Intestinal and renal uptake of oxalate in nutritional vitamin deficiencies with special reference to pyridoxine

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Nutritional deficiencies of vitamins A, B_{1} and B_{6} have been implicated as a cause of hyperoxaluria, leading to calculus formation in man and experimental animals. Marginal deficiency of vitamin B_{6} has been reported in recurrent stone formers (1), and hyperabsorption of oxalate from the gut has been implicated in calculus formation in vitamin B_{6} deficiency (2). Oxalate transport across the renal tubulus is still debatable because net reabsorption (3) and net secretion (4) have been reported. The present study aims to evaluate the molecular mechanisms involved in hyperoxaluria of pyridoxine deficiency using intestinal and renal brush border membrane vesicles (BBMV).

Materials and Methods

Male weanling rats (40–50 g body weight) of Wistar strain were fed a pyridoxine-deficient diet (Nutritional Biochemicals, Ohio, Cleveland) for a period of 45 days. Paired controls were maintained which were supplemented with 100 μg pyridoxine per day per rat by gastric intubation. At the end of the experimental period, pyridoxine deficiency was biochemically confirmed by measuring erythrocyte alanine transaminase activity in the two groups. The intestinal and renal BBMV were prepared by the method of Schmitz et al (5). Purity of BBMV was ascertained by an enrichment in the marker enzymes, such as sucrase, maltase, leucine amino peptidase (6) and a decrease in the activity of basolateral enzyme Na^{+} K^{+}-ATPase (7). The vesicularity and integrity of BBMV was assessed by D-glucose uptake under Na^{+}-gradient conditions (8).

Oxalate uptake assay

Aliquots of BBMV (20 μl containing 100–150 μg protein) were incubated with different concentrations of oxalate, containing 0.1 to 0.2 μCi of ^{14}C-oxalate, in 0.1 ml buffer (300 mM D-mannitol + 2 mM Tris-Hepes, pH 7.5) for 20 min in intestinal uptake studies, and in buffer (300 mM D-mannitol + 5 mM Tris-Hepes, pH 7.5) for 15 min in renal uptake studies both at 25°C. The reaction was terminated by the addition of 5 ml cold saline buffered with 2 mM Tris-Hepes, pH 7.5 and the reaction mixture was filtered on 0.45 μm filter (9). The radioactivity retained by the vesicles was used as an index of oxalate uptake.
Results and Discussion

The effect of oxalate concentration, in the range 0.1 mM - 1.0 mM, on its uptake by intestinal BBMV in pair-fed rats is shown in Fig. 1. The rate of uptake increased linearly with the oxalate concentration of the medium, suggesting that oxalate uptake by pair-fed rat intestine follows a passive diffusion process. On the other hand oxalate uptake by pyridoxine-deficient intestinal BBMV follows a biphasic mechanism comprising of saturable hyperbolic uptake in the concentration range 0.1 mM to 0.6 mM and linear uptake, thereafter, upto 1.0 mM (fig. 2). The results suggest the involvement/in-

![Fig. 1. Oxalate uptake by intestinal brush border membrane vesicles (BBMV) from pair-fed rats. Each point represents the mean ± S.E. of six observations](image1)

![Fig. 2. Oxalate uptake by vitamin B₆ deficient rat intestinal BBMV. Each point represents the mean ± S.E. of six observations](image2)