The allopurinol load test lacks specificity for primary urea cycle defects but may indicate unrecognized mitochondrial disease

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Summary: Thirty-three children ranging from 2 weeks to 12 years of age were selected for allopurinol loading, 16 on the basis of an increased urinary orotate excretion detected by routine organic acid analysis (group A), and 17 for clinical reasons suggesting a urea cycle defect (group B). The allopurinol load test proved positive in 13 of 16 patients from group A, mean peak orotate 64.0 μmol/mmol creatinine (upper limit of reference range, 13.2) and 11 of 17 patients from group B, mean peak orotate 41.0 μmol/mmol creatinine (upper limit of reference range, 13.2). Thorough investigation of these patients including urinary and plasma amino acid analysis and, in 17 cases, liver biopsy for histology and measurement of ornithine carbamyltransferase (OCT) and carbamyl-phosphate synthetase (CPS) activity failed to identify any evidence of a urea cycle disorder. However, muscle biopsies performed in 11 patients showed some evidence of mitochondrial disease in four cases, two defined on the basis of reduced respiratory chain enzyme activity and two on the basis of mtDNA abnormalities. These findings indicate that an increased excretion of orotate in sick children may not be uncommon and that a positive allopurinol load test result may not indicate a specific inherited urea cycle defect. In addition, these results raise the interesting possibility that defective ureagenesis may be a feature of mitochondrial disease in some individuals.
Carbamyl phosphate accumulation associated with inherited defects of the urea cycle can stimulate \textit{de novo} pyrimidine synthesis and result in an increased production of orotate and orotidine. This is more pronounced in the presence of allopurinol, which is believed to inhibit orotidine monophosphate decarboxylase and consequently to prevent the incorporation of orotidine in nucleotide synthesis (Fox et al 1970). Indeed, demonstration of an increased plasma concentration of orotidine and increased urinary excretion of orotate and orotidine following oral allopurinol administration has been proposed as a safe and reliable means of identifying carrier status for ornithine carbamyl-phosphate transferase (OCT) deficiency in adult females (Hauser et al 1990; Naylor et al 1977). More recently, several reports have also advocated its use in children (Burlina et al 1992; Riudor et al 1995; Sebesta et al 1994b) suspected of having a urea cycle defect or as part of the further investigation of increased orotate excretion in a randomly obtained urine sample. In most urea cycle disorders, orotidine accumulates and orotate excretion is markedly increased following allopurinol loading (Burlina et al 1992; Riudor et al 1995; Sebesta et al 1994a). The test appears to have a high sensitivity for detecting such conditions. However, a recent study of two female patients (Carpenter et al 1997) concluded that it may lack specificity and the authors suggest that positive results should be interpreted with caution.

We report the results of allopurinol loading in 33 children, 24 of whom gave a positive result. Despite thorough laboratory investigation and liver biopsy in 17 cases, no convincing evidence of a recognizable urea cycle defect could be found in any of these patients. However, 11 were subsequently investigated to exclude a mitochondrial disorder and 4 were found to have evidence of previously unsuspected mitochondrial disease.

**PATIENTS**

Thirty-seven patients were investigated over a 3-year period.

\textit{Group A — increased orotate excretion identified on urinary organic acid analysis:} Twenty patients, 10 female, 10 male, mean age 30 months, range 3 month–12 years, were identified on the basis of qualitatively increased orotate excretion following examination of urinary organic acids by gas chromatography–mass spectrometry (GCMS) (Table 1).

Allopurinol loading was performed in 16 cases. Percutaneous liver biopsy was performed for histological studies and measurement of OCT and carbamylphosphate synthetase activity in 10 of the children with an abnormal response to allopurinol loading. Muscle biopsy material was also obtained in 7 patients for histology and measurement of respiratory chain enzyme activity and investigation of mitochondrial DNA.

\textit{Group B — suspected for clinical reasons:} Seventeen patients, 11 female, 6 male, mean age 44.3 months, range 0.5 months–12 years, were investigated to exclude a possible urea cycle defect because of suggestive clinical features. These included children with cyclical vomiting, ataxia or hyperammonaemia and the siblings of