

# Effect of Pyridoxine Administration on the Urinary Excretion of Oxalic Acid, Pyridoxine, and Related Compounds in Mongoloids and Nonmongoloids

STANLEY N. GERSHOFF, PH.D.,\* AGNES L. MAYER, S.B.,† AND LUCAS L. KULCZYCKI, M.D.‡

THE RESULTS of two unrelated studies in this laboratory stimulated the present investigation. Gershoff *et al.*<sup>1</sup> have reported evidence suggesting the possibility of a methylating defect in mongoloids. In this same study a slight but statistically significant decrease in xanthurenic acid excretion following a tryptophan load test was observed in mongoloids as compared to nonmongoloid controls.

In another study Gershoff *et al.*<sup>2</sup> observed that in vitamin B<sub>6</sub>-deficient cats large quantities of oxalic acid were excreted in the urine, which resulted in severe kidney damage. In the present work the effect of load tests of pyridoxine and folic acid in mongoloids and non-mongoloids has been studied.

## EXPERIMENTAL

The subjects used in these experiments were either mongoloid or mentally deficient patients without other obviously abnormal characteristics. They ranged in age from 15 to 23 years. All of them lived in the same dormitory at the

From the Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts, and the Massachusetts Department of Mental Health, Wrentham State School, Wrentham, Massachusetts.

\* Assistant Professor of Nutrition; † Research Fellow in Nutrition, Department of Nutrition, Harvard School of Public Health; ‡ Assistant in Pediatrics, Department of Pediatrics, Harvard Medical School.

This work was supported in part by grants-in-aid from the National Institute of Arthritis and Metabolism (Grant No. A-1540) Public Health Service, National Institutes of Health, Bethesda, Maryland, and the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.

Wrentham State School and ate in the same dining room. Urine collections were made between 9:00 P.M. and 6:00 A.M. Vitamin loads were administered orally just prior to bedtime at 9:00 P.M. During collection periods the subjects were under continuous supervision to assure complete collections of urine. In the first study a set of control urines was obtained and the next night urines were collected after the administration of 10 mg pyridoxine hydrochloride and 5 mg folic acid. In a second study conducted six months later, using many of the same subjects, two sets of control urines were obtained and a load test of 20 mg of pyridoxine hydrochloride was given on the third evening.

In the first study the urines were analyzed for creatinine,<sup>3</sup> pyridoxic acid,<sup>4</sup> free and total vitamin B<sub>6</sub>,<sup>5,6</sup> oxalic acid,<sup>7</sup> folic acid,<sup>8</sup> and inorganic sulfate.<sup>9</sup> Analyses for pyridoxic acid and oxalic acid were done on the urines collected in the second study.

## RESULTS

The results of these experiments are summarized in Table I. In both experiments urinary oxalate decreased following the administration of the vitamin loads. The mean values obtained in the mongoloids and nonmongoloids were not significantly different. Following the administration of 10 mg of pyridoxine and 5 mg of folic acid, all 24 subjects showed a decreased oxalate excretion which averaged 58 per cent and was statistically significant ( $p < .001$ ) by the *t* test. In the second study the feeding of 20 mg of pyridoxine was followed by

TABLE I  
The Effect of Pyridoxine and Folic Acid on Urinary Metabolites of Mongoloids and Nonmongoloids

	Mongoloids		Nonmongoloids	
	Before load	After load	Before load	After load
	NO. SUBJECTS = 12		NO. SUBJECTS = 12	
<i>Pyridoxine</i> 10 mg				
<i>Folic acid</i> 5 mg				
Creatinine (mg)	514 ± 65	451 ± 39	438 ± 32	411 ± 36
Pyridoxic acid (mg)	0.91 ± .10	3.22 ± .21	1.23 ± .13	2.72 ± .21
B <sub>6</sub> , total (μg)	95 ± 7	257 ± 27	88 ± 9	381 ± 43
B <sub>6</sub> , free (μg)	24 ± 3	91 ± 10	36 ± 5	145 ± 20
Inorganic sulfate S (mg)	258 ± 27	310 ± 31	291 ± 28	271 ± 28
Folic acid (μg)	3.0 ± .2	641 ± 83	3.1 ± .3	612 ± 68
Oxalic acid (mg)	12.4 ± 1.4	4.7 ± .6	12.1 ± .9	5.5 ± .8
	NO. SUBJECTS = 10		NO. SUBJECTS = 12	
<i>Pyridoxine</i> 20 mg				
Pyridoxic acid (mg)	1.26 ± .15	6.96 ± .42	1.19 ± .08	5.08 ± 1.02
Oxalic acid (mg)	11.7 ± .12	9.4 ± .8	11.8 ± .8	8.4 ± 1.3

All values include standard errors of the mean.

Vitamin B<sub>6</sub> values are expressed as pyridoxine hydrochloride.

decreased oxalate excretion in 18 of the 22 subjects. The average decrease of the entire group was 25 per cent and was statistically significant ( $p < .005$ ).

Differences in the excretion of vitamin B<sub>6</sub> and its metabolites by mongoloids and non-mongoloids following the load tests were observed. The differences in the pyridoxic acid and vitamin B<sub>6</sub> values in the urines before and after the load tests gives an estimate of the recovery of the vitamin. In the first test non-mongoloids excreted  $14.9 \pm 2.0$  per cent\* and mongoloids  $23.1 \pm 1.2$  per cent\* of the administered 10 mg of vitamin B<sub>6</sub> as pyridoxic acid in 9 hours. These values are significantly different ( $p < .01$ ).

In the second study nonmongoloids excreted  $19.5 \pm 5.2$  per cent and mongoloids  $28.6 \pm 2.0$  per cent of the 20 mg vitamin B<sub>6</sub> load as pyridoxic acid. The data obtained on the nonmongoloids included one excessively high value of 66.1 per cent. If this value was not included in the calculations the recovery of vitamin B<sub>6</sub> as pyridoxic acid in the non-mongoloids would have been  $15.2 \pm 3.3$  per cent and the differences in the recovery values would again yield a  $p$  value of less than .01.

The urinary excretion of free and total vita-

min B<sub>6</sub> unlike pyridoxic acid was greater in the nonmongoloids than mongoloids. Differences in free and total vitamin B<sub>6</sub> excretion between control urines and urines obtained following the 10 mg pyridoxine load were  $67 \pm 10$  and  $162 \pm 32$  μg, respectively, for mongoloids and  $109 \pm 19$  μg and  $294 \pm 40$  μg for nonmongoloids.

There were no significant differences in urinary folic acid and inorganic sulfate excretions between mongoloids and nonmongoloids.

#### DISCUSSION

The most striking observations in these studies were the difference in the excretion of vitamin B<sub>6</sub> and pyridoxic acid between mongoloids and nonmongoloids and the marked and rapid effect of vitamin B<sub>6</sub> in decreasing urinary oxalate, most of which is generally thought to originate from exogenous food sources.

All of the subjects, both mongoloid and non-mongoloid, are presumed to have been receiving adequate dietary vitamin B<sub>6</sub> and presented none of the known symptoms of vitamin B<sub>6</sub> deficiency. Although determinations of vitamin B<sub>6</sub> metabolites in the urine are not considered adequate tests of vitamin B<sub>6</sub> nutrition,<sup>10</sup> they may be used to approximate roughly the level of dietary pyridoxine. Linkswiler and Reynolds<sup>11</sup> found that in three subjects consuming 2.65 to 3.21 mg of vitamin B<sub>6</sub> per day an

\* All values include standard errors of the mean.

average of 140  $\mu\text{g}$  of vitamin B<sub>6</sub> and 3.54 mg of pyridoxic acid were excreted in the urine daily. Sarett<sup>12</sup> has reported that in his experiments and those of others, humans eating self-selected diets excreted an average of slightly more than 3 mg of urinary pyridoxic acid daily. Calculating the nine hour urinary excretion values of vitamin B<sub>6</sub> and pyridoxic acid observed in control urines in these experiments on a 24-hour basis gives comparable values. Thus, it is reasonable to assume that the subjects studied were receiving more than 1 to 2 mg of vitamin B<sub>6</sub>, which is the amount suggested as the necessary daily intake by the Food and Nutrition Board.<sup>13</sup>

In vitro studies have shown that the activity of enzymes associated with sulfur metabolism is rapidly reduced in vitamin B<sub>6</sub> deficiency.<sup>14</sup> Gershoff *et al.*<sup>2</sup> have reported a decrease in urinary inorganic sulfate excretion in vitamin B<sub>6</sub>-deficient cats. Assuming that similar relationships exist in humans, it appears from this study that the rate of excretion of oxalate is more sensitive to dietary vitamin B<sub>6</sub> levels than that of inorganic sulfate.

It is difficult to interpret the differences in the excretion of vitamin B<sub>6</sub> and pyridoxic acid in mongoloids and nonmongoloids following the load tests. These differences were not observed in control urines. It appears that mongoloids oxidize pyridoxine to pyridoxic acid more rapidly than do nonmongoloids. This may account for the greater excretion of vitamin B<sub>6</sub> in the urine of the nonmongoloids following the load tests. There were no significant differences between groups in the excretion of folic acid before and after the load test. Other studies will be necessary to explain the difference in methylation previously reported.<sup>1</sup>

This is the first study to relate oxalate excretion in humans to dietary vitamin B<sub>6</sub>. In view of the role of oxalic acid in a number of human diseases,<sup>2</sup> it is of considerable interest to observe the marked decrease in urinary oxalate obtained in humans receiving an apparently adequate amount of vitamin B<sub>6</sub> when load tests of vitamin B<sub>6</sub> were given. The evaluation of urinary oxalate excretion as a functional test for adequacy of vitamin B<sub>6</sub> and the value of vitamin B<sub>6</sub> in the treatment of diseases as-

sociated with oxalate deposition or oxaluria, particularly oxalosis and various types of kidney disease, require further investigation.

#### SUMMARY

The excretion of various metabolites by mongoloid and nonmongoloid mentally deficient patients has been studied prior to and following the oral administration of pyridoxine and folic acid. The excretion of oxalic acid by both groups was markedly reduced by pyridoxine administration although the subjects had been receiving a diet apparently adequate in vitamin B<sub>6</sub>. Following pyridoxine administration, mongoloids excreted more pyridoxic acid and less vitamin B<sub>6</sub> than did nonmongoloids. No significant differences in folic acid excretion were observed in the two groups studied.

#### ACKNOWLEDGMENT

We wish to thank the staff of the Wrentham State School which made the collections of samples possible.

#### REFERENCES

1. GERSHOFF, S. N., HEGSTED, D. M., and TRULSON, M. F.: Metabolic studies of mongoloids. *AM. J. CLIN. NUTRITION* 6: 526, 1958.
2. GERSHOFF, S. N., FARAGALLA, F. F., NELSON, D. A., and ANDRUS, S. B.: B<sub>6</sub> deficiency and oxalate nephrocalcinosis in the cat. *Am. J. Med.* (in press).
3. CLARK, L. C. and THOMPSON, H. L.: Determination of creatine and creatinine in urine. *Anal. Chem.* 21: 1218, 1949.
4. HUFF, J. W. and PERLZWEIG, W. A.: Determination of pyridoxic acid in urine; in *Vitamin Methods*, Vol. II (ed. P. György). Academic Press, New York, 1951, p. 348.
5. ATKIN, L., SCHULTZ, A. S., WILLIAMS, W. L., and FREY, C. N.: Yeast microbiological methods for determination of vitamins. Pyridoxine. *Ind. Eng. Chem., Anal. Ed.* 15: 141, 1943.
6. SCUDI, J. V., BUHS, R. P., and HOOD, D. B.: The metabolism of vitamin B<sub>6</sub>. *J. Biol. Chem.* 142: 323, 1942.
7. POWERS, H. H. and LEVATIN, P.: A method for the determination of oxalic acid in urine. *J. Biol. Chem.* 154: 207, 1944.
8. CAPPS, B. F., HOBBS, N. L., and FOX, S. H.: A dehydrated experimental medium for the microbiological assay of folic acid. *J. Bact.* 55: 869, 1948.
9. FISKE, C. H.: The determination of inorganic sulfate, total sulfate and total sulfur in urine by the benzidine method. *J. Biol. Chem.* 47: 59, 1921.

10. UNGLAUB, W. G. and GOLDSMITH, G. A.: *Evaluation of Vitamin Adequacy: Urinary Excretion Tests. Methods for Evaluation of Nutritional Adequacy and Status.* Advisory Board on Quartermaster Research and Development, Committee on Foods, Nat. Acad. Sci., Nat. Res. Council, 1954, p. 69.
11. LINKSWILER, H. and REYNOLDS, M. S.: Urinary and fecal elimination of B<sub>6</sub> and 4-pyridoxic acid on three levels of intake. *J. Nutrition* 41: 523, 1950.
12. SARETT, H. P.: A study of the measurement of 4-pyridoxic acid in urine. *J. Biol. Chem.* 189: 769, 1951.
13. *Food and Nutrition Board, Recommended Dietary Allowances.* Nat. Acad. Sci., Nat. Res. Council. Pub. #302, 1953.
14. SNELL, E. E.: *Vitamin Adequacy and Specific Metabolic Functions. Methods for Evaluation of Nutritional Adequacy and Status.* Advisory Board on Quartermaster Research and Development, Committee on Foods, Nat. Acad. Sci., Nat. Res. Council, 1954, p. 89.

