Oxaluria

R. W. E. WATTS, MD, FRCP, Division of Inherited Metabolic Diseases, M R C Clinical Research Centre, Harrow

Calcium oxalate is a major constituent of at least two-thirds of urinary calculi in Western Europe and North America. Industrialisation of the community appears to be associated with a change from predominantly juvenile ammonium acid urate bladder stones to calcium oxalate renal stones in adults (Lonsdale *et al.*, 1968a, b; Sutor and Wooley, 1969, 1970; Sutor, 1970a). Even apparently pure calcium oxalate stones commonly contain a small central region of calcium phosphate (Hodgkinson and Nordin, 1971), and the epitaxial growth of calcium oxalate crystals on uric acid crystals is well known (Lonsdale, 1968).

The oxalate anion is a normal urinary constituent, the excretion of which increases with age, adult values being reached by about fourteen years (Gibbs and Watts, 1969). The effect of age is abolished if the results are corrected to a standard body surface area, the upper limit of the normal range being equivalent to about 55 mg anhydrous oxalic acid/24 h/1·73 m^2 as shown by Hockaday *et al.* (1965) and Gibbs and Watts (1969). Both these groups of workers used the method of isotope dilution analysis with ^{14}C oxalic acid but with completely different chemical procedures, and obtained very good agreement between their two laboratories. Isotope dilution analysis is considered to give the most accurate results, but is not convenient for routine use. Fortunately, the larger differences that have to be detected in order to establish a diagnosis of primary hyperoxaluria can be measured satisfactorily by a simple precipitation method (Archer *et al.*, 1957), but this method gives low results in and near the normal range compared with the isotope dilution method. Hodgkinson and Williams (1972) have developed a colorimetric method which agrees more closely with the isotope dilution procedure. Small post-prandial and diurnal variations in the urinary oxalate excretion, as well as longer term cyclic fluctuations within the normal range, have been reported (Hodgkinson, 1970). The post-prandial increases appear to be more marked in some patients with recurrent calcium oxalate urinary stones and contribute to the over-saturation of the urine with calcium oxalate which is necessary for stone formation. Such patients sometimes also have slightly increased levels of calcium excretion (Robertson *et al*, 1969; Hodgkinson and Nordin, 1971; Hodgkinson *et al.*, 1971). Normal urine has the property of preventing

the growth, from single crystals or the minute aggregates that normally occur in urine, of large aggregates which lodge in the pelvi-calyceal system and grow into stones (Marshall *et al.*, 1972). In most cases, it is the combination of small fluctuations in urinary oxalate excretion with other factors that appears to contribute to stone formation.

BIOCHEMISTRY AND PHYSIOLOGY OF OXALATE ANION

The urinary oxalate is largely of endogenous origin and the metabolic pathways leading to oxalate are shown in Fig. 1. It is metabolically inert (Curtin and King, 1955; Elder and Wyngaarden, 1960) and, except for the C_1 and C_2 carbon atoms of ascorbate, (Hellman and Burns, 1958; Atkins *et al.*, 1964) glyoxylate is the only known immediate metabolic precursor in animals. The cyclic mechanism of glyoxylate-glycine-ethanolamine (GGE cycle) shown in Fig. 1 is joined to other major metabolic pathways at point *A* via 3-hydroxypyruvate, which is in equilibrium with L-glycerate, and possibly at point *B* because a thiamine pyrophosphate bound form of glycolaldehyde is transferred in the transketolase reaction and thereby brought into the pentose

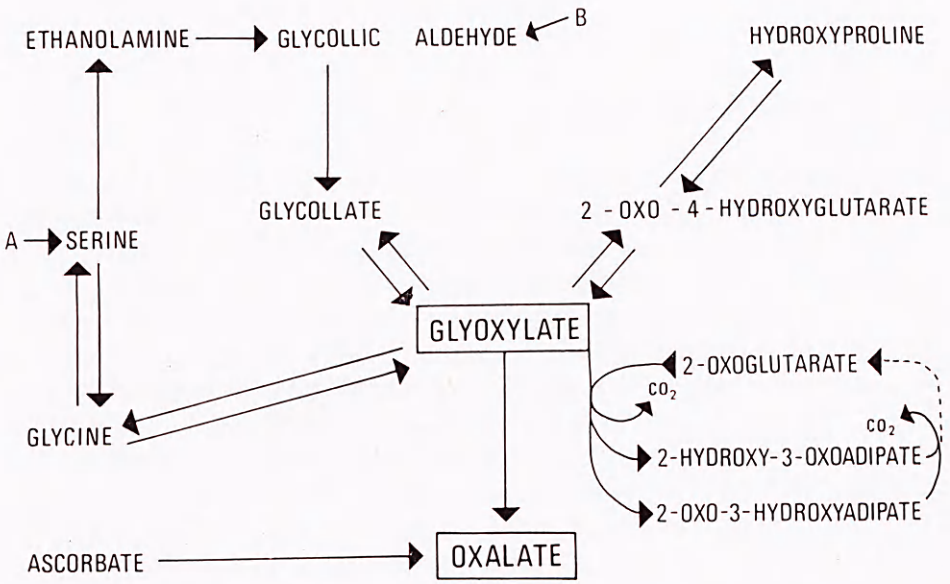

Fig. 1. The glyoxylate-glycine-ethanolamine (GGE) cycle. Glyoxylate is formed from glycine by transamination and by oxidative deamination, from L-hydroxyproline via α-hydroxy-γ-oxoglutaric acid and by the oxidation of glycollate. It is either oxidised to oxalate, reduced to glycollate, transaminated to glycine, or decarboxylated synergistically with 2-oxoglutarate by the enzyme 2-oxoglutarate: glyoxylate carbologase. The equilibrium of the transamination favours glycine formation.

phosphate pathway of glucose metabolism (Datta and Racker, 1959; Prochoroff *et al.*, 1962). Glycine itself links this cycle to the succinate glycine cycle (Shemin, 1955).

Satisfactory methods for the direct measurement of the blood oxalate are not available. The published values have decreased as methods have improved. The most recent values obtained by an enzymatic method are 80 to 148 μg/100 ml (Knowles and Hodgkinson, 1972). Lower values have been calculated from the results of studies with ^{14}C oxalate: 7 to 15 μg/100 ml (Elder and Wyngaarden, 1960); 16·5 μg/100 ml (Williams *et al.*, 1971); 5 to 22 μg/100 ml (Hodgkinson and Wilkinson, 1972).

The oxidation of glyoxylate is unidirectional, and lactic dehydrogenase (L-lactate: NAD^+ oxidoreductase EC 1.1.1.27) is the principal enzyme which catalyses this reaction in the soluble fraction of human liver (Gibbs, 1971) and heart (Gibbs and Watts, 1972), and in blood cells (Smith *et al.*, 1971). The other glyoxylate oxidising enzymes, glycollate oxidase (glycollate: oxygen oxidoreductase EC 1.1.3.1) and xanthine oxidase (xanthine: oxygen oxidoreductase EC 1.2.3.2), make only a minor contribution to the overall glyoxylate to oxalate oxidising ability of the soluble fraction of liver and heart. The metabolic interrelationships of lactate dehydrogenase and glycollate oxidase are shown in Fig. 2. Because the kidney, too, contains lactate dehydrogenase, these findings raise the possibility that some of the urinary oxalate may be formed there, particularly as the kidney contains D-amino acid oxidase (D-amino acid: oxygen oxidoreductase EC 1.4.3.3) which oxidatively deaminates glycine to glyoxylate. This would account for the extensive calcium oxalate deposits in this organ in primary hyperoxaluria and possibly for the usual finding that the renal clearance is a little greater than the glomerular filtration rate (*see below*). Other enzymes besides lactate dehydrogenase may have a physiological role in oxalate biosynthesis. Gibbs (1972) showed that rat liver cell peroxisomes and nuclei contain enzymes that catalyse the oxidation of glyoxylate to oxalate, whereas mitochondria and lysosomes do not. Vandor and Tolbert (1970) showed that glyoxylate is reduced to glycollate and transaminated to glycine by rat liver peroxisomal enzymes; they suggested that liver peroxisomes function as a substrate mediated electron shuttle for the oxidation of cellular NADH. Thus, the availability of glyoxylate for oxidation to oxalate and the rate of oxalate biosynthesis could reflect the redox state of the peroxisomes.

The renal clearance of oxalate has been investigated by infusing ^{14}C oxalate in the dog (Cattell *et al.*, 1962) and in man (Williams *et al.*, 1971). The ratio of ^{14}C oxalate clearance to glomerular filtration rate was significantly greater than unity in both cases, indicating net tubular secretion of oxalate.

Synthesis of oxalate from glycine and glyoxylate within the kidney could have contributed to this result. Cattell *et al.* (1962) also showed that drugs that block renal tubule transport mechanisms reduce the renal clearance and could in the extreme case convert a net tubular excretion into a net tubular reabsorption. A forced diuresis slightly increased the renal clearance of oxalate but changing the urinary pH did not alter the oxalate clearance. The effect of tubule transport blockers, diuresis and pH changes have not been investigated in man.

PRIMARY HYPEROXALURIA

Primary hyperoxaluria is a rare autosomal recessively inherited disorder in which the patients usually present during the first decade with recurrent urinary calculi and a sustained increase in urinary oxalate excretion. Such cases rarely survive beyond the third decade, although occasional patients are encountered first at a later age. The terminal uraemic illness is usually relatively brief and may be complicated by cardiac conduction defects and ischaemic lesions on the extremities. Occasional cases have presented in infancy and died within a few months. The true incidence of primary hyperoxaluria is difficult to establish but it is probably considerably less than one per cent of patients with calcium oxalate urinary stones. At post-mortem examination the kidneys are shrunken and gritty, the parenchyma is destroyed by interstitial calcium oxalate crystals, and the pelvicalyceal system is distorted by multiple calculi. Systemic deposits of calcium oxalate (oxalosis) are striking features. These occur in the myocardium, where they are associated with heart block (Stauffer, 1960; Coltart and Hudson, 1971), the tunica media of the small and medium-size muscular arteries, the rete testis, and at sites of active bone turnover (Scowen *et al.*, 1959).

Primary hyperoxaluria has recently been divided into two subtypes (Types I and II) on biochemical grounds (Williams and Smith, 1968). The urinary excretions of glycollate and glyoxylate as well as oxalate are increased in Type I cases; in Type II cases the excretion of L-glycerate is increased but the glycollate and glyoxylate excretions are normal. Koch *et al.* (1967) showed that patients with Type I primary hyperoxaluria lack the enzyme 2-oxoglutarate: glyoxylate carbologase that catalyses the synergistic decarboxylation of these two acids (Fig. 1) in the soluble fraction of the liver and other tissues, but that the corresponding mitochondrial enzyme is unaffected. Glyoxylate accumulates, is oxidised to oxalate, and reduced to glycollate on an equimolar basis (Fig. 2). The metabolic lesion in Type II primary hyperoxaluria is lack of D-glycerate dehydrogenase (D-glycerate: NAD^+ oxidoreductase EC 1.1.1.29). Williams and Smith (1971) suggest that reduction of accumulated 3-hydroxy-

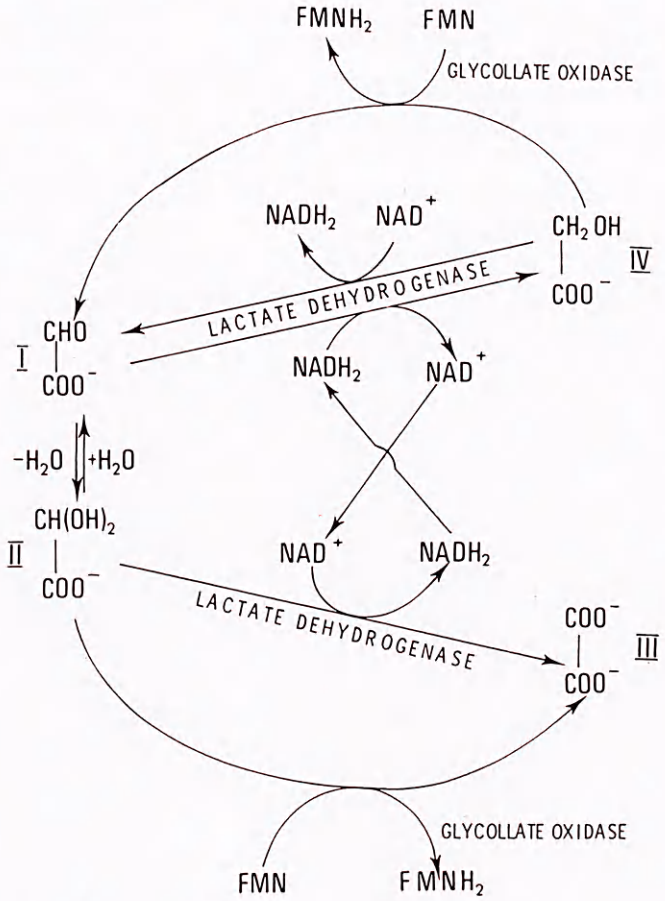

Fig. 2. The actions of lactate dehydrogenase and glycollate oxidase. I = Glyoxylate (aldehyde form); II = Glyoxylate (hydrated form); III = Oxalate; IV = Glycollate.

pyruvate, which is catalysed by lactate dehydrogenase and NADH, promotes the oxidation of glyoxylate to oxalate by increasing the supply of NAD^+. The NADH thereby generated is used again in the reduction of 3-hydroxypyruvate rather than of glyoxylate to glycollate (Fig. 2).

It is of interest to attempt to explain the selective distribution of calcium oxalate crystals which occurs in oxalosis on a biochemical basis. The factors to be considered are:

(i) the lactate dehydrogenase activity of the tissue;
(ii) the isoenzyme composition of the tissue lactate dehydrogenase;

(iii) the concentration of glyoxylate and of other possibly competing lactate dehydrogenase substrates;
(iv) the relative concentrations of NAD^+ and NADH;
(v) the availability of calcium ions.

The myocardial isoenzymes (predominantly isoenzymes 1 and 2) favour oxidative reactions, whereas the liver and skeletal muscle isoenzymes (predominantly isoenzymes 4 and 5) favour the reductive reactions. Impaired metabolism of glyoxylate as in Type I primary hyperoxaluria leaves more of this substrate available for oxidation to oxalate and reduction to glycollate by lactate dehydrogenase; however, the calcium oxalate deposits in primary hyperoxaluria do not follow lactate dehydrogenase activity. The ratio NAD^+/NADH is an important determinant of the activities of alternate metabolic pathways (Krebs, 1967). An increase in the value of the ratio would favour glyoxylate oxidation, making NADH available for the simultaneous reduction of glyoxylate to glycollate on an equimolar basis, unless it were used in another reaction as suggested in Type II primary hyperoxaluria. Myocardium, skeletal muscle and liver contain about the same concentration of total calcium (Long *et al.*, 1961), but in cardiac and smooth muscle calcium ions have the special function of current carrier. Thus, the calcium ion flux associated with muscle action potential is much greater in these tissues than in skeletal muscle (Naylor, 1965; Bulbring and Tomita, 1970; Beeler and Reuter, 1970; Lullman, 1970). This physiological difference in association with lactate dehydrogenase activity may determine the selective deposition of calcium oxalate in cardiac and smooth muscle as opposed to skeletal muscle. Local high concentrations of calcium ions have been demonstrated in the lateral sacs of the sarcoplasmic reticulum and over the I bands of cardiac muscle which has been fixed *in vivo*. The absence of calcium oxalate from the liver in spite of its importance in the oxidation of glyoxylate to oxalate is explained by an absence of local high concentrations of calcium ions. It is suggested that glyoxylate, as well as oxalate, accumulates when renal function is grossly impaired, and that it is oxidised to oxalate by lactate dehydrogenase. Calcium oxalate precipitates if the tissue also contains a high concentration of calcium ions. Anephric non-hyperoxaluric patients are hyperoxalaemic (Zarembski *et al.*, 1966) but they do not develop oxalosis because they can metabolise glyoxylate to oxalate by mechanisms other than oxidation, which are not operative in primary hyperoxaluria, so that there is no increased rate of oxalate biosynthesis at sites of high calcium ion concentration.

There is no specific treatment for primary hyperoxaluria but the following general principles should be followed:

(i) early detection and treatment of intercurrent urinary tract infections;
(ii) prompt surgical intervention to relieve urinary obstruction, with the conservation of as much renal tissue as possible;
(iii) measures to inhibit crystal formation in the urine;
(iv) measures to reduce the degree of over-saturation of the urine with calcium oxalate.

A combination of magnesium oxide or hydroxide to inhibit crystallisation, together with hydration and a low calcium, low oxalate diet to reduce the concentration of both calcium and oxalate in the urine, appears to be the most promising basic palliative regime, although the effect of reducing dietary oxalate intake is usually only small. Sodium orthophosphate may be used in place of the magnesium compounds, as this increases the excretion of pyrophosphate, which inhibits crystallisation; it also reduces calcium absorption, an effect that can be achieved by giving cellulose phosphate. Each component of the basic regime needs to be used to the limit of tolerance and in a sufficiently large dose to produce a measurable chemical effect. Thus, the dose of magnesium oxide or hydroxide, and sodium orthophosphate should be just insufficient to cause diarrhoea, and the urinary magnesium or phosphate excretions should be increased during the treatment. The urinary calcium excretion should be kept low, although children need sufficient calcium for normal bone growth. Large doses of pyridoxine hydrochloride (e.g. 50 to 150 mg every 8 hours) reduce the urinary oxalate excretion in some cases (Smith and Williams, 1967; Gibbs and Watts, 1970) and can be added to the basic regime. Sutor (1970b) has presented experimental data suggesting the potential value of methylene blue as an inhibitor of calcium oxalate crystallisation in primary hyperoxaluria.

Except in the case of pyridoxine, the effect of treatment can be assessed only by its effect on the rate of urinary stone formation. This is an unsatisfactory end-point because of the variable frequency with which stones are passed, which does not necessarily parallel their rate of formation, and it is difficult to judge radiologically small changes in the sizes and numbers of stones. However, untreated primary hyperoxaluria usually has such a bad prognosis that Dent and Stamp (1970) felt justified in concluding from their follow-up studies that magnesium hydroxide had improved the outcome of their series of nine cases. Williams and Smith (1972) concluded that phosphate supplements seemed to reduce stone formation in their patients. Although glycine is an important metabolic precursor of oxalate, attempts to reduce glycine availability for oxalate synthesis by dietary protein restriction (Gibbs and Watts, 1967) or by administering sodium benzoate (Archer *et al.*, 1958; Smith

and Williams, 1967) have not helped therapeutically. Attempts to inhibit the oxidation of glyoxylate to oxalate *in vivo* by means of drugs known to inhibit some of the enzymes which were thought to catalyse the oxidation were unsuccessful. The drugs tried, and the relevant enzyme, were:

(i) allopurinol (xanthine oxidase EC 1.2.3.2);
(ii) disulfiram and calcium carbimide (aldehyde dehydrogenase EC 1.2.1.3);
(iii) acetomenaphthone (aldehyde oxidase EC 1.2.3.1) (Gibbs and Watts, 1967, 1968).

These negative results are not surprising in the light of more recent studies showing the importance of lactate dehydrogenase for the oxidation of glyoxylate to oxalate. Kitamura *et al.* (1971) reported a case of hereditary deficiency of sub-unit H of lactate dehydrogenase in which the patient's serum lactate dehydrogenase was about one-third of the lower limit of the normal range and he presented with only mild diabetes mellitus. Thus, it might be therapeutically practicable to inhibit the enzyme.

Renal transplantation has been unsuccessful in the treatment of primary hyperoxaluria because of the rapid accumulation of calcium oxalate in the grafted kidney (Deodhar *et al.*, 1969; Klauwers *et al.*, 1960). Peritoneal and haemodialysis can remove oxalate from the circulation but are not successful as long-term treatments of primary oxaluria.

POISONING BY ETHYLENE GLYCOL

Ethylene glycol ($HO \cdot CH_2 \cdot CH_2 \cdot OH$) is commonly used as an 'antifreeze' agent and acute poisoning following its ingestion is well recognised. The patients die from acute renal failure associated with obstruction of the renal tubules by calcium oxalate crystals (Pons and Custer, 1946; Friedman *et al.*, 1962). Oxidation to oxalate via glycolaldehyde and glyoxylate is a minor metabolic pathway for ethylene glycol in experimental animals. It accounted for less than two per cent of the ingested dose at all of the dose levels studied by Gessner *et al.* (1961) although the proportion excreted as oxalate tended to increase at the higher doses. These workers also showed that ethylene glycol was most toxic for the animal species which oxidised it most readily to oxalate and that most of it was excreted unchanged or oxidised to carbon dioxide.

METHOXYFLURANE NEPHROPATHY

The use of the volatile anaesthetic methoxyflurane (2,2-dichloro-1,1-fluoromethyl ether) is sometimes associated with postoperative renal failure. Taves *et al.* (1970) suggested that this effect may be directly related to the dose of anaesthetic used. Hyperoxaluria and intratubular deposits of calcium

oxalate have been observed in some of the severe and fatal cases (Frascino *et al.*, 1970; Aufderheide, 1971). The uraemia is characteristically associated with an increased output of dilute urine and haemoconcentration. Most cases have occurred after long operations in the elderly, and the administration of tetracycline has been said to predispose to the complication. Pitressin does not correct the polyuria, and there is no specific association with hypotension during the period of the operation (Mazze *et al.*, 1971; Lawson and Eggers, 1971). Wilson *et al.* (1972) investigated seven women who had been given 0·1 per cent methoxyfluorane for 10 to 20 minutes during Caesarian section, and whose urinary fluoride excretion was markedly increased. Urinary oxalate excretion was increased in three of them only. The pathophysiologic mechanisms underlying the complication are poorly understood. The carbon

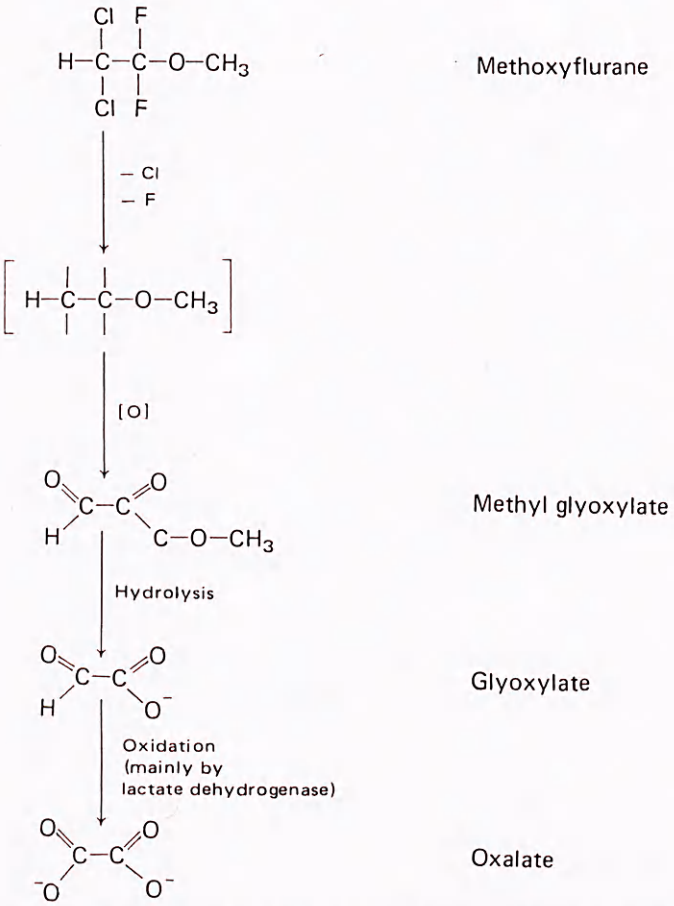

Fig. 3. Hypothetical mechanism for the conversion of methoxyflurane to oxalate.

skeleton of the anaesthetic could be metabolised to glyoxylate and oxalate as shown in Fig. 3, and the findings of Wilson *et al.* (1972) suggest that there may be some individual variation in the extent to which this happens. Acute fluoride poisoning would explain the polyuria with dehydration and azotaemia (Bond and Murray, 1952; Cousins and Mazze, 1972), and further damage would occur if calcium oxalate precipitation added an obstructive element to the basic lesion. The anaesthetic agent or fluoride ions might also increase oxalate production by inhibiting glyoxylate metabolism by pathways other than oxidation to oxalate. The observed and hypothetical interrelationships are shown in Fig. 4.

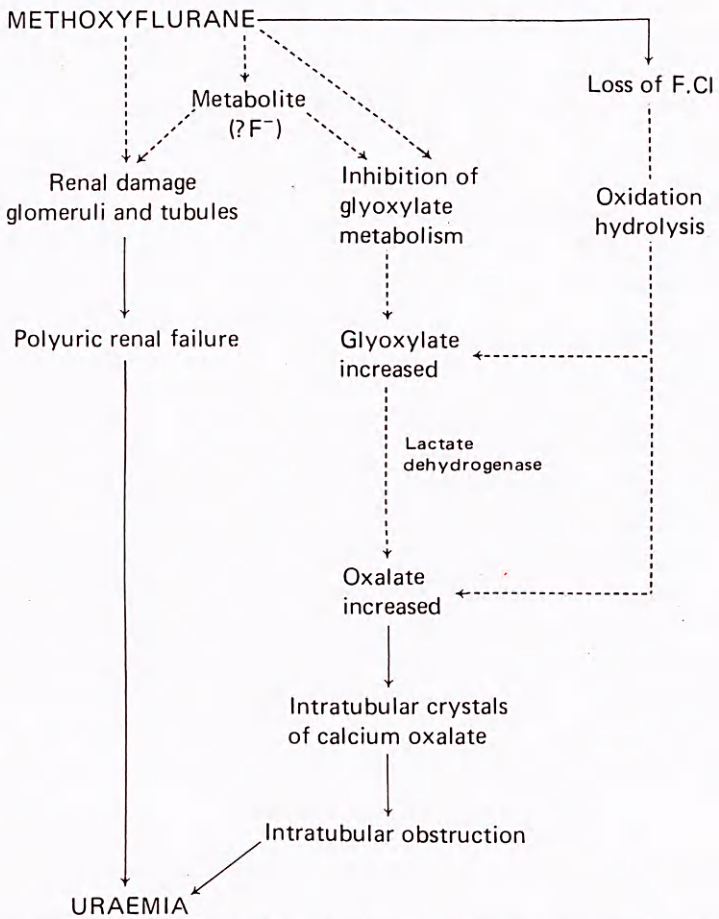

Fig. 4. The possible pathophysiologic basis of methoxyflurane nephropathy. The relationships, which are based on reported observations, are shown by solid lines. The broken lines are hypothetical.

HYPEROXALURIA AND INTESTINAL MALABSORPTION

Hofman *et al.* (1971) and Dowling *et al.* (1971) reported hyperoxaluria and calcium oxalate urolithiasis in patients with extensive disease of the small intestine, and after extensive small bowel resections. These patients excrete excessive amounts of bile salts in the faeces, and it is suggested that bacterial degradation of glycocholate liberates glycine which is oxidatively deaminated to glyoxylate, this being absorbed and oxidised to oxalate. Dowling *et al.* (1971) found that feeding large amounts of taurine in order to lower the glycocholate/taurocholate ratio in the faeces abolished the hyperoxaluria, and they interpreted this as indirect evidence supporting their theory of the causation of hyperoxaluria. The problem has been further investigated by Smith *et al.* (1972) and by Williams *et al.* (1972). Cholestyramine reduces the oxalate excretion in some cases. The enhanced oxalate synthesis from glyoxylate occurs without glycollic aciduria as in Type II primary hyperoxaluria. Williams *et al.* (1972) suggest that this may be related to increased NADH:NAD^+-linked bile acid synthesis which generates NAD^+ and thereby favours the oxidation of glyoxylate to oxalate in the liver.

PYRIDOXINE AND HYPEROXALURIA

The urinary oxalate excretion is increased in severe experimental pyridoxine deficiency in animals (Andrus *et al.*, 1959; Gershoff *et al.*, 1959a). Gershoff *et al.* (1959b) reported that pyridoxine reduced the urinary oxalate excretion of a group of institutionalised mental defectives who were not taking a pyridoxine-deficient diet, and Faber *et al.* (1963) demonstrated increased urinary oxalate excretion by normal subjects in whom a combined pyridoxine and pantothenic acid deficiency had been produced. Ludwig (1963) reported a patient with 'relative deficiency' of pyridoxine and a mild degree of hyperoxaluria which was corrected by pyridoxine treatment. Very large non-physiological doses of pyridoxine reduce the urinary oxalate excretion in some cases of primary hyperoxaluria; Gibbs and Watts (1970) showed that this was not due to the correction of pyridoxine deficiency. There have been no reports of increased oxalate production in the known vitamin B_6 dependency states: cystathioninuria, sideroblastic anaemia, xanthinurenic aciduria, and the cases of infantile convulsions associated with suboptimal pyridoxine intake (Scriver, 1967).

THIAMINE AND GLYOXYLATE METABOLISM

Thiamine deficiency in the rat is associated with increased concentrations of glyoxylate in the blood and urine (Liang, 1962a, b). Buckle (1963) reported the presence of glyoxylate, 61 to 286 μg glyoxylic acid per 100 ml, in the blood

of two cases of occidental dietary thiamine deficiency with cardiac failure, but not in thiamine deficiency with dominantly neurological manifestations. The detection limit of this analytical method was equivalent to 12·5 μg glyoxylic acid/100 ml plasma. The urinary oxalate excretion was not measured in these cases. Glyoxylate: 2-oxoglutarate carbologase requires thiamine pyrophosphate as a co-enzyme but further analytical work using sensitive analytical techniques is needed before gross dietary thiamine deficiency can be regarded as the nutritional analogue of Type I primary hyperoxaluria.

ENDEMIC BLADDER STONE IN SOUTH-EAST ASIA

The problem of endemic calculus disease has been especially well studied in north and north-east Thailand (Dhanamitta *et al.*, 1967; Halstead and Valyasevi, 1967; Valyasevi *et al.*, 1967; Valyasevi and Dhanamitta, 1967; Valyasevi *et al.*, 1969; Dhanamitta and Valyasevi, 1972). Here, the condition is associated with the practice of partly replacing breast feeds with premasticated glutinous rice (*Oryza glutinosa*) from the early neonatal period onwards. The supplements are sufficient to supply about half the infant's total calorie requirements. They increase the intake of L-hydroxyproline, which is a metabolic precursor of glyoxylate and, hence, of oxalate, as well as reducing the intake of breast milk and total fluids. The infants have increased urinary oxalate and L-hydroxyproline excretions, with aggregated calcium oxalate crystals in the urine. Their urinary phosphate excretion is low. Oral therapy with orthophosphate reduced the clumping of the calcium oxalate crystals and lowered the urine calcium concentration. The prevalence in boys rather than girls is explained by the short, straight female urethra, which permits the passage of aggregates of calcium oxalate that are retained in the male bladder and form centres for stone development.

It is unlikely that all cases of endemic vesical calculus are caused by the same factors, so that these conclusions will not be wholly applicable to the problem in other parts of the world. However, they have great general value as a classic example of what may come to be called biochemical epidemiology.

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