THE EFFECT OF SERIAL UTERINE BIOPSIES AND HYSTERECTOMY ON PERIPHERAL BLOOD LEVELS OF TOTAL UNCONJUGATED OESTROGEN AND PROGESTERONE IN THE BITCH

J. C. HADLEY

*Department of Surgery and Obstetrics, Royal Veterinary College Field Station,
Hawkshead Lane, North Mymms, Nr Hatfield, Hertfordshire*

(Received 11th March 1975)

The role of ovarian steroids in the aetiology of cystic endometrial hyperplasia in the bitch has been under discussion for some time. Earlier workers thought that this condition was due to abnormal oestrogen production (Hetzel, 1935; Bloom, 1954; Schulze, 1955). Teunissen (1952) and Dow (1959), however, produced evidence suggesting that prolonged high levels of progesterone were the cause of cystic endometrial hyperplasia in the bitch. The present work reports the changes in total unconjugated oestrogen and progesterone concentrations in the peripheral blood of bitches subjected to serial biopsies, which result in the development of uterine lesions without hormone treatment (Hadley, 1975a), and the effect of hysterectomy on the levels of these steroids.

One multiparous beagle, Bitch A, and two nulliparous mongrels, Bitches F and J, were used in this study. The animals were examined daily for signs of vulval swelling or bleeding, and each bitch was tested daily with a male dog from the first day of pro-oestrus until the end of oestrus. Vaginal smears were prepared daily from Day 1 of pro-oestrus until 2 to 4 days after the end of acceptance of the male. After premedication with promazine (Sparine: John Wyeth & Brother Ltd, Havant) and the induction of anaesthesia with thio-pentone (Pentothal: Abbott Laboratories Ltd, Queenborough), uterine biopsies were taken under halothane (Fluothane: I.C.I. Ltd, Macclesfield) and oxygen anaesthesia from Bitches A and J at various stages of the oestrous cycle (Table 1) by the technique described by Hadley (1975a). Using the same anaesthetic procedure, a hysterectomy was performed on Bitch F on Day 4 of oestrus.

Heparinized blood samples (5 ml) were collected from the cephalic vein of each bitch: during the second cycle of Bitch A, and in Bitch J, samples were taken daily until progesterone levels fell below 1 ng/ml and then twice weekly for the next 6 weeks. Five single samples were withdrawn during the first cycle of Bitch A. Bitch F was bled daily from Day 1 of pro-oestrus for the next 95 days. The samples were frozen and stored at -20°C until assayed. Total unconjugated oestrogen and progesterone concentrations were determined by the method of Challis *et al.* (1971) as described by Hadley (1973). The anti-serum used in the oestrogen assay was prepared against oestradiol-17 β and showed significant cross-reactions with oestrone (92%), oestriol (52%) and

Table 1. The schedule of uterine biopsies performed on Bitches A and J

Bitch	Age (years)	Oestrus	Days in relation to Day 1 of pro-oestrus when biopsy performed							
			5	12	19	30	47	75	106	
A	2	First	5	12	19	30	47	75	106	
		Second	6	13	18	31	49	72	98	
		Third	47							
	5	First	3	7	11	24	38	52	67	
		Second	192

TEXT-FIG. 1. Total unconjugated oestrogen (a) and progesterone (b) concentrations during three oestrous cycles in which uterine biopsies were performed (Bitches A and J). Vertical bars represent mean $\pm$ S.E.M., the remaining points being single observations. P-O, pro-oestrus; O, oestrus.

oestradiol-17 α (36%) (Challis *et al.*, 1971). The progesterone assay gave blank values and recovery figures of 0.3 ng/ml and 89% respectively, and the corresponding values for the oestrogen assay were 9 pg/ml and 77.5%.

The levels of total unconjugated oestrogen during the biopsy period are shown in Text-fig. 1(a). The oestrogen concentrations in the biopsied bitches rose from 7.0 pg/ml on Day 1 of pro-oestrus to give the highest mean $\pm$ S.E.M. daily value of 29.7 ± 4.7 pg/ml on the last day of this phase, and then declined rapidly during the first 2 days of oestrus. From Day 3 of oestrus to the end of oestrus, the mean oestrogen levels were usually between 10 and 14 pg/ml. During the first 7 days of metoestrus the oestrogen levels were either similar to, or slightly lower than, those of late oestrus. The mean concentrations increased from Days 8 to 17 of metoestrus, with the two highest values occurring on Days 10 and 14. The mean peak metoestrous value was 22.2 ± 2.4 pg/ml on Day 10. Levels subsequently fell to below 12 pg/ml by 18 days after the end of oestrus, and they generally remained between 6 and 12 pg/ml for the remainder of metoestrus. The changes in oestrogen concentrations in the hysterectomized bitch were similar to those seen in the biopsied bitches, although the levels were lower.

The progesterone levels in the biopsied bitches are shown in Text-fig. 1(b). The low pro-oestrous level rose to 1.3 ± 0.3 ng/ml on the last day of this phase, and increased slowly during the first 3 days of oestrus. A short period followed during which concentrations rose rapidly; the rate of increase then dropped, and there was little change in the second half of oestrus. On the last day of oestrus the progesterone level was 11.1 ± 1.3 ng/ml. Progesterone levels continued to rise slowly during metoestrus until the peak value of 18.4 ± 2 ng/ml was attained 11 days after the end of oestrus. Individual peak values were obtained of 25.1 ng/ml on Day 10 (Bitch A) and 16.2 ng/ml on Day 11 of metoestrus (Bitch J). A steady decline in progesterone levels occurred from Day 11 to Day 25 of metoestrus when the value was 1.3 ± 0.3 ng/ml. All except two of the subsequent samples were below 1 ng/ml. The progesterone concentrations during the cycle of the hysterectomized bitch are shown in Text-fig. 2. A peak value of 16.3 ng/ml was reached on Day 2 of metoestrus. Progesterone concentrations fell for 2 days after this and then remained fairly constant at 9 to 10 ng/ml for 8 days before rising to 11 to 14 ng/ml for a further 7 days. This was followed by a rapid decline to 1.0 ng/ml 28 days after oestrus, and all subsequent values were below this figure.

The oestrogen pattern in the biopsied bitches during pro-oestrus and oestrus was similar to that seen in the normal cycle (Hadley, 1975b), but the peak metoestrous level was attained 8 days earlier than in normal bitches. The maintenance value of unconjugated oestrogens at 6 to 12 pg/ml was slightly higher than that (3 to 7 pg/ml) observed in the second half of metoestrus in normal cycles.

The progesterone levels in the biopsied bitches were similar to those reported in normal non-pregnant bitches (Hadley, 1975b) from the onset of pro-oestrus until the attainment of the progesterone peak. The levels in normal bitches then fell and became fairly constant between Days 24 and 30 of metoestrus, reaching values below 1 ng/ml on Day 61 after oestrus; the values in the biopsied and the

hysterectomized bitches fell from their peak level to 1 ng/ml on the 25th and 28th days of metoestrus, respectively. This premature fall to 1 ng/ml and less 35 days earlier than in normal bitches is contrary to the suggestion of Sokolowski (1973) that the pseudopregnancy changes in the uterus as a result of surgical trauma are related to luteal retention in this species. The pattern of progesterone levels seen after surgery may indicate that a luteotrophic factor, possibly of uterine origin, may be active in the normal cycle at about 24 days of metoestrus, and that it was either not produced or not effective in the biopsied or hysterectomized animals.

TEXT-FIG. 2. Daily progesterone concentrations in an oestrous cycle during which a hysterectomy (arrow) was performed (Bitch F). P-O, pro-oestrus; O, oestrus.

Hadley (1975a) described the development of cystic endometrial hyperplasia in bitches in which serial uterine biopsies were performed. In the present study, the latest histologically normal biopsy was obtained 20 days after oestrus when progesterone concentrations had declined to 6.2 ng/ml, and levels were below 1 ng/ml 5 days before cystic endometrial hyperplasia was observed at Day 30 of metoestrus. The appearance of the endometrium did not return to normal until 55 days later, although progesterone levels remained below 1 ng/ml. Dow (1959) concluded that a high level of progesterone was necessary for the development of cystic endometrial hyperplasia and that this condition required the continued action of progesterone for its maintenance. This conclusion was not supported by progesterone concentrations found in the peripheral circulation in bitches with naturally occurring uterine disease (Christie *et al.*, 1972) and pyometra (Hadley, 1975c). The discrepancy between these findings and those of Dow (1959) may be related to the fact that the bitches in Dow's study were subjected to either prolonged progesterone treatment or frequent artificial cycles in which the blood progesterone level probably exceeded normal physiological concentrations. A possible explanation for the extensive changes seen in the uteri of the

biopsied bitches may be that the sudden, premature fall in peripheral progesterone concentrations in these animals resulted in an abnormally high oestrogen: progesterone ratio in the luteal phase, which was perhaps conducive to the development of cystic endometrial hyperplasia subsequently seen in these uteri.

The author would like to thank Professor G. H. Arthur for his encouragement and for providing research facilities, and Dr D. E. Noakes for his criticism of the text. Technical assistance was given by Miss M. A. Lemon, Mrs J. D. Sutton and Mrs G. E. Clarke. Financial support was kindly provided by Glaxo Laboratories Ltd, and Mrs M. Hughes typed the manuscript.

REFERENCES

- BLOOM, F. (1954) *Pathology of the Dog and Cat*. Evanston, Illinois.
- CHALLIS, J.R.G., HEAP, R.B. & ILLINGWORTH, D.V. (1971) The plasma concentrations of oestrogen and progesterone in non-pregnant, pregnant and lactating guinea-pigs. *J. Endocr.* **51**, 333-345.
- CHRISTIE, D.W., BELL, E.T., PARKES, M.F., PEARSON, H., FRANKLAND, A.C.L. & RENTON, J.P. (1972) Plasma progesterone in canine uterine disease. *Vet. Rec.* **90**, 704-5.
- DOW, C. (1959) Experimental reproduction of the cystic hyperplasia pyometra complex in the bitch. *J. Path. Bact.* **78**, 267-78.
- HADLEY, J.C. (1973) *A study of ovarian and uterine histology and of reproductive endocrinology in normal and in some genitally abnormal bitches*. Ph.D. thesis, University of London.
- HADLEY, J.C. (1975a) The development of cystic endometrial hyperplasia in the bitch following serial uterine biopsies. *J. small Anim. Pract.* **16**, 263-271.
- HADLEY, J.C. (1975b) Total unconjugated oestrogen and progesterone concentrations in peripheral blood during the oestrous cycle of the dog. *J. Reprod. Fert.* **44**, 445-451.
- HADLEY, J.C. (1975c) Total unconjugated oestrogen and progesterone concentrations in the peripheral blood of bitches with false pregnancy and pyometra. *Vet. Rec.* (in press).
- HETZEL, H. (1935) Die Hyperplasia glandularis cystica endometrii bei den Fleischfressern. *Wien. tierärztl. Mschr.* **22**, 609-622.
- SCHULZE, W. (1955) Zur pyometra der Hündin. *Dt. tierärztl. Wschr.* **62**, 504-506.
- SOKOLOWSKI, J.H. (1973) Reproductive features and patterns in the bitch. *J. Am. anim. Hosp. Ass.* **9**, 71-81.
- TEUNISSEN, G.H.B. (1952) The development of endometritis in the dog and the effect of oestradiol and progesterone on the uterus. *Acta endocr., Copenh.* **9**, 407-420.