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Combined Deficiency of Xanthine Oxidase and Sulphite Oxidase: A Defect of Molybdenum Metabolism or Transport?

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A child is described who presented in the neonatal period with feeding difficulties, severe neurological abnormalities, lens dislocation of the eyes and dysmorphic symptoms of the head. Routine laboratory investigations revealed a decreased serum urate and a positive sulphite reaction of the urine. Subsequent chromatographic examinations showed xanthinuria and increased excretion of *S*-sulphocysteine and taurine to be present. In addition, high thiosulphate and low sulphate excretions in the urine were observed. Xanthine oxidase deficiency was demonstrated in a jejunal biopsy specimen, whereas the excretion of sulphur containing substances was considered to be characteristic of sulphite oxidase deficiency.

This new combination of defects may be the result of malfunctioning of both enzymes, possibly caused by alterations in the essential molybdenum containing active centre of the enzymes, which they share in common.

Sulphite oxidase deficiency is a rare inborn error of cysteine metabolism (see Figure 1), only three patients with remarkable similar symptoms having been described (Irreverre *et al.*, 1967; Shih *et al.*, 1977). These symptoms include mental retardation, severe neurological abnormalities and lens dislocation. Probably also lethal neonatal cases occur. Chemically, these patients were characterized by an increased urinary excretion of sulphite, thiosulphate, *S*-sulphocysteine and taurine and a decreased sulphate excretion. Until now no good explanation has been found for the clinical symptoms in this disorder, although one may think that the toxicity of sulphite plays a major role. Xanthinuria, a defect of purine metabolism affecting the xanthine oxidase catalysed conversion of hypoxanthine to xanthine and the formation of uric acid from the latter compound, is a relatively benign disorder. After the original description by Dent and Philpot (1954) some 40 cases have been reported (Cartier and Perignon, 1978). About half of the patients have complaints of urolithiasis, the other ones

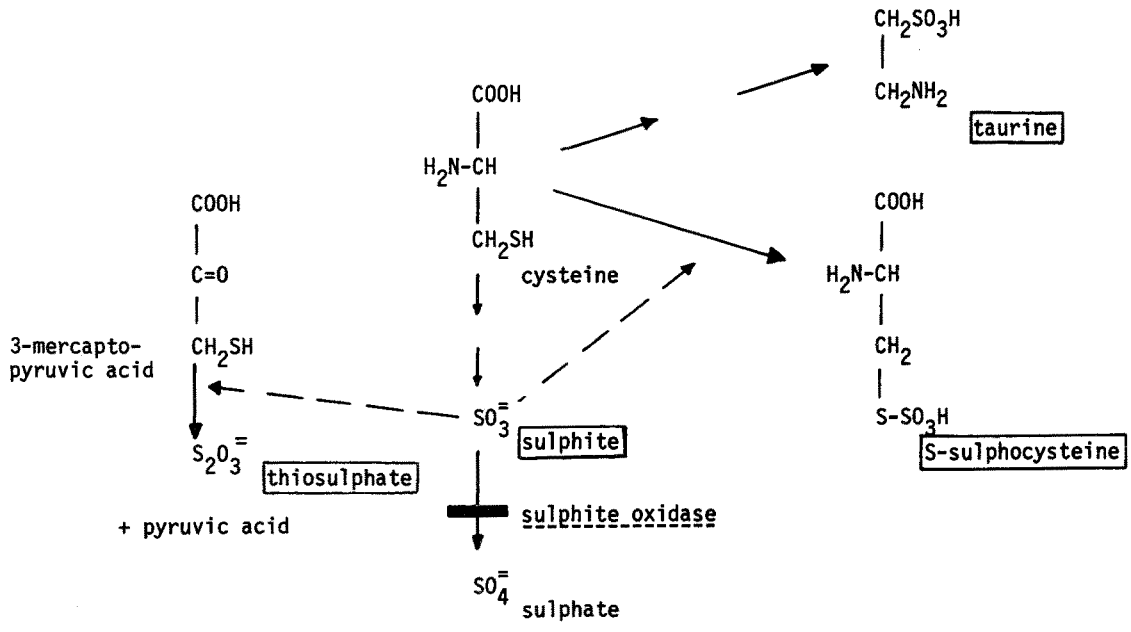

Figure 1 Abnormal cysteine metabolism in sulphite oxidase deficiency. Outlined compounds are excreted in excess. Sulphate excretion is strongly diminished

being mainly without symptoms, although mental retardation and myopathy have been reported in isolated cases. Molybdenum is incorporated in the active centre of both sulphite oxidase and xanthine oxidase (Figure 2) (Johnson *et al.*, 1974). In this report we will describe a girl in whom both defects were demonstrated. The implications of this finding with respect to the functioning of the active molybdenum centre in both enzymes will be discussed.

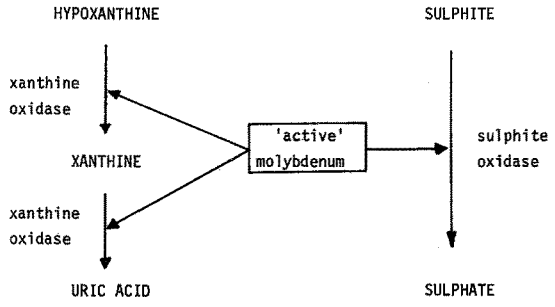

Figure 2 Essential role of molybdenum in adequate functioning of sulphite oxidase and xanthine oxidase

METHODS

Neutral and basic amino acids were analysed by micro two-dimensional thin-layer chromatography (Wadman *et al.*, 1975). Acidic amino acids were analysed by automated column chromatography (Technicon TSM 1 procedure) with the following modification: the samples were applied on sampling cartridges loaded with a strong anion-exchange resin (Technicon type S Chromobeads).

Urinary and serum uric acid were measured enzymatically. Urinary oxypurines were measured by two-dimensional thin-layer chromatography (van Gennip *et al.*, 1978) and by quantitative cation-exchange column chromatography (Stoop *et al.*, 1977). Thiosulphate was determined by a modified colorimetric procedure (Sörbo, 1957) after conversion to thiocyanate. Urinary sulphate was measured by a radiochemical technique: precipitation with labelled barium and counting of the excess radioactivity in the supernatant. The sulphite reaction in the urine was carried out with Merckoquant Sulfit-test.

Molybdenum concentrations in serum were measured with neutron activation analysis.

CASE REPORT

The female patient E.V. was the second child of healthy parents. An older brother is healthy and the mother has had one miscarriage. At the age of 10 days she was admitted to our hospital because of feeding difficulties. On admission a small body (length and weight according to the 10th percentile) was seen. There were many dysmorphic signs of the head: frontal bossing, asymmetry of the skull and a slight medio-facial dysplasia. Enophthalmus, nystagmus and a ring of Brushfield spots were observed. Both lenses were dislocated supero-temporally. The sucking reflex was poor, other reflexes being unremarkable. The EEG showed diffuse irregularities and hypofunctional and irritative disturbances at the right hemisphere. Typical tonic-clonic seizures were present. The PEG showed widened ventricles and periventricular atrophy.

Routine laboratory investigations were unremarkable with the exception of a decreased serum urate (0.01–0.07 mmol/l, normals: 0.12–0.35 mmol/l) and a positive sulphite test of the urine. Subsequent chromatographic analyses revealed an increased excretion of xanthine, hypoxanthine, *S*-sulphocysteine and taurine. The urinary excretion of thiosulphate proved to be increased whereas inorganic sulphate in the urine was strongly decreased. No mental progress was observed. Upon suspicion of sulphite oxidase deficiency the child was given a diet low in *S*- amino acids, but consisting of natural proteins.

At the age of 14 months small brownish concrements were noticed in her diapers. These calculi were proven to be xanthine stones. Alkalinization with 4 × 250 mg sodium bicarbonate and administration of 10 mg/kg allopurinol did not reverse the production of this gravel, nor did the latter drug change the hypoxanthine/xanthine ratio in the urine.

Now, at the age of almost 2 years, this girl is extremely hypertonic. Her head control is poor. She does react to painful stimuli and sounds, but not to light. Her length and weight are at the 10th percentile.

RESULTS

After the initial finding of a low serum urate, quantitative column liquid chromatography of urinary purines and pyrimidines revealed high excretions of xanthine and hypoxanthine. Typical excretory values are given in Table 1. Xanthine oxidase, as measured in an intestinal biopsy specimen, was proven to be absent, while several disaccharidases, which were used as reference enzymes, showed a completely normal activity.

The sulphite reaction, tested with Merckoquant Sulfit-test, was always positive in the patient's urine (i.e. > 10 mg/l), although the sulphite concentration varied considerably. This may for a part be due to the extreme instability of sulphite in aqueous solution. Allowing a urine sample to stand for a few hours may result in falsely negative results. Preservation of sulphite upon storage in a refrigerator is somewhat better, although losses cannot be completely avoided. The sulphite concentration is stable in the deep-freeze. The excretions of sulphur-containing metabolites, which are considered to be characteristic of sulphite oxidase deficiency, viz. *S*-sulphocysteine, taurine, thiosulphate and sulphate, are listed in Table 1. The excretion of inorganic sulphate was extremely low, which is compatible with the proposed defect. A decrease of the 'abnormal' sulphur-containing metabolites was observed after introduction of a low sulphur diet, with a concomitant decrease of inorganic sulphate (Table 1). Determination of the serum molybdenum concentration showed a completely normal value: 1.7 ng/ml (control: 1.0 ng/ml).

Administration of extra molybdenum in the form of oral supplements of ammonium molybdate (up to

Table 1 Excretion of characteristic metabolites in patient E.V. with combined xanthinuria and sulphituria

Compound	*mmol/l*	*mmol/g creatinine*	*Comments*
Xanthine	0.5–1.2	3.7–7.6	controls 0.06–0.25 mmol/g creatinine*
Hypoxanthine	1.4	0.1–1.1	controls 0.02–0.07 mmol/g creatinine*
S-sulphocysteine	0.4–0.8	2.3–2.6	control: n.d.
S-sulphocysteine	0.3–0.5	1.4–2.0	low sulphur diet
Taurine	0.3–2.6	1.9–7.6	
Taurine	0.3–0.8	1.1–1.6	low sulphur diet
Thiosulphate	0.28	0.80	controls: 0.02 mmol/l
Thiosulphate	0.06–0.14	0.30–0.36	low sulphur diet
Sulphate	0.74–1.85	1.91–13.70	control: 11.4 mmol/l
Sulphate	0.39	1.81	low sulphur diet

n.d.: not detected.
* : Balis *et al.*, 1967.

200 μg/day) did not lead to clinical or biochemical improvement.

Sulphite oxidase activity has still to be assayed, preferably in liver tissue.

DISCUSSION

The diagnosis xanthinuria is in many cases an accidental one, because most patients are without symptoms. In our case the suspicion of xanthine oxidase deficiency arose upon the finding of an extremely low serum uric acid, a compound which is routinely analysed in our hospital in children with mental disorders. Several other inherited defects leading to decreased uric acid levels are known, e.g. purine nucleoside phosphorylase deficiency (Stoop *et al.*, 1977) and the tubular reabsorption defect of urate. Thus the investigation of serum uric acid is a valuable screening test in the investigation of metabolic disease.

The biochemical diagnosis of sulphite oxidase deficiency is considered to be rather difficult (Shih *et al.*, 1977). The sulphite reaction has to be carried out in fresh urine or in urine which has been frozen immediately after voiding. False negative results of this test may be obtained if the patient's protein intake is extremely low. Analysis of the characteristic amino acid *S*-sulphocysteine may be troublesome because of its hydrophilic properties. This amino acid is not retained on a normal cation-exchange resin and is therefore lost in any prepurification step making use of this kind of resin. On a normal amino acid analyser *S*-sulphocysteine is eluted in the first emerging peak, together with cysteic acid. We therefore analysed the urine samples before and after acid hydrolysis and calculated the difference as *S*-sulphocysteine. No definite metabolic route is known along which the formation of *S*-sulphocysteine takes place. It is even questionable if the absence of sulphite oxidase is essential for its formation, as in some East African animals (blotched Kenya Genet) *S*-sulphocysteine is a normal urinary constituent (Crawhall and Segal, 1965). The clinical symptoms in the here described patient are remarkably similar to those described in the other patients with sulphite oxidase deficiency (Irreverre *et al.*, 1967; Shih *et al.*, 1977). However, the onset of the symptoms started at different moments in the various patients. These differences may indicate variations in the severity of the metabolic defect, although no evidence for this was obtained by studying excretory values. In our patient dislocation of the eye lenses was already present at the age of 10 days, suggesting that the lesion was already present at birth as a result of intrauterine effects of sulphite oxidase deficiency. At the age of 14 months our patient started to show the common symptoms of xanthine oxidase deficiency, i.e. the excretion of urinary tract stones. To our knowledge this is the youngest age at which these stones have been found (Cartier and Perignon, 1978). Our data indicate that the administration of the xanthine oxidase inhibitor allopurinol in xanthinuric patients is probably useless as no change in the hypoxanthine/xanthine ratio occurred and also the total production of oxypurines did not diminish. The remaining treatment for xanthine calculi is therefore increasing the fluid intake and alkalinization because the solubility of xanthine increases markedly with pH (Seegmiller, 1968).

The combination of xanthinuria and sulphituria is a unique, although not completely unexpected, variation of nature. It has been known for several years that xanthine oxidase and sulphite oxidase are virtually the only two enzymes in the human body requiring active molybdenum centres as a cofactor (Johnson *et al.*, 1974). The exact functioning of this 'active' molybdenum in combination with the haem groups present in both enzymes has not yet been clarified (Johnson and Rajagopalan, 1976). The biological function of both enzymes can be reversibly destroyed by tungsten, as was demonstrated in tungsten-treated rats (Johnson *et al.*, 1974). Also a deficiency of molybdenum resulting from absence of this trace element from the diet might lead to the same consequence. This was in part demonstrated in sheep grazing on low-molybdenum soils. These animals passed xanthine stones in the urine.

Several hypotheses remain open for the underlying defect in our patient. As already pointed out an intestinal transport defect is improbable, because a normal serum molybdenum concentration was found. Other possibilities are: a defective transport of molybdenum into the cell or a disturbance in the incorporation of molybdenum into the active centre. Theoretically this might mean that there exists a molybdenum dependency, in analogy to the

vitamin B_{12} dependency which exists in several forms of methylmalonic acidaemia. We tested this theory in our patient by giving extra molybdenum. However, there was no clinical or biochemical response. The cause of this lack of effect is still under investigation: one may think that molybdenum administered as ammonium molybdate is not resorbed or does not enter the cell.

A more direct approach for treatment would be administration of 'active molybdenum', which is present in the xanthine oxidase-containing fraction of milk protein. Experiments in this direction are in progress.

References

Balis, M. E., Krakoff, I. H., Berman, P. H. and Dancis, J. (1967). Urinary metabolites in congenital hyperuricosuria. *Science*, **156**, 1122

Cartier, P. and Perignon, J-L. (1978). Xanthinurie. *La Nouvelle Presse Med.*, **7**, 1381

Crawhall, J. C. and Segal, S. (1965). Sulphocysteine in the urine of the blotched Kenya genet. *Nature*, **208**, 1320

Dent, C. E. and Philpot, G. R. (1954). Xanthinuria, an inborn error (or deviation) of metabolism. *Lancet*, **i**, 182

Van Gennip, A. H., van Noordenburg-Huistra, D. Y., de Bree, P. K. and Wadman, S. K. (1978). Two-dimensional thin-layer chromatography for the screening of disorders of purine and pyrimidine metabolism. *Clin. Chim. Acta*, **86**, 7

Irreverre, F., Mudd, S. H., Heizer, W. D. and Laster, L. (1967). Sulfite oxidase deficiency: study of a patient with mental retardation, dislocated ocular lenses, and abnormal urinary excretion of *S*-sulfo-L-cysteine, sulfite, and thiosulfate. *Biochem. Med.*, **1**, 187

Johnson, J. L. and Rajagopalan, K. V. (1976). Purification and properties of sulfite oxidase from human liver. *J. Clin. Invest.*, **58**, 543

Johnson, J. L., Rajagopalan, K. V. and Cohen, H. J. (1974). Molecular basis of the biological function of molybdenum. Effect of tungsten on xanthine oxidase and sulfite oxidase in the rat. *J. Biol. Chem.*, **249**, 859

Seegmiller, J. E. (1968). Xanthine stone formation. *Am. J. Med.*, **45**, 720

Shih, V. E., Abroms, I. F., Johnson, J. L., Carney, M., Mandell, R., Robb, R. M., Cloherty, J. P. and Rajagopalan, K. V. (1977). Sulfite oxidase deficiency. Biochemical and clinical investigations of a hereditary metabolic disorder in sulfur metabolism. *N. Engl. J. Med.*, **297**, 1022

Sörbo, B. (1957). A colorimetric method for the determination of thiosulfate. *Biochim. Biophys. Acta*, **23**, 412

Stoop, J. W., Zegers, B. J. M., Hendrickx, G. F. M., Siegenbeek van Heukelom, L. H., Staal, G. E. J., de Bree, P. K., Wadman, S. K. and Ballieux, R. E. (1977). Purine nucleoside phosphorylase deficiency associated with selective cellular immunodeficiency. *N. Engl. J. Med.*, **296**, 651

Wadman, S. K., de Bree, P. K., van Sprang, F. J., Kamerling, J. P., Haverkamp, J. and Vliegenthart, J. F. G. (1975). N^{ε}-carboxymethyllysine, a constituent of human urine. *Clin. Chim. Acta*, **59**, 313