Effects of Vitamin B_{12} and Penicillin on Thiouracil Action in Chicks.* (19381)

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Retardation of growth in young rats fed 0.1% thiouracil(1) or following parathyroidectomy(2) on a vit. B_{12}-deficient diet was at least partially counteracted by vit. B_{12} supplementation. Survival rate in the latter rats was also significantly increased by vit. B_{12}. Although thyroid hyperplasia in some of the thiouracil-treated rats appeared to be prevented by vit. B_{12}, subsequent work in this laboratory and by Greer(3) showed that the vitamin does not modify this action of goitrogens.

Vit. B_{12} and penicillin have both been shown to stimulate growth in chicks. The vitamin is believed to enhance protein synthesis(4,5) and increase nitrogen retention (6), while penicillin has recently been demonstrated to spare vit. B_{12}, folic acid, choline, riboflavin, and pantothenic acid in the chick (7). Since the hypothyroidism accompanying thiouracil administration results in decreased body growth and appetite, it was of interest to see whether vit. B_{12} and penicillin would counteract these effects in chicks. It was also pertinent to determine whether the vitamin and antibiotic would modify the action of thiouracil on thyroid and comb weight.

Procedure. In the first experiment, day old, single comb, male White Leghorn chicks were divided into 8 uniform groups of 12 each, and were fed the vit. B_{12}-deficient ration of Miller and Groschke(8) for one week. For the next 4 weeks, the ration was supplemented with 0.1% thiouracil and/or 0.5 to 4.0 μg % vit. B_{12},‡ according to the grouping shown in Table I. Body weight and food consumption were measured weekly. At the end of 4 weeks, all birds were sacrificed and the thyroids and combs were removed and weighed. In the second experiment, day-old Rhode Island Red chicks were approximately evenly divided between males and females into 6 groups of 12 each. They were initially placed on the vit. B_{12}-deficient diet for 2 weeks, and for the following 3 weeks the diet was supplemented with 0.1% thiouracil, 4.0 μg % vit. B_{12} or 2 mg of procaine penicillin G per pound of food. The optimal level of vit. B_{12} for growth in Rhode Island Red chicks had previously been shown to be about 2.0 μg %(8), and hence the chicks in this experiment were receiving about twice the optimal level. At the end of 3 weeks, the birds were killed and the thyroids were removed and weighed. The chicks in both experiments were maintained on wire floors in battery brooders, with feed and water available at all times.

Results. The data from the first experiment are summarized in Table I. The addition of 0.5 to 4.0 μg % vit. B_{12} (Groups 2, 3, 4) to the basal ration increased body weight and food intake above the controls (Group 1). No explanation can be offered as to why 2.0 μg % vit. B_{12} proved less effective in these respects than 0.5 μg % vit. B_{12}. The vitamin did not alter thyroid or comb weight. Thiouracil (Group 5) markedly reduced average body weight gains and appetite, and more food was required per g of gain in body weight. Thyroid weight was increased about 12-fold while the combs weighed only about 1/8th as much as in the controls. The latter indicates that thiouracil drastically reduced androgen levels in the body. Vit. B_{12} (Groups 6, 7, 8) partially counteracted the depression of growth and appetite by thiouracil, but did not alter its action on thyroid and comb weight. It can be seen that the larger doses of vit. B_{12} were more effective in preventing growth

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TABLE I. Effects of Vit. B_{12} on Thiouracil Action in Male Leghorn Chicks, 8 Groups.

Treatment	Avg body wt, g		Avg food intake		Avg thyroid wt per 100 g body wt, mg	Avg comb wt per 100 g body wt, mg
	Initial	Gain	Daily	Per g gain body wt		
Controls	59.7	270.4	22.3	2.3	8.3 ± .4*	575 ± 19.8*
.5 μg % B_{12}	59.1	324.6	25.7	2.4	8.5 ± .3	655 ± 21.3
2 "	61.5	290.5	24	2.4	8.7 ± .4	569 ± 16.8
4 "	58.8	344.5	26.9	2.2	7.6 ± .4	537 ± 22.5
T†	59.6	151.3	13	3.2	99.9 ± 21	67 ± 6.4
T + .5 μg % B_{12}	58.5	161.3	16.6	3.4	89.8 ± 14.5	66.4 ± 7.9
T + 2 "	59	177.1	17.4	3.3	86.5 ± 10.4	64.2 ± 10.2
T + 4 "	59	192	20.2	2.9	115.8 ± 19.6	48.2 ± 7.5

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

† T = Thiouracil.

TABLE II. Effects of Vit. B_{12} and Penicillin on Thiouracil Action in Rhode Island Red Chicks, 6 Groups.

Treatment	Avg body wt, g		Food/gain ratio	Avg thyroid wt/100 g body wt, mg
	Initial	Gain		
Controls	88	125.5	3.27	15.7
4 μg % B_{12}	86	140.1	3.08	17.5
T*	88.7	97.1	4.07	153.4
T + 4 μg % B_{12}	88.5	123.7	3.05	143.4
T + penicillin	76	137.3	2.81	138.4
T + 4 μg % B_{12} + penicillin	75.2	154.2	2.59	159

* Thiouracil.

inhibition than the smaller doses.

In the second experiment (Table II), 4.0 μg % vit. B_{12} (Group 2) enhanced body growth and appetite of the Rhode Island Red chicks, but apparently this vitamin level was not as effective as in the Leghorn chicks. This suggests that the Rhode Island Red chicks had greater initial reserves of vit. B_{12} in their bodies than the Leghorn chicks. Thiouracil (Group 3) reduced average body weight gains and appetite, and increased the food/gain ratio. Vit. B_{12} (Group 4) and penicillin (Group 5) completely overcame the thiouracil-induced inhibition of growth, while the combination of the vitamin and antibiotic was considerably more effective than either alone. Thyroid hyperplasia in the thiouracil-treated chicks was not prevented by the vitamin or antibiotic.

Discussion. In general, these data on chicks are in agreement with those previously reported in rats(1,2). Thiouracil reduced body growth and appetite in both species, and these were at least partially counteracted by vit. B_{12}, or by penicillin in the chicks. It is believed to be significant that the greater doses of vit. B_{12} were more effective than the lower doses in overcoming growth inhibition by thiouracil in the Leghorn chicks. This suggests that still higher levels of vit. B_{12} than used might have resulted in more complete counteraction of growth inhibition. The favorable action of penicillin on growth in the Rhode Island Red chicks cannot be attributed entirely to a sparing of vit. B_{12} since it proved to be more effective than twice the optimal dose of the vitamin. It appears probable that the antibiotic also made larger quantities of other vitamins available to the chicks.

Neither the normal thyroids of the control chicks nor the greatly hyperplastic thyroids of the thiouracil-treated chicks were influenced by vit. B_{12} or penicillin. Similarly, vit. B_{12} or antibiotics failed to alter thyroid activity in euthyroid(9), hypothyroid(1) or hyperthyroid rats(9-11), despite stimulating body growth in these animals. This suggests that the favorable actions of vit. B_{12} and anti-

biotics on growth are exerted independently of any effect on thyroid function. However, it does not preclude the possibility that marked changes in thyroid activity can alter normal nutritional requirements of the body. Thus hypothyroidism generally decreases body growth and is believed to reduce dietary requirements, while severe hyperthyroidism also reduces body growth but is believed to increase nutritional requirements(12). If one considers normal body growth as a criterion of adequate dietary intake, then the favorable effects of vit. B_{12} or antibiotics can be considered as indicative of increased nutritional needs under either thyroid condition.

Summary. 1. White Leghorn male and Rhode Island Red chicks of both sexes were fed a vit. B_{12}-deficient ration which was supplemented with 0.1% thiouracil and/or 0.5 to 4.0 μg % vit. B_{12} or 2 mg procaine penicillin G per lb of food. After a preliminary depletion period of one and 2 weeks, respectively, on the vit. B_{12}-deficient diet, the chicks were fed the above supplements for an additional 4 or 3 week period. 2. Thiouracil markedly reduced body weight gains and food intake in both breeds of chicks, and increased the grams of food required for each gram of gain in body weight. Vit. B_{12} and penicillin partially or completely counteracted this growth depression and increased appetite. The larger doses of vit. B_{12} were more effective in these respects than the smaller doses. 3. The large increase in thyroid weight and severe inhibition of comb growth induced by thiouracil were not influenced by the vitamin or antibiotic. 4. In general, these results corroborate previous data on the effects of vit. B_{12} and antibiotics on rats fed thiouracil or following parathyroidectomy.

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Volume of Distribution of Radiosulfate as a Measure of the Extracellular Fluid. (19382)

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Virtual equilibrium following the injection of certain tracer ions, such as Na^+ and Cl^-, requires several hours or days, presumably because exchange rates with some of the intracellular unlabelled ion are very slow(1,2). On the other hand, substances such as mannitol, which are largely excluded from body cells, may attain equilibrium throughout the extracellular fluid within 50 minutes, even though slow cell penetration occurs thereafter(3). Sulfate, as a consequence of its rapid capillary exchange rate(4), might be expected to attain equilibrium throughout the extracellular fluid even more rapidly. Studies with unlabelled sulfate indicated that this is the case(5).

Studies were undertaken of the distribution and excretion of radiosulfate in normal human subjects. S-35 has a convenient half-life of 87 days and can be administered to man without hazard.* A dose of 100 μc† was injected in-