Contribution of Dialysis to Endogenous Oxalate Production in Patients with Chronic Renal Failure

Necia C. France,1 Patrick T. Holland,2,4 Martin R. Wallace3

We tested the possibility that the buffering agents in dialysis bath fluid might contribute to increased endogenous oxalate production in dialyzed patients. Using stable isotope dilution mass spectrometry, we obtained oxalate production rates and pool sizes directly for 10 patients in chronic renal failure, 5 of whom were undergoing continuous ambulatory peritoneal dialysis (lactate-buffered fluid). All peritoneal dialysis patients had either increased oxalate production rates or expanded oxalate pools when compared with undialyzed patients in renal failure. From a further four patients receiving maintenance hemodialysis we took blood samples immediately before and after three consecutive dialysis sessions in which the bath-fluid buffering agent (bicarbonate or acetate) was alternated; we analyzed these samples for oxalate and key precursors by capillary gas chromatography. Plasma glycine and serine concentrations remained within the physiological range. Glycolate and oxalate concentrations decreased, but the oxalate remained above normal after dialysis. All changes were independent of the bath-fluid buffering agent. We suggest that dialysis might stimulate the formation of oxalate by removing product inhibition of a late catabolic step.

Indexing Terms: chromatography, gas capillary/mass spectrometry, isotope dilution

Oxalate is an end product of the catabolism of amino acids and ascorbate. Chronic renal failure results in retention of oxalate and possibly also in the deposition of pathologically significant amounts of crystalline calcium oxalate in renal, cardiac, or peripheral vascular tissues (1).

In 1985 we reported the paradox of finding higher serum oxalate concentrations in chronic renal failure patients maintained on hemodialysis or receiving continuous ambulatory peritoneal dialysis than in patients in renal failure not on dialysis (2). Later reports have confirmed this observation both for peritoneal dialysis patients (3) and for hemodialysis patients (4). In one study, administration of pyridoxine (which catalyzes a "salvage" pathway that removes the oxalate precursor, glyoxylate) reduced serum oxalate concentrations significantly in dialyzed patients (5). More recently, Costello et al. (6) monitored over a period of 2.1–2.5 years plasma oxalate and oxalate removal rates in 18 renal-failure patients receiving hemodialysis and found significant increases in both indices unrelated to ascorbate or pyridoxine status. These workers concluded that dialysis per se increases endogenous oxalate production.

Theoretically, the lactate load after lactate-buffered peritoneal dialysis or the acetate load after acetate-buffered hemodialysis (7) could stimulate gluconeogenesis, thereby increasing concentrations of the glycolysis intermediate 3-phosphoglycerate, and consequently flux through the metabolic pathways to form serine and glycine. These in turn have been cited as important precursors of glycolate, glyoxyxlate, and their end product, oxalate (8), although several alternative precursors and links to the central metabolic pathways have been suggested (9). In Fig. 1 we summarize some of these metabolic interrelations.

We evaluated this possibility of endogenous oxalate production in two ways. First, we measured oxalate production rates directly by using stable isotope dilution in 10 patients in chronic renal failure, half of whom were undergoing peritoneal dialysis. Second, we measured some of the key intermediates on the pathway suggested above in the serum of four hemodialysis patients dialyzed thrice weekly, before and after consecutive dialysis sessions that differed only in the bath-fluid buffering agent (acetate or bicarbonate).

Materials and Methods

Subjects. Patients who received [U-13C]oxalate injections for the estimation of pool size and production rate were all in clinically assessed chronic renal failure unrelated to primary hyperoxaluria. Five (one woman, four men) had been on peritoneal dialysis for at least 6 months. The remaining five (three women, two men) were not yet on dialysis. Experiments took place after patients had had a light breakfast and a recent dialysis bag change. Of the four patients followed on hemodialysis, two (one woman, one man) were usually dialyzed with acetate-buffered bath fluid, and two (both men) with bicarbonate-buffered bath fluid. All had been on standard, thrice-weekly single-pass hemodialysis (4-h sessions; blood flow 200 mL/min; dialysate flow 500 mL/min) for at least 6 months. No subject took ascorbate supplements. Informed consent was obtained from all subjects, and the project was approved by the Waikato Hospital Ethical Committee.

Procedures and samples. For the [U-13C]oxalate procedures, a butterfly needle was inserted intravenously, and a baseline blood sample (8 mL) was withdrawn by syringe and transferred to an evacuated collection tube containing heparin. A sterile injection of 73.53 μmol of [U-13C]sodium oxalate (99% atom excess; MSD Isotopes,
Pointe Claire, Canada) in 2 mL of isotonic saline was given over 2 min and washed with another 1 mL of isotonic saline. Six further samples were withdrawn (after discarding the first 2 mL) at 15-min intervals from the start of the injection. The hemodialysis patients were venipunctured immediately before and after each of their regular thrice-weekly dialysis sessions, and 8-mL blood samples were taken into evacuated collection tubes containing heparin. Alternative bath-fluid buffering agents (acetate or bicarbonate) were substituted on the Monday and Friday sessions. All blood samples were centrifuged immediately at 4°C; two portions of the plasma obtained were acidified to pH 2.5 and frozen at −20°C until dialysis. For the amino acid analyses, a third aliquot (not acidified) was processed further on the day of collection.

Sample preparation. Samples for amino acid analysis were purified on a column of Dowex 50 resin as described by Adams (10). Eluates were held at 4°C and processed further within 10 days. All eluates from a given patient were prepared for gas chromatography in a single batch, including one glycine-plus-serine-supplemented specimen (supplemented with 500 μmol/L of each compound). The dried eluates were transferred to derivatization vials in 100 μL of 0.1 mol/L HCl, redried, and dissolved in 30 μL of N-methyl-N-(tert-butylidemethylsilyl)trifluoroacetamide (Pierce, Rockford, IL). The sealed vials were heated to 70°C for 60 min. After the addition of 20 μL of ethyl acetate (dried over anhydrous sodium sulfate), the sealed vials were left overnight at 50°C. This is a modification of the method of MacKenzie et al. (11). The extraction and derivatization of acidified samples for capillary gas chromatography of oxalate and glycinate have been described previously (12). Again, all specimens from a given patient were processed in one batch. For isoito dilution mass spectrometry of the specimens containing [U-13C]oxalate, the n-propyl derivatives were prepared as follows: 200 μL of 100 mM/L sulfuric acid in n-propanol was added to the dried acetate extract, and the vials were sealed and heated at 85°C for 90 min. After cooling, 1 mL of hexane and 500 μL of 0.2 mol/L sodium bicarbonate were added, and the contents of the vials were mixed. The hexane layer was removed and dried over anhydrous sodium sulfate before injection into the gas chromatograph-mass spectrometer.

Gas chromatography. Baseline specimens from the 10 patients for oxalate pool size determination were analyzed as previously described (12). For the hemodialysis specimens, a Series-7A gas chromatograph, fitted with flame-ionization detector and Chromatopac Series-1B integrator (all from Shimadzu, Kyoto, Japan), was used, with the gas chromatograph modified for capillary use by fitting a 5-mm on-column adaptor, a detector-connector kit, and a 25 m × 0.53 mm FSOT column coated with a 1-μm-thick film of BP5 as the stationary phase (all from Scientific Glass Engineering, Melbourne, Australia). Splitless injections (1 μL) were made at 80°C with the helium linear flow rate at ~26 cm/s. After 1 min, the oven temperature was programmed upwards at 2°C/min to 150°C. Between injections, the oven was taken to 270°C for 8 min before returning to 80°C.

Gas chromatography–mass spectrometry. (a) Isotope dilution analyses. The instrumentation has been described previously (12). In this instance, the mass spectrometer was used in chemical ionization mode with isobutane as reagent gas at ~0.02 Pa (1.5 × 10−4 Torr) source pressure, 64 eV electron energy, and 1.5 mA emission current. The gas chromatograph included a 15 m × 0.32 mm fused-silica open tubular column coated to 1 μm with DB5, and operated isothermally at 100°C (oxalate retention time 5 min 25 s). Injections (2 μL) were made in split mode (1:15, 250°C). Measured peaks at m/z 177 and m/z 175 were monitored with a 1-s cycle time. The method was calibrated as described by Duggan et al. (13) by using the peak area ratios.

(b) Confirmation of oxalate and glycinate derivatives. The tert-butylidemethylsilyl (TBDMS)-derivatized extracts were injected in splitless mode, and full-scan electron impact spectra were acquired (1-s cycle, m/z 800–30) during temperature programming of the column (80°C for 1 min, then 1°C/min to 280°C).

Calculations. Log percent [14C]oxalate vs time graphs were plotted by computer (HP 85; Hewlett-Packard, Avondale, PA). Pool sizes were obtained from the intercepts, and turnover rates (or fractional change per minute) and half-lives from the slopes, as illustrated by Duggan et al. (13). Oxalate production rates are the product of pool size and turnover rate. Measured gas chromatogram peak height or area component/average standard ratios were corrected with supplemented duplicates as described by Wolthers and Hayer (14); our "concentration correction factors" are analogous to k in their formula. Only one supplemented sample/batch was run for the amino acid analyses. The t-test for paired data was used to compare the mean percentage changes in metabolites after bicarbonate- vs acetate-buffered dialysis, for the last two dialysis sessions of the week.

Results
Log percent [14C]oxalate was significantly correlated linearly with time, with 10 correlation coefficients in the
range 0.830 (0.05 > P > 0.01, n = 6) to 0.973 (0.005 > P > 0.001, n = 6). Table 1 summarizes the markers of oxalate metabolism measured on, and calculated for, these patients. With the exception of patients 6 and 10, oxalate production rate was significantly correlated with log{oxalate pool size} (r = 0.876, 0.005 > P > 0.001, n = 8).

Amino acid TBDMS derivatives yielded well-separated peaks on gas chromatography, with reproducible retention times and the following concentration correction factors: serine 1.144 (SD 0.068; n = 6), glycine 1.695 (SD 0.112; n = 6). Baseline separation of the glycolate and oxalate from plasma extracts was not achieved, so quantification relied on manual peak-height ratio measurement. However, full-scan gas chromatography-mass spectrometry confirmed the identity of the derivatives. The sequence of plasma acid TBDMS derivative peaks was similar to that obtained by Wolthers and Hayer (14), using trimethylsilyl derivatives. TBDMS-glycolate and -oxalate retention times and concentration correction factors were similar in both gas chromatography systems used for this work, but the correction factors for oxalate (0.66-0.87) and glycolate (1.06-1.42) were more variable than those found for the amino acids.

Results for analytes measured in the pre- and posthemodialysis specimens are summarized in Fig. 2. Patterns similar to those shown were also measured for the Monday dialysis session. For comparing the effects of buffering agents, however, only the Wednesday and Friday sessions were considered equivalent. Mean changes were: glycine −21%, −10%; serine +9%, +9%; glycolate −80%, −84%; and oxalate −28%, −34%, for acetate- and bicarbonate-buffered dialysis, respectively. Between-buffer differences were not statistically significant. Oxalate concentrations remained above normal.

**Discussion**

Compared with [1-{14}C]oxalate techniques, the stable isotope method for the estimation of oxalate pool size and production rate is simple to perform, requiring no constant infusion to maintain serum radioactivity. Being based on a single mass spectrometric measurement at each data point, this method should show improved precision and specificity. The oxalate pool sizes measured in our renal failure patients are close to those found by Constable et al. (15), who used [1-{14}C]oxalate dilution in nine patients in renal failure. However, our oxalate production rates are at least twofold higher than the oxalate removal rates calculated from their data, with some of our peritoneal dialysis patients having production rates within the range of removal rates found by Watts et al. (16) for patients with primary hyperoxaluria.
Using the stable isotope method as described above on a single healthy subject, we determined a pool size of 170 μmol and a production rate of 1.89 mmol/day. These values, though lower than those found in 9 of the 10 patients, are still higher than expected (15), as was the plasma oxalate at 5.7 μmol/L. All three results reflect some in vitro oxalogenesis from the postcollection oxidation of ascorbate, which, in our method, contributed an additional 3–5 μmol/L oxalate. This contribution is less important, proportionately, in uremic plasma (17). The slope of the regression line for this healthy subject yielded a turnover rate (0.0077/min) and half-life (89 min) well within normal limits (8), which supported the accuracy of our regression line slope estimates and, therefore, of the oxalate production markers listed in Table 1 for patients in chronic renal failure. Increased intestinal absorption might account for the apparently high oxalate production rate in renal failure patients, although Costello et al. (6) cite evidence that makes this explanation unlikely. Furthermore, we found in eight of our renal-failure patients that oxalate production rates paralleled pool sizes, suggesting that retained oxalate precursors might be contributing to their increased endogenous oxalate production rate. In the two remaining patients, both of whom were on peritoneal dialysis, a marked increase in turnover rate compensated for a lower pool size, resulting in unexpectedly high oxalate production rates.

Oxalate can be formed from glyoxylate in the liver cytosol in a reaction requiring NADH and lactate dehydrogenase and known to be product-inhibited. Peroxisosomal glycolate oxidase is also considered a major contributor to oxalate formation from glycolate and glyoxylate, which are in turn derived principally from carbohydrates and amino acids (18). Asker and Davies (19) proposed a model in which the two enzymes cooperate in oxalate production. A third enzyme, glycolate dehydrogenase, is thought to play a minor role (18).

Normal plasma concentrations of glyoxylate are thought to be very low (20). Alternative fates of glyoxylate include pyridoxine-dependent transamination (in both peroxisomes and cytosol), and the cytosolic, NADH-requiring conversion back to glycolate by lactate dehydrogenase (see Fig. 1). One factor affecting the balance between the oxidation and reduction of glyoxylate by lactate dehydrogenase will be feedback inhibition of the oxidation by oxalate (19). These considerations might explain the reciprocal relation between oxalate turnover rates and pool sizes noted in our two peritoneal dialysis patients, as well as the apparent inefficiency of dialysis in removing oxalate noted previously (2–4) and in this report. We suggest that, before dialysis in hemodialysis patients and in those peritoneal dialysis patients whose greatly expanded oxalate pool size provides a “reservoir” of oxalate, the predominant fates of glyoxylate are transamination and conversion to oxalate catalyzed by glycolate oxidase. In peritoneal dialysis patients whose tissue oxalate concentrations are near the inhibitory threshold, and in hemodialysis patients late in a dialysis session, cytosolic glyoxylate conversion to oxalate is increased, owing to the removal of product that would inhibit lactate dehydrogenase activity. Some buildup in the immediate precursors of oxalate, both between hemodialysis sessions and (for peritoneal dialysis patients) late in the peritoneal fluid dwell period, is implied in this explanation of the dialysis effect, and our finding of a steep decrease in glycolate concentrations during hemodialysis sessions is compatible with such a buildup.

Recent methods for plasma glycolate determination (14, 20) suggest a reference range close to that of plasma oxalate. Retrospective examination of the 14 chromatograms obtained for our oxalate reference range [4.93 μmol/L (SD 1.48; n = 14) (12)] suggested a corresponding glycolate reference range of 3.73 μmol/L (SD 1.78; n = 14; range 0.8–6.6) when the median correction factor of 1.24 was applied (glycolate-supplemented samples were not assayed at this time). This, together with the mass-spectrometric confirmation performed, supported the accuracy of our glycolate results. Petrarulo et al. (20), using HPLC determination of derivatized glyoxylate formed enzymatically from glycolate, measured glycolate before and after one hemodialysis session in four uremic patients, all of whom, unlike those with primary hyperoxaluria, were distinguished by low-normal glycolate concentrations unchanged by dialysis. We note that the same method applied to urine yielded higher values for glycolate in idiopathic calcium oxalate stone formers than in healthy subjects (21). Uremic plasma, however, is potentially inhibitory to a wide range of enzymes (22), which could explain the failure of their method to detect the relatively small predialysis glycolate increases seen by us in renal failure unrelated to primary hyperoxaluria.

Other published studies (6, 23, 24) have also obtained posthemodialysis oxalate values remaining well above the reference range. We note, however, that the mean percent of oxalate reduction measured in this study (31%) was comparatively small; the studies cited above commonly reported reductions of 60% or more. The additive contribution from in vitro generation of oxalate, to which our method is prone, will result in an underestimation of the percent oxalate reduction achieved by hemodialysis. In this regard, we have noted a tendency for the measured oxalate to increase on repeated freeze-thawing of specimens, suggesting that our practice of holding the acidified plasma at −20°C to process all specimens on one patient simultaneously might exacerbate oxalogenesis. The gas-chromatographic methods are also undoubtedly measuring the 30% of oxalate bound to serum protein in uremic patients (17), so that our postdialysis concentrations will seem high compared with results from methods that initially deproteinize plasma by ultrafiltration. Wothers et al. (23), although using a similar oxalate method, dialyzed their patients only twice weekly, whereas ours were routinely dialyzed three times a week; this might explain our less dramatic loss per dialysis session.

In our hemodialysis patients, plasma glycine and serine concentrations remained within the expected phys-
iological range (10) and we found no evidence of increased flux through these intermediates to oxalate after acetate-buffered hemodialysis. Additional or unusual pathways to glyoxylate might be operating in renal failure to produce the rates of oxalate production we measured; e.g., glucuronate, which reportedly accumulates in uremia (22), could be a candidate glyoxylate precursor through postulated pathways involving galactose or xylitol (9).

One explanation for our results is that a high rate of endogenous oxalate production from glyoxylate fueled by retained oxalate precursors might be partially impeded by product inhibition of lactate dehydrogenase, as well as by pyridoxine-mediated salvage, in patients with chronic renal failure. Efficient dialysis removes the product inhibition component (regardless of which buffering agent is used) so that oxalate pool sizes tend to increase with time or to stabilize at above-normal concentrations, whether or not dialysis is instituted.

We acknowledge the expert technical assistance of Tony McGhie in obtaining the mass spectrometric data. This project was supported by the Waikato Medical Research Foundation and the Waikato Hospital Renal Research Fund.

References
2. Wallace M, France N. Serum oxalate in patients with chronic renal failure is raised and is higher in patients on dialysis [Abstract]. Kidney Int 1986;28:867.