Failure of Vitamin B_{12} to Modify Goitrogenic Action of Thiouracil. (18706)

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Meites(1) has recently made the interesting observation that vit. B_{12} administered in doses of 40-80 μg per kilo of food markedly inhibited the development of goiter in thiouracil-fed rats. In view of the importance of this finding similar experiments were undertaken in this laboratory to confirm these results.

Experimental. In the first experiment, twenty-four 50 g male albino rats were divided into 4 equal groups. The animals were fed a basal diet of Purina mink chow to which 0.03% propylthiouracil and an aqueous solution of vit. B_{12} were variously added as indicated in Table I. The experiment was continued for 24 days when the animals were killed with chloroform and the thyroids dissected and weighed. The results are summarized in Table I. It can be seen that no appreciable inhibition of goitrogenesis was produced by the administration of vit. B_{12}.

In view of these results, a second experiment was carried out, duplicating Meites' conditions as exactly as possible. Forty 50-60 g male albino rats were divided into 4 equal groups and fed a basal diet consisting of: yellow corn meal, 35%; ground wheat, 25%; linseed oil meal, 10%; whole milk powder, 20%; alfalfa leaf meal, 6%; brewers yeast, 3%; and table salt, 1%. Crystalline vit. B_{12} as a triturate of sodium chloride† and

TABLE I.

Group	No. of animals	Diet	Avg Body Wt—Orig., g	Avg Body Wt—Final, g	Avg Thyroid Wt—Actual, mg	Avg Thyroid Wt—mg/100 g Body Wt
1	6	Basal	52.7	120.3	10.1	8.7 ± 0.58*
2	6	Basal + 80 γ B_{12}/kg food	52.0	128.4	9.8	7.6 ± 0.67
3	6	Basal + .03% propylthiouracil	49.8	79.6	34.3	43.1 ± 4.65
4	6	Basal + .03% propylthiouracil + 80 γ B_{12}/kg food	55.0	87.5	37.6	41.8 ± 3.28

* Stand. error of mean $= \sqrt{\frac{\Sigma d^2}{n\,(n-1)}}$.

TABLE II.

Group	No. of animals	Diet	Avg Body Wt—Orig., g	Avg Body Wt—Final, g	Avg Thyroid Wt—Actual, mg	Avg Thyroid Wt—mg/100 g Body Wt
1	10	Basal	53.6	134.3	11.3	7.9 ± 0.35
2	10	Basal + .03% propylthiouracil	59.5	96.0	38.9	40.5 ± 2.75
3	10	Basal + .03% propylthiouracil + 40 γ B_{12}/kg food	56.3	90.6	44.1	49.4 ± 3.61
4	10	Basal + .03% propylthiouracil + 80 γ B_{12}/kg food	57.4	84.3	38.9	47.3 ± 3.93

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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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