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TECHNICAL NOTE

Capillary gas chromatography measurement of oxalate in plasma and urine

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Oxalic acid can be measured reliably in urine by colorimetric methods [1] and is useful for the diagnosis of hyperoxaluric states that result in nephrolithiasis. The measurement of plasma oxalic acid has been far more difficult due to its very low concentration in normal plasma and a variety of methodologic problems. Normal plasma oxalic acid, measured by in vivo isotopic dilution, has been reported in the range of ~1 μmoles/liter (~9 μg/dl) [2–4]. Far higher values, obtained by most published methods [5, 6] suggest a lack of specificity that would obscure variations in normal levels. The difficulty in preserving specimens, the lack of a way of monitoring interference, and the need for very large sample volumes makes enzymatic methods [7] less than ideal.

Recently, Wolthers and Hayer [8] reported a capillary gas chromatographic method for measuring plasma oxalic acid that gave a normal range closer to that obtained by in vivo isotopic dilution [4]. We have adapted and verified this method for measuring urinary and plasma oxalic acid and applied the method to normal adults and patients with primary hyperoxaluria. These patients have been studied during renal insufficiency, during hemodialysis, and with various levels of renal function after renal transplantation.

Methods. Urine specimens were collected for 24 hr on ice, acidified to a pH of less than 2.0 with HCl (1:100 v/v), and frozen at −20°C or −70°C until analysis. To triplicate 0.5 ml samples we added malonic acid (15 μg), a further two drops of 6 N HCl, and 1 g NaCl. Heparinized blood was collected on ice, the plasma separated (×2000 *g*, 20 min) within 1 hr of collection, and 0.5-ml aliquots acidified with two drops of HCl and frozen (−70°C) in microfuge tubes (Sarsted). Samples were partially thawed to transfer of the frozen/precipitated sample, containing 2 μg of malonic acid and 1 g NaCl, with one rinse of 0.5 ml of 0.1 N HCl. For either urine or plasma aliquots, a 4-min vigorous solvent extraction with 6 ml of ethyl acetate was performed twice. Ethyl acetate was evaporated at 35°C under anhydrous N_2, placed under P_2O_5 in vacuo for 30 min and organic acids derivatized in the same tube with 50 to 150 (urine) or 30 μl (plasma) of 30:1 (v/v) mixture of bis-trimethylsilyl-trifluoroacetamide (BSTFA) (Regis Chemical Co., Morton Grove, Illinois, USA) with bromotrimethylsilane (TMBS) (Sigma Chemical Co., St. Louis, Missouri, USA) prepared freshly each day. Tubes were tightly capped, heated at 80°C for 15 min, and 2 μl injected into the column within 6 hr.

Capillary gas chromatography (CGC) was performed on a gas chromatograph (Model 5880-A, Hewlett-Packard, Elkhart, Indiana, USA) equipped with an automatic sample injector, two split-mode capillary injection ports, and two-flame ionization detectors. Peak areas were integrated automatically from the detector signals. The di-TMS derivatives of oxalic (mw 234) and malonic (mw 248) acids from urine samples were separated on a fused silica capillary column (25 m × 0.31 mm I.D.) with a film 0.52 μm thick and a stationary phase of 5% phenylmethyl silicone, Ultra-2 (Hewlett-Packard). Because of an interfering substance in plasma, these were analyzed on an Ultra-1 column (Hewlett-Packard) with a film thickness of 0.17 μm, and a stationary phase of 5% methyl silicone, Ultra-1 (Hewlett-Packard). The carrier helium flow rate (linear velocity) was 21 cm/sec, the injection port temperature was 250°C, and the detector temperature was 300°C. The split ratio was 1:50 for urine and 1:10 for plasma. The oven temperature was raised from 50°C to 140°C over 18 min, then to 300°C and held for 10 min.

Results. The standard curves used for urine oxalic acid determination, for the ranges of 1.25 to 20 and 6 to 100 μg of oxalic acid were completely linear, with a regression equation of: peak area/20 equals μg oxalic acid per sample. For the plasma standards, the completely linear relationship was: peak area/80 equals μg oxalic acid, over the range of 0.125 to 5 μg.

Extraction efficiency and recovery studies. Solutions of ^{14}C oxalic (0.25 μ Ci/ml) (New England Nuclear Corp., Boston, Massachusetts, USA) and ^{14}C malonic (0.125 μ Ci/ml) acids in 0.1 N HCl were added to triplicate normal urine aliquots, documenting a maximum extraction efficiency under the conditions described, of 83 ± 4.6% for oxalic and 88 ± 4.6% for malonic acids. For plasma, in the presence of added unlabelled oxalic and malonic acids at levels of 0, 0.2, 0.5, 2.5 and 5 μg, extraction efficiencies ranged from 70 to 85%. There was no greater than a 6% difference between malonic and oxalic acids.

The overall recovery of unlabelled oxalic acid (at 6, 12.5, 25, 50, and 100 μg) and malonic acid (100 μg) measured in normal urine samples by CGC, for malonic acid was 93 ± 6.3% and for oxalic acid was 89 ± 8%. A separate series of recovery studies over the range of (added) oxalate of 25 to 200 μg gave 93 ± 11%,

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and for the range of 50 to 150 μg, 95 ± 7%. The overall oxalic acid recovery, over the range of 125 ng to 4 μg, after the correction of the peak area for the malonic acid internal standard area, was 0.83 ± 19% on nine samples for plasma. There was complete stability of triplicate samples run four times, from 6 hr to 6 months after acidification and freezing at −70°C.

Urinary oxalic acid was measured on 24-hr urine samples from a clinic population with recurrent nephroliathias. The population mean for the CGC method was 36.1 ± 19.7 (SD) mg/24 hr excretion, $N = 19$. Duplicate samples (courtesy of Dr. Charles Smith) analyzed by the method of Hodgkinson [1] gave 36.70 ± 14.2 mg/24 hr; $r = 0.636$, $P < 0.003$. The coefficient of variation for the CGC method was ± 12%, $N = 12$.

Normal plasma oxalate was determined on triplicate samples from 16 healthy adult volunteers. The mean oxalate concentration (corrected for 83% recovery) without an internal standard correction was 352 ± 70 (SD) ng/ml in the range 246 to 540 ng/ml with the median value of 341 ng/ml. Corrected for the malonic acid internal standard, the normal plasma oxalate was 443 ± 74 ng/ml, range 309 to 550 ng/ml, median 449 ng/ml (0.045 mg/dl). The coefficient of variation was ± 11.4% ($N = 9$), with or without correction for the internal standard.

In patients with primary hyperoxaluria with stable renal function [9], plasma oxalate was correlated with serum creatinine, corrected for 1.73 m^2 body surface area: $r = 0.818$, $P = 0.002$, $N = 11$. The most likely linear relationship is: oxalate (mg/dl) = Corr Cr (mg/dl)/5.2.

Endogenous oxalate and creatinine clearances were measured seven times in five patients with primary hyperoxaluria. The clearance ratios of oxalate to creatinine varied from 0.26 to 2.7. Elevated plasma oxalate (0.16 mg/dl) was found even in a patient with good renal function (creatinine clearance 67 ml/min) 7 years after transplantation. A proportionately high ratio of plasma oxalate to creatinine (in mg/dl) within 1 month of transplantation (three measurements), (0.32/.9, 0.42/1.0, 1.2/1.5) suggests the slower excretion of the pool of oxalate than that of creatinine. This was also reflected in the high (> 500 mg/24 hr) urinary oxalate excretion in these patients. Continued high plasma oxalate to creatinine ratios (0.95/2.8, 1.2/1.4) months after transplant in two patients were accompanied by recurrent deposition of oxalate in the transplanted kidney [9].

Dialysance of oxalate was determined for three adults and one infant with hyperoxaluria from simultaneous arterial/venous plasma determinations and calibrated hemodialysis blood flow. For five clearances in adults using a cupraphane hollow-fiber dialyzer, at flow rates from 150 to 350 ml/min, the oxalate/creatinine clearance ratio was 0.88 ± 0.09 (SD), the oxalate/urea clearance ratio was 0.73 ± 0.03. The creatinine/urea clearance ratio was 0.83 ± 0.09. For four clearances in the infant, using the unipuncture technique and a plate dialyzer at flows of 10 to 40 ml/min, the oxalate/creatinine clearance ratio was 0.95 ± 0.03, the oxalate/urea clearance ratio 0.85 ± 0.06 and creatinine/urea clearance ratio was 90 ± 0.04.

Discussion. The present study documents a CGC assay for plasma and urinary oxalic acid. Normal plasma oxalic acid values (4.9 ± 0.8 μM, 0.044 ± 0.007 mg/dl) are close to the range of those obtained by isotopic dilution methods and far lower than those by most previous methods [7, 10]. The method has the sensitivity necessary to follow, in small sample sizes, the changes in plasma oxalate in normal subjects, in hyperoxaluric patients at different levels of renal function after transplantation, and in measuring renal and dialysis oxalate clearances.

The CGC method measures the di-TMS derivative of oxalic acid. The variability of results (± 12%) derives from the use of malonic acid di-TMS as an internal standard, which can break down to the mono-TMS derivative, most notably beyond 6 hr after derivatization. This variability is similar to those of most other methods [11]. Samples can be preserved successfully, without special preservatives [12], and interfering substances can be identified on the chromatogram. CGC can also provide data on the important precursors of oxalic acid (glycollic, glyoxylic, and glyceric acids) that can assess the effects of metabolic manipulations on the synthesis and excretion of oxalic acid.

A practical application of dialysis clearances is the ability to customize dialysis for oxalosis [9]. In an adult patient with a plasma creatinine of 10 mg/dl, corresponding to an oxalate value of 1.5 mg/dl, a urea clearance of 160 ml/min, and an oxalate clearance of 120 ml/min, 540 mg of oxalic acid could be removed in a 6-hr dialysis, which represents approximately twice the daily production in hyperoxaluric patients. With a plasma creatinine of 4 mg/dl and a plasma oxalate of 0.75 mg/dl, half that amount would be removed. Thus, daily dialysis could remove more than the daily production of oxalate, both in infants and adults. Hemodialysis clearance can be optimized for hyperoxaluric patients whose usual course during dialytic therapy has been catastrophic. The degree to which intensive hemodialysis can deplete exchangeable stores of oxalate may be reflected by the decrease in plasma oxalate, which then may predict the success of transplantation.

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