Urinary outputs of oxalate, calcium, and magnesium in children with intestinal disorders

Potential cause of renal calculi

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Ogilvie, D., McCollum, J. P. K., Packer, S., Manning, J., Oyesiku, J., Muller, D. P. R., and Harries, J. T. (1976). *Archives of Disease in Childhood*, 51, 790. **Urinary outputs of oxalate, calcium, and magnesium in children with intestinal disorders: potential cause of renal calculi.** 24-hour urinary outputs of oxalate, calcium, and magnesium have been determined in a total of 62 children aged 3 months to 17 years who fell into the following groups: (i) 16 normal controls, (ii) 3 with primary hyperoxaluria, (iii) 9 with small and/or large intestinal resections, (iv) 9 with untreated coeliac disease, (v) 5 with pancreatic dysfunction, and (vi) a miscellaneous group of 20 children with a variety of intestinal disorders.

Taken as a whole, 58% of patients with intestinal disorders had hyperoxaluria, and of these 7% had urinary outputs of oxalate which fell within the range seen in primary hyperoxaluria. The proportion of children with hyperoxaluria in the different diagnostic groups was as follows: intestinal resections (78%), coeliac disease (67%), pancreatic dysfunction (80%), and miscellaneous (45%). 35% of the patients with hyperoxaluria had hypercalciuria, whereas magnesium excretion was normal in all subjects studied. In 2 patients treatment of the underlying condition was accompanied by a return of oxalate excretion to normal.

These results indicate that hyperoxaluria and hypercalciuria are common in children with a variety of intestinal disorders, and that such children may be at risk of developing renal calculi without early diagnosis and treatment.

Hyperoxaluria is common in adult patients with ileal disease or resection, and a proportion of these patients develop oxalate renal calculi (Dowling, Rose, and Sutor, 1971; Smith, Fromm, and Hofmann, 1972; Chadwick, Modha, and Dowling, 1973). Studies on urinary oxalate excretion in adults with other malabsorptive states are limited, and no systematic studies have been reported in children. The relation between urinary outputs of oxalate, calcium, and magnesium in children with different intestinal disorders has not previously been reported.

We have investigated the urinary excretion of oxalate, calcium, and magnesium in children with a variety of untreated intestinal disorders; in addition,

the effects of treatment have been sequentially studied in 2 patients. Our results indicate that hyperoxaluria and hypercalciuria are common in children with intestinal dysfunction and that treatment of the underlying disorder may reduce the risk of developing renal calculi later in life.

Subjects (Table)

24-hour urinary outputs of oxalate were determined in a total of 62 children aged 3 months to 17 years; the 16 normal controls were matched for age with the patient groups. The patients with intestinal disease were made up of four groups: 9 with intestinal resections (4 ileal resections, 2 jejunal resections, 1 ileal and caecal resection, 1 colectomy, and 1 ascending colon resection), 9 with untreated coeliac disease, 5 with pancreatic disease (2 cystic fibrosis, 2 Shwachman's syndrome, and 1 congenital isolated lipase deficiency), and a miscel-

TABLE
Details of subjects studied

Subjects	No.
Controls	16
Primary hyperoxaluria	3
Intestinal resections	
Ileum	4
Ileum and caecum	1
Jejunum	2
Whole colon	1
Ascending colon	1
Untreated coeliac disease	9
Pancreatic disease	
Cystic fibrosis	2
Shwachman's syndrome	2
Congenital isolated lipase deficiency	1
Miscellaneous	20

laneous group of 20 children with a variety of intestinal disorders including ulcerative colitis, Crohn's disease, and protracted diarrhoea of undetermined cause.

All the patients with untreated coeliac disease had a flat mucosa on biopsy, and the institution of a gluten-free diet was followed by prompt clinical improvement. It is likely that these children have true coeliac disease (i.e. permanent intolerance to gluten) but this has not yet been confirmed by gluten challenge. For comparative purposes 3 children with primary hyperoxaluria and renal calculi were also studied. 24-hour urinary calcium and magnesium outputs were determined simultaneously with oxalate in 35 of the patients with intestinal disorders.

Methods

In all subjects studied a fixed low dietary intake of oxalate was maintained for 2 to 3 days before study, and during urine collections. Whenever possible the children were asked to completely empty their bladders immediately before timed urine collections, and again immediately before completing the 24-hour collections. Samples were discarded if there was any doubt regarding the completion of the collections. Urine was collected into plastic containers containing a small volume of added merthiolate as a preservative, stored at 4°C, and acidified within 7 days of collection; storage at 4°C for up to 7 days did not affect oxalate concentrations.

Urinary oxalate was determined by the colorimetric method of Hodgkinson and Williams (1972), with minor modifications to permit batch analyses and incorporation of a quality control. All reagents were of Analar grade except for the chromotropic acid which was supplied by Hopkins and Williams Ltd. as 'purified for formaldehyde determination'. The electrolytic zinc wire (3 mm diameter) was kindly supplied by Dr. A. Hodgkinson of the General Infirmary, Leeds.

Within 7 days of collection urine samples were acidified to ensure complete solubilization of calcium oxalate crystals, and oxalate was then precipitated as its calcium salt from duplicate samples (0.5 ml) of urine. With each assay a quality control equivalent to 25 µg of

anhydrous oxalic acid per ml of 0.05 mol/l phosphate buffer (pH 7.4) was similarly acidified and precipitated in triplicate. The solutions were left to precipitate overnight, after which the precipitate was removed and stored at -20°C. Precipitates could be stored for at least 16 days before assay without significant changes in oxalate content, e.g. repeated analysis of a specimen of urine gave results of 11.5, 11.7, and 11.0 µg/ml before, and after 7 and 16 days storage, respectively. In practice, assays were usually performed within one week of precipitation. Using this procedure of storage, 20 sequential analyses of the quality control (25 µg oxalic acid/ml) over a 6-month period gave a mean oxalate concentration of 24 µg/ml $\pm$ 0.4 (SE) and a coefficient of variation of the method of 1.5%. The assay reaction was linear up to the maximum standard concentration of oxalate used (50 µg/ml), and addition of 10 to 50 µg oxalate to samples of urine resulted in recoveries of greater than 85%.

Using this method preliminary studies indicated that all of the 8 children with liver disease had apparent hyperoxaluria, and in two instances urinary outputs of oxalate approached those seen in primary hyperoxaluria (McCollum *et al.*, 1975). Oxalic acid is a dicarboxylic acid and, since the bilirubin molecule contains two carboxylic groups, the possibility that bilirubin might interfere with the assay was investigated. When 0.1 mg conjugated bilirubin was added to 0.5 ml of urine an apparent recovery of 124% was obtained, indicating that conjugated bilirubin interferes with the assay. Whole blood (10 µl), sodium taurocholate (0.25 and 0.50 mg), and whole human bile (0.1 ml) added to a similar volume of urine gave oxalate recoveries of 104, 96, and 108, and 88%, respectively. These results suggest that increased urinary excretion of conjugated bilirubin in patients with liver disease may result in spuriously high urinary outputs of oxalate using this method. Attempts were made to remove the interference but all proved unsuccessful, e.g. deconjugation with acid or β -glucuronidase followed by solvent extraction, degradation with ultraviolet light in an alkaline solution, and separation by XAD chromatography.

The expression of urinary oxalate values poses certain problems since the urinary excretion of oxalate increases with age, and adult values are attained by the age of about 14 years (Gibbs and Watts, 1969). Creatinine excretion increases more slowly reaching adult values by 20 years; thus urinary oxalate cannot be related to creatinine. When urinary oxalate output per unit of surface area is converted to the corresponding value for the adult standard body surface (1.73 m²), the values for children are the same as those obtained in adults, and there is no significant difference between the sexes (Gibbs and Watts, 1969). For these reasons our oxalate data is expressed as mg oxalate per 1.73 m² per 24 hours throughout the paper.

Calcium and magnesium were determined by atomic absorption spectrophotometry. The upper limit of urinary calcium and magnesium outputs have been taken to be 4 and 5 mg per kg body weight per 24 hours, respectively (Ghazali and Barratt, 1974).

The significance of differences between mean values was assessed by Student's 't' test.

Results

Oxalate excretion in untreated disease.

Fig. 1 shows urinary oxalate excretion rates in 16

FIG. 1.—Urinary excretion of oxalate in patients with primary hyperoxaluria compared to patients with a variety of untreated intestinal disorders. Broken line indicates upper limit of normal range (mean $\pm$ 2SD).

normal children, in children with a variety of untreated intestinal disorders, and in 3 patients with primary hyperoxaluria. The mean oxalate excretion in the control group was 24.1 with a range of 16.1 – 30.1 $\text{mg}/1.73 \text{ m}^2$ per 24 h; the upper limit of normal (33.1) was taken as 2 SDs above the mean. Using these criteria the proportion of children with hyperoxaluria in the different diagnostic groups was as follows: intestinal resections (78%), coeliac disease (67%), pancreatic dysfunction (80%), miscellaneous (45%). Taken as a whole, 58% of the patients with intestinal disorders had hyperoxaluria and of these, 7% had urinary outputs of oxalate which fell within the range seen in primary hyperoxaluria. Of the 9 children with intestinal resections only 2 had normal urinary outputs of oxalate; one with a total colectomy and the other who had the ascending colon removed. The one patient with normal oxalate excretion in the group with pancreatic dysfunction had congenital isolated lipase deficiency.

Calcium excretion in untreated disease.

Fig. 2 compares urinary calcium excretion rates in 35 children with intestinal disorders, with and without hyperoxaluria. The mean and (range) of calcium excretion in children with intestinal disorders without hyperoxaluria compared with

FIG. 2.—Urinary excretion of calcium in patients with intestinal disorders, with and without hyperoxaluria. Broken line indicates upper limit of normal range (>4 mg/kg per 24 h).

those with hyperoxaluria was 1.15 (0.1 – 3.6) and 3.22 (0.66 – 6.58) mg/kg body weight per 24 hours respectively; this difference was highly significant ($P=0.0005$), and 35% of the latter group had hypercalciuria (>4 mg/kg per 24 h).

Magnesium excretion in untreated disease.

Urinary magnesium excretion was determined in 34 children with intestinal disorders, and there was no significant difference ($P>0.05$) between those with hyperoxaluria compared to patients without hyperoxaluria. Excretion of magnesium was normal in all subjects studied.

Effects of treatment on oxalate excretion.

The effect of treatment on urinary oxalate excretion was investigated in 2 patients, both of whom had marked hyperoxaluria. After 3 months of a gluten-free diet oxalate excretion had returned to normal in a patient with coeliac disease and this was accompanied by an improvement in the morphological mucosal abnormalities (Fig. 3). In the second child with congenital pancreatic hypoplasia (Shwachman's syndrome) a similar response fol-

FIG. 3.—Effect of a gluten-free diet on urinary oxalate excretion in coeliac disease. Broken line indicates upper limit, of normal range (mean $\pm 2SD$). SVA, PVA, subacute and partial villous atrophy.

lowed treatment with pancreatic extract, a low fat diet, and medium-chain triglycerides (Fig. 4); correction of the steatorrhoea was accompanied by urinary oxalate excretion becoming normal.

FIG. 4.—Effect of low-fat diet and pancreatic extract on urinary oxalate excretion in Shwachman's syndrome. Broken horizontal line indicates upper limit of normal range for oxalate excretion (mean $\pm 2SD$), and continuous horizontal line upper limit of normal for faecal fat (> 4.5 g/24 h).

Discussion

It is well established that hyperoxaluria is common in adults with ileal disease or resection, and that a proportion of affected patients develop renal oxalate stones (Dowling *et al.*, 1971; Smith *et al.*, 1972; Chadwick *et al.*, 1973). In children, however, there is only one published report on the incidence of hyperoxaluria in patients with intestinal dysfunction. Valman, Oberholzer, and Palmer (1974) investigated 10 children who had had ileal resections, and reported that 4 had hyperoxaluria. No studies on the relation between urinary

oxalate, calcium, and magnesium outputs have been previously reported in children with ileal resections, or in children with other intestinal disorders.

Our study clearly shows that hyperoxaluria is common in children with a variety of malabsorptive states. Calcium and oxalic acid form an almost insoluble salt at neutral or alkali pH, and the solubility of calcium oxalate in aqueous solutions is increased in the presence of magnesium ions (Hodgkinson, 1970). It was therefore of interest to determine simultaneously urinary oxalate, calcium, and magnesium in our patients. Urinary outputs of magnesium were no different in children with intestinal dysfunction who had hyperoxaluria compared with those who did not have hyperoxaluria. In contrast, 35% of children with hyperoxaluria also had hypercalciuria, whereas calcium excretion was normal in all the patients with normal oxalate values. Since calcium oxalate is the major constituent of 'noninfectious' renal calculi in Western countries (Watts, 1972/1973), these findings further emphasize the risk that such children take of developing calculi. The mechanism of the hypercalciuria has not been defined in the present study.

Although none of our patients had evidence of stone formation our findings indicate that children with a variety of intestinal disorders may be at risk of developing renal calculi particularly if hyperoxaluria is associated with hypercalciuria. Treatment of one patient with coeliac disease and one with Shwachman's syndrome, who had urinary oxalate outputs similar to those seen in primary hyperoxaluria, resulted in a return to normal of urinary oxalate. These findings emphasize the importance or early diagnosis and treatment in the prevention of this potential complication.

Little information is available on the mechanisms of oxalate absorption from nondiseased intestine. *In vitro* studies in rat duodenum, jejunum, ileum, and colon suggest an energy-independent, unsaturable transport process (Binder, 1974). Thus oxalate may be absorbed by a process of simple passive diffusion and, since the dicarboxylic acid is a metabolic end-product, will be quantitatively excreted in the urine. A significant proportion of the normal absorption of oxalate occurs from the colon in both man (Saunders, Sillery, and McDonald, 1975) and primates (Chadwick *et al.*, 1973), and an intact colon is a necessary prerequisite to the development of hyperoxaluria in patients with intestinal dysfunction. For example, patients with extensive ileal and colonic resection do not have hyperoxaluria even when consuming a high oxalate diet (Earnest *et al.*, 1974); similarly patients with ileostomy do not develop hyperoxaluria. In this

context it is of interest that both of our patients with colonic resections had normal oxalate outputs.

Hyperoxaluria in patients with intestinal dysfunction is due to enhanced absorption of dietary oxalate (Chadwick *et al.*, 1973), but the precise cause of the enhanced absorption is controversial. Hofmann *et al.* (1970) suggested that in patients with intestinal disease such as ileal resection or bacterial overgrowth, there was bile salt malabsorption or increased bile salt deconjugation and that the liberated glycine was converted by intestinal bacteria to glyoxylate or oxalate. Subsequently, however, the same workers (Hofmann *et al.*, 1973) and others (Chadwick *et al.*, 1973) showed that bile salt glycine was not a precursor of urinary oxalate. More recent studies have focused on the effects of different fatty acids and bile salts on the colonic absorption of oxalate. Linoleic acid (18:2) enhanced colonic absorption of oxalate in the rat *in vivo*, whereas 10 mmol/l taurocholate depressed absorption (Saunders *et al.*, 1975). Using a similar *in vivo* perfusion technique in the rat colon, Dobbins and Binder (1975) showed that 5 mmol/l deoxycholic acid and ricinoleic acid markedly enhanced absorption; taurocholate did not increase oxalate absorption. Using urea as a marker of mucosal permeability they concluded that enhanced absorption of oxalate by deoxycholic and ricinoleic acids resulted from changes in mucosal permeability.

These findings suggest that unabsorbed fatty acids, and the products of colonic bacterial metabolism of fat and conjugated bile salts, increase colonic absorption of oxalate and in this way result in hyperoxaluria. Several lines of evidence support this possibility. Smith *et al.* (1972) reported that treatment of patients with ileal resections with cholestyramine reduced urinary excretion of oxalate, and in one patient with renal stones 2 of the 3 calculi disappeared during treatment. Cholestyramine binds bile salts and its effect might be explained by it preventing bile-salt induced enhancement of colonic oxalate absorption. In our patients the magnitude of oxalate excretion was greatest in patients with pancreatic dysfunction, and such patients not only have steatorrhoea but have also been shown to excrete faecal bile salts to a degree similar to that seen in children with ileal resections (Weber *et al.*, 1973). Thus, in these patients both fatty acids and bile salts may stimulate colonic absorption of oxalate.

The absence of hyperoxaluria in the patient with congenital lipase deficiency is of interest. Despite a complete absence of pancreatic lipolytic activity this patient absorbed more than 70% of her dietary intake of fat (Muller *et al.*, 1975), and this may be of

relevance to her normal output of urinary oxalate. There is a good correlation between faecal fat and urinary oxalate output (Earnest *et al.*, 1974; Stauffer, Stewart, and Bertrand, 1974; Andersson and Jagenburg, 1974; McDonald, Earnest, and Admirand, 1975); moreover, increased dietary intake of fat increases excretion of oxalate in patients with hyperoxaluria and intestinal dysfunction.

The available evidence suggests that hyperoxaluria and renal calculi in patients with intestinal dysfunction are secondary to increased colonic absorption of oxalate. This probably results from an effect of degraded bile salts and fatty acids on the permeability of the colonic mucosa to oxalic acid.

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REFERENCES

- Andersson, H., and Jagenburg, R. (1974). Fat-reduced diet in the treatment of hyperoxaluria in patients with ileopathy. *Gut*, **15**, 360.
- Binder, H. J. (1974). Intestinal oxalate absorption. *Gastroenterology*, **67**, 441.
- Chadwick, V. S., Modha, K., and Dowling, R. H. (1973). Mechanism for hyperoxaluria in patients with ileal dysfunction. *New England Journal of Medicine*, **289**, 172.
- Dobbins, J., and Binder, H. J. (1975). Bile salts and hydroxy fatty acids increase colonic oxalate absorption: a possible mechanism of hyperoxaluria? *Gastroenterology*, **68**, 864.
- Dowling, R. H., Rose, G. A., and Sutor, D. J. (1971). Hyperoxaluria and renal calculi in ileal disease. *Lancet*, **1**, 1103.
- Earnest, D. L., Johnson, G., Williams, H. E., and Admirand, W. (1974). Hyperoxaluria in patients with ileal resection: an abnormality in dietary oxalate absorption. *Gastroenterology*, **66**, 1114.
- Ghazali, S., and Barratt, T. M. (1974). Urinary excretion of calcium and magnesium in children. *Archives of Disease in Childhood*, **49**, 97.
- Gibbs, D. A., and Watts, R. W. E. (1969). The variation of urinary oxalate excretion with age. *Journal of Laboratory and Clinical Medicine*, **73**, 901.
- Hodgkinson, A. (1970). Determination of oxalic acid in biological material. *Clinical Chemistry*, **16**, 547.
- Hodgkinson, A., and Williams, A. (1972). An improved colorimetric procedure for urine oxalate. *Clinica Chimica Acta*, **36**, 127.
- Hofmann, A. F., Thomas, P. J., Smith, L. H., and McCall, J. T. (1970). Pathogenesis of secondary hyperoxaluria in patients with ileal resection and diarrhea. *Gastroenterology*, **58**, 960.
- Hofmann, A. F., Tacker, M. M., Fromm, H., Thomas, P. J., and Smith, L. H. (1973). Acquired hyperoxaluria and intestinal disease. *Mayo Clinic Proceedings*, **48**, 35.
- McCollum, J. P. K., Ogilvie, D., Muller, D. P. R., Manning, J., Packer, S., and Harries, J. T. (1975). Hyperoxaluria in children with hepatic, pancreatic, and intestinal dysfunction. *Archives of Disease in Childhood*, **50**, 824.
- McDonald, G. B., Earnest, D. L., and Admirand, W. H. (1975). Hyperoxaluria correlates with steatorrhea in patients with celiac sprue. *Gastroenterology*, **68**, 949.

- Muller, D. P. R., McCollum, J. P. K., Trompeter, R. S., and Harries, J. T. (1975). Studies on the mechanism of fat absorption in congenital isolated lipase deficiency. *Gut*, **16**, 838.
- Saunders, D. R., Sillery, J., and McDonald, G. B. (1975). Regional differences in oxalate absorption by rat intestine: evidence for excessive absorption by the colon in steatorrhoea. *Gut*, **16**, 543.
- Smith, L. H., Fromm, H., and Hofmann, A. F. (1972). Acquired hyperoxaluria, nephrolithiasis, and intestinal disease. *New England Journal of Medicine*, **286**, 1371.
- Stauffer, J. Q., Stewart, R. J., and Bertrand, G. (1974). Acquired hyperoxaluria: relationship to dietary calcium content and severity of steatorrhea. (Abst.) *Gastroenterology*, **66**, 783.
- Valman, H. B., Oberholzer, V. G., and Palmer, T. (1974). Hyperoxaluria after resection of ileum in childhood. *Archives of Disease in Childhood*, **49**, 171.
- Watts, R. W. E. (1972/1973). Oxaluria. *Journal of the Royal College of Physicians of London*, **7**, 161.
- Weber, A. M., Roy, C. C., Chartrand, L., Morin, C. L., and Cordeau, J. R. (1973). Malabsorption of bile acids in children with pancreatic insufficiency. *Gastroenterology*, **64**, 819.

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