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Studies on Certain Drug-Metabolizing Enzymes in Deoxypyridoxine-Treated Rats

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Abstract—The effect of deoxypyridoxine on the activities of drug-metabolizing enzymes was investigated in male rats. Phenylbutazone oxidase and aminopyrine N-demethylase decreased in both liver and kidney of deoxypyridoxine-treated rats that received either an 18% or 8% casein diet. However, the magnitude of decrease in activities was more when the rats received an 8% casein diet. The NADPH oxidase activity remained unchanged following deoxypyridoxine treatment. The diminished activities of phenylbutazone oxidase and aminopyrine N-demethylase noted after deoxypyridoxine treatment were restored by pyridoxine supplementation. The decreased activities of drug-metabolizing enzymes in deoxypyridoxine treated rats were not reversed by thyroxine supplementation. It is suggested that pyridoxine in the form of pyridoxal phosphate might be involved in the regulation of drug-metabolizing activities.

The metabolism of drugs has been shown to be markedly influenced by various factors such as age, sex, strain, species, stress, hormones, general nutritional status, as well as by vitamins (1, 2). Studies so far done with respect to vitamins showed that ascorbic acid, riboflavin and α-tocopherol play a role in the drug-metabolizing systems. Thus, investigations by several workers demonstrated that ascorbic acid deficiency results in a decrease in the metabolism of a variety of pharmacological agents (2) and steroids (3). Ascorbic acid deficiency also decreased the quantities of hepatic microsomal electron transport components which are involved in the metabolism of drugs and ascorbic acid supplements increased the quantities of such components and overall drug metabolism activities (4). Riboflavin deficiency produced in adult rats was also found to cause a significant decrease in certain drug metabolism activities (5). This diminished drug metabolism activity in riboflavin deficiency was also found to be accompanied by a decrease in the quantity of microsomal electron transport components (5). Likewise it was found that vitamin E deficiency has a depressing effect on hepatic drug metabolism (4, 6–8). However, very little information is available regarding the impact of vitamin B_6 on drug metabolism. Vitamin B_6 in the form of pyridoxal phosphate participates in a variety of enzymatic reactions involving transfer of groups (9). Moreover, dietary lack of pyridoxine reduces the body resistance to infections and also decreases the immunoglobulin formation (10–12). It is, therefore, worthwhile to note the status of drug-metabolizing enzyme systems in pyridoxine deficiency, and accordingly, the activities of certain drug-metabolizing enzymes were studied in deoxypyridoxine-treated rats.

Materials and Methods

Animals and diets

Male Wistar rats, 60 days old, were divided into four groups of equal average body weight. The animals of one of the groups received the 18% casein diet, while those of another group received the 18% casein diet containing 2 mg % deoxypyridoxine, an antagonist of vitamin B_6. The animals of one of the remaining groups were

fed on an 8% casein diet, while those of the other group received the 8% casein diet containing 2 mg % deoxypyridoxine. The 8% casein diet was made isocaloric by raising proportionally the dietary level of carbohydrate. The other ingredients of the diets were the same as reported elsewhere (13–15). Fat-soluble and water-soluble vitamins were furnished in the diet according to Berg (16). The animals receiving the 18% or 8% casein diet served as pair-fed controls for the corresponding deoxypyridoxine-ingested groups. The animals were maintained on these dietary regimens for 28 days.

In another set of experiments, the rats receiving the 18% casein diet with or without deoxypyridoxine were supplemented with pyridoxine at a dose of 0.5 mg/100 g body weight per day for 5 days prior to sacrifice.

Yet for another set of experiments, rats receiving the 18% casein diet with or without deoxypyridoxine were maintained for 28 days, and some of the rats receiving deoxypyridoxine were injected intraperitoneally with L-thyroxine at a dose of 50 μg/100 g body weight per day for 5 days prior to sacrifice.

After the experimental period was over, the animals were fasted overnight and sacrificed under light ether anesthesia. Blood was removed from the hepatic vein by means of a syringe. Liver and kidney tissues were excised, chilled in ice and deep-frozen until analysis.

Analytical procedure

Of the drug-metabolizing enzymes, NADPH and phenylbutazone oxidase activities were assayed in the microsomes isolated from the tissues, while the activity of aminopyrine demethylase was estimated in the 9000 g supernatant.

Isolation of microsomes: A 10% (W/V) homogenate of each of liver and kidney tissues was prepared in 0.05M Tris-HCl buffer containing 0.25 M sucrose at pH 7.4, using an all-glass homogenizer at 2–4°C. Microsomes were isolated from tissue homogenates by differential centrifugation in a preparative ultracentrifuge according to the method of Schneider and Hogeboom (17).

Assay of NADPH oxidase activity: The NADPH oxidase activity in the microsomes was assayed by the methods of Gillette et al. (18) and Conney and Burns (19) with slight modification.

Determination of phenylbutazone oxidase activity: The phenylbutazone oxidase activity in the microsomes was measured by the method described by Burns et al. (20), and the mixture of co-factors was included in the reaction medium incubated for 1 hr according to Conney et al. (21).

Determination of aminopyrine N-demethylase activity: A 10% (W/V) homogenate of each of liver and kidney tissues was prepared in 0.1M Tris buffer, pH 7.5, at 2–4°C. The homogenate was centrifuged at 9000 g to collect the supernatant. This supernatant was used for assaying the activity of aminopyrine N-demethylase according to the method of Gram et al. (22), and the formaldehyde formed as a result of the enzyme reaction for 1 hr was estimated by the method of Nash (23).

Determination of pyridoxal phosphate content of tissues: The pyridoxal phosphate content of liver and kidney was estimated by the method as reported elsewhere (24).

Determination of proteins: The protien contents of microsomal suspensions and supernatant fractions used for assaying of enzyme activity were estimated by the biuret reagent (25).

Results

As shown in Table 1, deoxypyridoxine-treated rats had a reduced level of pyridoxal phosphate in both liver and kidney.

The results presented in Tables 2 and 3 demonstrate that the phenylbutazone oxidase activity of both liver and kidney decreased significantly in deoxypyridoxine-treated rats that received either the 18% or 8% casein diet. The aminopyrine N-demethylase activity of both liver and kidney was also found to be diminished in deoxypyridoxine-treated rats, whether they received the 18% or 8% casein diet. However, the percentage decrease in the activity of these enzymes in deoxypyridoxine-treated rats was found to be more when the animals were fed on an 8% casein diet. The NADPH oxidase activity of neither liver nor kidney was affected in deoxypyridoxine-treated rats whether they were fed on an 18% or 8% casein diet.

Table 4 shows that the diminished activities of phenylbutazone oxidase as well as aminopyrine N-demethylase of both liver and kidney in deoxypyridoxine-treated rats were restored by pyridoxine supplementation.

The results presented in Table 5 demonstrate that thyroxine supplementation failed to restore the phenylbutazone oxidase and aminopyrine N-demethylase activities in deoxypyridoxine-treated rats.

Table 1. Effect of pyridoxine deficiency on pyridoxal phosphate content of tissues on an 18% casein diet

(The values are means±S.E.M.)

Dietary conditions	Pyridoxal phosphate content (μg/g)	
	Liver	Kidney
Pair-fed control (6)	6.90±0.31	5.80±0.23
Pyridoxine-deficient (Deoxypyridoxine-supplemented diet) (6)	3.80±0.40 P<0.001	3.30±0.36 P<0.001

The figures in the parentheses indicate the number of animals.

Table 4. Effect of pyridoxine supplementation on drug-metabolizing enzymes in pyridoxine-deficient rats

(The values are means±S.E.M.)

Dietary conditions	Phenylbutazone oxidase activity*		Aminopyrine N-demethylase activity+	
	Liver	Kidney	Liver	Kidney
Pair-fed control (6)	30.2±3.4	23.4±2.6	2.58±0.30	2.28±0.15
Pyridoxine-deficient (Deoxypyridoxine ingested) (6)	18.4±2.3 P^a<0.02	13.8±1.3 P^a<0.01	1.62±0.11 P^a<0.05	1.14±0.12 P^a<0.001
Pyridoxine-supplemented Pyridoxine deficient (7)	28.8±2.6 P^b<0.05	21.9±1.9 P^b<0.01	2.46±0.16 P^b<0.01	2.08±0.22 P^b<0.01

The figures in the parentheses indicate the number of animals. [a]=Compared with pair-fed control group. [b]=Compared with pyridoxine-deficient group. *μg of phenylbutazone oxidized/hr/mg protein. +μg HCHO formed/hr/mg protein.

Table 5. Activities of drug-metabolizing enzymes in thyroxine-supplemented pyridoxine-deficient rats receiving an 8% casein diet

(The values are means±S.E.M.)

Group of animals	Phenylbutazone oxidase activity*		Aminopyrine N-demethylase activity**	
	Liver	Kidney	Liver	Kidney
Pair-fed control (6)	13.7±3.2	10.1±2.0	2.52±0.42	1.79±0.18
Pyridoxine deficient (Deoxypyridoxine treated) (6)	5.96±1.2 P^a<0.05	4.72±0.66 P^a<0.05	1.05±0.16 P^a<0.01	0.67±0.05 P^a<0.001
Pyridoxine-deficient +Thyroxine 6)	6.12±1.9	5.06±0.98	1.16±0.13	0.72±0.07

The figures in the parentheses indicate the number of animals. [a]=Compared with pair-fed control group. *μg of phenylbutazone oxidized/hr/mg protein. **μg HCHO formed/hr/mg protein.

Table 2. Effect of pyridoxine deficiency on drug-metabolizing enzymes in rats receiving an 18% casein diet
(The values are means±S.E.M.)

Dietary conditions	Phenylbutazone oxidase activity*		NADPH oxidase activity**		Aminopyrine N-demethylase activity+	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Pair-fed control (6)	32.6±1.9	24.4±2.6	3.42±0.42	2.98±0.16	3.12±0.18	2.46±0.24
Pyridoxine-deficient	22.3±2.4	17.5±1.2	3.18±0.17	2.86±0.30	2.02±0.09	1.66±0.12
(Deoxypyridoxine-supplemented diet) (6)	$P<0.01$	$P<0.05$			$P<0.001$	$P<0.02$

The figures in the parentheses indicate the number of animals. *μg of phenylbutazone oxidized/hr/mg protein. **OD/min/100 mg protein. +μg HCHO formed/hr/mg protein.

Table 3. Effect of pyridoxine deficiency on drug-metabolizing enzymes in rats receiving an 8% casein diet
(The values are means±S.E.M.)

Dietary conditions	Phenylbutazone oxidase activity*		NADPH oxidase activity**		Aminopyrine N-demethylase activity+	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Pair-fed control (6)	28.7±2.8	22.3±2.5	2.84±0.62	2.12±0.50	2.22±0.30	1.66±0.24
Pyridoxine deficient	16.6±1.3	12.7±1.5	2.68±0.23	1.96±0.32	0.98±0.11	0.58±0.08
(Deoxypyridoxine-supplemented diet) (6)	$P<0.01$	$P<0.01$			$P<0.01$	$P<0.01$

The figures in the parentheses indicate the number of animals. *μg of phenylbutazone oxidized/hr/mg protein. **OD/min/100 mg protein. +μg HCHO formed/hr/mg protein.

Discussion

Nutritional deficiency caused by inadequate intake of protein was demonstrated to have a modifying effect on the response to pharmacological activities of drugs and foreign compounds (26). This is due in part to altered rates of metabolism (26–28). It was further demonstrated that the decreased metabolism of frugs and foreign compounds in protein deficiency is caused mostly by a reduction in the activities of specific drug-metabolizing enzymes (26–30). There is also increasing evidence that the activities of microsomal drug-metabolizing enzymes are affected by certain vitamins such as ascorbic acid, riboflavin and α-tocopherol (4). In ascorbic acid deficiency, it was found that there is a decrease in the quantity of hepatic microsomal electron transport components such as cytochrome P-450 and NADPH-cytochrome reductase, as well as decreases in a variety of drug enzyme reactions such as N-demethylation, O-demethylation and steroid hydroxylation (4, 31). It was also noted that prolonged riboflavin deficiency caused a significant decrease in drug metabolism activities with substrates such as aniline, acetanilide, aminopyrine and ethylmorphine (5). With riboflavin deficiency, there were also substantial decreases in microsomal electron transport components (4, 5).

In the present investigation, pyridoxine deficiency caused by ingestion of deoxypyridoxine resulted in diminished activities of phenylbutazone oxidase and aminopyrine N-demethylase of both liver and kidney. This indicates that lack of pyridoxine affects the metabolism of phenylbutazone and aminopyrine. The magnitude of reduction in the metabolism of these drugs was found to be greater when pyridoxine deficiency was produced in rats receiving a protein-deficient (8% casein) diet. This might be ascribed to a greater depletion of pyridoxine in deoxypyridoxine-ingested rats receiving a protein-deficient diet. The diminished activities of phenylbutazone oxidase and aminopyrine N-demethylase in deoxypyridoxine-ingested rats were not accompanied by a decreased activity of NADPH oxidase. This indicates that the effect of lack of pyridoxine on phenylbutazone oxidase and aminopyrine N-demethylase activities was not mediated through influence on NADPH oxidase activity.

Thyroxine has been known to influence the activity of certain drug-metabolizing enzymes (32–35), but the present investigation revealed that thyroxine supplementation to deoxypyridoxine-ingested rats failed to reverse the change in phenylbutazone oxidase and aminopyrine N-demethylase activities. This suggests that the deficiency of thyroid hormone could not be implicated for the decreased activity of phenylbutazone oxidase and aminopyrine N-demethylase in pyridoxine deficiency as produced by ingestion of deoxypyridoxine.

It is, therefore, possible that pyridoxine in the form of pyridoxal phosphate might be involved in one or more of the steps of enzymatic reactions involving metabolism of phenylbutazone and aminopyrine.

The possible involvement of pyridoxal phosphate-dependent enzymes in the metabolism of diphenylhydantoin and phenobarbital, as suggested by Hansson and Sillanpaa (36), might bear a relevance to the present study.

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References

1 **Kato, R.**: Drug metabolism in pathological and abnormal physiological states in animals and man. Xenobiotica **7**, 25–93 (1977)

2 **Zannoni, V.G. and Lynch, M.M.**: Role of ascorbic acid in drug metabolism. Drug Metab. Rev. **2**, 57–69 (1973)

3 **Ginter, E.**: Cholesterol: Vitamin C controls its transformation to bile acids. Science **179**, 702–704 (1973)

4 **Zannoni, V.G. and Sato, P.H.**: The effect of certain vitamin deficiencies on hepatic drug metabolism. Fed. Proc. **35**, 2464–2469 (1976)

5 **Patel, J.M. and Powar, S.S.**: Riboflavin and drug metabolism in adult male and female rats. Biochem. Pharmacol. **23**, 1467–1477 (1974)

6 **Carpenter, M.P. and Howard, C.N., Jr.**: Vitamin

E, steroids and liver microsomal hydroxylations. Am. J. Clin. Nutr. **27**, 966–979 (1974)
7 **Diplock, A.T.**: Possible stabilizing effect of vitamin E on microsomal, membrane bound, selenide containing proteins and drug metabolizing enzyme system. Am. J. Clin. Nutr. **27**, 995–1004 (1974)
8 **Murty, H.S., Caasi, P.I., Brooks, S.K. and Nair, P.P.**: Biosynthesis of heme in the vitamin E deficient rat. J. Biol. Chem. **245**, 5498–5504 (1970)
9 **Sauberlich, H.E.**: Biochemical systems and biochemical detection of deficiency. *In* The Vitamins, Edited by Sebrell, W.H., Jr. and Harris, R.S., Vol. **2**, p. 44–80, Academic Press, New York and London (1968)
10 **Axelrod, A.E. and Hopper, S.**: Effect of pantothenic acid, pyridoxine and thiamin deficiencies upon antibody formation to influenza virus PR-8 in rats. J. Nutr. **72**, 325–330 (1960)
11 **Axelrod, A.E., Hopper, S. and Long, D.A.**: Effect of pyridoxine deficiency upon circulating antibody formation and skin hyposensitivity reactions to diptheria toxoid in guinea pigs. J. Nutr. **74**, 58–64 (1961)
12 **Axelrod, A.E. and Trakatellis, A.C.**: Relationship of pyridoxine to immunological phenomena. *In* Vitamins and Hormones, Edited by Harris, R.S., Wool, I.G. and Loraine, J.A., Vol. **22**, p. 591–607, Academic Press, New York and London (1964)
13 **Chatterjee, A.K., Basu, J., Datta, S.C., Sengupta, K. and Ghosh, B.B.**: Effect of L-lysine administration on certain aspects of ascorbic acid metabolism. Int. J. Vitam. Nutr. Res. **46**, 286–290 (1976)
14 **Chatterjee, A.K., Datta, S.C. and Ghosh, B.B.**: Effects of emetine on the metabolism of ascorbic acid. Br. J. Pharmacol. **44**, 810–813 (1972)
15 **Datta, S.C., Chatterjee, A.K. and Ghosh, B.B.**: Metabolism of ascorbic acid in emetine-treated rats. J. Pharm. Pharmacol. **26**, 547–548 (1974)
16 **Berg, B.N.**: Maintenance of pregnancy in protein deprived rats by transitory protein supplements during early gestation. J. Nutr. **92**, 66–70 (1967)
17 **Schneider, W.C. and Hogeboom, G.H.**: Intracellular distribution of enzymes. V. Further studies on the distribution of cytochrome C in rat liver homogenates. J. Biol. Chem. **183**, 123–128 (1950)
18 **Gillette, J.R., Brodie, B.B. and LaDu, B.N.**: The oxidation of drugs by liver microsomes: On the role of TPNH and oxygen. J. Pharmacol. Exp. Ther. **119**, 532–540 (1957)
19 **Conney, A.H. and Burns, J.J.**: Induced synthesis of oxidative enzymes in liver microsomes by polycyclic hydrocarbons and drugs. *In* Advances in Enzyme Regulation, Edited by Weber, G., Vol. **1**, p. 189–214, Pergamon Press, Oxford (1963)
20 **Burns, J.J., Rose, R.K., Chenkin, T., Goldman, A.S., Schulert, A. and Brodie, B.B.**: The physiological disposition of phenylbutazone (butazolidin) in man and a method for its estimation in biological material. J. Pharmacol. Exp. Ther. **109**, 346–357 (1953)
21 **Conney, A.H., Davison, C., Gastel, R. and Burns, J.J.**: Adaptive increases in drug-metabolizing enzyme induced by phenobarbital and other drugs. J. Pharmacol. Exp. Ther. **130**, 1–8 (1960)
22 **Gram, T.E., Guarino, A.M., Green, F.E., Gigon, P.L. and Gillette, J.R.**: Effect of partial hepatectomy on the responsiveness of microsomal enzymes and cytochrome P-450 to phenobarbital or 3-methylcholanthrene. Biochem. Pharmacol. **17**, 1769–1778 (1968)
23 **Nash, T.**: The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J. **55**, 416–421 (1953)
24 **Chatterjee, A.K.**: Studies on the involvement of testicular hormone in the regulation of pyridoxal phosphate formation by the kidney tissue. Endocrinology **90**, 880–884 (1972)
25 **Gornall, A.G., Bardawill, C.J. and David, M.M.**: Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177**, 751–766 (1949)
26 **Campbell, T.C. and Hayes, J.R.**: Role of nutrition in the drug metabolizing enzyme system. Pharmacol. Rev. **26**, 71–97 (1974)
27 **Hayes, J.R. and Campbell, T.C.**: Effect of 3-methylcholanthrene induction on activity and binding kinetics. Biochem. Pharmacol. **23**, 1721–1731 (1974)
28 **Mgbodile, M.U.K., Hayes, J.R. and Campbell, T.C.**: Effect of protein deficiency on the inducibility of the hepatic microsomal drug-metabolizing enzyme system. II. Effect on enzyme kinetics and electron transport system. Biochem. Pharmacol. **22**, 1125–1132 (1973)
29 **Campbell, T.C. and Hayes, J.R.**: The effect of quantity and quality of dietary protein on drug metabolism. Fed. Proc. **35**, 2470–2474 (1976)
30 **Kato, N., Tani, T. and Yoshida, A.**: Effect of dietary level of protein on liver microsomal drug metabolizing enzymes, urinary ascorbic acid and lipid metabolism in rats fed PCB-containing diets. J. Nutr. **110**, 1686–1694 (1980)
31 **Sato, P.H. and Zannoni, V.G.**: Stimulation of drug metabolism by ascorbic acid in weanling guinea pigs. Biochem. Pharmacol. **23**, 3121–3128 (1974)

32 **Kato, R. and Takahashi, A.**: Thyroid hormone and activities of drug-metabolizing enzymes and electron transport systems of rat liver microsomes. Mol. Pharmacol. **4**, 109–120 (1968)

33 **Kato, R., Takahashi, A. and Omori, Y.**: Effect of thyroxine and thyroidectomy on the hydroxylation of testosterone by liver microsomes from male and female rats. Biochim. Biophys. Acta **208**, 116–124 (1970)

34 **Paul, S., Rifkind, A.B. and New, M.I.**: Changes in drug metabolism in children with thyroid disorders. J. Clin. Endocrinol. Metab. **42**, 155–159 (1976)

35 **Rumbaugh, R.C., Kramer, R.E. and Colby, H.D.**: Dose dependent actions of thyroxine on hepatic drug metabolism in male and female rats. Biochem. Pharmacol. **27**, 2027–2031 (1978)

36 **Hansson, O. and Sillanpaa, M.**: Pyridoxine and serum concentration of phenytoin and phenobarbitone. Lancet **1**, 256 (1976)