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Effect of Dietary Thyroid Powder on Urinary Excretion of Formiminoglutamic Acid and Methylmalonic Acid (40602)[1,2]

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The administration of large doses of thyroactive compounds such as thyroid powder (TP) or iodinated casein increases the requirement for vitamin B_{12} as measured by growth response (1–4) and increases the excretion of formiminoglutamic acid (FIGlu), formate (5, 6), and aminoimidazolecarboxamide. The increased FIGlu excretion resembles that produced by vitamin B_{12} deficiency in that it is markedly decreased by excess dietary methionine (6–8). Evidence of folic acid deficiency has been observed in thyrotoxicosis in man (9), and increased FIGlu excretion has been reported in cases of hyperthyroidism (10).

Vitamin B_{12} deficiency in rats produces both an increased excretion of FIGlu (11) and methylmalonic acid (MMA) (12, 13). The ability of high levels of protein intake to accentuate vitamin B_{12} deficiency in rats has been related to the increased intake of amino acids such as valine and isoleucine (14), and the excretion of methylmalonic acid in humans is markedly increased by valine and isoleucine loading (15).

Since thyroid hormones are known to stimulate the basal metabolic rate and food consumption, it is of interest to determine whether the increased urinary excretion of FIGlu in rats fed thyroid powder is due simply to an increased intake of histidine resulting from the increased food intake, or whether thyroxine modifies the metabolism of folic acid and vitamin B_{12}.

Materials and methods. A basal diet low in methionine (0.2%) containing 20% isolated soy protein supplemented with vitamin B_{12} (100 μg/kg) and folic acid (5 mg/kg) was used (Table I). Three-week-old male weanling rats of the Sprague–Dawley strain were housed individually in metabolism cages in a temperature- and light-controlled room. Both food and water intake were recorded twice a week. The animals were divided into four groups of 14 animals each which received the following treatments:

Group A: Basal diet.

Group B: Basal diet plus 3 g thyroid powder[5]/kg diet, which supplied about 9 mg thyroxine/kg diet.

Group C: High-protein diet. The protein content of this diet was periodically increased so that the protein intake/kg body wt for group C equaled the protein intake of group B. This was done by calculating the food consumed/100 g body wt during a given period for group B (which was elevated by the thyroid powder) and adjusting the protein content for group C so that the protein consumption/unit of body wt/day was equal in the two groups.

Group D: High-protein diet (diet C) plus thyroid powder (3 g/kg diet).

Group C, which received no thyroid powder, received the same protein intake/day as group B, which received thyroid powder. Group D, which received the high-protein diet plus thyroid powder, had an increased food intake and therefore received more protein/kg body wt than the other groups.

Periodically, 24-hr urine samples were collected for each animal, using two drops of 12 *N* HCl and five drops of toluene in the collection flask to avoid degradation of FIGlu.

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[2] Abbreviations used: FIGlu, formiminoglutamic acid; MMA, methylmalonic acid; TP, thyroid powder.

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[5] Mann Research Labs., Inc., N.Y. Thyroid powder nominally contains 0.2% iodine equivalent to approximately 0.3% thyroxine.

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Urinary FIGlu was assayed by the method of Tabor and Wyngarden (16) and urinary methylmalonic acid was determined by the method of Giorgio and Plaut (17) as modified by Thenen *et al.* (13). Urine samples were either analyzed immediately after collection or frozen and stored at −10°C until analyses were made.

Results and discussion. The effects of protein and thyroid powder on growth rate are shown in Figure 1. The levels of soy protein fed groups C and D during each period are indicated in the figure. The increased amounts of protein in the diets of groups C and D seem to have caused a slight growth depression of about 7% both in the absence and presence of thyroid powder (A vs C; B vs D). Feeding of thyroid powder at similar levels of protein produced a significant growth depression (as measured by final weight) of 32% on the normal protein diet and 33% on the high-protein diet.

TABLE I. COMPOSITION OF BASAL DIET[a]

Ingredient	Amount (g/kg diet)
Glucose monohydrate[b]	714
Soy assay protein[c]	200
Mineral salt mixture[d]	35
Water-soluble vitamin mixture[e]	10
Oil-soluble vitamin mix in corn oil[f]	10
Choline chloride[g]	1
Corn oil[h]	30

[a] Methionine content of diet: 0.2%. Histidine content: 3.37%.
[b] Cerelose Corn Products Co., Argo, Ill.
[c] General Biochemicals, Inc., Chagrin Falls, Ohio.
[d] Williams *et al.* (18).
[e] In glucose, provided in mg/kg diet: thiamin HCl, 15; riboflavin, 15; pyridoxine HCl, 15; Ca-pantothenate, 50; niacin HCl, 50; biotin, 0.2; menadione, 10; folic acid, 5; vitamin B_{12}, 0.1.
[f] In corn oil, provided per kg diet: vitamin A acetate, 15,000 IU; vitamin D, 2000 IU; and DL-α-tocopherol acetate, 50 IU.
[g] Nutritional Biochemicals Corp., Cleveland, Ohio.
[h] Mazola Corn Products Co., Englewood Cliffs, N. J.

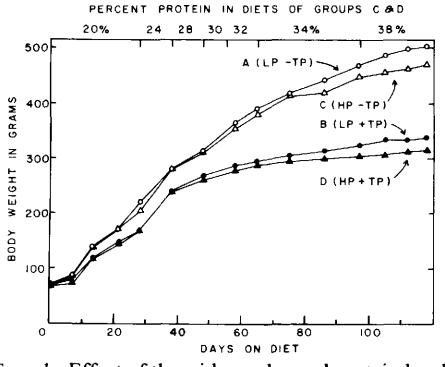

FIG. 1. Effect of thyroid powder and protein level on growth rate. Abbreviations: LP, low protein (20%); HP, high protein (increasing from 24 to 38%); TP, thyroid powder.

The food consumption data and the schedule of changing protein levels in groups C and D are given in Table II. The addition of thyroid powder increased food consumption by 22% at the beginning and 85% at the end of the experiment.

The urinary excretion of FIGlu as affected by thyroid powder and protein level are shown in Figure 2. Protein level had little effect on FIGlu excretion either in the absence (A vs C) or presence of thyroid powder (B vs D). Addition of thyroid powder increased FIGlu excretion by about fivefold on both the 20% protein and on the high-protein diets after the third week. The fact that increased protein, and thereby histidine, intake did not increase FIGlu excretion shows that the increase in FIGlu excretion produced by feeding thyroid powder is not due to its effect in stimulating food consumption. The effect of thyroid powder must, therefore, be due to its effect on the metabolism of folic acid, vitamin B_{12}, or histidine.

The effect of thyroid powder and increasing doses of soy protein on the excretion of MMA is shown in Figure 3. Either in the absence or presence of thyroid powder, the excretion of MMA appears to be increased by increasing the dietary level of protein. The excretion of MMA increased with increasing levels of protein intake expressed as g/kg body wt/day. Thus, on the 120th day, the protein intake of the basal group A was 11 g/kg body wt/day, and the excretion of MMA was 0.3 mmole/kg body wt/day. Groups B (low protein, +TP) and C (high protein, −TP) with intakes of 20 and 19 g protein were approximately the same with excretion levels of 1.4 and 1.0 mmole MMA. This is consistent with the effect of high-protein diets in producing a vitamin B_{12} deficiency characterized by impaired metabolism of MMA (11, 14, 15). This is the result of increased consumption of branched-chain amino acids such as valine and isoleucine which can be metabolized to methylmalonic acid (14, 15). The

TABLE II. EFFECT OF THYROID POWDER ON GROWTH AND FOOD CONSUMPTION AT DIFFERENT PROTEIN LEVELS[a]

Period (days)	Average body weight during period: Group A −TP 20% Pr (g)	Group B +TP 20% Pr (g)	Group C −TP high Pr (g)	Group D +TP high Pr (g)	Food consumption (per 100 g body wt/day): Group A −TP 20% Pr (g)	Group B +TP 20% Pr (g)	Group C −TP high Pr (g)	Group D +TP high Pr (g)	Protein level of diet: Groups A, B (%)	Groups C, D (%)
0–28	142	126	138	125	11.8	14.6	12.2	14.5	20	20
29–38	246	210	240	208	9.3	13.3	8.9	13.4	20	24
39–48	295	249	294	244	7.5	11.5	7.0	11.3	20	28
49–55	331	270	326	261	7.0	11.3	6.5	10.4	20	30
56–65	366	285	359	273	6.2	10.7	6.1	10.6	20	32
66–97	424	305	409	289	5.5	10.2	5.2	9.3	20	34
98–108	489	331	460	308	5.1	9.8	4.9	9.0	20	38

[a] The protein content of the high-protein diet was adjusted so that group C (−TP) would receive the same protein intake per 100 g body wt as group B (+TP, 20% protein). This was done by measuring food consumption of the various groups and adjusting the protein content. Accordingly, the levels of proteins in the basal diet of groups C and D were adjusted to 24, 28, 30, 32, 34, and 38% on the 28th, 38th, 48th, 55th, 65th, and 97th days, respectively.

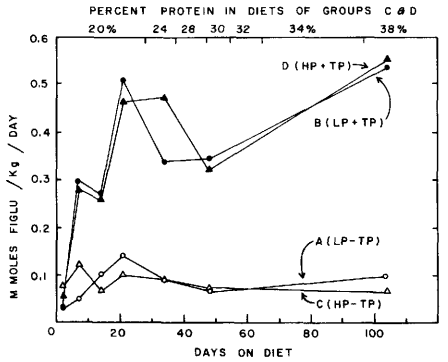

FIG. 2. Effect of thyroid powder and protein level on excretion of FIGlu. Abbreviations: LP, low protein (20%); HP, high protein (increasing from 24 to 38%); TP, thyroid powder.

effect of thyroid powder in increasing MMA excretion thus appears to reside mainly in its calorigenic effect resulting in increased food consumption with accompanying elevated protein intake. This is in contrast to the situation in FIGlu metabolism where there is no relationship between protein intake and FIGlu excretion. The question may be asked whether a 1.7-fold increase in protein intake in Group D (high protein, +TP) compared to Group C (high protein, −TP) can account for the 5-fold increase in MMA excretion. This much larger increase in MMA excretion than the increase in protein intake could be the result of overloading of the isomerase enzyme system. Similar overloading effects have been observed where a 2.3-fold increase in the level of soy protein (30 → 70%) caused a 10-fold increase in [^{3}H]propionate excretion following an i.p. dose of [^{3}H]propionate (12). Excretion of propionate provides a measure of the efficiency of the isomerase system in converting MMA to succinate. This overloading effect has also been observed in histidine metabolism where a 2-fold increase in histidine loading (200 → 400 μmole) caused a 150-fold increase in FIGlu excretion in the normal rat (20).

The effect of histdine loading and methionine supplementation on FIGlu excretion is shown in Table III. Histidine loading (10.4 mmole/kg body wt) caused a marked increase in FIGlu excretion in all groups and was most marked in group D (high protein, +TP) which had the highest intake of histidine (6.5 mmole/kg body wt) from dietary protein. The administration of methionine (2.0% in the diet) reduced FIGlu in all groups to very low levels (0.01–0.02 mmole/kg/day). This action of methionine in reducing the elevated FIGlu excretion induced by thyroid powder is similar to the action of methionine in reducing the elevated FIGlu excretion which occurs in vitamin B_{12} deficiency. These results also show that thyroxine does not produce a generalized vitamin B_{12} deficiency, as it does not markedly increase MMA excretion when protein intake is equalized. These data are consistent with the view that excess thyroxine interferes with either vitamin B_{12}-mediated synthesis of methionine or with its conversion to *S*-adenosylmethionine. The latter is the effective agent which exerts a feed-

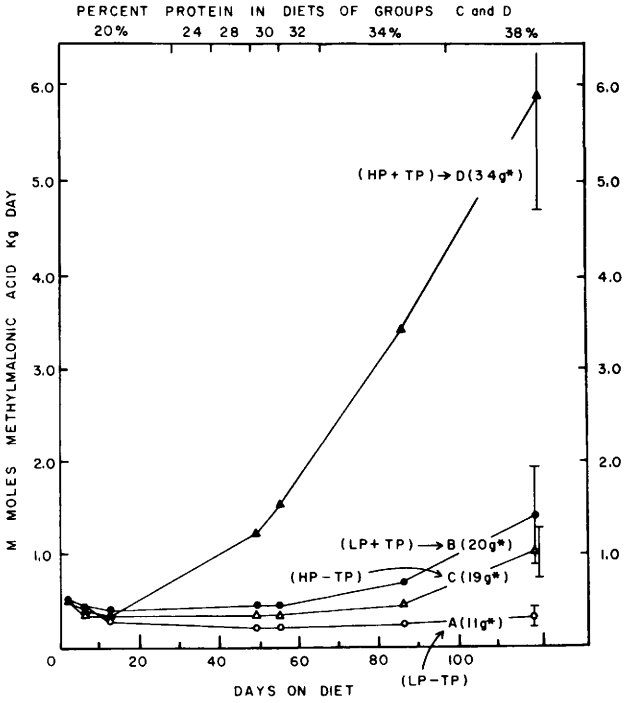

FIG. 3. Effect of thyroid powder and protein level on excretion of methylmalonic acid. Variability at 117 days expressed as standard error of the mean. Number of animals in each group at 117 days: A, 7; B, 5; C, 8; D, 11. (*) Grams of protein consumed per kg body weight per day in final period.

TABLE III. EFFECT OF HISTIDINE LOADING AND METHIONINE SUPPLEMENTATION ON EXCRETION OF FIGLu

Day of urine collection	Supplement	mmole FIGlu/kg body wt/day			
		Group A −TP 20% Protein	Group B +TP 20% Protein	Group C −TP high protein	Group D +TP high protein
103–104	—	0.10 ± 0.07[a]	0.53 ± 0.14	0.07 ± 0.04	0.55 ± 0.19
117–118	—	0.08 ± 0.03	0.43 ± 0.14	0.06 ± 0.02	0.44 ± 0.29
120–121	Histidine load[b]	2.66 ± 0.52	3.44 ± 1.20	2.13 ± 0.84	4.23 ± 0.65
121–122	—	0.25 ± 0.14	0.94 ± 0.40	0.15 ± 0.17	1.41 ± 0.60
124–125	—	0.06 ± 0.02	0.42 ± 0.12	0.08 ± 0.05	0.48 ± 0.11
125–126	Methionine 2%	0.05 ± 0.03	0.10 ± 0.06	0.05 ± 0.01	0.11 ± 0.10
127–128	Methionine 2%	0.01 ± 0.003	0.01 ± 0.004	0.01 ± 0.001	0.02 ± 0.007
129–130	—	0.05 ± 0.03	0.41 ± 0.07	0.06 ± 0.05	0.44 ± 0.17
Dietary histidine intake[c] per kg body wt on Day 100 (mmole)		1.8	3.3	3.5	6.5

[a] Standard deviation.
[b] 10.4 mmole L-histidine hydrochloride per kg body wt, by stomach tube.
[c] Calculated on basis of 2.4% histidine in soy protein.

back control on methionine synthesis by inhibiting the reduction of methylenetrahydrofolate to methyltetrahydrofolate (21, 22).

Summary. The possible role of increased food and protein intake in the action of thyroid powder in increasing the excretion of formiminoglutamic acid (FIGlu) and methylmalonic acid (MMA) was studied. The food intake of all animals was measured and the protein content of the diet of the control group (without thyroid powder) was increased so that the protein intake per day per kilogram of body wt was the same for the controls and for those receiving thyroid powder in the diet. The feeding of thyroid powder produced an increase in FIGlu excretion

which was not related to the increase in protein intake. The excretion of MMA which was produced by thyroid powder seemed related to the increase in protein intake caused by increased food consumption. Thyroid powder feeding had no effect on MMA excretion when protein intake was equalized. These data indicate that feeding thyroid powder affects the metabolism of vitamin B_{12} only as it is related to folic acid. It does not produce a generalized vitamin B_{12} deficiency which would have been expected to increase MMA excretion.

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