The Influence of Thyroxine and Thiouracil on Rats Fed Excess Tyrosine[1] (34572)

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Growing rats ingesting a low protein diet containing an excessive amount of tyrosine develop a toxicity syndrome which is characterized by eye and paw lesions and depressed food intake and growth (1–4). Signs of the toxicity are alleviated by daily injections of cortisol (5, 6) or by dietary supplements of certain amino acids (7). The beneficial effects of these treatments are associated with decreased plasma tyrosine concentration.

Tyrosine is a precursor of thyroxine and in protein-deficient rats a high tyrosine intake stimulates iodine uptake by the thyroid gland (8). Thiouracil, which inhibits thyroxine synthesis (9) and alters peripheral utilization (10) and increases deiodination (11) of thyroxine, prolongs the survival of rats fed a high tyrosine diet (12). Observations on the development of tyrosine toxicity in rats treated with thiouracil or thyroxine are reported in this paper.

Materials and Methods. Chemicals and diets. Male rats (Sprague-Dawley or Holtzman), weighing 50–55 g, were housed individually in suspended screen-bottom cages. They were fed the basal diet for 2 days, then were separated into groups and fed the experimental diets. The basal diet contained 6 % casein; L-methionine (L-Met), 0.2 %; fat, 5 %; adequate quantities of vitamins and minerals and carbohydrate to make 100 %. The diets were prepared as agar gels (7). L-tyrosine (3 % and 5 %) and thiouracil (0.05 % and 0.08 %) replaced an equal weight of carbohydrate. Body weights and group food intakes were recorded every other day and daily, respectively. The animal room temperature was 24° and lights were on from 8:00 AM to 8:00 PM. Rats for the various determinations were killed about 9:00 AM.

Treatment. L-thyroxine–sodium pentahydrate (thyroxine) was dissolved in 0.005 *N* sodium hydroxide to give solutions containing 25 or 5 μg/0.2 ml and was administered intraperitoneally at 4–5 PM daily. At the end of the experimental period rats were anesthetized with ether, and blood was removed by heart puncture using a heparinized syringe. Liver was excised and kept frozen at −20° until analyzed.

Enzyme assays. A 20 % liver homogenate was prepared in 0.14 *M* potassium chloride solution containing 0.005 *N* sodium hydroxide. The homogenate was centrifuged for 45 min at 25,000*g*. The clear supernate was used for enzyme assays. In the study of the effect of thiouracil, tyrosine transaminase (EC 2.6.1.5)[4] activity was measured in the crude homogenate at 38° using the method adopted by Rosen *et al.* (13), which is a modification of that of Canillakis and Cohen (14). In the study of the effect of thyroxine, tyrosine transaminase, and *p*-HPP-hydroxylase (EC 1.14.2.2)[4] activities in the supernate were assayed at 30° using the enol–borate method (15).

Plasma amino acids. Equal volumes of

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[4] Enzyme Commission on Nomenclature identification number.

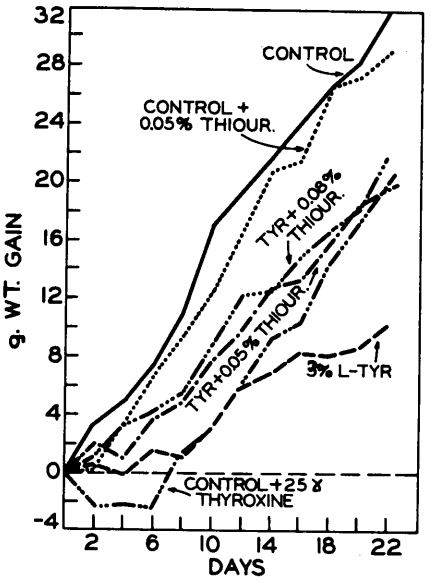

FIG. 1. Effect of thiouracil on growth of rats fed a low protein (6% casein) diet containing 3% of L-tyrosine.

plasma from each of the rats of a group were pooled. Plasma proteins were precipitated by the addition of 15 % sulfosalicylic acid to give a final concentration of 3% and the supernate was analyzed for amino acid content using a Technicon amino acid analyzer.

Results. Effect of thiouracil. Growth rate of rats fed a diet containing 3 % of L-tyrosine was depressed (Fig. 1), and half the animals developed pathological lesions typical of tyrosine toxicity. Additions of 0.05 and 0.08 % of thiouracil were equally effective in improving the growth rate but a few animals still developed mild lesions toward the end of the experiment.

The dietary tyrosine content was increased to 5 % in subsequent experiments to provide a more severe test of treatments that alleviated the toxicity. All rats fed the 5 % tyrosine diet developed characteristic external lesions much earlier than those fed only 3 %; they lost body weight, their food intake was severely depressed, and 2 of 6 rats died during the 2-week experimental period (Table I). Addition of 0.08 % thiouracil to the 5 % tyrosine diet prevented body weight loss and mortality without improving food intake; pathological lesions were mild and occurred later than in rats not receiving thiouracil. Thyroxine injections increased the severity of the lesions in animals fed the high tyrosine diet and 3 of 6 rats died within 2 weeks.

Plasma tyrosine concentration of rats fed the 5 % L-tyrosine diet for 2 weeks increased more than 60-fold (Fig. 2); thiouracil treatment had little effect but thyroxine treatment increased the concentration to double that for the untreated high tyrosine group. Liver tyrosine transaminase activity followed the same trend as plasma tyrosine concentration.

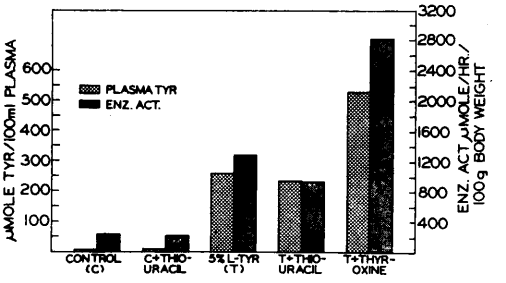

FIG. 2. Effects of thiouracil (0.08%) and thyroxine (5 μg/day) on plasma tyrosine concentration and liver tyrosine transaminase activity of rats fed low protein (6% casein) diet containing 5% of L-tyrosine.

TABLE I. Effect of Thiouracil and Thyroxine on Growth and Food Intake of Rats Fed a Diet Containing 5% of L-Tyrosine for 2 Weeks.

Diet	Av total food intake (g)	Av total wt gain[a] (g)	Mortality
6% Casein (C)	157	39.6 ± 2.4	0/6
C + 0.08% thiouracil	110	28.5 ± 2.3	0/6
C + 5% L-tyr (T)	55	−7.0 ± 1.7	2/6
T + 0.08% thiouracil	54	2.3 ± 0.6	0/6
T + thyroxine (5 μg/day)	54	−17.3 ± 1.5	3/6

[a] Mean ± standard error.

TABLE II. Effects of Thiouracil and Thyroxine on Some Plasma Amino Concentrations of Rats Fed a High Tyrosine Diet for 2 Weeks.

	Treatment				
Amino acid	Control	Control + 0.08% thiouracil	+ 5% L-Tyr	+ 5% L-Tyr + 0.08% thiouracil	+ 5% L-Tyr + 5 μg of thyroxine
	(micromoles per 100 ml of pooled plasma)				
Thr	16.3	7.8	31.2	37.1	17.3
Ser	43.9	46.4	63.5	69.1	28.5
Glu	23.2	12.9	31.2	32.2	17.0
Gly	28.3	55.0	43.3	62.0	17.4
Ala	51.9	75.0	83.0	103.3	28.9
Val	11.6	11.6	17.5	13.8	16.4
Ile	7.5	5.7	5.8	8.1	5.7
Leu	8.6	8.5	9.4	13.0	9.4
Phe	9.4	5.9	11.0	8.8	9.7
Lys	40.3	44.6	46.0	27.9	49.7
His	10.3	10.2	7.9	9.5	6.6
Total dispensable amino acids[a]	147.4	189.3	221	266	91.8
Total indispensable amino acids	104.0	94.3	97.6	81.1	114.8

[a] Tyrosine omitted.

Changes in plasma amino acids must be viewed with reservation as the food intake of the high tyrosine groups was severely depressed. Nevertheless, plasma tyrosine values for rats fed the high tyrosine diets were elevated despite food intake depression and even weight loss (Table I, Fig. 2). The total dispensable amino acids, excluding tyrosine, were elevated in plasma of rats fed the basal and the high tyrosine diets containing thiouracil and also in plasma of untreated rats fed the high tyrosine diet (Table II).

Effect of thyroxine. This experiment was terminated after 9 days when 2 of 5 rats fed the high tyrosine diet and injected with thyroxine had died. Thyroxine treatment depressed liver weight of rats fed the basal diet but not of those fed the high tyrosine diet (Table III). Liver enzyme activities are expressed per unit of body weight to reflect the relative degradative capacity of each group. Tyrosine transaminase activity of rats fed the 5 % L-tyrosine diet for 9 days increased about 6-fold (Table III) and thyroxine treatment increased it further. *p*-HPP-hydroxylase activity increased about 25 % in rats fed the high tyrosine diet for 9 days. Thyroxine treatment decreased liver *p*-HHP-hydroxylase activity of rats fed the control or the high tyrosine diet somewhat although the values are within the range of diurnal variations for this enzyme (6).

Discussion. Administration of thyroxine aggravated tyrosine toxicity whereas administration of thiouracil alleviated signs of toxicity and prolonged the survival of rats fed a high tyrosine diet. Schweizer (12) reported that thiouracil lessened the weight loss of rats fed a high tyrosine diet. Since food intake of rats fed the high tyrosine diet was not depressed by thiouracil treatment, the beneficial effect on tyrosine toxicity cannot be attributed to lower tyrosine intake.

High plasma tyrosine concentrations were accompanied by high tyrosine transaminase activity in liver, whereas one might expect plasma tyrosine concentration to be low when this enzyme activity is high. The ratio of tyrosine transaminase to *p*-HPP-hydroxylase activity was elevated in the rats fed the high tyrosine diet and thyroxine injection increased it further (Table III), the hy-

TABLE III. Effect of Thyroxine Treatment[a] on Tyrosine Transaminase and *p*-Hydroxyphenyl Pyruvate Hydroxylase of Rats Fed a High Tyrosine Diet for 9 Days.

Diet	Treatment	Mortality	Liver wt (g/100 g of body wt)	Enzyme act/100 g of body wt[b]		
				TT	*p*HPPH	TT/*p*HPPH
6% Casein (C)	Saline	0/5	5.14	108 ± 26	268 ± 25	0.40
6% Casein (C)	Thyroxine	0/5	4.18	128 ± 7	181 ± 17	0.71
C + 5% L-tyr	Saline	1/5	5.48	625 ±180	352 ± 52	1.78
C + 5% L-tyr	Thyroxine	2/5	5.36	941	298	3.16

[a] Five μg of L-thyroxine injected daily intraperitoneally.

[b] Both tyrosine transaminase activity (TT) and *p*-hydroxyphenylpyruvate hydroxylase (*p*HPPH) activity were determined by methods of Lin *et al.* (1958). Activities are expressed as μmoles of *p*-hydroxyphenylpyruvic acid formed or consumed per hour, respectively, ± standard error of the mean.

droxylase would thus appear to be limiting for tyrosine catabolism. Plasma tyrosine concentration is increased by thyrotoxicosis in man (16) and the rat (17, 18); even in the euthyroid rat, an oral load of *p*-hydroxyphenylpyruvate results in accumulation of tyrosine in plasma (19).

Adrenalectomized (20) and thyroxine-treated (6, 20) rats are particularly susceptible to tyrosine toxicity and administration of cortisol (5, 6) or thiourical (6, 12) alleviate the toxicity. Tyrosine is reported to increase the uptake of radioactive iodine by the thyroid in rats fed a low-protein diet (8). High doses of cortisone are required to lower protein-bound iodine values in hyperthyroid subjects (22) and to increase tyrosine transaminase in hyperthryoid rats (23). Thyroxine treatment increases (6) and cortisol treatment (5) decreases plasma tyrosine concentration; administration of cortisol stimulates tyrosine oxidation (6) and hyperthyroidism increases cortisol degradation (21). Rivlin and Melmon (21) reported that cortisol depressed plasma tyrosine in human subjects administered an oral load of tyrosine but not in hyperthyroid subjects. Rivlin and associates (21, 24) have concluded that adrenal–thyroid interrelations are important in the control of tyrosine metabolism and the accumulated observations suggest that corticosterone–thyroxine balance may be important in the development of tyrosine toxicity.

Summary. Addition of thiouracil to a high tyrosine diet alleviated signs of tyrosine toxicity in the rat, whereas, daily injections of thyroxine aggravated them. Plasma tyrosine concentration and liver tyrosine transaminase activity were high in rats fed a high tyrosine diet; thyroxine administration increased them further, but depressed slightly the activity of liver *p*-hydroxyphenylpyruvate hydroxylase.

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