0.03% propylthiouracil were variously added as indicated in Table II. At the end of 30 days the animals were killed with chloroform and the thyroids weighed. In this experiment there was again no inhibition of the size of the goiters produced by thiouracil in the B_{12}-fed animals. In both experiments, the thyroids of the B_{12}-fed animals were just as large, vascular, and friable as those of the controls. No satisfactory explanation is in evidence to account for the discrepancy in findings between the two laboratories.

Summary. It was not possible to confirm the antigoitrogenic effect of vit. B_{12} in thiouracil-fed rats.

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Urinary Excretion of Neutral 17-Ketosteroids by Normal Dogs. (18707)

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The Zimmermann reaction with *m*-dinitrobenzene is used with considerable success for the assay of neutral 17-ketosteroids in human urine. Engstrom and Mason(1) and Talbot and coworkers(2) have shown that errors in the colorimetric determination caused by the presence in urine of colored substances and chromogenic material other than 17-ketosteroids may be eliminated either by a preliminary Girard separation or, if the reaction is carried out according to the technic of Callow *et al.* (3), by application of a color correction equation. Both methods give essentially the same results. There have been few reports in which this technic has been applied to the urine of experimental animals. Davis *et al.* (4) and Kimeldorf(5) used it for rabbit urine, Henriques *et al.*(6) for Cebus monkey and Dorfman *et al.*(7) for chimpanzee urine. Dog urine was analyzed for 17-ketosteroids by Paschkis and coworkers(8) and by Lawrence (9). In both cases crude urine extracts were used and no correction for overestimation due to non-ketosteroid chromogens was given.

We have analyzed the urine of normal dogs for neutral 17-ketosteroids, using both the isolation technic with Girard's reagent(10) and the color correction equation(11) and obtained values consistently lower than Paschkis(8) and Lawrence(9). Moreover, spectrochemical evidence indicates that even these values may be too high.

Method. Normal mongrel dogs were kept in metabolism cages and their urine was collected in 3 ml concentrated HCl as a preservative. The urine was stored in the refrigerator and analyzed within 48 hrs. after collection. Each urine sample was made up to 1 liter, acidified to 15 vol. % with concentrated HCl and hydrolyzed by boiling for 10 min. Ten g NaCl was added while hot, the urine was immediately cooled to 60°C and transferred to a modified Hershberg-Wolfe extractor(12). One 1 CCl_4 was used, 500 ml in the extractor and 500 ml in the boiling flask. The urine was extracted for exactly 1.5 hours and the extract purified and analyzed according to Talbot's method

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TABLE I.
Urinary Excretion of Neutral 17-Ketosteroids by Normal Dogs, mg/24 hr.

Dog. No. & Sex	No. of Specimens	Crude Extract	Corrected Value	Ketonic Fraction	K_S
13 ♀	4	1.2	.4	.3	1.1
19 ♀	2	1.1	.6	.6	1.6
29 ♀	2	0.8	.5	.4	1.5
30 ♀	2	0.6	.6	.6	1.2
20 ♂	2	1.5	.5	.4	1.1
58 ♂	2	1.5	.6	.7	1.7
64 ♂	2	1.5	.6	.5	1.4

(10). After taking aliquots for the analysis of the crude urine extract, the remainder was evaporated to dryness and the residue fractionated with Girard's reagent-T(10). Following reaction with *m*-dinitrobenzene, the crude extract, ketonic and non-ketonic fractions were read on a Coleman Universal Spectrophotometer, Model 14, at 520 mμ and then at 420 mμ. All analyses were carried out in duplicate, 2 samples of standard solution of dehydroisoandrosterone* and 3 blanks being included in every series of analyses.

Results. The spontaneous 24-hr. urinary excretion of neutral 17-ketosteroids by normal dogs is given in Table I. All values are the mean of at least two 24-hr. specimens. The values from crude urine extracts were corrected by use of the Gibson-Evans equation (11). Corrected $E_G = \frac{K_I E_G - E_V}{K_I - K_S}$, where E_G and E_V are the extinctions at 520 mμ and 420 mμ, respectively, $K_I = 1.7$ from the mean ratio of the extinction at 420 mμ to the extinction at 520 mμ for the non-ketonic fraction in 47 measurements on 10 normal dogs, and $K_S = 0.4$ from the same ratio of extinctions for crystalline dehydroisoandrosterone. The K_S values in the table are extinction ratios for the ketonic fractions and show a large deviation from the K_S value of pure 17-ketosteroid. Nevertheless, there is good agreement between the 17-ketosteroid concentration determined in the ketonic fraction and the value calculated by applying the correction equation to the crude urine extract.

The high K_S values for the ketonic fractions of dog urine led to an investigation of the absorption spectra of the colored products of the Zimmermann reaction. Fig. 1 shows a comparison of the spectra given by the products of the *m*-dinitrobenzene reaction of different dog urine extracts with that of dehydroisoandrosterone and human urine extracts. Only the crude extract from dog urine to which 5 mg dehydroisoandrosterone per 1 had been added shows the absorption maximum at 520 mμ, characteristic for 17-ketosteroids. In contrast to human urine extracts, which exhibit an absorption peak at 520 mμ, neither the crude extract nor the ketonic fraction of dog urine give the characteristic color reaction.

The analytical method was tested by parallel determinations with the addition of pure ketosteroid to normal urine. Table II gives

TABLE II.
Recovery of 5 mg Dehydroisoandrosterone Added to Human and Dog Urine, in mg.

Urine	DHA Added	Crude Extr.	Corrected Value	% Recovery
Pooled human ♂	—	16.0	11.8	
,, ,,	+	21.0	16.8	100
24-hr dog ♀	—	1.3	0.5	
,, ,,	+	6.5	5.8	106
,, ,,	—	1.8	1.0	
,, ,,	+	7.8	6.4	108

TABLE III.
Urinary Excretion of Neutral 17-Ketosteroids by Normal Dogs after ACTH, in mg/24 hr.

Dog. No. & Sex	Treatment	Crude Extract	Corrected Value	Ketonic Fraction	K_S
19 ♀	Pretreatment	1.4	.6	.7	0.9
,,	25 mg ACTH	0.8	.3	.4	0.8
,,	Posttreatment	1.2	.3	.5	0.9
30 ♀	Pretreatment	1.1	.5	.6	1.3
,,	25 mg ACTH	2.3	.6	.7	1.6
,,	,, ,,	1.0	.5	1.0	1.5
,,	,, ,,	1.0	.5	.6	1.2
,,	Posttreatment	0.8	.3	.7	1.2

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* Generously supplied by the Schering Corporation through the courtesy of Robert E. Waterman.

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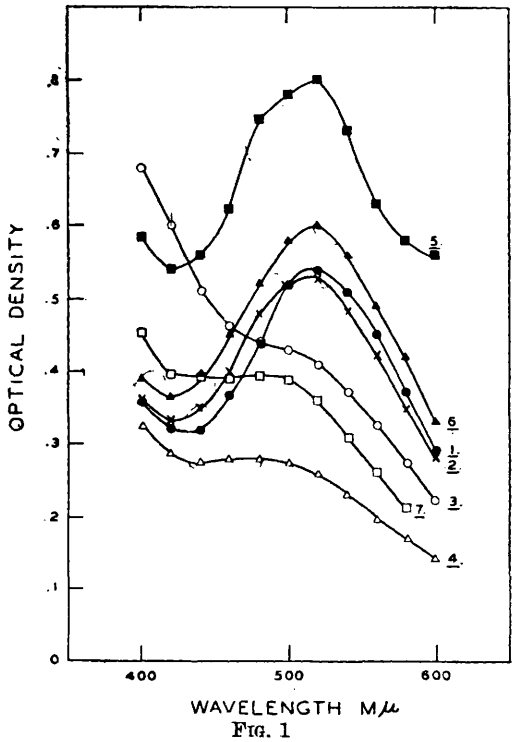

FIG. 1

Absorption spectra of urine extracts after Zimmermann reaction.

1. Dehydroisoandrosterone (DHA); 2. dog urine + DHA, crude extract; 3. crude extract of dog urine; 4. ketonic fraction of dog urine; 5. crude extract of human urine; 6. ketonic fraction of human urine; 7. crude extract of urine from ACTH-treated dog.

the results of recovery experiments on human and dog urines. To a 1 liter aliquot of pooled normal human male urine and to aliquots of two 24-hr. urine specimens from normal female dogs 5 mg dehydroisoandrosterone were added. The values from the crude urine extracts were corrected by use of the Gibson-Evans equation, uring $K_I = 1.0$ for the human urine. This value was obtained as the mean of 20 analyses of urine samples from 10 different human subjects. Recoveries of added ketosteroid were uniformly good.

The ketosteroid excretion of the normal dog after adrenal stimulation with adrenocorticotrophic hormone is recorded in Table III. Two female dogs were given 25 mg ACTH per day intramuscularly in 3 equal doses at 3-hr. intervals. One dog was treated for one day, the other for 3 days. The urine was collected on the day prior to, during, and following injection and analyzed as before. No significant change in the urinary excretion of ketosteroids occurred, nor was there any change in the absorption spectrum of the urine extract of a treated dog after reaction with *m*-dinitrobenzene (Fig. 1).

Discussion. The evidence presented indicates that the normal dog excretes only negligible quantities of neutral 17-ketosteroids. The higher values reported by Paschkis and coworkers (0.5-1.5 mg/day) and by Lawrence (0.2-5.4 mg/day) are undoubtedly due to interference of urinary pigments and chromogens other than 17-ketosteroids with the colorimetric assay. Urine extracts purified with Girard's reagent do not give the characteristic color reaction and even the low values given in the present paper are probably too high. In confirmation of Paschkis *et al.*(8) no sex difference was found in the ketosteroid excretion of dogs. Also in contrast to humans, dogs did not respond to adrenal stimulation by ACTH with an increase in urinary ketosteroids. This was also observed by Lawrence (9), but no data were given. Furthermore, dogs do not respond to the administration of testosterone by increased urinary excretion of androgens, as shown by Kochakian(13), by Dingemanse and Tyslowitz(14) and by Paschkis *et al.*(8). The explanation for the negligible excretion of ketosteroids may be that dogs efficiently convert androgens to estrogens(8).

Summary. 1. Normal dogs excrete less than 1 mg neutral 17-ketosteroids per day. 2. No sex difference exists in the urinary ketosteroid excretion of dogs. 3. Administration of ACTH to dogs does not result in increased excretion of ketosteroids.

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