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Effect of Dietary Threonine Supplementation on Tyrosine Toxicity in the Rat

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ABSTRACT The objective of this study was to determine the effect of threonine supplementation on tyrosine metabolism in rats fed a low protein diet with excess tyrosine. The growth retardation and the development of eye and paw lesions that occur in rats ingesting a basal plus 3% or 5% L-tyrosine diet could be alleviated partially by the addition of 0.5% or 1.0% L-threonine to the diet. An increased blood tyrosine level in rats fed excess tyrosine was also lowered by threonine supplementation. In tyrotoxic conditions, the activities of liver tyrosine transaminase (EC 2.6.1.5) and threonine dehydratase (EC 4.2.9.16) were elevated, but *p*-hydroxyphenyl pyruvic acid oxidase (EC 1.13.11.27) which is also intimately associated with tyrosine toxicity was found to be inactivated. Furthermore, biosynthesis of ascorbic acid in liver was significantly lowered in this condition. However, addition of L-threonine in the diet, not only could cure the signs developed due to excess tyrosine, but also could affect the levels of enzymes studied. J. Nutr. *107:* 1575–1582, 1977.

INDEXING KEY WORDS tyrosine toxicity · threonine alleviation · tyrosine transaminase · *p*HPP oxidase · threonine dehydratase ascorbic acid biosynthesis

It was reported earlier that as a result of high tyrosine intake, the tyrotoxic signs, particularly, increased blood tyrosine level and urinary excretion of *p*-hydroxyphenylpyruvic acid (*p*HPP), could be overcome by threonine supplementation to the diet (1–5). Tyrosine toxicity can also be counteracted by increasing the level of dietary protein or free amino acids (5–8). Although the reasons for the toxicity are not clear, it is well established that tyrosine transaminase in the rat can be increased either by tyrosine feeding (9–10) or by injection of hydrocortisone or glucagon (11–12). However, under these conditions there is no stimulation of *p*HPP oxidase activity (6). Furthermore, *p*HPP oxidase is highly susceptible to substrate inactivation (13, 14) and this substrate inactivation can be reversed by ascorbic acid either in vivo (15) or in vitro (16, 17). In order to understand the effect of threonine on tyrosine toxicity, the tyrosine catabolizing enzymes, particularly tyrosine transaminase and *p*HPP oxidase and threonine dehydratase were studied. The level of ascorbic acid synthesis and its relation with *p*HPP metabolism were also investigated in tyrotoxic rats.

MATERIALS AND METHODS

Animals and diets. Weanling albino (Holtzman strain) rats weighing between 25 and 40 g were used in these experiments. Two or 3 days before being divided into groups, they were fed a basal diet containing 9% casein, prepared according

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to Benton et al. (1), and a modification was done according to Alam et al. (2). The composition of the basal diet was: (in percent) vitamin free casein 9.0; salt mixture (18) 4.0, peanut oil 5.0; L-methionine 0.3; DL-tryptophan 0.1; starch 81.6; vitamin mixture (18), 0.5; Choline chloride, 0.2. L-Tyrosine (3 or 5%) and L-threonine (0.5 or 1%) were added to the basal diet at the expense of equivalent amount of starch. The diets were stored at 4° throughout the experimental period. Food and water were supplied ad libitum. All the rats were kept in a light and temperature controlled room.

Enzyme assay. At different days during the experimental period, rats were killed by decapitation. Livers were rinsed with ice-cold 0.9% NaCl solution, excised, blotted and weighed. A part of the liver was homogenized with 2 volumes of ice-cold 0.15 M KCl solution in a glass homogenizer. The homogenate was then diluted with 0.2 M potassium phosphate buffer, pH 7.0 at a ratio of 1:8, and the diluted homogenate was centrifuged at 20,000 × g for 30 minutes. A part of the supernatant was used for assaying tyrosine transaminase activity according to the method of Chan and Cohen (19).

In order to determine *p*HPP oxidase activity, the routine assay mixture contained (in μmoles) in a total volume of 4.0 ml; sodium phosphate buffer pH 6.5, 200; α-α'-dipyridyl, 40; *p*HPP, 8 and enzyme fraction. After incubation for 10 and 20 minutes at 28°, the reaction was stopped by adding 0.5 ml of 30% metaphosphoric acid and the clear supernatant was assayed for the amount of *p*HPP left by a modification of Millons reaction (20). Since this enzyme is inactivated in the presence of excess substrate within 8 to 10 minutes (15, 16), *p*HPP oxidase activity was expressed in terms of percent inactivation as measured on the basis of relative utilization of *p*HPP in 10 to 20 minutes of incubation.

Ascorbic acid biosynthesis in liver homogenate was assayed according to the method of Chatterjee et al. (21). Threonine dehydratase activity in the supernatant was determined by the method of Bottomley et al. (22). Blood tyrosine level was estimated according to the method of La Du and Howell (23). Protein was estimated by the method of Lowry et al. (24).

Rats were kept in metabolic cages for 24 hours, and urine was collected in 10 ml of 6 N HCl. After diluting to 100 with distilled water, urinary *p*HPP content was measured by the method of Bernhart and Zilliken (25).

RESULTS AND DISCUSSION

Tyrotoxic signs and threonine supplementation. Tyrosine toxicity in rats was developed either by feeding 3% or 5% tyrosine in the diet. From table 1, it was evident that the rats fed the diet containing 3% tyrosine, lost weight and had a low

TABLE 1

Effect of DL-threonine supplementation on weight gain, food intake and mortality of rats fed excess tyrosine

Dietary L-tyrosine	Supplementation DL-threonine	Weight gain per rat	Food intake per rat	Development of lesions	Mortality rate
%	%	g/week	g/5 days		
—	—	9.8±1.6[1]	35.0±2.0	0/4	0/10
—	0.5	10.9±0.7	37.5±1.2	0/4	0/10
3	—	3.9±0.9[a]	24.5±1.0[a]	10/10	0/10
3	0.5	8.9±1.5	32.5±1.8	5/10	0/10
3	1.0	9.9±1.7	37.5±0.7	2/10	0/10
5	—	−1.5±0.6[a]	19.5±0.75[a]	10/10	6/10
5	0.5	4.3±0.6[a]	25.5±0.9[a]	8/10	3/10
5	1.0	8.9±1.4	31.4±0.9	7/10	1/10

[1] Mean values ±SD of eight experiments each consists of at least four rats. Significantly different from control rats, [a] $P < 0.001$.

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TABLE 2

Effect of DL-*threonine supplementation on blood tyrosine level in rats fed excess tyrosine*

Dietary supplementation		Blood tyrosine level[1] at different days of experiment			
L-tyrosine	DL-threonine				
%	%	*1 day*	*6 days*	*9 days*	*12 days*
—	—	40± 1.0	46± 1.6	39±2.5	46±3.0
—	0.5	33± 2.0	45± 2.6	43±1.0	54±5.0
3	—	822±510[a]	834± 2.0[a]	807±1.9[a]	923±2.5[a]
3	1.0	116± 5.0[a]	166± 3.0[a]	138±3.0[a]	215±1.9[a]
5	—	1,221± 7.0[a]	1,331±10.0	1,409±6.0[a]	1,525±5.0[a]
5	1.0	381± 2.0[a]	436± 2.0[a]	380±1.0[a]	350±3.0[a]

[1] Blood tyrosine level is expressed as μM of tyrosine. [2] Mean values of eight experiments±SD, each group in each experiment contained at least four rats. Significantly different from control rats, [a] $P < 0.001$.

level of food intake which was accompanied by the development of external pathological lesions in the eyes and paws. A more pronounced effect was observed when the tyrosine content in the diet was increased to 5%; some rats died within 2 weeks. However, improvement in growth depression and partial disappearance of pathological lesions were observed in the high tyrosine groups supplemented with 0.5% or 1.0% of L-threonine.

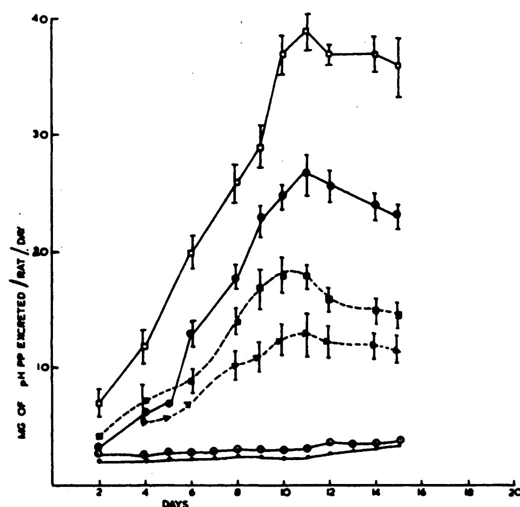

Fig. 1 Effect of threonine supplementation to tyrosine fed rats on the urinary excretion of *p*HPP (●——●——● 9% casein, ⊙——⊙——⊙ 9% Casein + 0.50% DL threonine, ●——●——● 3% tyrosine, □——□——□ 5% tyrosine, ▲——▲——▲ 3% tyrosine + 0.5% threonine, ■---■---■ 5% tyrosine + 0.50% DL threonine). Urine samples were pulled from 3 rats/group for 24 hours and there were at least 24 rats in each group of each experiment. The experiment was repeated three times. The vertical bars represented SEM. On the initial days of the experiment, SEM was not represented in each group.

The results on blood tyrosine level of the rats fed the excess tyrosine diet with or without threonine supplementation are shown in table 2. The data show that the blood tyrosine level was increased in rats fed excess tyrosine and this increase in blood tyrosine was related to the dietary tyrosine level. Although threonine supplementation prevented this increase, the level was higher than that of rats fed the basal diet.

Effect of threonine supplementation on pHPP metabolism. Effect of threonine supplementation to the excess tyrosine diet on urinary excretion of *p*HPP is represented in figure 1. The excretion of *p*HPP was increased by increasing tyrosine level in the diet, and it was maximum on days 10 to 12 of the experiment. It was also correlated with the onset of toxic signs. Threonine supplementation to tyrosine excess diet effectively reduced the excretion of *p*HPP compared to tyrosine fed rats although it remained at high level compared to that of controls.

The activity of liver tyrosine transaminase isolated from tyrotoxic and threonine fed rats was determined (table 3). The results show that tyrosine transaminase activity was increased on the very first day of the experiment in the tyrosine fed group and remained elevated throughout the period. The increase in this enzyme activity was dependent on the dietary level of tyrosine. However, threonine supplementation to excess tyrosine diet effectively

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TABLE 3

Effect of threonine supplementation on tyrosine transaminase activity in rats fed excess tyrosine

Dietary supplementation		Tyrosine transaminase activity[1] at different days of experiment			
L-tyrosine	L-threonine				
%	%	*1 day*	*6 days*	*9 days*	*12 days*
—	—	30.1±0.7[2]	25.9±1.5	29.3±1.6	26.1±1.4
—	0.5	31.4±1.4	27.3±1.4	26.1±1.5	28.1±1.4
3	—	96.3±0.8[a]	60.9±1.2[a]	50.9±1.2[b]	41.9±1.5
3	0.5	59.7±0.5[a]	42.3±1.3[c]	33.9±0.9	30.5±0.3
3	1.0	44.7±0.4[b]	41.9±1.3[c]	31.2±0.3	34.0±0.5
5	—	115.9±1.1[a]	78.9±0.9[a]	60.9±1.1[a]	45.6±1.9[b]
5	0.5	80.9±1.0[a]	61.3±0.4[a]	49.2±0.3[b]	42.3±1.7[c]
5	1.0	72.1±1.6[a]	55.0±0.7[b]	42.9±0.5[c]	35.0±0.8

[1] Tyrosine transaminase activity is expressed as μmoles of *p*HPP formed/mg of protein/hour. [2] Mean values ±SD of eight experiments of at least 10 rats in each group, significantly different from control rats, [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$.

prevented part of the rise in the level of tyrosine transaminase activity.

The percent inactivation of *p*HPP oxidase (the enzyme which is responsible for the degradation of *p*HPP) between incubation for 10 and for 20 minutes was also determined in different experimental groups (table 4). This result indicates that *p*HPP oxidase became more susceptible to inactivation in tyrosine fed rats as compared to controls. The degree of inactivation of *p*HPP oxidase was also dependent on the dietary tyrosine level. Threonine supplementation (0.5% or 1%) partially alleviated the substrate inactivation of *p*HPP oxidase; however, normal level was achieved only in rats fed the 3% tyrosine diet on day 12, but not in rats fed the 5% tyrosine diet.

The ratio of activities of tyrosine transaminase to *p*HPP oxidase in different experimental groups is shown in figure 2. In control rats, the ratio remained constant (approximately 1) throughout the experiment. In tyrotoxic conditions, the ratio of these two enzymes reached the highest level within 6 to 9 days, and then started to decline. However, the ratio of these two enzymes in livers of rats fed the threonine supplemented tyrosine diet continued

TABLE 4

Effect of threonine supplementation on the rate of inactivation of pHPP oxidase in rats fed excess tyrosine

Dietary supplementation		Rate of inactivation of *p*HPP oxidase[1] between 10 and 20 minutes at different days of experiments			
L-tyrosine	L-threonine				
%	%	*1 day*	*6 days*	*9 days*	*12 days*
—	—	54±0.8[2]	58±0.9	60±0.6	62±1.0
—	0.5	55±0.6	59±0.6	61±0.6	60±0.9
3	—	56±0.9	78±0.6[a]	80±0.8[a]	71±0.6[b]
3	0.5	59±0.9	67±1.4[c]	71±0.9[b]	59±0.9
3	1.0	56±0.9	69±0.6[c]	70±0.9[b]	61±0.6
5	—	58±0.9	88±0.8[a]	92±0.5[a]	84±0.3[a]
5	0.5	60±0.9	76±0.4[a]	82±0.2[a]	83±1.0[a]
5	1.0	56±0.5	71±0.4[b]	70±0.9[b]	71±0.3[b]

[1] Rate of inactivation of *p*HPP oxidase is expressed in terms of percent inactivation, which has been measured, on the basis of relative utilization of *p*HPP after 10 and 20 minutes of incubation at 28°. [2] Mean values ±SD of 8 experiments, significantly different from control rats, [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$.

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TABLE 5

Effect of threonine supplementation on ascorbic acid biosynthesis in rats fed excess tyrosine

Dietary supplementation		Ascorbic acid biosynthesizing capacity[1] at different days of experiments			
L-tyrosine	L-threonine				
%	%	*1 day*	*6 days*	*9 days*	*12 days*
—	—	3.55±0.2[2]	4.21±0.2	3.85±0.4	3.70±0.5
—	0.5	3.9 ±0.1	4.15±0.25	4.01±0.1	3.8 ±0.3
3	—	3.95±0.1	2.63±0.29[a]	2.27±0.4[a]	2.91±0.1[b]
3	0.5	4.19±0.1	3.83±0.1	3.10±0.2[c]	3.65±0.3
3	1.0	3.92±0.9	3.71±0.2	3.52±0.1	3.72±0.15
5	—	3.78±0.2	2.03±0.2[a]	1.95±0.2[a]	2.52±0.1[a]
5	0.5	3.83±0.1	3.42±0.2	2.63±0.2[a]	2.83±2[b]
5	1.0	3.95±0.2	3.31±0.2	2.75±0.1[b]	2.91±0.2[b]

[1] Ascorbic acid biosynthesizing capacity is expressed as μg of ascorbic acid formed/mg of protein/1.5 hour.
[2] Mean values ±SD of 8 experiments, significantly different from control rats, [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$.

to be higher than that of the controls although it was significantly lower than that of the rats fed the tyrosine diet. This result suggests that threonine partially reduces the level of tyrosine transaminase and *p*HPP oxidase in tyrotoxic rats. The present study suggests that high dietary tyrosine ingestion which induces the toxic syndrome, may also alter *p*HPP metabolism. Furthermore, the rate of inactivation of *p*HPP oxidase was increased in the later stage of experimental period (9–12 days) in rats fed the 5% tyrosine diet and as a consequence, the appearance of toxic signs and elevation of urinary excretion of *p*HPP occurred. It appears therefore, that the high level of tyrosine in the diet produces some metabolic reorientation and as a result, the usual equilibrium existing between the metabolism of the two enzymes may be altered. However, this can be overcome by threonine supplementation to the tyrosine diet either by decreasing formation or increasing utilization of *p*HPP.

Effect of threonine supplementation on biosynthesis of ascorbic acid. The data on the ascorbic acid biosynthesizing capacity in liver tissues of different experimental groups are shown in table 5. In tyrosine fed rats, the marked reduction in ascorbic acid biosynthesis started from the sixth day of experiment and this could be cor-

TABLE 6

Effect of threonine supplementation on threonine dehydratase activity in rats fed excess tyrosine

Dietary supplementation		Threonine dehydratase activity[1] at different days of experiments			
L-tyrosine	L-threonine				
%	%	*1 day*	*6 days*	*9 days*	*12 days*
—	—	1.48±0.12[2]	1.77±0.2	1.61±0.1	1.47±0.3
—	0.5	1.50±0.2	1.39±0.15	1.71±0.2	1.52±0.2
3	—	3.95±0.2[a]	2.53±0.3[b]	1.91±0.2	1.75±0.3
3	0.5	2.72±0.2[a]	2.19±0.1[b]	1.85±0.2	1.79±0.1
3	1.0	2.15±0.1[b]	1.81±0.2	1.65±0.1	1.51±0.09
5	—	4.12±0.2[a]	2.70±0.2[a]	1.91±0.2	1.81±0.1
5	0.5	3.71±0.1[a]	1.79±0.1	1.85±0.2	1.79±0.2
5	1.0	3.01±0.2[a]	1.78±0.1	1.63±0.3	1.9 ±0.2

[1] Threonine dehydratase activity is expressed in terms of μg of α-ketobutyric acid formed/mg of protein/0.5 hours. [2] Mean values ±SD of 8 experiments, significantly different from control rats, [a] $P < 0.001$; [b] $P < 0.01$.

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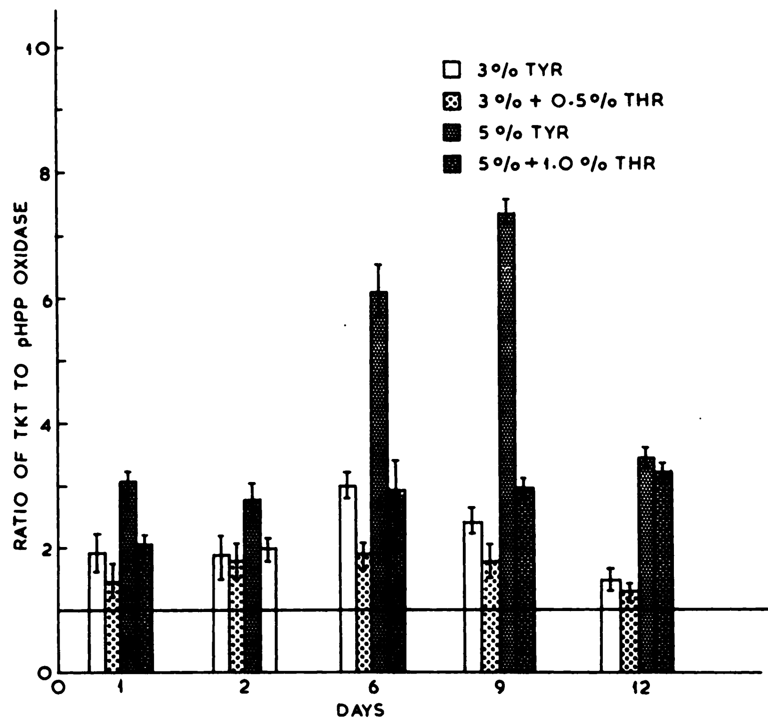

Fig. 2 Ration of tyrosine transaminase (TKT) and *p*HPP oxidase in threonine supplemented tyrosine fed rats. Livers were isolated from normal tyrotoxic and threonine supplemented rats on different days of experimental period and the activities of TKT and *p*HPP oxidase were determined according to the methods described. The ratio of these two enzymes in normal and only 0.5% L-threonine supplemented rats remains about one throughout the experiment (not shown in Fig. 2). The vertical bars represented SEM.

related with the susceptibility of *p*HPP inactivation as well as with the appearance of toxic signs. In threonine supplemented tyrosine fed rats, the biosynthesizing capacity of ascorbic acid was not significantly decreased until day 9. It seems, therefore, that in threonine supplemented tyrosine fed rats, ascorbic acid was available and it protected *p*HPP oxidase from its substrate inactivation. This result also agrees with the finding of Ghosh et al. (26) that in tyrotoxic conditions, liver ascorbic acid biosynthesizing capacity is reduced.

Effect of threonine supplementation on threonine dehydratase activity. As shown in table 6, the level of threonine dehydratase activity in tyrosine fed rats reached a maximum on the very first day and then it declined gradually; however, threonine supplementation prevented the increase. Both threonine dehydratase and tyrosine transaminase are hormone-induced enzymes (11, 12, 27, 28). Threonine dehydratase activity can also be regulated by dietary constituents, but threonine alone is ineffective (29–31). It seems therefore, that dietary tyrosine may be responsible for the release of corticosteroids and as a result, induction of both threonine dehydratase and tyrosine transaminase occur in this stress condition.

The present study suggests that biochemical lesions developed as a result of triggering of sequential events by the high level of tyrosine in blood, can be overcome by threonine supplementation. Furthermore, threonine does not enhance the tyrosine catabolism since there is no increase in tyrosine transaminase and *p*HPP oxidase activity in threonine supplemented tyrotoxic rats. This result also supports the

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findings of Godin (32) that in threonine supplemented tyrotoxic rats, the conversion of ^{14}C-tyrosine to $^{14}CO_2$ is not accelerated. It might be due to competition between tyrosine and threonine for intestinal absorption. Such competition between structurally unlike amino acids has been reported earlier (33).

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Errata

The date of death for Dr. John W. Bratzler was incorrectly listed in the Proceedings of the 1977 Annual Meeting of the American Institute of Nutrition as published in the August 1977 issue of the JOURNAL OF NUTRITION. Dr. Bratzler died on March 8, 1975. Also listed incorrectly in the list of Committees, was Dr. R. V. Swift as a member of the Ad Hoc Committee on the History of Nutrition. Dr. R. W. Swift, a former member of this Committee, is deceased.

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