UR-3490-1550

MASTER

MAMMARY TUMORS AND SERUM HORMONES IN THE BITCH TREATED WITH MEDROXYPROGESTERONE ACETATE OR PROGESTERONE FOR FOUR YEARS

MAR 1979
Patent Office Counsel

D. W. Frank, D.V.M., Ph.D.,+ K. T. Kirton, Ph.D.,*
T. E. Murchison, D.V.M., Ph.D.,Δ W. J. Quinlan, ,†
M. E. Coleman, Ph.D.,Δ T. J. Gilbertson, Ph.D.,*
E. S. Feenstra, D.V.M., Ph.D.,* and F. A. Kimball, Ph.D.*

NOTICE
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

653 9800 +The Upjohn Company, Kalamazoo, Michigan 49001, present address Dawson Research Corporation, P.O. 8272, Orlando, Florida 32856.

*The Upjohn Company, Kalamazoo, Michigan 49001.

950 9249 ΔDawson Research Corporation, P.O. Box 8272, Orlando, Florida 32806

554 0000 †Department of Radiation Biology and Biophysics School of Medicine and Dentistry, University of Rochester, Rochester, N.Y. 14642

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Synopsis

After four years of a long term contraceptive steroid safety study, the incidence and the histologic type of mammary dysplasia produced is similar in beagles treated with medroxyprogesterone acetate (medroxyprogesterone) or progesterone.

Serum insulin, thyroid stimulating hormone (TSH), triiodothyronine, growth hormone, prolactin, 17β-estradiol, progesterone, and cortisol were determined by radioimmunoassay on samples collected after 45 months of treatment. Serum growth hormone and insulin concentrations were elevated in a dose related manner in both treatment groups. Triiodothyronine, cortisol, and estradiol-17β (medroxyprogesterone only) were lowered. TSH and prolactin concentrations were not changed. Pituitary-gonadal hormone interaction in the pathogenesis of mammary neoplasia of the dog is discussed. Prolonged treatment of the beagle with massive doses of progesterone or medroxyprogesterone results in a dose related incidence of mammary modules.

INTRODUCTION

The hormone dependence of mammary cancer was hypothesized by Beatson[1] in 1896 who noted remission of tumors in patients following ovariectomy. The experimental induction of mammary tumors in mice bearing pituitary isografts[2] suggested a relationship between steroid and protein hormones in tumor development and permitted manipulation of the prolactin estrogen interaction in the laboratory study of mammary neoplasia. The pathogenesis of mammary neoplasia has been intensively investigated in rodents, and is only partly understood in women[3,4]. However, in the dog, a species with a high spontaneous incidence of mammary dysplasia[5,6] and neoplasia[7,8], it remains incompletely characterized. In order to relate mammary tumorogenesis to hormonal alteration in the bitch, we determined the incidence of mammary tumors and assayed serum levels of prolactin, growth hormone, 17β-estradiol, progesterone, cortisol, insulin, thyroid stimulating hormone and triiodothyronine during the course of a study comparing medroxyprogesterone acetate with progesterone in the beagle.

Development of steroidal contraceptives calls for administration of new drugs at multiples of the human dose for two years in the rat, seven years in the dog and ten years in the monkey[9]. The 17α-hydroxyprogesterone derivatives used alone have shown no increase in mammary tumor incidence in rats[10], mice[10] or monkeys[11], and no evidence of mammary dysplasia has been found in women given medroxyprogesterone acetate when used alone in clinical contraceptive testing[12].

Contraceptive doses of medroxyprogesterone have not been linked to alterations of prolactin[13] or growth hormone secretion[14] or abnormally elevated serum 17β-estradiol[15] in clinical trials of the drug. However, an increased incidence of mammary dysplasia has been found in the dog.

MATERIALS AND METHODS

Animals and Drug Administration

One hundred forty (140) virgin beagle bitches were hysterectomized at 4-6 months of age and divided into six treatment groups. Drug treated groups of twenty bitches each received 30, 180 or 690 mg medroxyprogesterone acetate (medroxyprogesterone) per dog every 3 months for 36 months and 30, 300 or 735 mg per dog every 3 months from 36 to 48 months. Medroxyprogesterone was administered intramuscularly as an aqueous suspension. These latter doses correspond to 1, 10 and 25x the human contraceptive dose. Approximately 50 mg per dog is the efficacious contraceptive dose in the bitch[16]. Two other groups of 20 dogs each received intramuscularly an aqueous suspension of 46 or 1140 mg progesterone per dog per week. Preliminary evaluation suggested that these progesterone doses resulted in approximately 20 or 500 ng/ml serum progesterone which corresponds to approximately 1x or 25x luteal phase concentrations. Forty dogs were administered aqueous vehicle every 3 months through the 29th month of study. After 29 months, the controls were divided equally, with one-half receiving the vehicle volume equivalent to the high dose progesterone group, i.e. 11.4 ml per dog per week, and the other half receiving vehicle volume equivalent to that administered to the high dose medroxyprogesterone group, i.e. 4.6 ml per dog per three months.

The animals were housed individually in screened outdoor runs and allowed water *ad libitum*. The standard laboratory chow ration was adjusted to maintain a desirable body weight.

Routine Observations

Clinical observations were conducted daily and estrus and general health examination were conducted weekly. The mammary glands were examined monthly by inspection and palpation for changes in numbers and character of mammary nodules and the character of discharge. Since the study inception, the same person has conducted these examinations and updated schematics and descriptions of the nodules. Mammary nodules which were less than 2 mm in diameter and, therefore, no longer palpable, were deleted the following month. As the individual nodules became confluent, thus losing their individual identity, they were scored as originally found. Tissue for examination was derived from unscheduled deaths; the nodules were not biopsied. (The mammary nodules were histologically classified based upon the guidelines proposed by Hampe[17] as modified by Dr. Robert Kwapien, Director Contraceptive Steroid Project*, Armed Forces Institute of Pathology, Washington, D.C.). Routine hematology, serum chemistry, ophthalmology, bromsulfophthalein retention, and oral glucose tolerance tests were performed at regular intervals.

Hormone Determinations

Sera were collected and frozen during the 45th study month from all surviving dogs. All hormones were quantitated by radioimmunoassay. Canine prolactin was determined by a heterologous assay[18] employing an ovine prolactin standard. Canine growth hormone was determined by an assay employing rhesus antibody to canine pituitary growth hormone[19].

*Contributors to the Contraceptive Steroid project include industrial pathologists, staff of the AFIP and FDA. One of the project goals was to develop a consensus for the diagnostic criteria for mammary tumors of the bitch.

Thyroid stimulating hormone (TSH) was determined by radioimmunoassay described by Quinlan[20]. Progesterone[21], cortisol[22], and triiodothyronine (T-3)[23], and 17β-estradiol[24] were determined by previously published procedures. Insulin was quantitated with a Schwarz/Mann kit (Orangeburg, N.J.).

Statistical Analysis

Analysis of variance was used to test for overall differences among treatment group responses. Where applicable for statistically significant endpoints, the response of each treatment group was compared to the control. When treatment group variances were not homogeneous, a log transformation of the data was utilized. If variances remained heterogeneous, the Kruskal-Wallis test was used to test for overall differences among treatment group responses. For statistically significant endpoints in this case, each treatment group was compared to the control using the Wilcoxon rank sum test[25]. It should be noted that when the Wilcoxon test was used, the probabilities presented refer to pairwise comparisons and do not account for experiment-wise error rates except when the results of the medroxyprogesterone groups pooled were compared to the results of the progesterone groups pooled.

RESULTS

The results of the 48th month mammary gland clinical examinations are summarized in Table 1 and the histologic diagnosis of nodules from dogs which have died is found in Table 2. The incidence (Table 1) and type (Table 2) of tumors produced by medroxyprogesterone and progesterone was similar. Peripheral serum hormone concentrations in samples collected during the 45th month from dogs treated with medroxyprogesterone or progesterone are illustrated in Tables 3 and 4, respectively. Serum prolactin, growth hormone, and insulin responses were similar in the two treatment groups. Prolactin concentration did not change with treatment, while growth hormone and insulin concentrations increased in a dose-related manner in both progestin treated groups. Triiodothyronine was depressed in dogs treated with either progestogen, and thyroid stimulating hormone was significantly depressed in the high dose medroxyprogesterone treated group. Serum progesterone was increased in the progesterone treated groups, as expected, and in the low dose medroxyprogesterone treated groups as well. Serum estradiol was similar in all progesterone treated groups but depressed in a dose-related manner in medroxyprogesterone treated dogs. Serum cortisol was decreased significantly in dogs treated with progesterone and the high dose medroxyprogesterone treated group. There were no significant differences in the hormone values of vehicle control dogs for progesterone versus vehicle control dogs for medroxyprogesterone with the exception of significantly increased serum cortisol levels in the progesterone control group ($p<.05$). The pooled progesterone treated groups compared to the

pooled results of medroxyprogesterone treated groups had significantly increased estradiol and progesterone levels in dogs treated with progesterone ($p<.01$). Other responses were similar in groups of dogs treated with either progestogen.

Table 1

Mammary Nodule Incidence in Beagles Treated with Medroxyprogesterone Acetate and Progesterone for Four Years

Dose Group	Number of Surviving Dogs*	Number of Dogs With Nodules	Number of Nodules Present	Number of Nodules No Longer Present**
Vehicle Controls Medroxyprogesterone acetate	17	2	2	1
Vehicle Controls Progesterone	18	0	0	1
3 mg/kg Medroxyprogesterone acetate	19	13	29	6
30 mg/kg Medroxyprogesterone acetate	18	15	93	15
75 mg/kg Medroxyprogesterone acetate	14	12	105	26
5 mg/kg Progesterone	20	13	26	14
125 mg/kg Progesterone	4	4	45	10

* Initially 20 dogs per group
** Nodules no longer clinically detected

Table 2

Histopathology⊛ of Mammary Nodules in Beagles Treated With Medroxyprogesterone Acetate and Progesterone for Four Years*

	Vehicle Control		Medroxyprogesterone acetate			Progesterone		
	Medroxy-progesterone acetate	Progesterone	3 mg/kg	30 mg/kg	75 mg/kg	5 mg/kg	125 mg/kg	Total
Number of dogs examined	3	2	1	2	2	0	16	
Hyperplasias								
Simple Lobular Hyperplasia	0	2	1	3	9	0	11	26
Complex Lobular Hyperplasia	0	0	0	3	13	0	33	49
Simple Papillary Cyst	0	0	0	5	4	0	4	13
Complex Papillary Cyst	0	0	0	2	0	0	0	2
Total	0	2	1	13	26	0	48	90
Tumors								
Simple Adenoma	0	0	0	1	1	0	1	3
Complex Adenoma	0	0	0	4	12	0	9	25
Mixed Mammary Tumor	0	0	0	0	4	0	1	5
Complex Duct Papilloma	0	0	0	0	1	0	1	2
Simple Duct Papilloma	0	0	0	0	1	0	0	1
Basiloid Adenoma	0	0	0	0	0	0	1	1
Intralobular Non-infiltrating Solid Carcinoma, Comedo-Type**	0	0	0	0	0	0	1	1
Focus of Noninfiltrating Carcinoma*	0	0	0	0	0	0	1	1
Total	0	0	0	5	19	0	15	39
Total	0	2	1	18	45	0	63	129

*Unscheduled deaths only. See Table 1 for number of surviving dogs at 4 yrs.
** Not a nodule

⊛Nodules classified after Hampe[17]

Table 3

Serum Hormone Determinations in Beagles Given Medroxyprogesterone Acetate for 45 Months

Hormone	Vehicle Control	Medroxyprogesterone acetate 3 mg/kg	30 mg/kg	75 mg/kg	Overall Significance
Insulin μu/ml	5.98 ± 4.55† (15)	15.60 ± 5.60**L (18)	23.42 ± 17.14**L (20)	28.39 ± 18.19**L (16)	**L
TSH ng/ml	6.15 ± 3.23 (17)	5.60 ± 3.92 (20)	6.05 ± 3.57 (20)	3.01 ± 1.68**L (16)	**L
Thyroxin (T_3) ng/100 ml	130.52 ± 25.79 (17)	116.25 ± 41.59 (20)	104.09 ± 47.01*W (20)	104.75 ± 22.29**W (16)	*K
Growth Hormone ng/ml	5.67 ± 1.27 (14)	6.77 ± 5.23*W (12)	42.45 ± 48.84**W (16)	52.14 ± 47.49**W (12)	**K
Prolactin ng/ml	1.60 ± 0.63 (15)	2.20 ± 0.95 (20)	1.70 ± 0.65 (20)	2.06 ± 1.48 (16)	
Estradiol 17β pg/ml	29.12 ± 7.29 (16)	22.65 ± 7.00* (20)	18.22 ± 6.86** (20)	12.68 ± 4.77** (16)	**
Progesterone ng/ml	9.9 ± 15.2 (17)	34.3 ± 70.9**L (20)	3.3 ± 4.5 (19)	4.9 ± 4.6 (16)	**L
Cortisol μg/100 ml	2.27 ± 1.04 (17)	2.08 ± 1.18 (20)	2.89 ± 2.12 (20)	0.57 ± 0.42**K (16)	**K

† Mean ± S.D., number of observations in parenthesis
* $p<0.05$
** $p<0.01$
W Significance determined by Wilcoxon Rank Sum test
L Significance determined by log transformation of data
K Significance determined by Kruskal-Wallis test

Table 4

Serum Hormone Determinations in Beagles
Given Progesterone for 45 Months

Hormone	Vehicle Control	Progesterone 5 mg/kg	Progesterone 125 mg/kg	Overall Significance
Insulin μu/ml	6.63 ± 5.71[†] (15)	13.51 ± 9.14[*L] (20)	27.24 ± 15.46[**L] (7)	**L
TSH ng/ml	5.67 ± 2.22 (17)	6.63 ± 6.16 (20)	4.40 ± 3.38 (7)	
Thyroxin (T_3) ng/100 ml	125.88 ± 38.13 (18)	115.39 ± 39.03 (20)	74.00 ± 35.06[**W] (7)	*
Growth Hormone ng/ml	6.92 ± 1.89 (11)	5.37 ± 2.09[*W] (20)	89.20 ± 88.96[**W] (5)	**K
Prolactin ng/ml	1.70 ± 0.68 (17)	1.80 ± 0.61 (20)	1.85 ± 0.37 (7)	
Estradiol 17β pg/ml	26.20 ± 9.35 (18)	24.55 ± 7.72 (20)	29.69 ± 13.09 (7)	
Progesterone ng/ml	13.8 ± 25.2 (18)	62.06 ± 159.7[**W] (20)	156.3 ± 67.1[**W] (7)	**L
Cortisol μg/100 ml	3.11 ± 0.87 (18)	2.02 ± 0.98[**] (20)	1.45 ± 1.21[**] (6)	**

[†] Mean ± S.D., number of observations in parenthesis
* $p<0.05$
** $p<0.01$
[W] Significance determined by Wilcoxon Rank Sum test
[L] Significance determined by log transformation of data
[K] Significance determined by Kruskal-Wallis test

DISCUSSION

The incidence of mammary nodules in bitches treated with progesterone and medroxyprogesterone for 4 years was similar and related to the dose of the compound administered. Microscopic examination of the mammary nodules indicated a similar histologic spectrum and distribution of change in both progesterone and medroxyprogesterone treated bitches. Lobular hyperplasia was the most frequent observed microscopic lesion, and complex adenomas were the neoplasms found most frequently. No morphologic evidence was found that tumors associated with medroxyprogesterone treatment differed from those produced by progesterone except that histologic evidence of malignancy was confined to two dogs treated with 125 mg/kg progesterone weekly for 4 years.

The biological significance of progestogen stimulation to mammary tumorogenesis of the bitch is indicated by the strong dose relationship between the treatment and nodule incidence. Prolonged progesterone treatment at massive dose levels is tumorogenic in the beagle.

Similarly, medroxyprogesterone acetate (17α-hydroxy-6α-methylprogesterone acetate) is tumorogenic in the beagle when administered at multiples of the human dose prescribed for steroid contraceptive safety assessment. This is in keeping with the previously reported responsiveness of the canine mammary gland to stimulation by progestational agents.

In his recent review McGuire[26] indicates that the relationship of progesterone with anterior pituitary hormones in the genesis of

mammary neoplasia is unclear. Evidence from studies of rats and mice suggests a primary relationship between estrogen and prolactin in the development of mammary neoplasia while progesterone plays an important but uncertain role. Though approximately 40% of the breast cancer in women is responsive to steroid chemotherapy, the role of pituitary hormones in the genesis of mammary neoplasia in women is based largely upon circumstantial evidence. Higher serum prolactin levels have been reported in families with an increased incidence of breast cancer; however, prolactin receptors have not been demonstrated in human breast cancer tissue[26]. Hormonally responsive tumors are thought to be primarily estrogen dependent. Estrogen, however, increases progesterone receptors in mammary tissue, and the clinical remission rate reflects an advantage of combined steroid chemotherapy upon demonstration of estrogen and progesterone receptors. Contraceptive doses of medroxyprogesterone in women do not produce alterations of prolactin[13] or growth hormone secretion[14] or result in abnormal levels of serum 17β-estradiol[15].

In the present study, serum prolactin was similar in all groups of dogs, which suggests that chronic progestational stimulation does not result in increased prolactin secretion in the bitch. Growth hormone was increased by both progestogens in a dose related fashion. Because many high dose dogs showed alterations in carbohydrate metabolism, elevated serum insulin and acromegalic change, this increase in growth hormone was not unexpected. Serum prolactin shows no apparent relationship to the incidence of progestationally induced mammary nodules of the bitch, while serum growth hormone shows a close relationship to the

incidence of mammary nodules. Graf[27] reported similar findings in beagles given norethisterone enanthate. (In spontaneous mammary carcinoma of the dog Hamilton[28] found no difference in serum prolactin between 32 cases with neoplasia and 22 normal dogs). From direct analysis of the dog pituitary in various physiological and pathological states, Saluja[29] concluded that estrogen is prolactin responsive in the dog as well as in the rat. Increased prolactin occurred in the pituitary of 9/12 bitches with aggressive mammary carcinoma; however, peripheral prolactin concentrations were not determined. Pituitary growth hormone content also was not reported.

Our observations and those of others[30] indicate that the mammary tumors in the bitch may be expressed as a result of prolonged stimulation with massive doses of progesterone or closely related 17α-OH-derivatives. Similar results have not been reported in other species with these compounds. No evidence of treatment related mammary dysplasia has been seen in clinical trials with medroxyprogesterone acetate.

Results of these studies reveal several significant differences, and some similarities, between the beagle and human in the hormonal response to exogenous progestational compounds. In the present study, serum prolactin and cortisol were not changed significantly in dogs receiving 3 mg/kg medroxyprogesterone, similar to previous reports for the human[13,15]. Growth hormone was elevated and estradiol depressed ($p<0.05$) in the dog, while these parameters were not changed in the human[14,15]. Progesterone was elevated in the low dose medroxyprogesterone treated dogs, which may reflect continued ovarian cyclicity. This is in

contrast to human studies[15]. Basal serum insulin was elevated in the dog ($p<0.01$), but only following a glucose tolerance test in the human[14].

ACKNOWLEDGEMENTS

The authors would like to thank H. S. Greenberg of the Biostatistics unit of The Upjohn Company for his analysis of the data. The laboratory assistance of Susan Porteus of the Fertility Research unit and Donald Brink and Richard Smith of the Experimental Medicine and Clinical Research Laboratory unit of The Upjohn Company is gratefully appreciated.

REFERENCES

1. Beatson GT: On the treatment of inoperable cases of carcinoma of the mamma: Suggestions for a new method of treatment, with illustrative cases. Lancet 2:104, 1896.

2. Loeb L, Kirtz MM: The effects of transplants of the anterior lobe of the hypophysis on the growth of the mammary gland and on the development of mammary gland carcinoma in various strains of mice. Amer J Can 36:56, 1939.

3. Gullino PM: Considerations on the preneoplastic lesions of the mammary gland. Amer J Path 89:413, 1977.

4. Smithline F, Sherman L, Kolodny HD: Prolactin and breast carcinoma. New Eng Med J 292:784, 1975.

5. Cameron AM, Faulkin LJ: Hyperplastic and inflammatory nodules in the canine mammary gland. J Natl Can Instit 47:1277, 1971.

6. Wadsworth PF, Heywood R, Prentice DE, Van Abbe J: The incidence of mammary tumors in a group of 40 8-year-old female beagle dogs used in a toxicity study. Toxicol. Letters 1:285, 1978.

7. Schneider R: Comparison of age, sex and incidence rates of human and canine breast cancer. Cancer 26:419, 1970.

8. Frye FL, Dorn CR, Taylor D, Hibbard HH, Klauber MR: Characteristics of canine mammary gland tumor cases. Anim Hosp. 3:1, 1967.

9. Berliner VR: US Food and Drug Administration requirements for toxicity testing of contraceptive products. Pharmacologic models in contraceptive development, Proceedings of World Health Organization 1973, Edited by MH Briggs and E Diczfalusy, World Health Organization 1974, p. 240.

10. Leonard BJ: The use of rodents for studies of toxicity in contraceptive research. Pharmacologic models in contraceptive development, Proceedings World Health Organization, September 1973, Edited by MH Briggs and E Diczfalusy, World Health Organization, 1974, p.34.

11. Finkel MJ, Berliner VR: The extrapolation of experimental findings (animal to man). The dilemna of the systemically administered contraceptive. Lab Invest 28:383, 1973.

12. McDaniel EB, Pardthaisong T: Incidence of breast nodules in women receiving multiple doses of medroxyprogesterone acetate. J. Biosoc Sci 5:83, 1973.

13. Spellacy WN, Buhi WC, Birk SA: Stimulated plasma prolactin levels in women using medroxyprogesterone acetate or an intrauterine device for contraception. Fertil Steril 26:970, 1975.

14. Spellacy WN, McLeod AGW, Buhi WC, Birk SA, McCreary SA: The effects of medroxyprogesterone acetate in carbohydrate metabolism: Measurements of glucose, insulin, and growth hormone after twelve months' use. Fertil Steril 23:239, 1972.

15. Briggs M, Briggs M: Plasma hormone concentrations in women receiving steroid contraceptives. Obstet Gynec Brit Cmwlth 79:946, 1972.

16. Sokolowski JH, Zimbelman RG: Canine reproduction: effects of multiple treatments of medroxyprogesterone acetate on reproductive organs of the bitch. Am J Vet Res 35:1285, 1974.

17. Hampe JF, Misdorp W: Tumors and dysplasias of the mammary gland. Bull Wld Hlth Org 50:111, 1974.

18. McNeely AS, Fricsen H: Heterologous radioimmunoassay for rabbit prolactin. Endocrinology, in press, 1978.

19. Dramer MW: Radioimmunoassay for dog growth hormone:Its application to the endocrine study of normal and the head X-irradiated dog. Thesis submitted for partial fulfillment for degree of Doctor of Medicine for distinction in research. University of Rochester School of Medicine and Dentistry, Rochester, New York, 1973.

20. Quinlan WJ, Michaelson SM: The importance of TRH on thyroid gland hemostasis in normal and X-irradiated beagles as determined by radioimmunoasay for TSH. The Physiologist 16:428, 1973.

21. DeVilla GO, Robert K, Wiest WG, Mikhail G, Fleckeniger G: A specific radioimmunoassay of plasma progesterone. J Clin Endocrinol Metab 35: 458, 1972.

22. Colburn WA: Radioimmunoassay for cortisol using antibody against pregnisilone conjugated at the third position. J Clin Endocrinol Metab 41:868, 1975.

23. Hüffner M, Hesch RD: Radioimmunoassay for triiodothyronine in human sera. Acta Endocrinol 72:464, 1974.

24. England BG, Niswender GD and Midgley (Jr.) AR: Radioimmunoassay of estradiol 17β without chromatography. J. Clin Endocrinol Met 38:42-50, 1974.

25. Hollender M, Wolfe DA: Non-Parametric Statistical Methods, New York, John Wiley, 1973, p. 115.

26. McGuire WL, Chamness GC, Horwitz KB, Zava DT: Hormones and their receptors in breast cancer. Receptors in Hormone Action 11:401, 1978.

27. Gräf KJ: The role of the anterior pituitary gland in progestagen induced proliferative mammary gland changes in the beagle. Drug Res 28:54, 1978.

28. Hamilton JM, Knight PJ, Beevers J: Serum prolactin concentrations in canine mammary cancer. Vet Rec 102:127, 1978.

29. Saluja PG, Hamilton JM, Grnow M, Misdorp W: Pituitary prolactin levels in canine mammary cancer. Eur J Can 10:63, 1974.

30. Nelson LW, Weikel JH, Reno FE: Mammary nodules in dogs during four years treatment with megesterol acetate or chlormadinone acetate. J Natl Can Instit 51:1303, 1973.