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NUTRITIONAL FACTORS AFFECTING DRUG-METABOLIZING ENZYMES OF THE RAT*

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Abstract—Feeding diets rich in thiamin depresses aniline hydroxylase, cytochrome P-450 and cytochrome b_5 within 9–14 days. Pair-feeding experiments suggest that the depression of aniline hydroxylase, cytochrome *c* reductase and ethylmorphine demethylase is due to the thiamin; however, the depression of cytochrome P-450 and b_5 may be due primarily to the increased amount of carbohydrate ingested by rats fed the enriched diet. When starch was substituted for sucrose, cytochrome P-450 was not lowered by high thiamin ingestion, although cytochrome b_5 and NADPH cytochrome *c* reductase were depressed similarly to that of rats fed high thiamin levels in a sucrose-based diet. Although aniline hydroxylase and ethylmorphine demethylase activities were significantly depressed by both high thiamin diets, this effect was more pronounced in rats fed the sucrose-based diet.

The ingestion of a synthetic diet composed of protein (casein 16 per cent), carbohydrate (sucrose 73 per cent), fat (corn oil 3 per cent) and necessary vitamins and minerals plus high levels of thiamin hydrochloride has been shown to depress selectively the metabolism of aniline [1], heptachlor [1], zoxazolamine [2] and aminopyrine [2] in male rats. Associated with the decreased rates of metabolism of these agents are decreased microsomal cytochrome P-450 content [2], decreased cytochrome *c* reductase activity, decreased apparent binding affinity of aniline [3] and a change in the difference spectrum of ethylisocyanide [3]. Glucose 6-phosphate dehydrogenase activity of the soluble cytoplasm was elevated [2]. Neither hexobarbital metabolism, its binding affinity to microsomes nor hexobarbital sleeping time was altered in the male rat [2, 3]; however, high thiamin ingestion prolonged hexobarbital sleep time in females [2]. Other dietary variables such as starvation, quality and quantity of dietary protein, quality of carbohydrate, other vitamins, quality and quantity of dietary fat, and dietary minerals have also been implicated in altering metabolism and toxicity of drugs.

The present work was conducted in order to define more clearly the roles of thiamin and carbohydrate ingestion in the complex system of drug hydroxylation.

MATERIALS AND METHODS

Animals. Male and female Sprague–Dawley rats (Holtzman Co., Madison, Wis.) weighing 50–65 g were placed on a laboratory test diet containing "vitamin free" casein (16 per cent), non-nutrient cellulose (4 per cent), Jones–Foster Salt mixture (4 per cent), corn oil (3 per cent) and necessary vitamins [4]. Sucrose (73 per cent) or corn starch (73 per cent) was incorporated as the carbohydrate source. Thiamin hydrochloride (Nutritional Biochemicals Co.) was omitted from one diet (thiamin-deficient diet) and added at the concentration of 20 μg/g of food in the other (high thiamin diet). Control rats received Purina Laboratory Chow. Pair-fed animals were provided the same daily quantity of high thiamin diet as their thiamin-deficient pairmates.

Enzyme assays. At the termination of each experiment, rats were decapitated and their livers quickly removed, chilled, weighed and microsomes prepared as previously described [5]. The following assays were conducted: aniline hydroxylase [6], ethylmorphine demethylase [7], cytochrome P-450 [8], cytochrome b_5 [8], NADPH cytochrome *c* reductase [9], glucose 6-phosphate dehydrogenase [10] and protein [11]. Data from these assays were subjected to statistical analyses using the Student's *t*-test. P values of <0.05 represent minimal significant differences between means.

RESULTS AND DISCUSSION

Male rats placed on sucrose-based diets deficient in thiamin or containing a high concentration of thiamin show progressive changes in the hepatic enzymes involved in drug metabolism (Table 1). After 14, 16 and 21 days on a high thiamin diet, aniline hydroxylase is significantly lower than in rats receiving the thiamin-deficient diet, whereas the concentrations of cytochromes P-450 and b_5 are lower after 9, 14, 16 and 21 days on this diet. Glucose 6-phosphate dehydrogenase activity of the soluble fraction was elevated at day 14 and thereafter. The synthetic diet, containing either high or low levels of thiamin, reduces microsomal aniline hydroxylase, cytochrome P-450 and cytochrome *c* reductase compared to values obtained from rats fed lab chow until such times as signs of thiamin deficiency (inhibition of growth) are induced (14–16 days).

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Table 1. Influence of dietary thiamin on hepatic enzymes of male rats*

Enzyme	Diet†	Days on diet 5	9	14	16	21
Aniline hydroxylase (μmoles/g liver/hr)	HT	0·56 ± 0·11	0·81 ± 0·06	0·57 ± 0·05	0·92 ± 0·11	0·99 ± 0·07
	TD	0·50 ± 0·09	0·87 ± 0·09	1·32 ± 0·18‡	1·44 ± 0·12‡	2·06 ± 0·21‡
	LC	2·40 ± 0·17‡	2·48 ± 0·11‡	1·95 ± 0·12‡	2·17 ± 0·12‡	1·44 ± 0·06‡
Cytochrome P-450 (nmoles/mg protein)	HT	0·25 ± 0·02	0·17 ± 0·01	0·19 ± 0·01	0·26 ± 0·04	0·32 ± 0·02
	TD	0·25 ± 0·02	0·26 ± 0·01‡	0·43 ± 0·05‡	0·51 ± 0·05‡	0·64 ± 0·06‡
	LC	0·62 ± 0·02‡	0·44 ± 0·05‡	0·54 ± 0·04‡	0·65 ± 0·05‡	0·72 ± 0·05‡
Cytochrome b_5 (nmoles/mg protein)	HT	0·58 ± 0·04	0·32 ± 0·02	0·46 ± 0·04	0·37 ± 0·01	0·40 ± 0·02
	TD	0·55 ± 0·06	0·52 ± 0·02‡	0·73 ± 0·02‡	0·80 ± 0·07‡	1·05 ± 0·09‡
	LC	0·64 ± 0·03	0·49 ± 0·05‡	0·76 ± 0·07‡	0·63 ± 0·03‡	0·88 ± 0·02‡
NADPH cytochrome *c* reductase (nmoles reduced/mg/min)	HT	89·7 ± 17·1	58·5 ± 9·5	88·3 ± 13·3	67·9 ± 20·9	72·2 ± 10·1
	TD	73·9 ± 14·6	68·4 ± 9·4	104·8 ± 18·6	87·9 ± 21·0	106·6 ± 13·4
	LC	145·9 ± 27·7	157·7 ± 19·1‡	171·9 ± 13·0‡	215·4 ± 11·0‡	165·0 ± 9·3‡
G-6-P dehydrogenase (μmoles NADP reduced/g protein/min)	HT	55·7 ± 9·3	15·3 ± 1·7	41·1 ± 6·9	48·0 ± 6·8	71·5 ± 16·7
	TD	71·0 ± 18·6	14·7 ± 3·6	16·3 ± 4·3‡	10·1 ± 1·3‡	13·2 ± 1·9‡
	LC	23·8 ± 3·0‡	34·2 ± 3·4‡	10·1 ± 0·5‡	11·8 ± 1·1‡	7·3 ± 0·7‡

* Values are mean ± S.E.M. Number of animals per group: 5 days, $n = 6$; 9 days, $n = 7$; 14 days, $n = 7$; 16 days, $n = 6$; 21 days, $n = 9$. All animals were fed sucrose-based diets.
† HT = high thiamin; TD = thiamin deficient; LC = lab chow.
‡ Significantly different from rats fed HT diet.

Table 2. Influence of dietary thiamin on hepatic enzymes of female rats*

Enzyme	Diet†	Days on diet 5	9	14	16	21
Aniline hydroxylase (μmoles/g/liver/hr)	HT	0·67 ± 0·18	0·54 ± 0·03	1·06 ± 0·15	0·87 ± 0·09	0·89 ± 0·08
	TD	0·73 ± 0·09	0·72 ± 0·08	1·56 ± 0·29	0·89 ± 0·07	2·09 ± 0·17‡
Cytochrome P-450 (nmoles/mg protein)	HT	0·25 ± 0·01	0·14 ± 0·01	0·23 ± 0·03	0·24 ± 0·03	0·28 ± 0·03
	TD	0·24 ± 0·01	0·13 ± 0·01	0·44 ± 0·05‡	0·37 ± 0·04‡	0·62 ± 0·02‡
Cytochrome b_5 (nmoles/mg protein)	HT	0·52 ± 0·06	0·86 ± 0·04	0·61 ± 0·10	0·42 ± 0·02	0·45 ± 0·02
	TD	0·41 ± 0·05	0·99 ± 0·08	1·18 ± 0·11‡	0·68 ± 0·07‡	0·99 ± 0·01‡
NADPH cytochrome *c* reductase (nmoles reduced/mg/min)	HT	86·0 ± 17·8	43·6 ± 17·6	48·2 ± 10·6	64·6 ± 5·6	60·0 ± 14·4
	TD	46·2 ± 11·2	54·0 ± 13·6	60·4 ± 9·6	112·4 ± 13·0‡	146·4 ± 7·8‡
G-6-P dehydrogenase (μmoles NADP reduced/g protein/min)	HT	83·8 ± 8·0	61·4 ± 3·9	56·2 ± 7·8	93·7 ± 15·4	37·6 ± 3·8
	TD	79·3 ± 12·5	42·4 ± 5·1	22·6 ± 2·8‡	49·3 ± 9·6‡	11·7 ± 2·4‡

* Values are mean ± S.E.M. Number of animals per group: 5 days, $n = 3$; 9 days, $n = 3$; 14 days, $n = 6$; 16 days, $n = 6$; 21 days, $n = 5$. All animals were fed sucrose-based diets.
† HT = high thiamin; TD = thiamin deficient.
‡ Significantly different from values obtained from rats fed HT diet.

Table 3. Effect of progressive food deprivation on various liver parameters*

Period of food deprivation	Liver wt / body wt	Microsomal protein	Cytochrome P-450	Cytochrome b_5	Cytochrome *c* reductase	G-6-PD	Metabolic rate: Aniline hydroxylase	Metabolic rate: Ethylmorphine demethylase
Male rats								
6	95 ± 2	100 ± 3	90 ± 6	116 ± 5†	83 ± 5	96 ± 13	99 ± 6	114 ± 7
12	71 ± 1†	87 ± 3	140 ± 6†	112 ± 3	147 ± 17	97 ± 7	183 ± 5†	161 ± 6†
24	67 ± 1†	87 ± 2†	129 ± 6†	165 ± 7†	122 ± 12	49 ± 9†	198 ± 6†	152 ± 13
48	68 ± 1†	95 ± 2	160 ± 5†	141 ± 3†	184 ± 17†	49 ± 11†	189 ± 12†	153 ± 9†
72	67 ± 1†	77 ± 3†	179 ± 12†	170 ± 3†	137 ± 11	53 ± 3†	295 ± 29†	119 ± 6†
Female rats								
72	65 ± 4†	83 ± 1†	251 ± 21†	135 ± 12	156 ± 3†	65 ± 4†	494 ± 12†	305 ± 25†

* Expressed as percentage of the mean of three–six control rats fed a diet high in thiamin for 5 days prior to decapitation and killed at the time each group listed in this table was killed. (Each value represents mean ± S.E.M. of six rats.)
† Statistically different from fed controls.

Table 4. Influence of dietary restriction on liver and drug-metabolizing enzymes of thiamin-fed male rats*

Liver parameter	Thiamin deficient†	High thiamin pair-fed†	High thiamin *ad lib.*†
Liver wt/body wt (× 100)	4·21 ± 0·05‡ (47)	4·21 ± 0·09‡ (24)	5·10 ± 0·07 (48)
Microsomal protein (mg/g liver)	21·42 ± 0·39‡,§ (24)	23·73 ± 0·80 (14)	24·20 ± 0·34 (23)
Aniline hydroxylase (nmoles/mg protein/hr)	64·3 ± 1·8‡,‖ (24)	45·7 ± 1·9 (22)	41·6 ± 2·0 (25)
Cytochrome P-450 (nmoles/mg protein)	0·591 ± 0·031‡ (28)	0·599 ± 0·061‡ (8)	0·349 ± 0·016 (30)
Cytochrome b_5 (nmoles/mg protein)	0·920 ± 0·036‡ (6)	0·836 ± 0·061‡ (8)	0·455 ± 0·026 (7)
NADPH cytochrome *c* reductase (nmoles/mg/min)	212·3 ± 3·6‡,‖ (23)	149·4 ± 3·0 (16)	137·1 ± 3·8 (24)

* Number in parentheses = n; all animals were fed sucrose-based diets.
† All rats were fed diet for 21 days.
‡ Significantly different from rats fed high thiamin *ad lib.* ($P < 0·01$).
§ Significantly different from pair-fed rats ($P < 0·05$).
‖ Significantly different from pair-fed rats ($P < 0·01$).

Similar changes in the above parameters were observed in female rats, although they appear to develop somewhat slower than in the males (Table 2). In addition, NADPH cytochrome *c* reductase activity is enhanced in female rats fed a thiamin-deficient diet for 16 and 21 days.

Since animals on the deficient diet begin to lose weight between 14 and 21 days on regimen, the effects observed could be partially if not entirely due to food deprivation. Male rats were placed on a diet rich in thiamin and, after 5 days, food was withheld from some for 6, 12, 24, 48 and 72 hr; several parameters were then measured and compared to those of fed animals killed at the same time. These results, expressed as per cent of the mean for fed controls, appear in Table 3. Correlation coefficients (r) indicated that time of starvation has a progressive effect on cytochrome P-450 ($r = 0·92$), aniline hydroxylase ($r = 0·87$) and possibly the liver weight/body weight ratio ($r = 0·79$) and cytochrome b_5 content ($r = 0·74$); however, there appears to be little relationship between starvation and progressive change in cytochrome *c* reductase, microsomal protein content or ethylmorphine demethylase ($r = 0·08$), even though food deprivation appears to enhance ethylmorphine metabolism somewhat. One group of female rats was starved 72 hr and, as shown in Table 3, their response was essentially the same as that of male rats except that ethylmorphine demethylase was greatly enhanced by this length of starvation.

That some of the changes observed in male rats fed high or deficient thiamin diets are related to the quantity of diet ingested is again suggested by the data derived in pair-feeding experiments (Table 4). The activities of aniline hydroxylase and NADPH cytochrome *c*

Table 5. Influence of dietary restriction on liver and drug-metabolizing enzymes of thiamin-fed female rats*

Liver parameter	Thiamin deficient†	High thiamin pair-fed†	High thiamin *ad lib.*†
Liver wt/body wt (× 100)	4·12 ± 0·13‡ (13)	3·84 ± 0·14‡ (14)	5·14 ± 0·15 (15)
Microsomal protein (mg/g liver)	20·4 ± 1·2§,‖ (13)	24·4 ± 1·2 (14)	30·1 ± 3·0 (15)
Aniline hydroxylase (nmoles/mg protein/hr)	76·8 ± 7·2‡,¶ (7)	30·2 ± 6·3 (6)	25·6 ± 4·3 (7)
Ethylmorphine demethylase (nmoles/mg protein/hr)	767·5 ± 63·0‡,¶ (6)	282·5 ± 54·1 (5)	162·3 ± 87·7 (5)
Cytochrome P-450 (nmoles/mg protein)	0·334 ± 0·022‡ (5)	0·309 ± 0·016‡ (8)	0·228 ± 0·01 (8)
Cytochrome b_5 (nmoles/mg protein)	1·294 ± 0·024‡,§ (4)	0·990 ± 0·069‡ (6)	0·598 ± 0·032 (8)
NADPH cytochrome *c* reductase (nmoles/mg/min)	162·0 ± 12·8‡,¶ (6)	117·6 ± 3·3‡ (6)	87·9 ± 3·1 (5)
G-6-P dehydrogenase (μmoles NADP reduced/g protein/min)	4·7 ± 0·4‡,¶ (6)	67·7 ± 3·1‡ (6)	34·3 ± 1·7 (7)

* Numbers in parentheses = n; all animals were fed sucrose-based diets.
† All rats were fed diet for 21 days.
‡ Significantly different from rats fed high thiamin *ad lib.* ($P < 0·01$).
§ Significantly different from pair-fed rats ($P < 0·05$).
‖ Significantly different from rats fed high thiamin *ad lib.* ($P < 0·05$).
¶ Significantly different from pair-fed rats ($P < 0·01$).

Table 6. Influence of carbohydrate source on drug-metabolizing enzymes of female rats*

Parameter	Thiamin deficient (sucrose based)	Thiamin deficient (starch based)	High thiamin (sucrose based)	High thiamin (starch based)
Terminal body wt (g)	63·3 ± 2·1† (7)	66·9 ± 1·6† (8)	161·5 ± 5·6 (5)	151·3 ± 4·3 (6)
Liver wt/body wt (× 100)	4·0 ± 0·1† (7)	3·8 ± 0·1 (8)	4·7 ± 0·1‡ (5)	4·0 ± 0·1 (6)
Microsomal protein (mg/g liver)	21·0 ± 0·5§ (6)	20·4 ± 0·7† (6)	23·7 ± 1·0 (5)	25·9 ± 0·6 (6)
Cytochrome P-450 (nmoles/mg protein)	0·432 ± 0·018† (6)	0·405 ± 0·022 (7)	0·301 ± 0·026‖ (5)	0·379 ± 0·010 (6)
Cytochrome b_5 (nmoles/mg protein)	0·688 ± 0·035† (6)	0·621 ± 0·033† (7)	0·331 ± 0·015‖ (5)	0·406 ± 0·017 (6)
Cytochrome c reductase (nmoles/mg/min)	204·0 ± 14·3† (6)	207·0 ± 6·4† (7)	105·7 ± 4·3 (5)	125·0 ± 6·7 (6)
Aniline hydroxylase (nmoles/mg protein/hr)	54·8 ± 2·8† (6)	55·2 ± 1·7† (7)	18·3 ± 1·2‖ (5)	25·9 ± 1·7 (6)
Ethylmorphine demethylase (nmoles/mg protein/hr)	396·8 ± 46·5† (6)	355·1 ± 29·7† (7)	36·6 ± 5·7‖ (5)	71·6 ± 8·8 (6)
G-6-P dehydrogenase (μmoles NADP reduced/g protein/min)	19·8 ± 2·1† (6)	23·9 ± 1·9† (7)	102·0 ± 11·4‡ (5)	49·6 ± 2·8 (6)

* Number in parentheses = n.
† Significantly different from values derived from animals fed high thiamin diet of same carbohydrate base ($P < 0·01$).
‡ Significantly different from values derived from animals fed same vitamin level but corn starch base ($P < 0·01$).
§ Significantly different from values derived from animals fed high thiamin diet of same carbohydrate base ($P < 0·05$).
‖ Significantly different from values derived from animals fed same vitamin level but corn starch base ($P < 0·05$).

reductase are clearly altered by the thiamin in the diet and not by the ingestion of large amounts of carbohydrate, whereas the content of cytochrome P-450 and cytochrome b_5 may be influenced primarily by the amount of carbohydrate ingested. Although microsomal protein is greater in both groups eating the diet rich in thiamin, only the group fed the thiamin-rich diet *ad lib.* had increased liver weight to body weight ratios. This enlargement of the liver could be due to the presence of extra lipid and glycogen.

In the female rat it is again clearly demonstrated that high thiamin consumption in pair-fed rats is responsible for depressing aniline hydroxylase activity (Table 5). Ethylmorphine demethylase was included in these experiments and its activity also was depressed by thiamin. Unlike the male, cytochrome b_5 content in the female is depressed by thiamin in pair-fed animals, although not to the same extent as in those fed the diet *ab lib.* Again pair-feeding of a diet rich in thiamin significantly reduced the activity of NADPH cytochrome *c* reductase, while glucose 6-phosphate dehydrogenase activity was elevated in a manner similar to that found in rats fed the high thiamin diet *ad lib.*

There are reports that high concentrations of dietary sucrose and monosaccharides such as glucose depress barbiturate metabolism in mice without affecting aniline hydroxylase [12, 13] and that these carbohydrates, when fed to rats, increase liver weight and decrease some drug-metabolizing enzymes and cytochrome P-450 in comparison to rats fed similar quantities of starch [14]. As shown in Tables 4, 5 and 6, liver weight as per cent of body weight increased in both male and female rats when fed high levels of thiamin *ab lib.* in the sucrose-based diet, but liver weights were not elevated in animals fed this level of thiamin in the starch-based diet or when the sucrose intake was limited by pair-feeding. In starch-fed male rats there was no significant alteration in microsomal protein induced by thiamin; however, high thiamin ingestion in female rats significantly elevated microsomal protein per gram of liver (Tables 5 and 6).

Cytochrome P-450 content of hepatic microsomes is

Table 7. Influence of carbohydrate source on drug-metabolizing enzymes in male rats*

Parameter	Thiamin deficient (sucrose based)	Thiamin deficient (starch based)	High thiamin (sucrose based)	High thiamin (starch based)
Terminal body wt (g)	85·1 ± 1·9† (11)	75·8 ± 3·6† (14)	164·3 ± 4·9 (12)	175·7 ± 9·0 (12)
Liver wt/body wt (× 100)	4·13 ± 0·08† (11)	3·81 ± 0·12 (14)	4·83 ± 0·09‡ (12)	4·18 ± 0·13 (12)
Microsomal protein (mg/g liver)	17·6 ± 1·1 (6)	18·9 ± 0·9 (6)	20·7 ± 1·1 (6)	24·4 ± 2·3 (6)
Cytochrome P-450 (nmoles/mg protein)	0·734 ± 0·039† (10)	0·698 ± 0·027 (7)	0·506 ± 0·041 (12)	0·611 ± 0·038 (12)
Cytochrome b_5 (nmoles/mg protein)	0·845 ± 0·051† (10)	0·751 ± 0·033† (9)	0·431 ± 0·038 (12)	0·539 ± 0·048 (12)
Cytochrome c reductase (nmoles/mg/min)	189·6 ± 4·4†,‡ (5)	224·5 ± 5·9† (6)	129·0 ± 6·7 (6)	146·8 ± 4·9 (6)
Aniline hydroxylase (nmoles/mg/hr)	78·3 ± 9·8† (10)	73·4 ± 4·3† (11)	36·6 ± 3·0‡ (12)	52·7 ± 3·4 (12)
Ethylmorphine demethylase (nmoles/mg/hr)	445·9 ± 23·2† (10)	488·7 ± 26·8† (10)	313·5 ± 11·3§ (12)	354·8 ± 11·3 (10)
G-6-P dehydrogenase (μmoles NADP reduced/g protein/min)	22·9 ± 1·7† (6)	29·1 ± 2·3† (6)	99·1 ± 4·1‡ (6)	64·5 ± 6·6 (6)

* Number in parentheses = n.
† Significantly different from values derived from animals fed high thiamin diet of same carbohydrate base ($P < 0·01$).
‡ Significantly different from values derived from animals fed same vitamin level but corn starch base diet ($P < 0·01$).
§ Significantly different from values derived from animals fed same vitamin level but corn starch base diet ($P < 0·05$).

significantly depressed in rats fed high thiamin in sucrose-based diets, but is not significantly depressed in either male or female rats fed high levels of thiamin in starch-based diet (Tables 6 and 7). This further supports the finding that sucrose, not thiamin, is responsible for depressing cytochrome P-450. Depression of cytochrome b_5, on the other hand, occurs in rats fed high levels of thiamin whether fed the sucrose- or starch-based diet.

NADPH cytochrome *c* reductase in both male and female rats is depressed significantly by feeding high levels of thiamin in either starch- or sucrose-based diets. Since it is depressed approximately equally in the pair-fed male rats receiving high levels of thiamin, this effect appears to be due to the thiamin ingestion rather than carbohydrate. In the female, NADPH cytochrome *c* reductase depression may be caused by both thiamin and sucrose (Tables 5 and 6).

Aniline hydroxylase and ethylmorphine demethylase activities are lower in both starch- and sucrose-based high thiamin diets in male and female rats than in those on the thiamin-deficient diets (Tables 6 and 7). Sucrose-based high thiamin diets, however, appear to depress both pathways more than starch-based diets. The effect appears to be mediated through thiamin ingestion, since it is also evident in the animals pair-fed high levels of thiamin.

Glucose 6-phosphate dehydrogenase activity of the 105,000 *g* supernatant is consistently elevated in animals fed high levels of thiamin. The fact that it is also elevated in those rats pair-fed the same quantity of sucrose as the deficient animals raises the question of carbohydrate absorption in thiamin deficiency states. It has been reported that thiamin deficiency states result in reduced carbohydrate absorption from the gastrointestinal tract [15]. Although the evidence gathered in these experiments strongly suggests that drug metabolism and cytochrome *c* reductase activity are depressed by high thiamin ingestion, another factor which might affect these drug-metabolizing enzymes is the quantity of carbohydrate actually absorbed from the gastrointestinal tract. The findings that glucose ingestion by mice reduces the hexobarbital metabolic rate [13] and perhaps prolongs barbiturate sleep time [12] while not altering aniline hydroxylase or cytochrome *c* reductase activity [13], and that sucrose, glucose or fructose fed to rats may depress cytochrome P-450 and biphenyl 4-hydroxylase [14] support the concept that some components of the drug-metabolizing enzyme system may be altered selectively by quantity or quality of carbohydrate. However, the fact that rats fed high thiamin diets for 14 days exhibit less ability to metabolize aniline even though they have not yet begun to lose weight supports the findings from pair-feeding experiments that thiamin, not carbohydrate, is responsible for depressing this drug-metabolizing function.

Results from these and other studies indicate that correlation between drug-metabolizing activity and concentration of cytochrome P-450 does not always exist. The type of cytochrome P-450 formed during various dietary states may provide an explanation for the differences in drug metabolism by male and female rats. Wade *et al.* [3] reported that thiamin deficiency states in male rats induce a cytochrome P-450 with spectral binding characteristics similar to those of the cytochrome P_1-450 of Sladek and Mannering [16]; however, this does not appear to occur in the female (unpublished results). Cytochrome P_1-450 does not appear to be involved in hexobarbital metabolism; thus thiamin deficiency states in the male produce minimal effects on hexobarbital metabolism. Thiamin deficiency in female rats reduces hexobarbital sleep time [2] without altering this in the male.

Since pair-feeding experiments suggest that drug metabolism is depressed by thiamin ingestion and that the concentration of cytochrome P-450 is depressed by sucrose ingestion, the site of thiamin interaction may not involve synthesis or maintenance of total cytochrome P-450. Several alternatives for thiamin interaction appear possible: (1) thiamin may interact with a component such as cytochrome *c* reductase to inhibit its normal action on substrate; (2) thiamin, by enhancing carbohydrate absorption from the gastrointestinal tract, may shift biochemical reactions away from drug hydroxylation to carbohydrate utilization and storage; (3) thiamin may interfere with substrate binding to the terminal oxidase; or (4) thiamin may induce alteration in the biochemical composition of the hepatic endoplasmic reticulum or in its physical conformation.

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