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Hormonal Hypercholesterolemia in the Dog: Influence of Niacin, Niacinamide, Benzmalecene, and Cholestyramine Resin. (28229)

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Plasma cholesterol levels of human females fluctuate during the menstrual cycle, and increase considerably during pregnancy(1). The influence of ovarian cyclical changes on cholesterol levels in dogs, has not, to our knowledge, been reported. Canine reproductive physiology differs from the human in that following ovulation in dogs the corpus luteum persists as during the pregnant state, and unmated females commonly exhibit a period of pseudopregnancy.

We report here the occurrence in dogs of high plasma cholesterol level during pseudopregnancy, production of hypercholesterolemia in males and females by injection of progesterone and prolactin, and the influence of niacin, niacinamide, sodium benzmalecene (2), and cholestyramine resin(3,4) on these cholesterol levels.

Methods. Adult beagle dogs, 8 to 15 kg, were used. They were fed a mixture of approximately equal weights of Purina dog meal and Big Time canned dog food in sufficient quantity to maintain body weight. This diet contained, by analysis, 783 mg of cholesterol per average daily portion. Dogs were weighed and blood was drawn once a week. Plasma was prepared by mixing 4.0 ml whole blood with 0.7 ml of citrate-dextrose (ACD) solution, and analyzed for total cholesterol by the procedure of Abell *et al.*(5). Because of dilution with anticoagulant, all reported cholesterol concentrations are about 77% of serum values. Plasma cholesterol concentrations were determined in all dogs for 3 to 10 weeks before experimental regimens were begun. The above analytical procedure did not detect progesterone or its metabolites pregnane-3α-ol-20-one, pregnane-3α-20β-diol, or pregnane-3α-20α-diol.

Progesterone and prolactin† were injected subcutaneously in aqueous suspension 7 times a week. Injections were given twice on Fridays and twice on Mondays, and were omitted over the weekend. Niacin, niacinamide, sodium benzmalecene, and cholestyramine resin, when given, were mixed in the daily diet.

Results. 1. *Pseudopregnancy.* Plasma cholesterol concentrations in 3 female dogs during pseudopregnancy are presented in Table I. Levels increased to approximately

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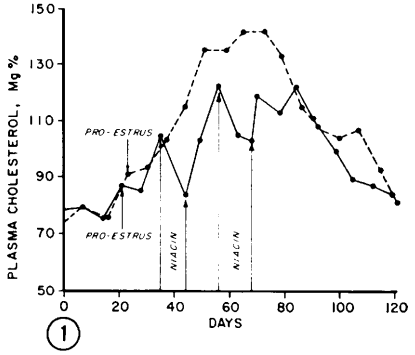
† Prolactin, containing 3 electrophoretic components (approximately 20 I.U./mg) was prepared in Merck Sharp & Dohme Research Laboratories from sheep pituitaries, using a modification of the method of Cole and Li(11).

TABLE I. Plasma Cholesterol Concentrations in Pseudopregnant Female Dogs.

Period	No. 1961		No. 1962		No. 1964	
	Total cholesterol	Body wt	Total cholesterol	Body wt	Total cholesterol	Body wt
	(mg% ± S.D.)	(kg)	(mg% ± S.D.)	(kg)	(mg% ± S.D.)	(kg)
Anestrus	76 ± 3	8.7	122 ± 6	10.1	93 ± 1	8.5
2 weeks after pro-estrus observed	103	9.3	160	10.2	153	9.1
Pseudopregnancy peak period	137 ± 4 (5 wk)	9.5	211 ± 15 (3 wk)	10.1	179 ± 5 (4 wk)	8.9
Anestrus	81 ± 4	10.7	123	10.3	93	9.0

double the anestrous values in 4 weeks after the first signs of pro-estrus, maintained this peak concentration for 2 to 4 additional weeks, and then gradually declined, reaching normal levels 98 days after the pro-estrous period had begun. Niacin feeding (200 mg/kg/day) counteracted this hypercholesterolemia. Cholesterol levels fell during periods of niacin feeding, and rose again when it was withdrawn (Fig. 1).

2. *Progesterone injection.* A. *Female dogs.*



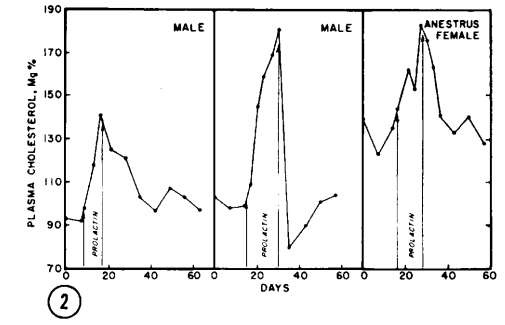


FIG. 1. Influence of niacin on hypercholesterolemia of pseudopregnancy in the dog. Dotted line—Plasma cholesterol concentrations during normal pseudopregnancy. Solid line—Plasma cholesterol concentrations in the same dog fed niacin for 2 periods during pseudopregnancy.

FIG. 2. Effect of prolactin on plasma cholesterol concentrations in dogs.

TABLE II. Effect of Progesterone on Plasma Cholesterol Concentrations in Anestrous Female Dogs.

Dog No.	Total cholesterol	Body wt	Treatment
	(mg% ± S.D.)	(kg)	
1962	124 ± 6	10.9	None
(P 19 da)	128	11.4	P 6 da
	151	11.3	P 13 da
	172	11.2	P 18 da
	218 ± 19	11.0	Post-injection peak period (4 wk)
	123	10.8	Post-injection 63 da
1964	93 ± 1	8.5	None
(P 15 da)	119	9.4	P 6 da
	163	9.9	P 13 da
	200 ± 9	9.4	Post-injection peak period (4 wk)
	96	8.5	Post-injection 54 da
1738	83 ± 5	8.9	None
(P 22 da)	111	9.3	P 6 da
	119	9.9	P 14 da
	189	9.9	P 21 da
	244 ± 2	10.0	Post-injection peak period (3 wk)
	107	10.4	Post-injection 69 da

P = 100 mg progesterone per day, subcutaneously.

Three normal, anestrous female dogs were injected with progesterone, 100 mg/day, for 15 to 22 days. Their plasma cholesterol rose to levels as high as those seen during pseudopregnancy (Table II). When progesterone injection was discontinued, cholesterol concentrations continued to rise, and did not decline for 4 or 5 weeks thereafter. They returned to normal within 8 to 10 weeks after injections were stopped. All 3 dogs gained some weight while hypercholesterolemic; 2 of them returned to their initial weights as cholesterol levels fell. One of these dogs, No.

TABLE III. Effect of Daily Progesterone Injections on Plasma Cholesterol Concentrations in Male Dogs.

Weeks of treatment	Total cholesterol – mg%						
	150 mg progesterone, S.C.			200 mg progesterone, S.C.			
	No. 1940	No. 1956	No. 1958	No. 1941	No. 1985	No. 1988	No. 1990
Control	89 ± 5*	102 ± 3*	111 ± 7*	67 ± 2*	71 ± 5*	68 ± 5*	81 ± 3*
1	92	116	124	73	74	68	90
2	100	102	138	79	75	78	110
3	93	129	138	80	76	78	112
4	98	114	154	98	86	77	105
5	86	121	173	90	93	77	105
6	91	130	193	91	90	81	110
7	94	128	191	93	107	84	
8	118	145	202	98	117	88	
9	128	148	174	103	121	87	
10	164	154	211	105		104	
29-52	129 (52 wk)	151 (52 wk)	206 (48 wk)	120 (48 wk)	139 (29 wk)	115 (44 wk)	149 (35 wk)

* Standard deviation.

1962, was subsequently ovariectomized. Plasma cholesterol concentration (± S.D.) was 124 ± 8 mg% prior to surgery, and was 119 ± 5 mg% during a 6-week postoperative period. When this dog was given progesterone, 100 mg/day, cholesterol level rose to 152 mg% in 7 days. Feeding of niacin (200 mg/kg/day) for 11 days, while continuing progesterone injection, resulted in a drop in plasma cholesterol to 108 mg%. When niacin treatment was stopped, cholesterol level rose again, reaching 162 mg% after 3 weeks. Niacinamide (200 mg/kg/day) was then added to the diet for 12 days. Cholesterol level continued to rise, reaching 215 mg%. Withdrawing niacinamide and feeding sodium benzmalecene (25 mg/kg/day) for 13 days lowered plasma cholesterol to 165 mg%. One week after benzmalecene was withdrawn, during treatment with progesterone alone, cholesterol concentration rose to 182 mg%.

B. *Male dogs.* Male dogs responded to progesterone injection more slowly and to a smaller extent than did females. Daily injection of 100 mg of progesterone had no effect in 3 weeks on the plasma cholesterol of one male, produced a transitory 10% rise during 4 weeks of injection in a second, and increased cholesterol level of a third male 21% after 7 weeks. Larger doses and longer courses of injection produced greater rises (Table III). Degree of hypercholesterolemia attained was not directly related to amount of hormone administered. Differences in response of individual animals could not be attributed to initial age or body weight. Injection was continued as long as a year in some of the dogs with little change in weight and no visible signs of toxicity. As in females, cholesterol concentrations continued to rise for several weeks after progesterone injections were stopped, then slowly declined to pretreatment levels.

A progesterone-treated (150 mg/day) male dog was fed cholestyramine resin (25 g dry weight/day) for 30 days, while progesterone injections were continued. Plasma cholesterol fell from 149 mg% to 95 mg% in 6 days, and remained at this lowered level during the resin treatment period. When resin feeding was stopped, cholesterol level returned to 148 mg% in 2 weeks. The same dog was then fed sodium benzmalecene (25 mg/kg/day) and plasma cholesterol dropped from 168 mg% to 106 mg% in 7 days, and to 71 mg% in 14 days. Cholesterol level rose again to 163 mg% in 13 days after benzmalecene feeding was stopped.

Niacin feeding, 200 mg/kg/day for 33 to 35 days, produced no discernible effect on cholesterol levels of 3 normal and of 3 progesterone injected hypercholesterolemic male dogs. Feeding of niacinamide (200 mg/kg/day), however, increased cholesterol levels in 2 of 3 normocholesterolemic males, and in 3 long term progesterone treated male dogs. Control cholesterol levels of 102 ± 2 and 103 ± 5 mg% rose to 134 and 124 mg% during 3 weeks of feeding niacinamide to 2 normal dogs. In a third dog the control level of 98 ± 7 mg% was not changed in 13 days of treatment. Plasma cholesterol levels in 3

progesterone-injected male dogs rose from 127 ± 6, 126 ± 5, and 116 ± 3 mg% to 147, 186, 158 mg% during 20 to 33 days of niacinamide feeding.

3. *Prolactin injection.* Subcutaneous injection of prolactin, 15 mg/day for 8 to 15 days, produced prompt rises in plasma cholesterol in 2 males and in an anestrous female dog (Fig. 2). The female exhibited mammary development and secreted milk. Cholesterol levels dropped as soon as prolactin injections were discontinued, and were at or below pretreatment levels in 5 to 25 days. In an ovariectomized female (No. 1964) prolactin injection increased blood cholesterol from 82 ± 3 mg% to 132 mg% in 7 days. Niacin (200 mg/kg/day) was then added to the diet, while prolactin injections continued, and cholesterol level dropped to 97 mg% in 11 days. When niacin was withdrawn, it rose to 141 mg% in 3 weeks. Niacinamide (200 mg/kg/day) was then fed for 6 days. Plasma cholesterol continued to increase to 160 mg%. Feeding of sodium benzmalecene (25 mg/kg/day) instead of niacinamide, lowered plasma cholesterol to 124 mg% in 6 days. Cholesterol concentrations rose to 142 mg% in 2 weeks after benzmalecene treatment was stopped. This ovariectomized female dog also showed signs of mammary development and lactation during prolactin treatment.

Discussion. The hypercholesterolemia observed here in pseudopregnant female dogs, in which both placenta and fetus are absent, was similar in magnitude to that seen in pregnant dogs in this laboratory. The presence of a functioning corpus luteum in both states suggested that progesterone might contribute to the elevated cholesterol levels. Daily injection of progesterone did raise cholesterol levels in an ovariectomized female and intact female dogs, and also in male dogs. The course of treatment here was longer than that reported by Oliver and Boyd in men(1).

Daily injections of prolactin also raised cholesterol levels. Again, the course of treatment was longer than that in previously reported work(6). Prolactin produced its effect in males and in an ovariectomized female, as well as in an intact female, and thus its action cannot be mediated through the ovary.

Progesterone and prolactin differed in their cholesterol-raising effects. The action of progesterone was slower, it persisted when treatment was stopped, and its effect was greater in females than in males. The post-injection cholesterol elevation ascribed to progesterone can be at least partially explained by the absorption of the steroid from accumulated depots. The action of prolactin was relatively rapid, it ceased when treatment was stopped, and its effect was equally great in females and males.

The feeding of niacin has been reported to lower blood cholesterol levels in normal or hypercholesterolemic rabbits(7) and men(8). There is disagreement concerning its action in dogs(9,10). In the present work, niacin feeding lowered cholesterol levels in pseudopregnant dogs, and in progesterone-injected and prolactin-injected ovariectomized female dogs, but had no effect on cholesterol levels in normal or progesterone-injected male dogs.

The feeding of niacinamide either had no effect or increased cholesterol levels in normal and progesterone-injected male dogs. Niacinamide appeared to increase plasma cholesterol in progesterone or prolactin-injected ovariectomized female dogs, but the data are not sufficient to allow this increase to be attributed to the niacinamide alone.

Summary. Pseudopregnant female dogs exhibited a characteristic hypercholesterolemia that was similar to that seen in true pregnancy in this laboratory. Daily subcutaneous injection of progesterone or prolactin raised blood cholesterol concentrations in male, anestrous female, and ovariectomized female dogs. The two hormones differed in their action. Niacin feeding counteracted the hypercholesterolemia of pseudopregnancy, and lowered plasma cholesterol levels in progesterone-injected or prolactin-injected ovariectomized female dogs. It had no effect on cholesterol concentrations of normal male or progesterone-injected male dogs. Niacinamide generally increased plasma cholesterol levels. Sodium benzmalecene lowered the blood cholesterol of a prolactin and a progesterone-injected female, and of a progesterone-

injected male dog. Cholestyramine resin decreased the elevated cholesterol level of a progesterone-injected male dog.

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Kinetic Properties and Adaptations of Liver Glucose-6-Phosphatase.* (28230)

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The adaptation of liver glucose-6-phosphatase has been observed in response to both dietary(1) and hormonal(2) manipulations, as well as in diabetes(3). It appears that the adaptation results in a synthesis of more enzyme since the increase in activity can be prevented by ethionine, both in the case of response to diet(4) and to hormone administration(5). In both of these experiments, it was shown that the suppression of the adaptation by ethionine could be counteracted by additional methionine. There has been little work on the nature of the new or "induced" glucose-6-phosphatase. One report on the sub-cellular localization of the enzyme after cortisone treatment indicated no differences in distribution of the enzyme in control and cortisone treated rats(6). However, the widespread appearance of activity in all fractions appears to leave something to be desired in establishing a single sub-cellular localization. It has also been reported that in diabetes, which increases the liver glucose-6-phosphatase activity, this enzyme has altered kinetic properties(7). This report indicated an increase in apparent K_m (K_m') of the enzyme in both whole homogenates and with isolated microsomal fractions. All differences between the enzyme from normal and diabetic animals disappeared after solubilization with digitonin or desoxycholate(8). There has also been a report indicating no differences in the kinetic properties of the enzyme in normal and diabetic rats(9).

This report concerns studies of the kinetic properties of glucose-6-phosphatase in normal rats and in animals with increased total liver glucose-6-phosphatase activities. Male Sprague-Dawley rats were used. The diets have been described previously(1,10). Diabetes was produced by injection of alloxan and hydrocortisone injected as a suspension of the acetate (5 mg/rat/day). Diabetes was confirmed by blood glucose levels of 300 mg% or greater. The following methods were used: glucose-6-phosphatase assay(1), determination of K_m'(11), tissue fractionation (12), and blood glucose(13). All animals

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