

Endogenous Oxalate Synthesis and Glycine, Serine, Deoxy pyridoxine Interrelationships in Vitamin B₆-deficient Rats*

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Attempts to study the etiology of diseases associated with oxaluria or oxalate deposition or both, have been handicapped by the misconception that very little urinary oxalate is endogenous in origin. However, Gershoff *et al.* (1) have demonstrated oxalate nephrocalcinosis and oxaluria in vitamin B₆-deficient cats. In vitamin B₆-deficient rats consuming diets free from oxalate, renal calculi of calcium oxalate monohydrate have been observed (2). Such renal calculi and the obstructive sequelae they produce, closely resemble the conditions found in human cases of kidney disease accompanied by oxalate stones. The possibility that these findings relating oxalate production and vitamin B₆ may be pertinent in human nutrition is indicated by the finding (3) that vitamin B₆ supplements decreased oxalate excretion in human subjects receiving diets thought to be more than adequate in vitamin B₆.

In the present paper, the effect of glycine, deoxy pyridoxine, and isonicotinic acid hydrazide supplements on oxaluria in vitamin B₆-deficient rats is reported. The effect of L-serine and glycine on deoxy pyridoxine toxicity has also been studied.

EXPERIMENTAL AND RESULTS

Male weanling rats of the Charles River CD strain were used. The diets fed contained 15 or 40 per cent casein, 4 per cent salts IV (4), 4 per cent corn oil, 1 per cent cod liver oil, and 0.3 per cent choline. In a number of instances different levels of glycine or L-serine were added to the diets. Sucrose was used to bring each diet to 100 per cent. All diets were supplemented with 4 mg. of thiamine, 8 mg. of riboflavin, 40 mg. of niacin, 20 mg. of Ca pantothenate, 1 mg. of folic acid, 1 mg. of menadione, 0.2 mg. of biotin, and 0.1 mg. of vitamin B₁₂ per kilogram of diet. When used, 4 mg. of pyridoxine HCl, 500 mg. of deoxy pyridoxine, and 2 gm. of isonicotinic acid hydrazide were added per kilogram of diet.

In the first experiment 12 groups of 9 rats were used. The diets fed contained 15 or 40 per cent casein with or without 3 per cent added glycine. Some of the vitamin B₆-deficient diets

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contained deoxy pyridoxine. At the end of each week during the 30-day experimental period, subgroups of 3 rats from each group were placed in metabolism cages and 48-hour urine collections made. The oxalic acid content of the urine was determined by the method of Powers and Levatin (5). The results of this experiment (Table I) clearly show that in vitamin B₆-deficient rats there is a marked increase in urinary oxalate excretion. The addition of 3 per cent glycine to the 15 per cent casein diets and of deoxy pyridoxine to both 15 per cent and 40 per cent casein diets, resulted in a further increase in oxalate excretion. Oxaluria was most pronounced when 40 per cent casein diets containing both added glycine and deoxy pyridoxine were fed. Although the addition of deoxy pyridoxine inhibited growth, as expected when added to the basal diet, the further addition of glycine provided considerable protection against the growth-inhibiting and lethal effects of the deoxy pyridoxine. Three per cent glycine had no significant effect on the growth of animals receiving vitamin B₆, but it caused some growth inhibition in deficient rats not receiving deoxy pyridoxine.

Isonicotinic acid hydrazide, commonly used in the treatment of tuberculosis, is known to increase vitamin B₆ requirements (6). Its effect on oxalate production in rats fed 15 per cent casein diets without added glycine has been studied in groups of 9 rats. At the end of the first and second experimental weeks, urines were collected (over a 48-hour period) for oxalate analysis. The mean results of this study shown in Table II, indicate that isonicotinic acid hydrazide is effective in increasing urinary oxalate excretion by rats particularly when they are vitamin B₆ deficient.

The protective effect of glycine on deoxy pyridoxine toxicity and the fact that glycine is rapidly converted to serine in animal tissues, led to the third study in which the effect of L-serine and glycine in sparing vitamin B₆-deficient rats from the toxic effects of deoxy pyridoxine was studied. The results of this experiment, Table III, demonstrate again the protective effect of glycine and that L-serine is more effective than glycine when fed on an equal molecular basis.

DISCUSSION

The quantity of endogenous oxalate produced in these experiments must be related to the amount of oxalate precursors ingested and the degree of vitamin B₆ deficiency produced. It appears from this study and that of Calhoun *et al.* (7) in vitamin B₆-deficient rats and the work of Archer *et al.* (8) and Scowen

TABLE I

Effect of glycine and deoxypyridoxine on oxaluria and weight changes in vitamin B₆-deficient rats

Diet	Average weight change per 28 days	Mg. urinary oxalic acid per 100 gm. body weight on days*			
		8 + 9	15 + 16	22 + 23	29 + 30
15% Casein					
+B ₆	105	1.0	1.1	0.6	0.5
+B ₆ + 3% glycine.....	102	1.3	1.3	0.8	0.7
-B ₆	31	1.9	2.3	1.3	1.3
-B ₆ + deoxypyridoxine.....		2.8	2.3 (8)	2.3 (6)	
-B ₆ + 3% glycine.....	15	3.1	3.2		2.3
-B ₆ + 3% glycine + deoxypyridoxine.....	26	4.5	2.9	2.6	2.4
40% Casein					
+B ₆	151	0.8	0.7	0.5	0.5
+B ₆ + 3% glycine.....	166	1.1	1.0	0.9	0.5
-B ₆	30	2.0	1.5	1.9	1.6
-B ₆ + deoxypyridoxine.....		4.2	3.1 (4)	3.6 (3)	
-B ₆ + 3% glycine.....	20	2.3	1.5	1.7	1.7
-B ₆ + 3% glycine + deoxypyridoxine.....		5.2	5.2	6.2	

* Except for groups in which rats died, each value represents the mean of 3 collections from groups of 3 rats. Numbers in parentheses represent survivors.

TABLE II

Effect of isonicotinic acid hydrazide on oxaluria in rats

Treatment	Mg. urinary oxalic acid per 100 gm. body weight per 48 hours*
+B ₆	0.7 ± 0.2
+B ₆ + INH†.....	1.1 ± 0.2
-B ₆	1.4 ± 0.2
-B ₆ + INH.....	2.8 ± 0.3

* Each value represents the mean of six determinations plus standard error of the mean.

† INH, isonicotinic acid hydrazide.

et al. (9) in cases of primary hyperoxaluria, that a considerable amount of urinary oxalate may be of endogenous origin and derived in great part from glycine. Oxalate has been shown to be formed from glycine via glyoxylic acid (10, 11) and inhibition of the system described by Cammarata and Cohen (12), *i.e.* glyoxylic acid plus glutamic acid ↔ glycine plus α-ketoglutaric acid, could result in an accumulation of glyoxylic acid with increased oxalate formation. The slightly decreased oxalate excretion of some of the deficient groups as the first experiment progressed, may have been due to lessened consumption of the glycine-containing diets when the deficiency became intensified.

TABLE III

Effect of L-serine and glycine on growth inhibition of B₆-deficient rats fed deoxypyridoxine*

Diet†	Average gained per 14 days
	gm.
+B ₆	38
-B ₆	27
-B ₆ + deoxypyridoxine.....	0
-B ₆ + deoxypyridoxine + 0.5% glycine.....	4
-B ₆ + deoxypyridoxine + 1.0% glycine.....	13
-B ₆ + deoxypyridoxine + 1.5% glycine.....	27
-B ₆ + deoxypyridoxine + 0.7% L-serine.....	15

* Five rats per group.

† Diets contained 15 per cent casein.

Two vitamin B₆ antagonists, deoxypyridoxine and isonicotinic acid hydrazide, have been used to accelerate the production of the deficiency state. The feeding of either of these compounds, particularly to animals not receiving dietary vitamin B₆, was accompanied by an increase in oxaluria. This is presumably due to an acceleration in the rate of development or severity of the deficiency. Some practical significance may be attached to this action of isonicotinic acid hydrazide, since it is widely used in the treatment of tuberculosis. It has not yet been determined whether the administration of isonicotinic acid hydrazide to patients results in increased oxalate production. It seems probable that this would occur unless adequate pyridoxine supplementation was provided.

Although serine and glycine are "nonessential" amino acids, they are necessary for the synthesis of body proteins and a number of essential metabolites. The use of deoxypyridoxine in these studies apparently resulted in a deficiency of glycine and serine since dietary supplementation with these amino acids reversed the growth inhibition caused by the antivitamin. Deoxypyridoxine may inhibit the formation of serine and glycine or enhance their degradation.

Excessive amounts of glycine are toxic (13) particularly when the diet is vitamin B₆ deficient (14). Thus, it would appear that whether glycine supplementation accentuates or ameliorates vitamin B₆ deficiency depends on the different degrees to which the vitamin B₆-dependent systems are depressed.

SUMMARY

Urinary excretion of endogenous oxalate by rats is markedly increased in vitamin B₆ deficiency. This effect can be enhanced by dietary supplementation with glycine, deoxypyridoxine, or isonicotinic acid hydrazide.

Deoxypyridoxine toxicity can be overcome by serine or glycine supplementation suggesting that deoxypyridoxine interferes with the availability of these amino acids.

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