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Enhancement of Experimental Atherosclerosis by ACTH in the Dog.* (25420)

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Prolonged treatment with large doses of ACTH or cortisone is known to elicit a hyperlipemic response in the rabbit(1-3) in man (4), and to a lesser extent in the dog(5). Cholesterol-induced hyperlipemia in rabbits and dogs is heightened markedly during administration of ACTH, cortisone, or hydrocortisone (5-7). The present studies show that prior short-term administration of ACTH enhances development of hyperlipemia in dogs subsequently given a standard thiouracil and cholesterol diet for 6 to 10 months.

Methods. Atherogenic diet: The standard diet consisted of 2 daily doses of 50 mg thiouracil/k of body weight and kibble containing 10% cholesterol (prepared with ether solvent), to provide a daily cholesterol intake of 750 mg/k of body weight(8). To insure complete intake, this diet was fed first, followed by a meat-kibble diet fed *ad lib*. Dogs were divided into 3 groups: *Group A*. Twenty-eight dogs (13 beagles, 3 "Kendall mongrels,"† and 12 ordinary mongrels) were normal controls throughout and fed a meat-kibble diet only. *Group B*. Fourteen dogs (8 beagles and 6 "Kendall mongrels") were maintained on standard atherogenic diet, plus meat-kibble. *Group C*. Seven dogs (4 beagles, one "Kendall mongrel," and 2 mongrels) were given one to 3 injections of 40 units of ACTH-gel subcutaneously at intervals of 6 to 12 days. Two to 4 months later, the 7 dogs were placed on the atherogenic diet. Three additional male beagles received 3 subcutaneous injections of 40 units ACTH-gel at 3 or 4 day intervals. After the third injection, the 3 dogs were started on the atherogenic diet. All dogs but the ordinary mongrels were approximately 1 year of age. All were maintained in kennels with outside runs. Each dog was used as his own control for at least one to 4 months. Each was weighed at 2-week intervals and fed the atherogenic diet individually on a body-weight basis. Blood samples drawn from fasting dogs at 2 to 4 week intervals were analyzed for serum cholesterol(9), phospholipids(10) and alpha and beta lipoproteins (11). At autopsy, tissues were fixed in 10% neutral formalin. Aortas were stained with Sudan IV to demonstrate lipid content of atheromatous lesions(12).

Results. Group A. Without treatment, serum lipid values of beagles and ordinary mongrels were comparable (Table I). Cholesterol and phospholipid values for the "Kendall mongrel" tended to be higher ($p = <0.05$‡) than in the normal beagle. C/P and lipoprotein ratios did not differ significantly between beagles and "Kendall mongrels."

Group B. The atherogenic diet fed 4 to 11 months tended to elevate serum cholesterol, phospholipids, and C/P ratio in all dogs, ($p = <0.05$) and a marked rise occurred in %

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† It has been suggested that breeds of dogs differ in susceptibility to atherosclerosis: beagles are considered to be relatively resistant while a strain, originally mongrels and inbred by Dr. Forrest Kendall, are relatively susceptible.

‡ The rank sum test was used for statistical evaluation of data.

TABLE I. Effect of ACTH Pretreatment on Lipid and Lipoprotein Response to Atherogenic Diet.

Group		Time*	No. dogs ♂	No. dogs ♀	Serum cholesterol and phospholipids: No. determinations†	Chol., mg % ♂	Chol., mg % ♀	P-lip., mg % ♂	P-lip., mg % ♀	C/P ratio ♂	C/P ratio ♀	Lipoproteins: No. determinations†	% β ♂	% β ♀
					Control diet, no ACTH									
A	Beagles	1–12	14	14	157	153	163	318	340	.48	.48	94	30	26
	Kendall mongrels	1–10	6	4	26	218	217	380	385	.57	.56	16	33	31
	Mongrels	2	6	11	22	149	161	295	301	.51	.53	14	34	30
					Atherogenic diet, no ACTH									
B	Beagles	11	4	4	122	515	510	477	513	1.08	.99	41	48	39
	Kendall mongrels	10	3	3	92	721	713	572	605	1.26	1.18	27	46	51
					Atherogenic diet, ACTH									
C	Beagles	8–10	5	2	61	1322	1325	849	764	1.56	1.73	12	83	67
	Kendall mongrels	9	1	0	10	2530		1049		2.41		5	83	
	Mongrels	6	2	0	12	988		637		1.55		7	53	

* Time: months on diet indicated, during which determinations were made.
† No. determinations: No. of determinations entering the total male-female averages shown for each column.
As an indication of normal variability in beagles the stand. dev. for cholesterol, phospholipids, the C/P ratio and the % β lipoprotein for males were 33, 53, .07 and 8, and for females 50, 38, .15 and 9, respectively.

beta lipoproteins of beagles and "Kendall mongrels" (Table I, Fig. 1-A and 2-A). The difference between the 2 strains of dog is exemplified in Figs. 1 and 2. This strain difference will be elaborated elsewhere.

Group C. Prior treatment with one to 3 subcutaneous injections of 40 units ACTH-gel§ heightened the hyperlipemic response ($p = 0.001$) of 7 dogs of 3 different breeds maintained for 6 to 10 months on the atherogenic diet (Table I). This effect persisted for 6 to 10 months of the study (Figs. 1 and 2).

Autopsy findings. Dogs in Group C *pretreated with ACTH* and fed the atherogenic diet for 6 to 10 months showed severe atherosclerosis in almost all vessels of the arterial tree. Coronary, thyroid, and cerebral vessels, and the bifurcations and terminal portion of the abdominal aorta were affected most severely. Fig. 3-B demonstrates the high lipid content of atherosclerotic plaques in the lower part of abdominal aorta of a dog from Group C. Death of one dog followed a cerebral hemorrhage near the posterior portion of the circle of Willis. Death of a second animal was asociated with far advanced atherosclerotic lesions in the circle of Willis and all other arteries of the body. Four of the 10 dogs in this group suffered single or multiple cerebral occlusions which produced symptoms similar to those following cerebral occlusions in man.

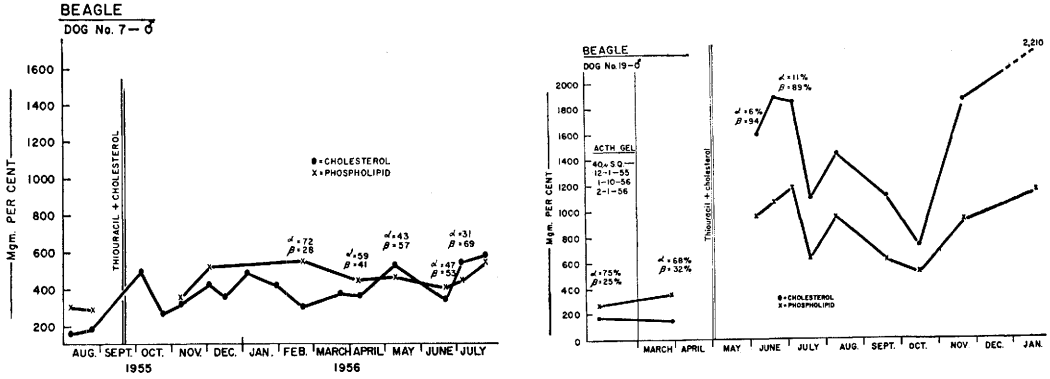
Dogs not *pretreated with ACTH* (Group B) developed scattered, small atherosclerotic plaques, found only in femoral and iliac arteries and in the abdominal aorta and its major branches, as in one case shown in Fig. 3-A.

Discussion. Pretreatment with one to 3 injections of ACTH-gel markedly enhanced subsequent hyperlipemia produced in dogs by feeding a thiouracil-cholesterol diet. This was associated with significant enhancement of the atherosclerotic process in larger arteries.

One of the prerequisites for producing experimental hyperlipmia in the dog has been the establishment of a hypothyroid state. It has been suggested that in the dog the adrenal cortical steroids exert their effect on lipid

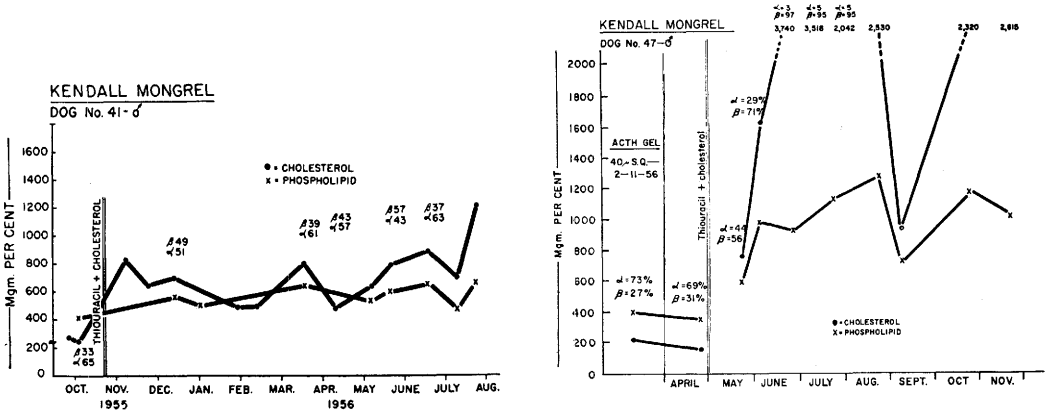
§ ACTH-gel used was made by Organon and Armour Labs.

metabolism, in part at least, through a depression of thyroid function(5). In normal patients, large doses of cortisone have been shown to produce a marked suppression of thyroid function as measured by P.B.I. levels and I^{131} uptake(13). In acute experiments, it has been shown that ACTH and adrenal steroids are capable of reducing rate of release of thyroidal radioiodine in rats and rabbits(14). Furthermore, in the hypophysec-



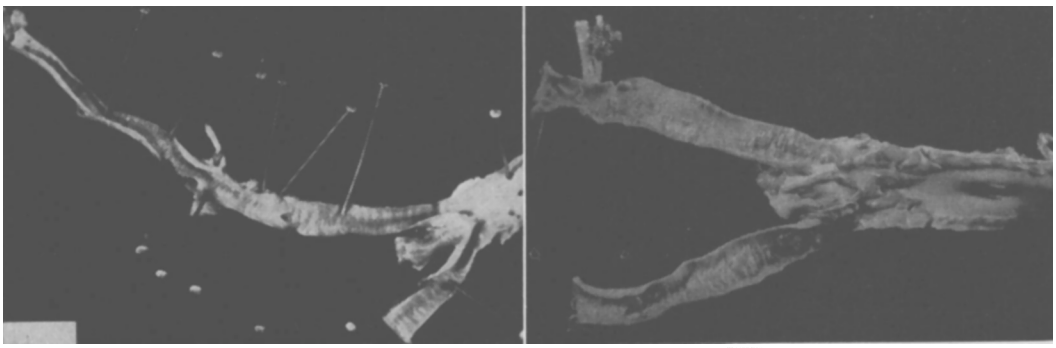
A. Without ACTH pretreatment B. With ACTH pretreatment

FIG. 1. Response of beagle to thiouracil-cholesterol regimen.



A. Without ACTH pretreatment B. With ACTH pretreatment

FIG. 2. Response of "Kendall mongrel" to thiouracil-cholesterol regimen.



A. Without ACTH pretreatment B. With ACTH pretreatment

FIG. 3. Sudan IV-stained aortas obtained from beagles maintained on thiouracil-cholesterol regimen.

tomized rat, ACTH and cortisone have been shown to significantly inhibit the increased thyroidal uptake of I^{131}, known to follow administration of thyrotropic hormone(15).

It has been shown that the level of circulating adrenal cortical steroids is one of the factors which regulates the ACTH content of the pituitary. Adrenalectomy causes a rise in pituitary content of ACTH and hypertrophy of the pituitary(16,17,18). Administration of cortisone produces a depletion of pituitary ACTH. The associated atrophy of the adrenal gland suggests that ACTH secretion is also decreased(18). Furthermore, they showed(18) that administration of ACTH-gel to the rat caused a significant rise in pituitary ACTH, suggesting that lasting derangement of the endogenous ACTH-regulating mechanism may have occurred with failure of high levels of circulating adrenal corticosteroids to suppress further ACTH production. Our preliminary studies reveal a trend for dogs pretreated with ACTH-gel to show increased amounts of urinary 17-hydroxycorticoids. Urinary corticoid excretion for normal beagles averaged 3.7 mg/24 hours (33 determinations); urinary corticoid excretion for thiouracil and cholesterol-fed beagles averaged 3.4 mg/24 hours (40 determinations); and urinary corticoid excretion for ACTH-pretreated dogs averaged 4.7 mg/24 hours (12 determinations).

Summary. One to 3 injections of 40 units of ACTH-gel significantly enhanced the elevated serum cholesterol and phospholipid levels produced by subsequent thiouracil and cholesterol feeding in the dog. This effect was associated with paralytic strokes in 4 of 10 dogs. Autopsy revealed markedly more widespread and more severe atherosclerosis in dogs pretreated with ACTH than in dogs who received only a high thiouracil and cholesterol diet.

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