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Glycolate determination detects type I primary hyperoxaluria in dialysis patients

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Glycolate determination detects type I primary hyperoxaluria in dialysis patients. The detection of type I primary hyperoxaluria is based on the finding of exceedingly high oxalate excretion which is associated with increased glycolate excretion. The differential diagnosis of this disease may become a difficult task once end-stage renal disease (ESRD) and anuria have supervened. The various procedures thus far proposed to obviate this circumstance are complex, inaccurate or not reproducible. In this paper we propose the accurate liquid chromatographic determination of glycolate in blood and dialysate as a means to detect type I primary hyperoxaluria in patients on maintenance hemodialysis (RDT). The method is based on the enzymatic conversion of glycolate to glyoxylate coupled with α-keto acid derivitization with phenylhydrazine. The resulting phenyhydrazone is then resolved by high-performance liquid chromatograph (HPLC). With this method, plasma glycolate in 12 healthy controls was 7.8 ± 1.7 μmol/liter, almost twentyfold less than previously reported. Five dialysis patients with high serum oxalate, of whom four with primary hyperoxaluria and one with Crohn's disease and presumed enteric oxalate hyperabsorption, were checked by this method and compared to nine patients with oxalosis-unrelated ESRD. The patients with hyperoxalemia were also evaluated for their response to pyridoxine therapy. The measurement of glycolate in blood drawn prior to and at the end of the dialysis session as well as in the dialysate soundly discriminated the patients with type I hyperoxaluria from all the other dialysis patients. Glycolate measurement was shown to be much more powerful than oxalate in that patients with oxalosis-induced ESRD exhibited an almost two hundred and fiftyfold increase compared to the oxalosis-unrelated patients. There was no overlapping at all irrespective of timing and type of sampling. Glycolate measurement represented a valuable tool to distinguish Crohn's disease related from genetically induced hyperoxalemia and to assess responsiveness to pyridoxine treatment.

Primary hyperoxaluria is a rare inborn error of metabolism involving oxalate by two distinct pathways: the more common type I primary hyperoxaluria has now been recognized to be due to deficiency of peroxisomal alanine:glyoxylate aminotransferase which is located in the liver cells [1]; the more rare type II is due to lack of D-glyceric dehydrogenase [2]. Both types are featured by a marked increase in oxalate endogenous production, which lead to progressive kidney destruction and to systemic tissue deposition of oxalate. Detection of these syndromes relies on the finding of persistent hyperoxaluria, with urine oxalate usually exceeding 1 mmol/24 hours. High urine oxalate is associated to increased excretion of both glycolate and glyoxylate in type I hyperoxaluria, and to L-glycerate in type II [2, 3]. Both types usually present with a very aggressive recurrent calcium oxalate nephrolithiasis leading to end-stage renal failure (ESRD). In these conditions detection and differential diagnosis by means of the usual ways, that is, their urine determination, are not allowed. Various procedures have been proposed to obviate this circumstance, including determination of oxalate in plasma [4, 5], measurement of oxalate dynamics by [^{14}C]-oxalate dilution [6], detection of calcium oxalate crystalline deposits in body tissues [7, 8], and demonstration of specific enzyme defects in liver tissue [1]. However, each of these methods have some disadvantage: the determination of oxalate in plasma requires complex sample manipulation to prevent spontaneous oxalate generation [9, 10]; furthermore, plasma oxalate raises not only in ESRD secondary to primary hyperoxaluria but also in oxalosis-unrelated diseases [11, 12]; pyridoxine deficiency [13] and high-dose ascorbate supplements [14] have been implicated as potential causes of hyperoxalemia in dialyzed patients. Labelled oxalate dilution methods require that radioactive material be handled. Studies of both crystalline tissue deposits and liver biopsies are invasive and poorly reproducible.

Since oxalate precursors can be expected to be retained in renal failure, their detection in body fluids could represent a useful approach to diagnosis. We have recently developed a high-performance liquid chromatographic (HPLC) procedure for the determination of glycolic acid in urine and plasma [15], whose sensitivity permits detection of glycolate at normal range levels. This procedure has been used for the measurement of glycolate levels in blood and dialysate of uremic patients on maintenance dialysis (RDT) for either oxalosis-induced or oxalosis-unrelated ESRD. It will be shown that glycolate determination can be a method of choice to identify type I primary hyperoxaluria as well as to assess efficacy of pyridoxine therapy in the course of RDT.

Methods

Subjects studied

Four patients undergoing dialysis for hystologically-proven systemic oxalosis were enrolled in this study.

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Patient B.M. was a 14-year-old boy who had been previously referred to as featuring typical X-ray changes of kidney and bone in the course of type I primary hyperoxaluria [16]. He had passed his first stone when he was four years old and had had several stone episodes until he was nine years old. At this time he presented to our institution with bilateral nephrocalcinosis associated to large-sized caliceal stones in both kidneys and multiple stones along the right ureter. Serum creatinine was 123 μmol/liter and creatinine clearance was 43 ml/min; urine oxalate was 3.5 mmol/24 hours (oxalate/creatinine, 0.46 mmol/mmol) and urine glycolate was 2.56 mmol/24 hours (glycolate/creatinine, 0.33 mmol/mmol). He was given pyridoxine (300 mg/day) associated to high fluid intake and was sent to the referring institution in Palermo. Renal function deteriorated progressively and he had to be started on hemodialysis when he was 12. The second study, to which these data refer, was performed 18 months later. He had discontinued pyridoxine after starting RDT and this drug was not restored during this study period.

Patient M.A. was a 14-year-old girl who had been dialyzed for 21 months. The onset of the disease had occurred at the age of six when she passed a renal stone. She was free of symptoms until age 12 when she was admitted to Ospedali Riuniti in Reggio Calabria with oliguria and uremia. X-ray films showed that renal pelvis in both kidneys were entirely filled with large radiopaque stones. Every attempt to remove urinary obstruction was unsuccessful and she was started on dialysis. Diagnosis of primary hyperoxaluria was based on renal biopsy depicting the typical features of renal oxalosis and by the finding of steep increases in plasma oxalate. Pyridoxine treatment (500 mg/day) was instituted and was being given when the present study was performed in December 1989. Her clinical condition progressively worsened during dialysis in that she developed severe anemia and experienced thromboses of several arteriovenous fistulas. Extensive calcifications of peripheral vessels prevented the creation of a definitive vascular access. She died in June 1990 of infectious complications due to subclavian catheterization.

Patient B.F. was a 21-year-old man who had been on renal replacement therapy for 72 months. Nephrolithiasis had begun when he was four years old; at that time he had been operated for bilateral pyelolithotomy. Bilateral renal stones recurred a few months later, and since then he had severely recurrent nephrolithiasis complicated by urinary tract infection, and ultimately by chronic pyelonephritis. Diagnosis of type I hyperoxaluria was established by increased urine oxalate and glycolate. ESRD had supervened when he was 15 years old and he had been treated with hemodialysis for few months until September 1984. At that time the patient underwent living-related donor kidney transplantation; afterwards he was advised to increase fluid intake and was given high dose pyridoxine (1200 to 1500 mg/day). Nonetheless renal function deteriorated progressively and he re-entered dialysis treatment in January 1986. Renal biopsy of the grafted kidney exhibited extensive deposition of calcium oxalate crystals in renal tubules and interstitium. In June 1986 he received a second cadaveric graft but he had to be explanted after a few weeks due to acute graft rejection. He was re-started on dialysis and was treated at the Ospedali Riuniti, Reggio Calabria, where he was studied in December 1989. He regularly took pyridoxine 300 mg/day which was maintained during the first study. Oxalate and glycolate measurements were carried out a second time 20 days after stopping vitamin B6.

Patient S.F. was diagnosed as affected by primary oxalosis after he had been on dialysis for five years, and has been previously referred to as an example of primary oxalosis with delayed onset and slow progression [17]. He had been healthy until the age of 18, when he passed his first stone composed of calcium oxalate. At the age of 28 he underwent surgery for large stones in the left ureter and bladder. At that time serum creatinine was 132 μmol/liter. Renal stones recurred thereafter and he progressed to ESRD, so that he was started on RDT when he was 33. During the subsequent five years on RDT his major problems were severe anemia, increasing bone pain unresponsive to vitamin D therapy and repeated thromboses of the vascular access. He was finally referred to the Nephrology Division of the University of Turin with a putative diagnosis of secondary hyperparathyroidism, which was suspected by finding subperiosteal reabsorption of the fingers and widespread vascular calcifications. Since blood biochemistries were poorly consistent with the diagnosis of hyperparathyroidism, bone biopsy was performed and revealed extensive deposition of calcium oxalate crystals filling medullary spaces. These findings suggested primary hyperoxaluria as the underlying primary disease. The patient was therefore enrolled in this study. First measurements of blood and dialysate were carried out while he was not taking pyridoxine. After diagnosis of type I primary hyperoxaluria had been established he was given pyridoxine, 900 mg/day. The second study was performed 12 months later while he was on pyridoxine.

Patient C.N. was a 32-year-old male undergoing dialysis for 18 months for renal and systemic amyloidosis secondary to Crohn's disease; two years before entering dialysis he had been operated on of extensive ileal resection. Since his plasma oxalate was found to be steeply increased he was presumed to represent a case of absorptive hyperoxalemia unrelated to enzymatic defects. However, plasma levels of oxalate and glycolate were poorly affected by changes in both dietary oxalate and calcium supplements, so he was tested for pyridoxine deficiency. Pyridoxine was given intravenously, 600 mg three times a week at the end of dialysis, plus 300 mg orally in the interdialytic days for 15 days, and he was restudied for plasma oxalate and glycolate levels.

Nine patients (5 males and 4 females, aged 40.8 ± 14.0 years) who had been on RDT for 63 ± 52 months were identified as cases of oxalosis-unrelated renal failure. All the patients were investigated while on their home diets and all were taking vitamin D supplements and calcium carbonate at usual doses; none was taking vitamin B6.

All these patients were being dialyzed by standard hemodialysis or soft hemodiafiltration, with KT/V of urea, estimated according to Sargent and Gotch single-pool-variable volume model [18] of 1.1 through 1.4.

Twelve healthy subjects (7 males and 5 females, aged 41.0 ± 14.4 years) were considered as controls.

Blood and dialysate sampling

Blood samples for glycolate, oxalate and creatinine determination were taken in the fasting state from the healthy subjects. Samples from uremic patients were drawn by their arteriovenous fistula prior to and at the end of a dialysis session.

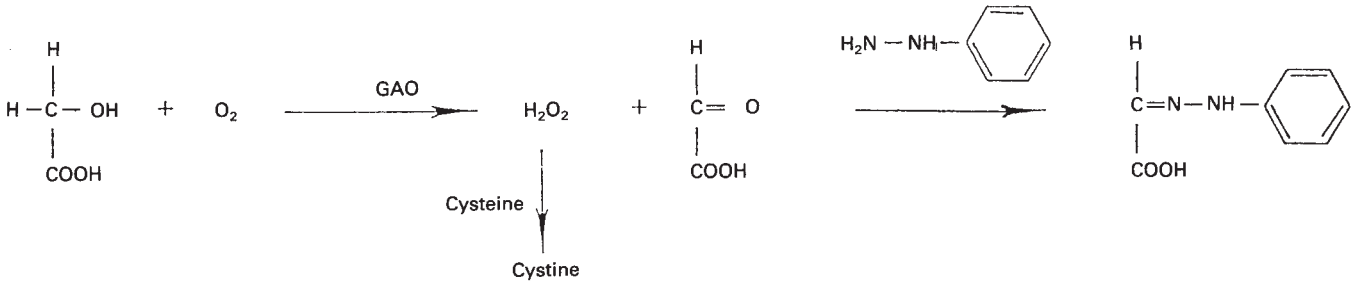

Fig. 1. *Reaction for glycolate HPLC assay.* Glycolate oxidase (GAO)-catalyzed oxidation of glycolate to glyoxylate; the resulting glyoxylate reacts with phenylhydrazine to produce the corresponding phenylhydrazone. The reaction is carried out at pH 8.3 and in the presence of cysteine as a reducing substrate to eliminate hydrogen peroxide.

Predialysis blood samples were taken from the three hyperoxalemic patients (namely patients B.F., S.F. and C.N.) who were restudied on pyridoxine. All the samples were handled in such a way as to prevent spontaneous generation of oxalate as previously described [19]. Soon after being taken, blood was placed on ice and processed without delay; blood was centrifuged at 1000 × g for 10 minutes at 4°C. One aliquot of whole serum was used for creatinine determination. A total of 1.0 ml aliquot of serum was acidified with 40 μl of concentrated HCl, vigorously vortex-mixed for six minutes and then ultrafiltered without delay using YMT membranes (Amicon) with 30,000 Daltons molecular weight cut-off, and analyzed for oxalate as soon as possible. One milliliter aliquot of serum for glycolate determination was ultrafiltered in the same way as described above, save that HCl addition was omitted. Ultrafiltrates were harvested and stored frozen at −20°C until analysis.

The whole dialysate from a single dialysis session was collected on plastic bags to which 10 ml of concentrated HCl had been added to prevent precipitation of calcium salts. Two 1 ml aliquots were stored frozen without further manipulation.

Samples of patients B.F. and M.A. were thoroughly processed by the laboratory of the referring Nephrology Division of Reggio Calabria, and were sent to our laboratory immediately.

Chemical methods

Oxalate was determined by ion chromatography using a Dionex QIC apparatus equipped with a conductimetric detector as previously described [12].

Glycolate was determined by means of a HPLC procedure derived by the original application in urine [15]. The method is based on the enzymatic conversion of glycolate to glyoxylate coupled with α-keto acid derivitization with phenylhydrazine; the resulting phenylhydrazone is then resolved by the HPLC procedure (Fig. 1). To eliminate any aspecific contribution to the peak, a pre-incubation step was carried out at room temperature for 120 minutes by mixing into 10 ml glass vials 25 μl of reconstituted (25 U in 1 ml phosphate buffer) glycolate oxidase from spinach (glycolate:oxygen oxidoreductase, EC 1.1.3.15, Sigma Co., St Louis, Missouri, USA), 1.0 ml of 0.1 mol/liter phosphate buffer and 50 μl of cysteine solution (439 mg of L-cysteine hydrochloride in 25 ml of water). The derivatives were prepared by adding 1.0 ml of pretreated phosphate buffer with 50 μl of 500 mmol/liter phenylhydrazine and 200 μl of standard, ultrafiltrate or dialysate samples. The blanks were prepared accordingly by adding 1.0 ml of the above phosphate buffer with phenylhydrazine. After vortexing, 25 μl of the suspended glycolate oxidase were added and the solution was vortexed again. The mixture was left at room temperature for 20 minutes, and the lightly yellow solution was thereafter injected into the liquid chromatograph. Chromatography was performed by a Varian 5500 LC, equipped with and UV variable wavelength detector and a Rheodyne 7126 (Berkeley, California, USA) automatic injection valve with a 50 μl loop. Peak eights were measured with a Shimadzu R1A (Kyoto, Japan) recorder/integrator. The detection was performed at 324 nm. An octadecylsylil 250 × 4 mm LiChrospher RP-18 10 μm column (Merck) was used throughout in connection with a 30 × 4 mm Perisorb RP-18 30 to 40 μm (Merck) guard column. In the described conditions glycolate peak eluted in about 5.6 minutes. Figure 2 shows typical chromatograms of a standard solution, a blank and plasma samples from a normal subject and a patient with primary hyperoxaluria. The chromatographic signal includes the glyoxylate concentration; however, when ten ultrafiltrates from normal subjects were analyzed for glyoxylate by a modification of a method described previously [20], their contents never exceeded 0.3 μmol/liter. Glyoxylate was also determined on all the predialysis ultrafiltrates from the patients with primary oxalosis yielding a mean of 23 ± 14 μmol/liter, so as to contribute only 1.5% to the glycolate peak. These findings prompted us to neglect glyoxylate contribution. The linearity of the chromatographic response was good in the range 0 through 200 μmol/liter (the regression equation of the standard curve being: $y = 1.36\ x - 1.45$, $r = 0.9987$, $P < 0.001$; where Y indicated peak eight as mm, and X glycolate concentration as μmol/liter). The sensitivity was of 0.3 μmol/liter. Samples with higher glycolate content were diluted before being analyzed. The intra- and inter-run CV at 5.7 μmol/liter were 2.6 and 11.3%, respectively. Recoveries of added glycolate to plasma ultrafiltrates averaged 98.9 ± 6.8%. The efficiency of the ultrafiltration was tested by spiking plasma samples with glycolate prior to ultrafiltration. Measured recoveries of 102 ± 7%, corrected for both volume reduction due to plasma proteins and Donnan effect, suggested that complete ultrafiltration occurred. Recoveries also tested on ultrafiltrates from uremic patients yielded comparable results. To test whether contaminants could interfere with the glycolate assay, blood samples from both normal subjects and hyperoxalemic patients were added with 0.5 mmol/liter of oxaloacetate, α-ketoglutarate, glutarate, L-lactate, pyru-

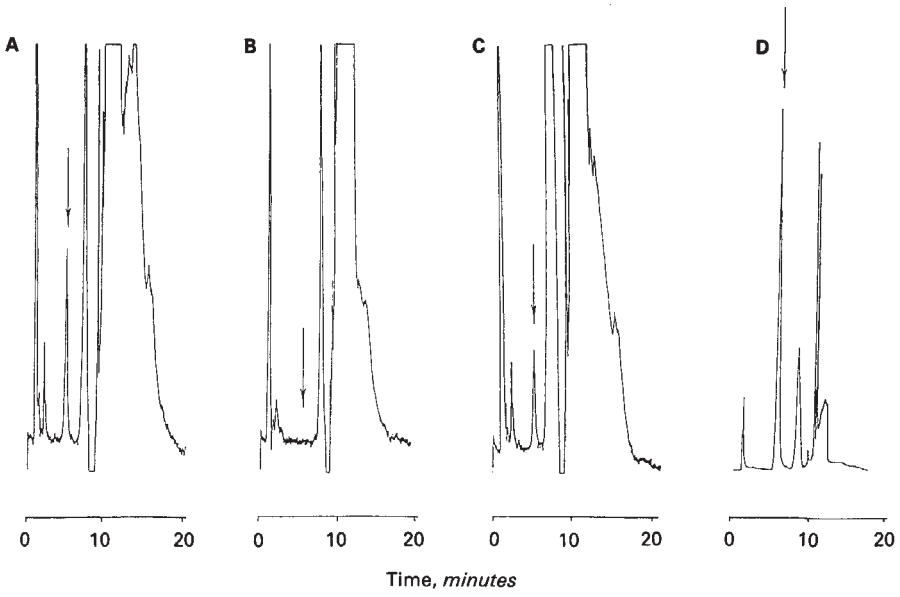

Fig. 2. *Typical HPLC chromatograms for the analysis of plasma glycolate: glycolate 20 μmol/liter aqueous standard, 0.002 absorbance units full scale* **(A)**; *aqueous blank, 0.002 a.u.f.s.* **(B)**; *sample from a healthy control, 0.002 a.u.f.s.* **(C)**; *predialysis sample from a patient with type I hyperoxaluria, 0.064 a.u.f.s.* **(D)**. The arrows indicate the glyoxylate phenylhydrazone peaks.

Table 1. Plasma levels of the considered parameters in normal subjects and uremic patients

	Normal Subjects	Oxalosis-unrelated ESRD	Crohn's-related hyperoxalemia	Type I primary oxalosis
Number	12	9	1	4
Creatinine	88 ± 17.5	1017 ± 179[a]	1275	816 ± 188[a]
Oxalate	6.1 ± 1.6	50.9 ± 18.4[a]	119	162.0 ± 24.0[b]
Glycolate	7.8 ± 1.7	6.1 ± 0.8	23.3	1536 ± 1062[b]

Means ± SD are shown. Values for the patient with Crohn's disease represent means of four replicated analyses performed off pyridoxine. All data are expressed as μmol/liter.

[a] $P < 0.001$ vs. healthy subjects

[b] $P < 0.001$ vs. both healthy subjects and oxalosis-unrelated ESRD

Table 2. Dialysis kinetics of oxalate and glycolate in patients with ESRD secondary to different renal diseases

	Oxalosis-unrelated ESRD	Crohn's-related hyperoxalemia	Type I primary oxalosis
Predialysis[a]			
Creatinine	1017 ± 179	1275	816 ± 188
Oxalate	50.9 ± 18.4	119	162 ± 24.0[c]
Glycolate	6.1 ± 0.8	23.3	1536 ± 1062[c]
Postdialysis[a]			
Creatinine	383 ± 151	433	342 ± 149
Oxalate	20.5 ± 11.8	35.9	60.4 ± 14.0[c]
Glycolate	6.5 ± 1.2	16.1	371 ± 138[c]
Dialysate[b]			
Creatinine	17090 ± 4760	23690	9910 ± 901[d]
Oxalate	603 ± 304	2379	1792 ± 356[c]
Glycolate	347 ± 77	798	11050 ± 7333[c]

[a] Data are expressed as μmol/liter

[b] Data are expressed as μmoles

[c] $P < 0.001$ and [d] $P < 0.05$ vs. patients with oxalosis unrelated ESRD

vate, oxalate, mesoxalate, L-citrate, L-glucose, L-ascorbate, L-tartrate, tartronate, malonate, maleate, malate, succinate as well as main physiological L-amino acids, which did not produce any significant interference.

Serum creatinine was determined by routine method.

Results

Values from predialysis samples of uremic patients and from fasting samples of normal subjects are listed in Table 1. All the uremic patients exhibited significant increases in plasma oxalate levels as compared to normal subjects. Means from patients with type I primary hyperoxaluria and from the one with enteric hyperoxalemia were higher than that from oxalosis unrelated patients. Plasma glycolate was enormously increased in the case of primary oxalosis, whereas it was unchanged or only mildly above control values in the other uremic patients.

Table 2 lists some dialysis related features in the uremic patients. The striking changes of glycolate levels were confirmed in blood samples taken at different times of the dialysis session as well as in dialysate collections. Interestingly, the ratio of means between hyperoxaluric patients and oxalosis-unrelated patients was about 3 for oxalate while it rose to over 250 for glycolate. None of the measured samples overlapped between the two groups of uremic people. Also, glycolate was mildly increased in blood and dialysate of the patient with renal amyloidosis; however, it remained far below the values observed in primary hyperoxaluria. Glycolate measurement represented a valuable means to distinguish Crohn's disease related from genetically induced hyperoxalemia.

To evaluate the impact of pyridoxine therapy on plasma levels of oxalate and glycolate, predialysis blood biochemistries off pyridoxine were compared to those on pyridoxine. As listed in Table 3 pyridoxine appeared to partially influence both oxalate and glycolate levels in only one patient with type I hyperoxaluria. We were unable to obtain measurements of pyridoxine for patient M.A.; however, her chemistries on pyridoxine suggested that she was not responsive to treatment. None of the three patients with primary oxalosis who were

Table 3. Effect of vitamin B6 administration on predialysis plasma levels of oxalate and glycolate[a]

	Oxalate		Glycolate	
	Off B6	On B6[b]	Off B6	On B6
Type I primary hyperoxaluria				
B.M.	185	—	786	—
M.A.	—	135	—	1441
B.F.	150	154	3066	2890
S.F.	180	94	980	685
Crohn's-related hyperoxalemia				
C.N.	119	55	23.3	4.6

[a] Data are expressed as μmol/liter

[b] Doses of pyridoxine: M.A., 500 mg/day; B.F., 300 mg/day; S.F., 900 mg/day; C.N., 600 mg/day intravenously three times a week plus 300 mg orally in the interdialytic days.

assessed on had treatment plasma levels of glycolate less than one hundredfold normal.

Conversely, plasma levels of glycolate in the patient with Crohn's disease were restored to normal upon pyridoxine substitution, which confirmed that vitamin B6 deficiency was contributing to altered oxalate metabolism in this subset.

Discussion

Type I primary hyperoxaluria always results in chronic renal failure due to the progressive destruction of renal tissue by crystalline deposits of calcium oxalate. As a rule this syndrome starts early in life and induces ESRD within the first two decades of age [21]. There were 221 patients recorded in the EDTA Registry up to December 1986 with oxalosis as the primary renal disease [22]. The identification of the syndrome is not difficult when patients are investigated before renal failure induces anuria, in that oxalate excretion raises to over 1 mmol/24 hr [21, 23] and the measurement of corresponding oxalate precursors in urine permits discrimination between the various forms of hyperoxaluria [23]. Once ESRD and anuria supervene, identification and differential diagnosis may become a complicated task. This issue has been updated by the recent recognition that a considerable heterogeneity exists in the presentation of primary hyperoxaluria, with a slow course and a delayed onset in some cases in which systemic oxalosis has been reported to develop three to five years after the initiation of RDT [24] or even after renal transplantation [25]. In these instances diagnosis is also urged by prognostic considerations, since the progressive development of systemic oxalosis, which is not prevented by either dialysis treatment [6] or kidney transplantation [7, 22], represents a serious threat to patient survival.

It has been stressed earlier that the methods for the detection and control of primary oxalosis in dialysis patients have many drawbacks. Bone or liver biopsies permit a correct diagnosis, but in addition to being invasive these techniques are poorly reproducible. The determination of oxalate in plasma and dialysate has been proposed as a means to detect primary oxalosis [5], but methods for measuring oxalate in plasma are not always available and their accuracy depends on delicate sample handling [10, 19]. Moreover, plasma oxalate rises in patients on dialysis for causes other than primary hyperoxaluria, and some overlapping may have a confusing effect. This has been pointed out in previous papers [11, 12] and is confirmed in the present study, which demonstrates that the differences between the two groups of dialysis patients may be cancelled out by different timing of the withdrawal. This misleading event is partially obviated by the measurement of the removed oxalate, but it requires that whole dialysate be collected.

In this study, we have explored whether this problem could be dealt with by means of the measurement of the main oxalate precursor, glycolate, in blood and dialysate of patient on RDT. Indeed, the determination of glycolate in serum and urines is claimed as being a method of choice for the detection of hyperoxaluria syndromes [23]; however, available data are not recent and generally refer to patients studied prior to the onset of anuric renal failure. We are not aware of any recent study of the use of these measurements in ESRD or RDT, therefore this investigation represents the first report on this issue. Our results outline that the accurate determination of glycolate is a valuable tool for the diagnostic approach to primary hyperoxaluria. In fact, the method herein proposed enabled us to observe extraordinarily high blood levels of glycolate in all the patients with histologically proven oxalosis, and the results were strongly corroborated by the dosage of glycolate in dialysate. The differences were so striking that no overlap at all was found between the two populations of dialysis patients irrespective of time and type of sampling. The specificity of glycolate increases for type I primary hyperoxaluria was also proven by the results of the patient in which persistent hyperoxalemia was associated with Crohn's disease and ileal resection, since glycolate levels in blood and dialysate were different in the two syndromes. Conversely, our method could even detect a mild increase in glycolate levels, which successfully revealed that pyridoxine deficiency was playing a subsidiary role in this context.

Comparing basal glycolate levels to those measured on pyridoxine appeared to be a useful means of assessing if and how patients responded to treatment. It has been previously reported that pyridoxine can decrease oxalate generation in about one third of the patients with the glycollic variant of primary hyperoxaluria [26], and sequential studies of plasma oxalate as well as of ^{14}C-oxalate dynamics have been proposed to evaluate pyridoxine efficacy [27]. We suggest that accurate sequential measurements of glycolate should similarly and perhaps more simply meet this purpose. It is also reasonable to predict that the changes of glycolate under pyridoxine substitution could be more prompt and sensitive than those of oxalate. In fact plasma (and dialysate) levels of the latter might be affected by a slow equilibration with tissue deposits of oxalate, and this could mask the ongoing decrease of oxalate generation. The present study was not originally intended to test this hypothesis, which is worth being investigated prospectively.

In our laboratory glycolate determination has been shown to represent a procedure much more powerful than so far supposed on the basis of previous methods [28, 29]. In fact our range for normal subjects are almost twentyfold lower than those established by previous authors who used the same enzymatic reaction of glycolate oxidase [28, 30]. The specificity of this reaction was significantly improved by the use of phenylhydrazine—which reacts rapidly only with very acti-

vated carbonyl moieties, that is, α-keto carboxylic groups—as a derivatizing agent and by the use of the HPLC separation of the corresponding phenylhydrazones. These steps efficiently prevented overestimations arising from use of hydrogen peroxide as a marker of the enzymatic reaction [15].

It remains to be explained why patients with oxalosis-unrelated ESRD had glycolate blood levels comparable to or mildly lower than those of normal subjects, in the fact of the loss of renal function. No definite conclusion can be put forward on the basis of available data. However, we suggest that in this context, that is, in the absence of any specific defect of its pathway, glycolate may behave similarly to other intermediary metabolites, such as lactate, pyruvate and acetoacetate. Blood profiles of these organic anions have been shown to be influenced by the acute changes in acid-base balance induced by RDT. In fact their concentration is close to the normal range in the predialysis samples and seem apparently unaffected by dialysis, simply because their concentration at the end of the session is the balance between metabolic generation and dialysis removal [31]. This hypothesis is in keeping with most of our findings for glycolate in patients with oxalosis-unrelated ESRD.

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