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THE EFFECT OF THIAMINE DEFICIENCY ON THE METABOLISM OF ACETAMINOPHEN (PARACETAMOL)

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Abstract—The effect of thiamine deficiency on the metabolism of acetaminophen (paracetamol) was studied in male and female rats. Deficiency of thiamine enhanced the rate of disappearance of the drug from the plasma which resulted in the apparent decrease in the plasma half-life. The alteration in the rate of acetaminophen metabolism was, in part, due to an increase in the formation of the water soluble metabolites characterized as glucuronide and sulfate conjugates. The effect of thiamine deficiency could be overcome by supplementation with thiamine by intraperitoneal injection. A single large dose of thiamine (650 μg) could reduce the rate to the normal level within 24 hr. However, a series of 5 low doses (260 μg/dose/day) was required to produce the same effect.

Acetaminophen (paracetamol) is an analgesic antipyretic drug which is widely used and available without prescription. It is considered 'safe' at therapeutic levels, but overdosage results in liver necrosis in experimental animals and in man [1–5].

Acetaminophen is eliminated mostly by metabolism and to a smaller extent by excretion [6, 7]. Major metabolites are excreted in the urine in the forms of glucuronide and sulfate conjugates [8–11]. Evidence has indicated that the hepatotoxicity of acetaminophen is due to the reactive metabolite likely to be the *N*-hydroxy derivative [12] generated by the cytochrome P-450, mixed function oxidase system. Covalent binding of this metabolite to cellular macromolecules is thought to be responsible for liver necrosis [13, 14]. The binding occurs when hepatic glutathione has been depleted [15, 16]. There appears to be a positive correlation among covalent binding of toxic metabolite to the macromolecules, depletion of liver glutathione and the severity of necrosis [17].

Nutritional status of animals has been shown to alter the metabolism of drugs. High level of thiamine intake has been reported to depress the metabolism of many drugs together with the decrease in cytochrome P-450 content and cytochrome *c* reductase activity [18–21]. Thiamine deficiency, on the other hand, has been found to increase the metabolism of drugs and a carcinogen [22, 23].

It is the purpose of this study to investigate the influence of thiamine deficiency on the metabolism of acetaminophen in the rat.

MATERIALS AND METHODS

Chemicals. Acetaminophen (*N*-acetyl-*p*-aminophenol) and β-glucuronidase were purchased from Sigma Chemical Co. (St Louis, MO). Radioactive ^{3}H-acetaminophen (specific activity 6.41 mCi/mmole) was obtained from New England Nuclear (Boston, MA). All dietary ingredients, vitamins and Rogers and Harper salt mixture were obtained from NB Co. Biochemicals, (Cleveland, OH).

Animals and diets. Weanling, Fischer rats, aged 21 days were housed individually with food and water provided *ad lib.* Semipurified agar-gel diet [24] was used throughout the study. The control diet contained all vitamins and minerals required by rats. In the thiamine deficient diet, thiamine hydrochloride was omitted from the vitamin mix. All animals were fed control diet until 30 days old; they were then divided into two groups and fed either control or thiamine deficient diet for the study periods. The animals were fasted 16 hr prior to the administration of acetaminophen (i.p. in 0.9% NaCl). They were killed by decapitation 1 hr following the drug administration, between 9.00 and 11.00 a.m.

Supplementation of thiamine. Thiamine hydrochloride dissolved in 0.9% NaCl was given to deficient rats, intraperitoneally, as a single or repeated treatments. A series of low dose, 260 μg/day, of thiamine hydrochloride, was given successively. At high dose, 650 μg of thiamine hydrochloride was given as a single injection. Acetaminophen was administered 24 hr after thiamine supplementation.

Assay method for erythrocyte transketolase. The erythrocyte transketolase activity was determined in 0.2 ml blood sample by the method of Brin *et al.* [25] which was based on the fact that inadequacy of thiamine resulted in an insufficient amount of thiaminepyrophosphate (TPP) coenzyme to bind with the apotransketolase, causing a decrease in transketolase (TK) activity of the red blood cells. The addition of TPP *in vitro* (TPP effect) would restore the TK activity by reconstituting the apotransketolase into active halotransketolase enzyme. A TPP stimulation effect from 0 to 15 per cent was considered to be normal. A TPP effect from 15 to 24 per cent was considered to be marginally deficient

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and if the TPP effect exceeded 25%, the animal was deficient.

Metabolism of acetaminophen. The rate of metabolism of acetaminophen *in vivo* was determined by measuring the levels of unchanged drug remaining in plasma and liver at one hour following the administration of acetaminophen. Liver was homogenized in 0.1 N HCl (1:5 w/v). The homogenate was extracted with isoamylalcohol–ether and the level of unchanged acetaminophen was determined by the method of Brodie and Axelrod [7]. Plasma acetaminophen was extracted and determined by the methods of Routh *et al.* [26] and Imai *et al.* [27].

When radioactive ^{3}H-acetaminophen was used, the unchanged drug in liver and plasma was extracted with ethylacetate by the method of Brodie and Axelrod [7] as modified by Mitchell *et al.* [15]. After the solvent was evaporated to dryness, the residue was redissolved in absolute ethanol (0.5 ml) and counted in 15 ml of Aquasol from New England Nuclear (Boston, MA).

The method of Lowenthal *et al.* [28] was employed in the determination of various metabolites formed. The presence of glucuronide and sulfate conjugates was determined by incubating the remaining aqueous phase of the extracted samples with β-glucuronidase (containing sulfatase) at 37°C for 24 hr. After enzymatic hydrolysis, the samples were reextracted with ethylacetate and assayed for radioactivity. Plasma and liver protein concentrations were determined by the method of Lowry *et al.* [29].

RESULTS

Effect of feeding thiamine deficient diet on food intake, growth rate, liver protein concentration and the thiamine status

Growth rate, food intake, liver protein concentration and erythrocyte transketolase activity of male rats throughout the 28 days on regimen are shown in Table 1.

The signs of thiamine deficiency evidenced by inhibition of growth became apparent after the animal was fed the deficient diet for 14 days. Significant weight loss was observed after 21 days on regimen. Erythrocyte transketolase activity expressed as per cent TPP effect showed that rats on this diet for 14 days were already deprived of thiamine and the severity of deficiency progressed with the length of the feeding period. A considerable decrease in food intake and growth of thiamine deficient rat was observed during the fourth week. Any effect, if appeared at this period might not be the manifestation of thiamine deficiency alone, but it could be partially due to food deprivation. The protein concentration of the liver was not affected by thiamine deficiency. Based on this study, the 21 days on regimen was selected as the experimental condition for most experiments in order to minimize the complication due to deficiencies of other nutrients which might result from anorexia.

Effects of thiamine deficiency on the rate of metabolism of acetaminophen in male and female rats

The alteration in the rate of metabolism of acetaminophen (91 mg/kg) during the progress of thiamine deficiency was studied in rats of both sexes at various intervals on regimen. The levels of acetaminophen in the plasma of the controls were higher than those of the deficient rats indicating that thiamine deficiency enhanced the disappearance of acetaminophen from the plasma (Table 2). This effect was observed in male rats upon feeding thiamine deficient diet for 14 days and it became more pronounced with the severity of deficiency. In female rats, the effect of thiamine deficiency on acetaminophen metabolism was slightly delayed. A significant increase in the rate of disappearance could be seen at 21 days feeding period and progressed with time.

Change in the levels of acetaminophen in the liver with feeding periods followed the same pattern as that of the plasma in both male and female rats (Table 3).

Figure 1 demonstrates the plasma profiles of acetaminophen (91 mg/kg, i.p.) in the control and thiamine deficient male rats fed 21 days on the diets. The absorption and distribution throughout the body appears to be relatively more rapid than the rate of elimination, indicating a monoexponential expression. The plasma half-life estimated from Fig. 1, including all points using manual CSTRIP method [30] was 34.31 min in the thiamine deficient rats

Table 1. Effect of thiamine deficiency on growth rate, food intake, liver protein concentration and transketolase activity in male rats*

Parameter	Diet	Feeding period (days)			
		7	14	21	28
Body weight (g)	Control	84.00 ± 4.35	117.90 ± 7.97	153.24 ± 9.08	169.20 ± 13.37
	Thiamine Deficient	84.70 ± 14.58	102.00 ± 7.41†	110.50 ± 13.54†	85.10 ± 8.09†
Food intake (g)	Control	19.16 ± 1.30	22.75 ± 1.12	26.45 ± 1.24	32.00 ± 2.00
	Thiamine Deficient	17.40 ± 1.81	19.50 ± 2.00	13.45 ± 0.70†	1.81 ± 0.31†
Liver protein (mg/g liver)	Control	248.60 ± 7.24	254.76 ± 8.21	255.24 ± 5.12	251.12 ± 8.72
	Thiamine Deficient	249.04 ± 8.13	240.57 ± 11.81	245.37 ± 6.33	258.72 ± 9.96
Transketolase activity (%TPP effect)	Control	–	7.32 ± 3.33	7.40 ± 1.50	7.49 ± 0.93
	Thiamine Deficient	–	53.39 ± 7.37†	68.94 ± 32.87†	70.38 ± 19.68†

* Values represented means ± S.D. of 5 rats.
† Significantly different from the control values ($P < 0.01$).

Table 2. The effect of thiamine deficiency on the rate of disappearance of acetaminophen from the plasma*

Sex	Diet	Plasma acetaminophen level (μg/ml)			
		Feeding period (days)			
		7	14	21	28
Male	Control	50.14 ± 10.30(4)	47.48 ± 10.36(10)	44.93 ± 6.17(10)	47.79 ± 5.76(4)
	Thiamine deficient	49.19 ± 21.99(5)	36.44 ± 10.35†(5)	30.17 ± 4.83‡(10)	26.30 ± 3.25‡(3)
Female	Control	45.21 ± 6.89(6)	43.53 ± 7.91(9)	44.93 ± 7.88(6)	47.18 ± 6.30(6)
	Thiamine deficient	46.59 ± 6.67(6)	40.78 ± 7.85(9)	34.86 ± 4.83‡(7)	32.18 ± 4.50‡(6)

* Groups of male and female rats fed on either the control or TD diet for 7, 14, 21 and 28 days were injected with acetaminophen (91 mg/kg bw) and were killed at 1 hr after the treatment. The levels of the unchanged drug in plasma were determined. Values in parentheses are numbers of animals. The results are given as mean values ± S.D.

† Significantly different from control ($P < 0.05$).

‡ Significantly different from control ($P < 0.005$).

Table 3. The effect of feeding thiamine deficient diet on the rate of disappearance of acetaminophen from the liver*

Sex	Diet	Concentration of acetaminophen in liver (μg/g)			
		Feeding period (days)			
		7	14	21	28
Male	Control	19.09 ± 8.29(4)	19.09 ± 2.84(5)	17.45 ± 2.40(5)	20.83 ± 0.84(4)
	Thiamine deficient	14.84 ± 7.85(5)	14.62 ± 3.38†(5)	11.67 ± 3.27‡(9)	5.89 ± 2.07‡(4)
Female	Control	18.92 ± 3.27(6)	19.17 ± 3.94(11)	20.29 ± 3.55(10)	18.32 ± 3.83(6)
	Thiamine deficient	21.31 ± 4.49(6)	17.23 ± 2.94(9)	13.70 ± 4.11‡(7)	8.74 ± 2.90‡(6)

* Groups of male and female rats fed on either the control or TD diet for 7, 14, 21 and 28 days were injected with acetaminophen (91 mg/kg bw) and were killed at 1 hr after the treatment. The levels of the unchanged drug in liver were determined. Values in parentheses are numbers of animals. The results are given as mean values ± S.D.

† Significantly different from control ($P < 0.01$).

‡ Significantly different from control ($P < 0.005$).

Table 4. The effect of thiamine deficiency on the *in vivo* metabolism of ^{3}H-acetaminophen in male rats*

Tissue	Unchanged acetaminophen		Total water soluble metabolite		Glucuronide and sulfate conjugates	
	Control	Thiamine deficient	Control	Thiamine deficient	Control	Thiamine deficient
Liver radioactivity (10^5 dpm/g)	6.80 ± 0.32	3.84 ± 0.96†	9.16 ± 2.38	19.18 ± 1.54†	7.12 ± 1.18	12.84 ± 1.24†
Plasma radioactivity (10^5 dpm/ml)	14.24 ± 1.68	8.36 ± 1.94‡	2.52 ± 0.18	5.20 ± 0.34†	0.82 ± 0.02	1.60 ± 0.10†

* Groups of control or thiamine deficient rats, 21 days on feeding regimen, were injected intraperitoneally with ^{3}H-acetaminophen (100 μCi/rat, 1000 mg/kg) and were killed 3 hr after the injection. Acetaminophen was extracted from the plasma and liver and assayed for radioactivity. The water soluble metabolites were hydrolysed to the parent compound by incubating with β-glucuronidase and sulfatase. The results are given as mean values ± S.D. of 3 rats.

† Significantly different from the control values ($P < 0.005$).

‡ Significantly different from the control values ($P < 0.01$).

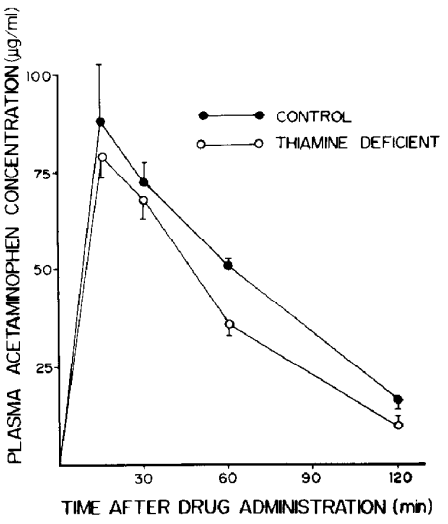

Fig. 1. Plasma concentration–time curve of acetaminophen following a single intraperitoneal administration. Groups of male rats fed the diets for 21 days were injected intraperitoneally with acetaminophen (91 mg/kg). Plasma concentrations of the unchanged drug were determined in samples collected at various intervals. Each point represents the mean ± S.D. of 3 rats.

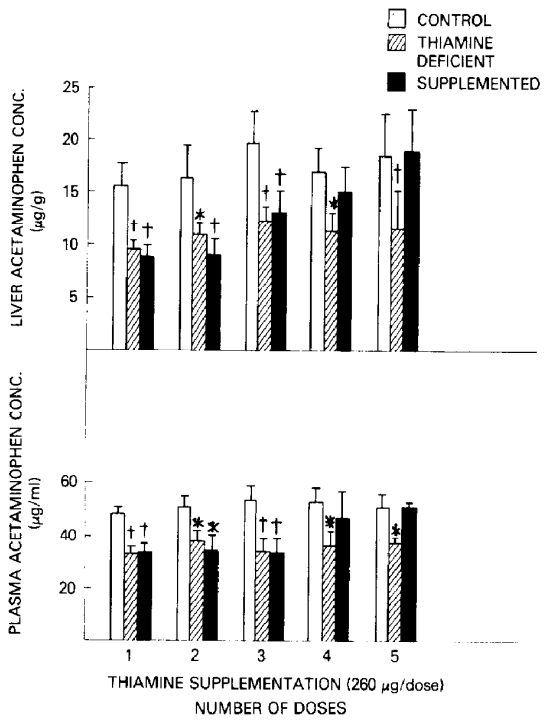

Fig. 2. Effect of thiamine supplementation, at low dose, on acetaminophen metabolism. Thiamine deficient rats, 21 days on feeding regimen were pretreated with thiamine hydrochloride (260 μg/dose/day) prior to the administration of acetaminophen (91 mg/kg, i.p.). Plasma and liver acetaminophen levels were determined one hour after the administration of the drug. Values are the means ± S.D. of 6 animals in each group. * Indicates that the difference from the control is significant at $P < 0.01$. † Indicates that the difference from the control is significant at $P < 0.005$.

whereas in the controls, this was slightly longer, being 43.86 min. This observation supported the previous findings that the rate of disappearance of acetaminophen from plasma was significantly enhanced in thiamine deficient rats.

The *in vivo* metabolism of ^{3}H-acetaminophen in male rats measured by the levels of unchanged drug and other water soluble metabolites extracted from the liver and plasma 3 hr after the administration was shown in Table 4. The amounts of the unchanged drug in both plasma and liver of the controls were nearly two folds higher than the deficient levels, indicating higher metabolic rate in the thiamine deficient rats. This possibility was supported by the findings that the level of total water soluble metabolites formed in thiamine deficient rats was considerably higher than the control values. Enzymatic hydrolysis of this fraction showed that increasing formation of the glucuronide and sulfate conjugates was partly responsible for the apparent increase in the levels of water soluble metabolites. However, failure of excretion may be an alternative explanation for high levels of metabolites in liver and plasma.

Table 5. Effect of high dose of thiamine supplementation on acetaminophen metabolism*

Pretreatment	Group	Acetaminophen levels: Liver (μg/g liver weight)	Acetaminophen levels: Plasma (μg/ml plasma)
Saline	Control	17.09 ± 4.12(6)	53.58 ± 4.78(6)
Saline	thiamine deficient	12.16 ± 1.51†(6)	42.66 ± 5.56§(6)
650 μg thiamine hydrochloride	Supplemented	18.14 ± 4.34‡(6)	54.24 ± 6.60‖(5)

* Thiamine deficient rats, 21 days on regimen, were pretreated with thiamine hydrochloride (650 μg) 24 hr prior to the administration of acetaminophen (91 mg/kg). Plasma and liver drug concentrations were determined one hour after the administration. Results are expressed as the mean ± S.D. Values in parentheses are numbers of animals.

† Significantly different from the control value ($P < 0.05$).

‡ Significantly different from the thiamine deficient value ($P < 0.005$).

§ Significantly different from the control value ($P < 0.005$).

‖ Significantly different from the thiamine deficient value ($P < 0.01$).

Reversal of thiamine deficiency

In an attempt to separate the possible effects of thiamine deficiency on the metabolizing enzyme system from its nutritional effects and to ascertain that the observed effect was solely the influence of thiamine deficiency, not complicated by deficiencies of other nutrients; a series of experiments was carried out wherein thiamine deficient rats were supplemented with thiamine. A single large dose of thiamine hydrochloride (650 μg) given to the deficient rats 24 hr prior to the administration of acetaminophen (91 mg/kg) could lower the rate of metabolism to the normal level (Table 5). However, when a smaller dose of thiamine hydrochloride (260 μg) was given, a series of successive daily administration was required to produce the same effect (Fig. 2). Three consecutive injections of thiamine caused no change in the rate of metabolism of acetaminophen which gradually decreased after 4 doses. The effect of thiamine deficiency was completely reversed by 5 daily doses of 260 μg thiamine hydrochloride.

DISCUSSION

The role of thiamine in drug metabolism appeared to be complex. Earlier evidence suggested that high level of thiamine consumption depressed the activities of enzymes responsible for the metabolism of many drugs [18, 19]. Deficiency of thiamine, on the other hand, enhanced drug metabolism [22, 23].

In this study, it was shown that thiamine deficiency increased the rate of disappearance of acetaminophen in male and female rats. However, the effects in the females appeared to develop somewhat slower than in the males. Wade *et al.* [21] also reported the delay of thiamine effects on aniline hydroxylation and on other components of microsomal enzyme system in female rats.

The metabolic pathways of acetaminophen involved the formation of glucuronide and sulfate conjugates, were considered to represent the nontoxic pathways. The other alternative was microsomal oxidation by the cytochrome-P-450 system, producing toxic intermediate(s) which reacted with reduced glutathione or with cellular macromolecules. Mitchell and co-workers [15] demonstrated that only a small fraction of acetaminophen was converted by this pathway to generate the reactive intermediate(s). The present results demonstrated that thiamine deficiency enhanced the *in vivo* disappearance rate of acetaminophen, which, in turn, resulted in the concomitant decrease of the plasma half-life. This might reflect a greater rate of metabolism and/or excretion of the drug under such condition.

Analysis of the liver and plasma drug metabolites showed a significant increase in the levels of glucuronide and sulfate conjugates which indicated that the enhanced rate of metabolism was, at least in part, associated with increasing formation of these conjugates thought to represent the nontoxic pathways. Thiamine deficiency may also cause the alterations in other metabolic pathway(s) involving cytochrome P-450 which may be associated with toxicity but further investigation will be required for a complete understanding. Decreasing the plasma half-life of acetaminophen in thiamine deficient rats also suggests that the duration of drug action may be less than normal.

Since the effect of thiamine deficiency could be completely reversed within 24 hr by supplementation with a single high dose of thiamine (650 μg), this clearly indicated that the alteration in the rate of acetaminophen metabolism was the result of thiamine deprivation and not secondary to deficiencies of other nutrients. When smaller doses of thiamine (260 μg) were supplemented, recovery appeared gradually after 3 daily injections. It took 5 successive doses of thiamine to reduce the rate to the normal level. An analogy can be drawn from these results and those of the transketolase enzyme. Nose *et al.* [31] reported that with the low daily doses of thiamine (40 μg), transketolase activity began to recover after 2 days and almost attained the normal level after 3 days. However, the thiamine content of the liver can be repleted on the first day. High dose of thiamine (20 mg/day) shortened the latent period to one day although complete recovery took 3–4 days. The results from the present study suggested that the presence of thiamine in the liver might cause inhibition of acetaminophen metabolism. This process probably took place in thiamine deficient rats when the pool of thiamine had been restored.

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