Urinary Excretion of Corticosteroids and Neutral 17-Ketosteroids in Adult Female Dogs. (20806)

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Chemical assays on urinary excretion of 17-ketosteroids in the dog are few(1-3) and studies of corticosteroid output by dogs are virtually nonexistant(4). In an investigation in which the crude urinary extract was fractionated with Girard's reagent-T, Glenn and Heftmann(3) found that normal dogs of either sex excreted less than 1 mg of neutral 17-ketosteroids per day. They found no increase in excretion of these compounds following administration of ACTH. No figures on the 24 hour excretion of corticosteroids in the dog have been published, to the authors' knowledge.

The present study was undertaken to establish more clearly the normal range of steroid excretion by female dogs and the extent to which this excretion was modified by factors generally recognized to influence adrenal hormone production or metabolism.

Method. Healthy adult female dogs were maintained on a stock diet, kept in metabolic cages and their urine collected using thymol as a preservative. All animals were catheterized at the beginning and end of each 24 hour period. The fresh urine was adjusted to pH 1 with concentrated HCl, an aliquot placed in a Hershberg-Wolfe extractor(5), and extracted continuously with redistilled methylene chloride for a period of 20 hours to obtain maximum yield(6). The methylene chloride containing the urinary extract was evaporated to about 50 ml, quantitatively transferred to a volumetric flask, and diluted to 100 ml. Twenty-five ml of the extract were transferred to a separatory funnel and 100 ml of redistilled ether added. Subsequent purification was essentially that of Heard, Sobel, and Venning(4). After washing with alkali and water, the purified extract was evaporated to dryness and taken up in methanol. Aliquots of this solution were used for corticoid and 17-ketosteroid estimation. Utilizing blue tetrazolium, estimation of reducing corticosteroids was carried out as previously described (7) with one simplification: acid alcohol (80 ml of methanol and 20 ml of concentrated HCl) was used instead of acid pyridine. The color of the reduced blue tetrazolium was not as long lasting as with pyridine but was stable for about one hour. The Zimmerman reaction with m-dinitrobenzene was used on extracts treated with Girard's reagent-T for neutral 17-ketosteroid assay(8).

Results, normal values. Significant amounts of neutral 17-ketosteroids are excreted by normal adult female dogs (Table I); the values are considerably lower than for adult human subjects(9-12). However, if expressed in terms of body weight, the daily excretion

TABLE I. Urinary Excretion of Neutral 17-Ketosteroids and Reducing Corticosteroids by Normal Female Dogs.

	17-ketosteroid excretion		Reducing corticosteroid excretion	
Dog wt	No. of specimens*	Mean ± Stand. dev.	No. of specimens*	Mean ± Stand. dev.
21	8	2.4 ± .80	11	1.3 ± .30
13	6	1.6 ± .63	4	1.3 ± .30
18	15	2.7 ± 1.23	18	1.6 ± .62
14			12	2.3 ± .63
			4	2.0 ± .45
16.5	9	1.2 ± .42		
17.5	10	2.7 ± .59	14	1.8 ± .49
18	14	2.2 ± .68	28	2.0 ± .90
Avg		2.1 ± .73		1.8 ± .53

* Excretion in 24 hr urine specimens expressed in mg.

TABLE II. Effect of I.M. and I.V. ACTH on Urinary Excretion of Neutral 17-Ketosteroids and Corticosteroids by Normal Female Dogs.

Dog No.	Total dose, mg/24 hr	17-ketosteroids, mg/24 hr		Corticosteroids, mg/24 hr	
		Pretreatment exer.	Exer. during treatment	Pretreatment exer.	Exer. during treatment
1*	90	2.6	2.3	—	—
490	65	1.2	1.6	—	—
492	70	3.1	3.3	1.8	1.3
496	75	2.5	1.9	—	—
1†	60	—	—	2.4	4.0
45	40	—	—	2.7	2.8
1‡	25	2.2	4.0	2.2	6.3
10	23	—	—	1.1	2.2
	20	—	—	—	3.6
23	50	3.0	3.6	2.0	3.4
	30	—	4.3	—	5.6
45	25	—	—	1.3	4.0
	25	—	—	2.6	6.0
	25	—	—	—	5.5
496	20	3.5	—	2.6	3.3
	20	—	5.0	—	4.8
	25	—	5.0	—	6.1

* Intramusc. inj. every 24 hr. † Continuous 24 hr intramusc. drip. ‡ Continuous 24 hr intrav. drip.

TABLE III. Effect of a Single Injection of Cortisone on Urinary Excretion of Neutral 17-Ketosteroids and Corticosteroids by Normal Female Dogs.

Dog No.	Total dose, mg	17-ketosteroids, mg/24 hr		Corticosteroids, mg/24 hr	
		Pretreatment exer.	Exer. following inj.	Pretreatment exer.	Exer. following inj.
23*	200	3.0	5.5	1.8	4.2
	200	—	—	1.8	4.2
		—	—	—	2.1‡
	200	—	—	2.7	5.9
		—	—	—	1.8‡
496†	75	3.5	5.4	3.0	3.8
	100	—	—	2.3	2.8
	200	—	—	2.2	1.2

* Dog No. 23, intramusc. inj. 24 hr following cortisone. † Dog No. 496, intrav. inj. ‡ Excretion during 2nd

would be quite similar to that of the adult human female, although somewhat more variable. The average excretion of neutral 17-ketosteroids for the 6 adult female dogs was 2.1 mg/24 hr. The range of corticosteroid excretion was similar to that obtained by the same methods on adult human subjects(13). There was less daily variation than observed for ketosteroid excretion. The average excretion for blue tetrazolium reducing substances for seven dogs was 1.8 mg/24 hr (Table I).

Effect of ACTH administration. Intermittent intramuscular injections of ACTH caused no detectable increase in 17-ketosteroid output (Table II). Continuous intramuscular infusions resulted in some elevation in the urinary corticosteroid level in one out of 2 dogs. Continuous intravenous administration of ACTH, however, caused a consistent rise in 17-ketosteroid and corticosteroid excretion. The increase in 17-ketosteroid output was never greater than 180% of the pretreatment excretion, whereas corticosteroid output was increased from 210 to 330% following I.V. ACTH.

Effect of cortisone (Table III). Intramuscular and intravenous administration of cortisone was followed by some increase in 17-

TABLE IV. Hepatic Bloodflow and Reducing Corticoid Excretion in the Dog.

Dog No.	Procedure	Time, P.O. days	Corticoid excretion, mg/24 hr	R.B. dye clearance‖
45	Normal	—	2.6 (9)*	102
	Eck fistula	5– 40	3.0 (3)	41
		41– 77	1.1 (9)	38
		93–128	.5 (5)	39
	Control	63	.8 (1)	
	I.V. ACTH†	63– 99	3.4 (4)	
	Control	129	.5 (1)	
	I.V. ACTH‡	129–130	.65(2)	
	Control	76	1.8 (1)	
	I.M. cortisone§	77	2.8 (1)	
496	Normal	—	2.1 (13)	105
	Reverse Eck	8– 71	.91(14)	85

* ( ) No. of determinations from which avg value was obtained.
† 16 mg/24 hr (Armour preparation).
‡ 45 mg/24 hr.
§ 335 mg, I.M.
‖ Rose Bengal dye clearance(17).

ketosteroid excretion in the 2 dogs studied. The effects of I.M. cortisone injection on corticosteroid output were irregular in one animal while I.V. administration of this hormone had no effect on corticosteroid excretion in a second animal.

Effect of operative procedures on corticoid excretion. Bloodflow through the liver was reduced and increased by Eck and reverse Eck fistula formation respectively. A reduced bloodflow through the liver did not significantly alter corticoid excretion for the first 40 days in the animal studied (Table IV). However, as the animal became debilitated, the corticoid excretion declined. The response to ACTH was similar to that of a normal animal until the health of the animal declined, at which time there was essentially no response to ACTH even though administered in larger doses than previously. Formation of a reverse Eck fistula was associated with a reduced corticoid excretion which remained at approximately half the normal value during the 71 days of observation. This animal remained in good health throughout the period of observation.

A few other miscellaneous observations were made on corticoid excretion in the dog. One dog that normally excreted an average of 1.3 mg of corticoids daily excreted 0.3 mg after adrenalectomy while receiving 1 mg of DOCA daily. One hypophysectomized male dog excreted 0.65 mg of corticoids daily one year after hypophysectomy. Common bile duct obstruction and diverting bile excretion into the urine did not significantly alter the 24 hour excretion of corticoids in the urine of one dog. A comparison of corticoid excretion following intrasplenic and intramuscular injections of cortisone gave inconclusive results on one dog with an exteriorized spleen.

Discussion. Female dogs excrete a substantial amount of corticosteroids in their urine. Spontaneous daily variation is small enough so that significant increases or decreases in corticosteroid output may be recognized with reasonable accuracy.

The normal values for 24 hour excretion of 17-ketosteroids reported here are higher than those obtained by Glenn and Heftmann(3). This difference is most probably due to the fact that their extraction time was limited to 1.5 hours. Pincus and Romanoff(6) have shown that, so far as corticosteroids are concerned, 4 hours of extraction will result in a 35% yield of the potential maximum.

Intramuscular ACTH did not produce consistent elevations in 17-ketosteroid or corticosteroid output. However, intravenous ACTH caused increased excretion of both groups of steroids.

The relatively small increases in urinary reducing steroids following large doses of cortisone indicate that this substance is largely altered in the normal animal prior to excretion.

The effect of adrenalectomy on the excretion of tetrazolium reducing substances in urine suggests that most of the material included in this estimation was adrenal in origin. Similar results have been obtained on adrenal insufficient human subjects using the same method(13). The urine of dogs contains vit. A in quantities up to 450 I.U. per 100 ml(14) which reduces phosphomolybdic acid(15). Investigation has shown that with the present method of corticosteroid estimation, errors due to vit. A reduction will be less than 2%.

Alteration of the blood supply to the liver and splenic injections of cortisone give no clear evidence concerning the role of the liver in the metabolism of corticosteroids(16) although the reduced excretion following reverse

Eck fistula in one animal suggests that the liver must play a part in the degradation of these hormones. No evidence was obtained that reducing steroids are excreted in the dog's bile.

The reduced excretion of corticosteroids that eventually occurs in the Eck fistula animal can best be explained on the basis of debility and inanition which developed during the postoperative period of study.

Summary. Normal values for neutral 17-ketosteroids and reducing corticoids have been presented for adult female mongrel dogs. Stimulation of the adrenal glands of the dog by continuous intravenous infusion of ACTH increased both the reducing corticoid and neutral 17-ketosteroid excretion. Additional observations are reported regarding factors that influence the excretion of reducing corticoids by the adult female dog.

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Received December 11, 1953. P.S.E.B.M., 1954, v85.

Antibiotic Combinations and Resistance: Response of *E. coli* to Antibiotics, Singly and in Pairs.* (20807)

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In a previous study(1) it was shown that the development of resistance in strains of staphylococci and in a strain of enterococcus to penicillin (Pen), streptomycin (S) or erythromycin (E) *in vitro* could be depressed and delayed by repeated subcultures in the presence of combinations of E with either Pen or S. Further observations(2) with strains of staphylococci and certain streptococci repeatedly subcultured in Pen, S, E, oxytetracycline (Terramycin, "T") and coloramphenicol (C) and in 8 additional pairs of these antibiotics confirmed these findings and indicated further that: 1) the rate and degree of resistance which developed against individual antibiotics to which the organisms were exposed varied with the antibiotics and sometimes with the strain of organism; 2) subcultures on the pairs of antibiotics resulted in increased resistance to the homologous mixture, but usually of lower degree than against the individual components when used separately; 3) when increases in resistance to the

* Aided by a grant from the U. S. Public Health Service.