Effect of Thyroxine on Folic Acid Metabolism. (31594)

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The feeding of thyroid powder or thyroxine-like active materials has been observed to induce vitamin B_{12} deficiency in rats(1,2) and chicks(3). In the study of the mechanism of thyroxine-like active materials in accentuating vit. B_{12} deficiency, we have found that the feeding of iodinated casein produced marked increases in the excretion of formiminoglutamic acid (FIGLU). Since the excretion of FIGLU(4,5,6) and formate(7) are increased in deficiencies of both folic acid and vit. B_{12}, it seemed of interest to investigate in more detail the effect of thyroxine-like active materials on the excretion of these two metabolites.

Methods. The basal diet contained 20% isolated soy protein, 64% glucose monohydrate,‡ 2% calcium carbonate, 2% salt mixture(6), 1% vitamin premix(6), and 1% corn oil containing vitamins A, D and E. The vitamin mixture furnished the following amounts per kg of diet: riboflavin 10 mg, Ca pantothenate 50 mg, niacin 50 mg, thiamine 10 mg, pyridoxine 10 mg, biotin 0.2 mg, and choline 1 g. The vitamins A, D and E supplement in corn oil furnished 15,000 units of vit. A, 2,000 units of vit. D, and 70 units of vit. E per kg of diet. No folic acid was added to the diet but the isolated soy protein furnished 1.8 mg of folic acid per kg of diet as determined by microbiological assay. The methionine content of the diet was 0.12%.

Male weanling rats weighing *ca* 60 g were maintained on the basal diet for 3 days before being placed on the experimental diets. Four rats in 2 groups of 2 animals each were used for each treatment. These were maintained in groups of 2 in metabolism cages when urine collections were being made. The urine was collected in bottles containing sufficient hydrochloric acid to insure that the sample of urine collected had a pH of 3 or lower in order to prevent decomposition of FIGLU. FIGLU was determined by the enzymatic method of Tabor and Wyndgarden(8). Formate was determined by a modification(7) of the method of Grant(9) which is based on the reduction of formate by magnesium in acid solution to give formaldehyde which, in turn, is estimated colorimetrically by heating with chromotropic acid. Iodinated casein (Protomone®) was used as a source of thyroxine-like activity. It had a thyroid activity which corresponds to 0.3% thyroxine(1) which is similar to that of U.S.P. thyroid powder.

Iodinated casein was fed at levels of 0.1, 0.3 and 1.0% in the diet with and without a supplement of vit. B_{12}. The vit. B_{12} was injected I.P. at a level of 5 μg twice a week, which corresponds to an intake of approximately 150 μg per kg of diet consumed.

Results. The effects of iodinated casein and of vit. B_{12} on growth and FIGLU excretion are given in Table I. The excretion curves for formate and FIGLU on the basal diet and on the 0.1% iodinated casein diet over a 44-day experimental period are shown in Fig. 1.

The addition of 0.1% of iodinated casein produced a growth depression which was partially prevented by the vit. B_{12} (Table I). The increased growth after 42 days due to vit. B_{12} was 21 g in the absence of iodinated casein and 53 g in the presence of 0.1% iodinated casein.

Iodinated casein produced a rapid increase in FIGLU excretion and its effect was most marked during the early part of the experimental period. After 10 days on the experimental diet (7 days after addition of iodinated casein), excretion of FIGLU had increased 300 to 600% in rats receiving vit. B_{12} and iodinated casein, and 50 to 100% in rats receiving iodinated casein but no vit. B_{12}. The effect of iodinated casein diminished with time and by 19 days FIGLU excretion by

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‡ Cerelose—Trade name for glucose monohydrate. Obtained from Corn Products Co.

TABLE I. Effect of Iodinated Casein and Vitamin B_{12} on Body Weight and Excretion of FIGLU and Formate. 4 rats per group.

Supplements		Avg body wt (g)			FIGLU (μmoles excr./day/kg body wt)		Formate (μmoles excr./day/kg body wt)
Iodinated casein	B_{12}*	Initial	21 days	42 days	10 days	19 days	19 days
None	—	62	133	208	210	550	950
"	+	59	144	229	50	95	220
.1%	—	58	113	143	345	545	1,190
"	+	61	139	196	355	395	440
.3%	—	58	128	176	400	740	
"	+	55	134	191	305	400	
1.0%	—	61	123	141‡	300	575	
"	+	64	133‡	190†	195	370‡	

* 5 μg given I.P. twice a week. † Two survivors. ‡ Three survivors.

vitamin B_{12} deficient animals was essentially the same with and without iodinated casein (Table I, Fig. 1). In the presence of vit. B_{12} iodinated casein continued to give a marked increase (4-fold) in FIGLU excretion after 16 days.

To determine whether a folic acid deficiency had been induced by iodinated casein, 3 mg of folic acid were given I.P. on the 17th and 20th days (Fig. 1). This produced a 60% drop in FIGLU excretion in rats receiving vit. B_{12} and iodinated casein, and a 27% drop in rats receiving iodinated casein but no vit. B_{12}. Thus, it appears that a massive dose of folate can partially correct the FIGLU excretion produced by iodinated casein. It should be noted that the diet contained no sulfonamide to inhibit bacterial synthesis by intestinal flora, and that the basal diet contained 1.8 mg of folate per kg (by microbiological assay) contributed by the isolated soy protein. This

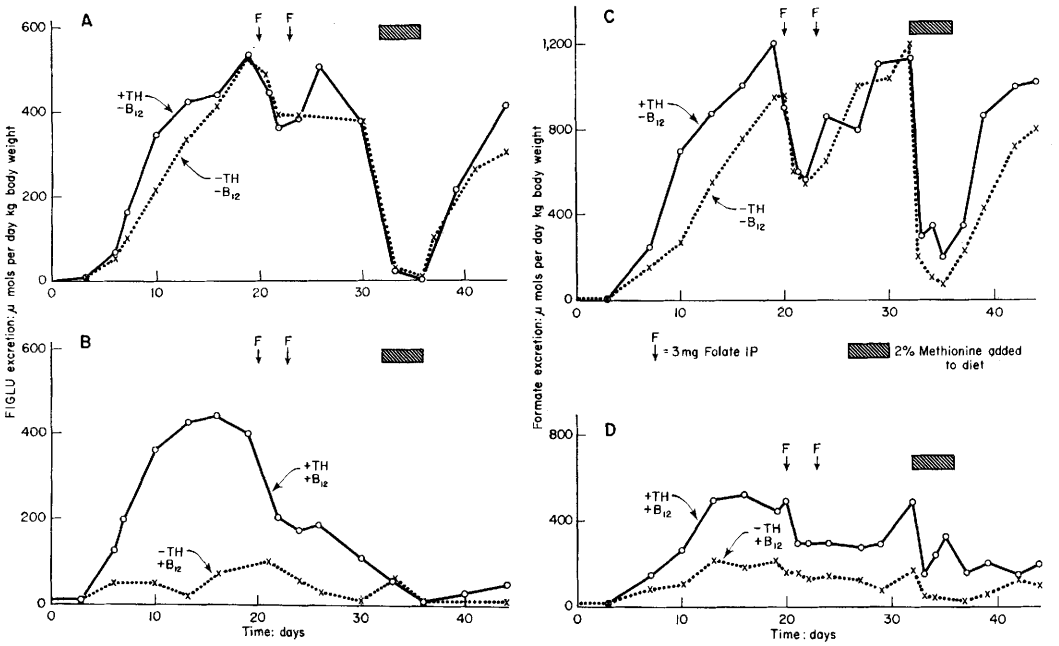

FIG. 1. Effect of vitamin B_{12}, methionine and iodinated casein as a source of thyroxine on excretion of FIGLU and formate.

would furnish *ca* 18 μg per day (based on a feed consumption of 10 g per day) which may be compared with the dose of 10 to 20 μg of folic acid for the cure of leucopenia in folic acid deficient rats (Daft and Sebrell, 10).

The administration of supplemental methionine has been reported to sharply decrease FIGLU excretion resulting from either folate or vit. B_{12} deficiency(4,6,11,12). Methionine at a level of 2% was added to the diet for 4 days beginning at 32 days. As can be seen from Fig. 1, the FIGLU excretion decreased to almost zero within 24 hours and rose immediately after the supplementary methionine was removed.

The effects of iodinated casein on formate excretion were similar to those on FIGLU excretion; however, the excretion of formate was *ca* 1.5 to 2 times that of FIGLU.

Discussion. Although the metabolism of FIGLU and formate is known to involve folic acid but not vitamin B_{12} dependent enzyme reactions, a deficiency of vit. B_{12} increases the excretion of FIGLU(4,5) and formate(7) similar to that produced by a deficiency of folic acid. The effect of vit. B_{12} deficiency in increasing the excretion of FIGLU has been postulated to be an indirect one resulting from an induced secondary deficiency of folic acid. One possible mechanism is that 5-methyltetrahydrofolate accumulates during vit. B_{12} deficiency, since its conversion to tetrahydrofolate by transmethylation with homocysteine to give methionine by the B_{12} dependent reaction is impaired. The metabolism of FIGLU and formate by folate dependent reactions requires tetrahydrofolate, and 5-methyltetrahydrofolate must be demethylated by methyltransferase before it can function as a coenzyme for formiminotransferase.

The feeding of iodinated casein can increase FIGLU excretion in the presence of large quantities of vit. B_{12} and folic acid. The injection of 5 μg vit. B_{12} two times per week corresponds to *ca* 150 μg per kg of diet, which is *ca* 5 times the minimum requirement of the rat for growth(13).

The metabolic pattern produced by feeding iodinated casein resmbles that of folate deficiency in that both FIGLU and formate excretions are increased, and the excretions of both are reduced by methionine supplementation. The main primary effect of thyroxine appears to be on vit. B_{12} as iodinated casein produces no further increase in FIGLU excretion on a vitamin B_{12} deficient diet.

Summary. The feeding of iodinated casein as a source of thyroxine-like activity increased excretion of formiminoglutamic acid and formate on diets containing folic acid and vitamin B_{12}. Large doses of folate given I.P. produced a partial reduction in FIGLU and formate excretion. Supplementation with 0.2% methionine produced a large and rapid decrease in both FIGLU and formate excretion even when iodinated casein was present in the diet.

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