, rats ovulating.

($P < 0.05$) less than the appropriate control by Duncan's New Multiple Range Test but not by Dunnett's Test (Steel & Torrie, 1960). The graphic distribution in Text-fig. 1 suggests a slight shortening of pseudopregnancy when the three rats with extremely long (20 to 22 days) pseudopregnancies are excluded.

OVARIAN WEIGHTS AT THE TERMINATION OF PSEUDOPREGNANCY

Statistical analysis of the data has indicated that mean ovarian weight in intact and hysterectomized rats at the end of pseudopregnancy was significantly increased ($P < 0.05$) as the result of the injection of oxytocin. Furthermore, pre-ovulatory ovaries weighed significantly less ($P < 0.01$) than those containing recent corpora lutea.

DISCUSSION

ACCURACY OF THE VAGINAL SMEAR TECHNIQUE

Daily examination of vaginal smears provided a relatively reliable method for detecting the termination of pseudopregnancy. However, the occurrence of a

pro-oestrous vaginal smear in the afternoon or evening was not always followed by ovulation the following morning. It would appear that ovulation in this colony of rats occurs during the early morning hours since ovaries of rats killed between 8 a.m. and 2 p.m. contained corpora lutea similar to those observed by other authors 5 to 15 hr postovulation (Boling, 1942; Bassett, 1943; Pederson, 1951). The lack of corpora lutea intermediate between the 5 to 15 hr and 24 to 36 hr classifications strongly suggests that ovulation always occurs at approximately the same time each morning.

EFFECT OF BOVINE ENDOMETRIAL EXTRACTS

The failure of the endometrial preparations to shorten the duration of pseudo-pregnant dioestrus in hysterectomized rats argues against, but does not disprove, the existence of a luteolytic uterine factor. These negative observations contradict the experimental results of Hechter *et al.* (1940) and Bradbury *et al.* (1950) who appeared to hasten luteal regression in hysterectomized rats by means of uterine implants and endometrial extracts. The data do not support the *in-vitro* luteolytic activity which Duncan *et al.* (1961) observed in Day 18 endometrial preparations from swine uteri. On the contrary, the Day 18 bovine endometrial extracts tended to prolong pseudopregnancy, but this tendency was of questionable statistical significance.

The ability of exogenous vasopressin to shorten the duration of vaginal dioestrus and to hasten ovulation in hysterectomized rats, but not in intact rats, suggests that vasopressin is acting to specifically reverse whatever changes occur following hysterectomy. The possibility that vasopressin acts directly on the ovary cannot be excluded, but such a direct action which would substitute exactly for absent neural or humoral uterine stimuli seems unlikely. The most common mechanism linking uterine stimuli in intact rats and exogenous vasopressin in hysterectomized rats is an action on the hypophysis. If stimuli from the nonpregnant uterus were to terminate luteal function on the 13th day of pseudopregnancy by acting through the hypophysis, exogenous vasopressin might affect the hypophysis of hysterectomized rats in the same manner and thereby shorten pseudopregnancy to 13 days. The ability of exogenous vasopressin to release corticotrophin (Nichols, 1961) and luteinizing hormone (McCann, Taleisnik & Friedman, 1962; Giuliani, Martini, Pecile & Fochi, 1961) supports the idea that vasopressin may have acted through the hypophysis in the present experiments. Silbiger & Rothschild (1963) suggest that the prolongation resulting from hysterectomy is mediated through the hypophysis.

The similar termination of pseudopregnant luteal function by uterine stimuli in intact rats and exogenous vasopressin in hysterectomized rats could occur in either of two ways. One possibility is the inhibition of hypophysial luteotrophin synthesis or release. Corpora lutea would then cease to function because they lacked sufficient luteotrophin. The second possible mechanism would involve the secretion of an hypophysial hormone inhibitory to corpus luteum function. Such a luteolytic hormone would then either inhibit the lutein cells directly or antagonize the action of luteotrophin on them. Rothchild (1964) reported that luteinizing hormone initiated regression of the persistent corpora lutea in rats

whose hypophyses had been autografted beneath the renal capsule. It is not clear whether luteinizing hormone is also the hormone whose secretion might be stimulated by uterine stimuli in intact rats and by exogenous vasopressin in hysterectomized rats. The ability of exogenous vasopressin to also induce luteal regression in autografted rats (Faulkner & Hansel, 1962) suggests further investigation of this possibility.

Exogenous oxytocin did not exert a definite luteolytic effect in pseudopregnant rats as it does in autografted rats (Faulkner & Hansel, 1962) and cattle (Armstrong & Hansel, 1959). There was a slight tendency for a hastening of ovulation in the hysterectomized but not the intact rats. The positive results of Faulkner & Hansel (1962) were obtained after prolonged twice daily administration. The failure of oxytocin to modify pseudopregnant dioestrus in intact rats agrees with the observations of Brinkley & Nalbandov (1963).

ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Mr T. Dale and Mr P. Kitzberg. The oxytocin used in these experiments was kindly supplied by M. E. Davenport, Armour Pharmaceutical Co. The experiments were supported in part by funds provided by the regional research project NE-41 entitled Endocrine Factors Affecting Reproduction in Dairy Cattle, a co-operative study by Agricultural Experiment Stations in the Northeast (U.S.), and the Dairy Husbandry Research Branch ARS-USDA.

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