Ethylene glycol induces hyperoxaluria without metabolic acidosis in rats

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Elevated urinary oxalate excretion (hyperoxaluria) is a clinical condition affecting some 30% of the U.S. stone-forming population (19). Hyperoxaluria can result from both genetic and nonheritable causes. For example, extreme hyperoxaluria may be observed in patients having a relatively rare genetic disorder known as primary hyperoxaluria (types 1 and 2), where hepatic oxalate production is enhanced by the absence of alanine-glyoxylate transferase or glycolate oxidase, whereas more benign hyperoxaluria may result from enhanced enteric absorption of dietary oxalate or oxalate precursors (22).

While there are several animal models (5, 33, 42) that are used to study hyperoxaluria and its consequences, the most commonly employed and simplest approach to induce hyperoxaluria is to provide ethylene glycol (EG) in an animal’s drinking water. EG is readily absorbed along the intestine and is metabolized in the liver to oxalate. Despite being one of the most popular animal models for studies of hyperoxaluria (20, 21), oxalate-induced renal tubular injury (2, 27, 35, 47), and calcium oxalate nephrolithiasis (9, 28, 33, 34), the use of EG as an oxalate precursor in experimental models has been criticized on the grounds that it produces metabolic acidosis (4, 5, 11), which clearly could confound interpretation of studies employing EG-induced hyperoxaluria. The acidosis hypothesis has been generally extrapolated from clinical observations of accidental or intentional overdoses (10, 15, 30–32) of antifreeze formulations, which may contain up to 95% EG. Remarkably, whether the dosage of EG commonly utilized to induce experimental hyperoxaluria (0.75% vol/vol provided in the drinking water) also results in metabolic acidosis has not been established. Given the substantial volume of information and conclusions derived from studies employing EG-induced hyperoxaluria regarding calcium oxalate nephrolithiasis and oxalate-induced renal injury, it is necessary that the issue of metabolic acidosis be experimentally resolved.

Accordingly, we chose to test the hypothesis that the standard EG-induced model of hyperoxaluria produces metabolic acidosis in male Sprague-Dawley rats by arterial blood-gas analyses and serum and urinary chemistries. We also evaluated these acid-base parameters in unilaterally nephrectomized rats (control and EG-treated), which is a more severe hyperoxaluria model (20). A fifth group of rats served as a metabolic acidosis reference group. Our results indicate that two-kidney rats made hyperoxaluric by administering of EG are not acidotic after 4 wk. However, signs of nascent acidosis are evident in rats that manifest compromised renal function as a consequence of the EG ingestion or by the renal insufficiency alone remains to be determined. We conclude that at the dosage schedules commonly employed, EG does not produce metabolic acidosis in Sprague-Dawley rats.

MATERIALS AND METHODS

Animals

A total of 80 male Sprague-Dawley rats (275–300 g) were utilized in the current study and were purchased from Harlan (Indianapolis, IN). All rats had free access to Purina Rat Chow 5001 during the entire course of the study. The chemical composition of the diet is provided in Table 1. All experimental protocols were conducted in accordance with the guidelines of the University of Florida Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental Models and Protocols

Two experimental models of EG-induced hyperoxaluria, together with their respective controls, were examined in the current study, which provided varying degrees of hyperoxaluria. A fifth group of rats...
was made acidic and served as positive controls for metabolic acidosis.

Control group. For the control group (CON), 17 rats were provided free access to food and normal drinking water served as controls.

Hyperoxaluria group. For the hyperoxaluric group (HYP), 17 rats were given free access to food and drinking water that contained 0.75% (vol/vol) EG (changed daily) for a period of 4 wk (21). The provision of this dose of EG has generated hyperoxaluria in as early as 3 days (28) and as long as 60 days (47) with no discernable effect on renal function as judged by creatinine clearance (21, 27, 29).

Unilateral nephrectomy group. For the unilateral nephrectomy group (UNI), 17 rats were unilaterally nephrectomized (see below) and given 1 wk to recover. These nephrectomized rats were provided free access to food and normal drinking water served as a nephrectomy control group.

Hyperoxaluria-induced chronic renal failure group. For the hyperoxaluria-induced chronic renal failure group (HRF), 17 unilaterally nephrectomized rats were given 1 wk to recover from surgery before being given free access to food and drinking water that contained 0.75% (vol/vol) EG for a period of 4 wk as previously described (20).

Metabolic acidosis group. For the metabolic acidosis group (MA), acidosis was produced by providing free access to food and drinking water that contained 0.28 M NH₄Cl plus 5% (wt/vol) sucrose for 4 (n = 6) or 14 days (n = 6). This is a well-established protocol for the induction of metabolic acidosis in the rat (1, 38, 39). An initial analysis indicated no significant differences between the two acidotic groups for any parameter examined, so the rats were combined into a single metabolic acidosis group (n = 12).

Unilateral Nephrectomy

To produce oxalate-induced chronic renal failure, unilateral nephrectomies were performed on 34 rats. Briefly, a surgical plane of anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (40 mg/kg body wt). A small dorsal incision, ~1.5–2 cm, was made along the upper flank overlying the left kidney. The kidney was exposed through the dorsal incision, decapsulated, and the renal vasculature was ligated before excision of the renal mass. The flanking musculature was sutured, and the skin was closed using Autoclip wound clips. Before treatments were initiated, all rats that underwent surgery were given 1 wk to recover.

Two weeks after the initiation of treatment and on the penultimate day of the study (4 wk), rats were placed in metabolic cages and 24-h urine collections were made. Urine was collected in 20 μl of 20% sodium azide as a preservative. Particulate matter was sedimented by low-speed centrifugation. A 5-ml aliquot was removed, and the remainder was acidified by the addition of 3 N HCl (~1 ml/5 ml urine volume). The acidified urine was used for the determination of citrate, calcium, and oxalate, whereas osmolality, phosphate, chloride, titratable acid, and ammonium excretion were determined from the non-acidified aliquot.

Blood Collection

At the end of their respective treatment period, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg body wt). Arterial blood samples (~1 ml) were drawn from the abdominal aorta (n = 11 rats/treatment group) with blood-gas sampling syringes (PROVENT®), Portex, Keene, NH), and the samples were immediately transported to the STAT lab of Shands Hospital at the University of Florida for blood-gas analysis with an ABL system 500 (Radiometer, Westlake, OH). Arterial blood samples were also drawn from the acidotic reference groups (MA; n = 12) and processed as described above. Base excesses were calculated with an algorithm provided by Radiometer and represent the concentration of titratable base when the blood is titrated with strong base or acid to a plasma pH of 7.4 at a PCO₂ of 40 Torr and 37°C at the actual oxygen saturation (37). A separate group of rats (n = 6 rats/treatment group) was utilized for analysis of serum electrolytes. Serum Na⁺, K⁺, and Cl⁻ and CO₂ were measured with a Roche Hitachi Modular P800 chemistry analyzer (Roche Diagnostics, Indianapolis, IN) in venous blood drawn from the anterior vena cava. Anion gaps were calculated from the serum electrolytes as follows: anion gap = serum [Na⁺] – (serum [Cl⁻] + serum [CO₃²⁻] + serum [HCO₃⁻]) (43). We did not perform serum electrolyte analysis on the MA reference group as it was well established that NH₄Cl causes a hyperchloraemic (normal anion gap) acidosis (4, 43). All rats were fully exsanguinated via cardiac puncture, and the serum was collected by centrifugation at 3,000 g for 15 min. An aliquot was immediately processed for oxalate determination with all precautions to prevent oxalogenesis (18), and the remainder of the serum was frozen for osmolality and creatinine determination.

Biochemical Determinations

Urinary chloride concentrations were determined with a chloridometer (Labconco, Kansas City, MO). Urine and serum osmolalities were measured with a freezing-point osmometer (Fiske Associates, Norwood, MA). Free orthophosphate (phosphate) was measured with a malachite green phosphate assay kit (BioAssay Systems, Hayward, CA).Creatinine was determined in the urine and serum samples using a modification of the Jaffe reaction as described by Slot (45) and further modified by Heinegard and Tiderstrom (24). Urinary ammonium was measured with an ammonium ion-selective electrode (detectION 3051, World Precision Instruments, Sarasota, FL). Calcium (Pointe Scientific, Lincoln Park, MI), citrate (R-Biopharm, Marshall, MI), and urinary oxalate (Trinity Biotech, St. Louis, MO) were measured with commercially available kits. Serum for oxalate determination was ultrafiltered using an Amicon Ultra-4 device, and oxalate was measured as previously described (18). Titratable acid was quantitated by titrating the urine samples to pH = 7.4 with either 1.0 N NaOH or 1.0 N HCl.

Statistical Analyses

All data were subjected to least-squares ANOVA using the general linear models procedures of the Statistical Analysis System (3). Significant treatment effects were separated by a Student-Newman-Keuls sequential range test (46). Main effects of EG and unilateral
nephrectomy were tested by orthogonal contrasts. When the main effect of unilateral nephrectomy was significant, comparisons were made between the UNI rats and the HRF rats. Differences between the MA group and the CON group were compared by an unpaired Student’s t-test. All data were tested for heterogeneity of variance with the Levene median test and for normality with the Kolmogorov-Smirnov test before ANOVA procedures (12). When normality and/or heterogeneity of variance tests failed, data were rank transformed before ANOVA. All data are expressed as means ± SE, and differences between means were considered statistically significant if P < 0.05.

**RESULTS**

Ingestion of excess non-carbonic acid loads or of compounds that are metabolized to such acids (e.g., EG and NH4Cl) may produce a metabolic acidosis characterized by a fall in arterial pH and HCO3− concentration at normal PCO2. Additionally, the anion gap [serum [Na+] − (the sum of serum [Cl−] + serum [CO3])]) produced by non-carbonic acid ingestion may be elevated in metabolic acidosis.

**Blood-Gas and Electrolyte Analyses**

Comparisons of arterial blood-gas analyses for the five groups of rats are shown in Fig. 1. Metabolic acidosis caused a significant reduction in arterial pH (Fig. 1A; 7.43 ± 0.01 vs. 7.33 ± 0.02 in CON vs. MA, respectively) and arterial bicarbonate concentrations (Fig. 1B; 29.1 ± 0.6 vs. 21.6 ± 1.1 meq/l in CON vs. MA, respectively). However, arterial pH and bicarbonate concentrations did not statistically differ among CON, HYP, UNI, and HRF, but the lowest numerical values for each were consistently observed in HRF rats. PCO2 (Fig. 1C) and PO2 (83.8 ± 4.8 Torr in CON) were unaffected by any of the treatments (data not shown). However, there was a tendency for PCO2 to be lower in the MA group, which may represent a respiratory compensation for metabolic acidosis. Base excess was normal in the HYP group relative to the CON group (Fig. 1D). In contrast, base excess tended to be reduced in the HRF rats relative to CON rats, whereas a base deficit of 1.4 ± 1.6 meq/l was observed in the MA rats.

Serum Na+, K+, and Cl− were also measured in all rats except the MA group (Table 2). Anion gaps were calculated from these data and the serum bicarbonates (Fig. 1B). As shown in Table 2, only the unilaterally nephrectomized rats treated with EG exhibited a significant elevation in anion gap, indicating the accumulation of anions other than chloride and bicarbonate (perhaps EG metabolites like glycolate, glyoxylate).

Thus these results demonstrate that chronic EG ingestion, at the dosages provided in this study, does not have an impact on the acid-base chemistries of rats with normal renal function (see below). The more severe model, coupling reduced renal mass with EG ingestion, appears to exhibit some, but certainly not all, of the characteristics of metabolic acidosis. In contrast, the conventional model of NH4Cl-induced metabolic acidosis exhibited the primary hallmarks of this state: decreased arterial pH with a fall in plasma bicarbonate and a base deficit.

**Urine Chemistries**

Metabolic acidosis may also be manifest in urinary chemistries as a reduction in urinary pH, an increase in the excretion of titratable acid (principally phosphate), and an increase in urinary ammonium ion excretion. Additionally, urinary citrate excretion, a principal organic anion of urine, is reduced in acidosis (16) and calcium excretion may be enhanced (6). Consequently, changes in urinary chemistries in the five experimental groups were evaluated using two 24-h urine collections. Collections for the CON, HYP, UNI, and HRF groups were made at 14 and 28 days and are depicted separately as noted below. In the MA group, there were no significant differences in the parameters measured in 4- and 14-day collections; hence these were combined and are depicted as noted below as a single time point (4–14).

Urinary pH after 2 and 4 wk of treatment followed a similar pattern and was not different among CON, HYP, and UNI rats, as illustrated in Fig. 2A. In contrast, urinary pH was significantly lower in the HRF rats than in the CON, HYP, and UNI rats at 2 and 4 wk, but this fall in urinary pH was not nearly as significant as in the MA rats.
striking as that observed in the acidosis reference group (MA), where urinary pH fell below 5.5. Titratable acid was not different among CON, HYP, and UNI rats with the mean titratable acid in all these groups at 2 and 4 wk not significantly deviating from zero. However, titratable acid was increased in the HRF rats relative to the CON rats at 2 and 4 wk, with titratable acid in HRF rats being 0.22 ± 0.09 and 0.13 ± 0.08 meq/24 h at 2 and 4 wk, respectively. Titratable acid was higher in the MA rats than in all other groups, averaging 1.41 ± 0.08 meq/24 h. Total acid excretion, defined as the sum of potentially ionizable H⁺ ions (titratable acidity) and bound (nonionizable) H⁺ ions in the form of ammonium, was similar among CON, HYP, UNI, and HRF rats, but was higher in MA rats (Fig. 2B). The lack of a significant acid load in CON rats from the current study is most likely due to the alkali load provided by the diet (8, 41). Twenty-four hour urinary excretion of ammonium was nearly 30-fold higher in MA rats than in CON rats (Fig. 3A). In contrast, urinary excretion of ammonium did not differ between CON and HYP rats at 2 or 4 wk. Unilateral nephrectomy caused a significant decline in urinary ammonium excretion that was not additionally affected by hyperoxaluria-induced renal failure.

Metabolic acidosis induced hyperphosphaturia, with 24-h phosphate excretion being about fourfold higher in the MA group than in the CON group (Fig. 3B). The hyperphosphaturia was not evident in the HYP group, again illustrating the dichotomy between the results obtained in the MA and HYP groups. Urinary excretion of phosphate was similar between UNI and HRF groups after 2 wk but was significantly higher in HRF rats than in UNI rats after 4 wk.

Urinary chloride excretion was similar among CON, HYP, UNI, and HRF groups after 2 and 4 wk (Table 2). In contrast, chloride excretion in the MA reference group was about threefold higher than in the CON group.

The MA group showed a significant decrease in urine citrate excretion compared with CON rats (Fig. 4A). In contrast, EG-induced hyperoxaluria (HYP group) had no effect on excretion of citrate in the urine after either 2 or 4 wk compared with the CON group. However, unilateral nephrectomy (UNI group) caused a modest rise in urinary citrate excretion, and this increase was attenuated in HRF rats, suggesting some trend toward development of metabolic acidosis after 4 wk on EG treatment which correlates with the reduction in renal function as judged by a twofold increase in serum creatinine and a 50% reduction in renal creatinine clearance as described in a subsequent section.

Urinary excretion of calcium (Fig. 4B) was significantly increased with metabolic acidosis (MA group) and unilateral nephrectomy (UNI group) but tended to be reduced in all hyperoxaluric groups compared with their appropriate controls (HYP vs. CON and HRF vs. UNI). This trend was apparent after both 2 and 4 wk on treatment.

**Oxalate Handling**

Consistent with our previous studies that utilized these rat models (20, 21), the EG-treated rats (HYP and HRF) exhibited significant hyperoxaluria, hyperoxalemia, and an increased renal clearance of oxalate compared with their respective controls (Fig. 5). By 2 wk, urinary oxalate excretion was increased about four- and sevenfold in HYP and HRF rats, respectively, compared with CON rats. Further significant increases were apparent at 28 days of EG treatment. The significant elevation in serum oxalate and the reduced renal clearance of oxalate in HRF compared with HYP, which we have previously reported (20), is confirmed here and is clearly a direct consequence of reduced renal function in these rats. This study also confirms an earlier report (20) demonstrating no differences in oxalate handling in rats with one kidney compared with healthy controls (both kidneys intact). Interestingly, we find here that metabolic acidosis is not associated with any significant alterations in oxalate homeostasis, as judged by results showing that urinary oxalate excretion, serum

### Table 2. Serum and urinary electrolyte measurements after 4 wk on ethylene glycol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON</th>
<th>HYP</th>
<th>UNI</th>
<th>HRF</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body wt, g</strong></td>
<td>389.2±0.8 (n=17)</td>
<td>390.8±6.6 (n=17)</td>
<td>384.0±5.3 (n=17)</td>
<td>381.8±5.5 (n=17)</td>
<td>368.9±5.0 (n=12)</td>
</tr>
<tr>
<td><strong>Kidney wt, g/kg</strong></td>
<td>2.63±0.07 (n=11)</td>
<td>2.85±0.03 (n=11)</td>
<td>3.75±0.12* (n=11)</td>
<td>5.15±0.34† (n=11)</td>
<td>2.77±0.07 (n=12)</td>
</tr>
<tr>
<td><strong>Osmolality, mosmol/kgH₂O</strong></td>
<td>329.0±6.6 (n=9)</td>
<td>341.6±7.8 (n=9)</td>
<td>320.7±9.5 (n=11)</td>
<td>331.2±9.1 (n=10)</td>
<td>329.0±3.6 (n=12)</td>
</tr>
<tr>
<td><strong>Na⁺, meq/l</strong></td>
<td>138.7±0.8 (n=6)</td>
<td>140.2±0.9 (n=6)</td>
<td>139.3±0.3 (n=6)</td>
<td>142.3±1.4* (n=6)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>K⁺, meq/l</strong></td>
<td>7.5±0.4 (n=6)</td>
<td>7.7±0.4 (n=6)</td>
<td>6.8±0.3 (n=6)</td>
<td>6.5±0.1* (n=6)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>CI⁻, meq/l</strong></td>
<td>99.3±0.6 (n=6)</td>
<td>100.0±0.5 (n=6)</td>
<td>99.5±0.6 (n=6)</td>
<td>101.3±1.5 (n=6)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Anion gap, meq/l</strong></td>
<td>15.5±0.6 (n=6)</td>
<td>17.7±1.8 (n=6)</td>
<td>14.7±0.6 (n=6)</td>
<td>21.8±1.7† (n=6)</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Volume, ml/24 h</strong></td>
<td>14.0±0.9 (n=17)</td>
<td>21.1±2.9* (n=17)</td>
<td>19.1±0.8 (n=17)</td>
<td>49.0±3.9* (n=17)</td>
<td>15.7±2.6 (n=12)</td>
</tr>
<tr>
<td><strong>Osmolality, mosmol/kgH₂O</strong></td>
<td>1.950±93 (n=11)</td>
<td>1.749±119 (n=11)</td>
<td>1.584±32 (n=11)</td>
<td>746±77* (n=11)</td>
<td>1.992±51 (n=12)</td>
</tr>
<tr>
<td><strong>Na⁺, meq/24 h</strong></td>
<td>2.14±0.14 (n=11)</td>
<td>2.62±0.14 (n=11)</td>
<td>2.55±0.08 (n=11)</td>
<td>2.95±0.54 (n=11)</td>
<td>1.97±0.16 (n=12)</td>
</tr>
<tr>
<td><strong>K⁺, meq/24 h</strong></td>
<td>3.83±0.22 (n=11)</td>
<td>4.38±0.19 (n=11)</td>
<td>4.41±0.15 (n=11)</td>
<td>3.75±0.22 (n=11)</td>
<td>3.98±0.23 (n=12)</td>
</tr>
<tr>
<td><strong>Cl⁻, meq/24 h</strong></td>
<td>3.09±0.12 (n=17)</td>
<td>3.57±0.11 (n=17)</td>
<td>3.63±0.10 (n=17)</td>
<td>3.38±0.12 (n=17)</td>
<td>10.93±0.49* (n=12)</td>
</tr>
<tr>
<td><strong>Unmeasured A⁻, meq/24 h</strong></td>
<td>2.96±0.21 (n=10)</td>
<td>3.41±0.22 (n=10)</td>
<td>3.30±0.15 (n=10)</td>
<td>2.97±0.48 (n=9)</td>
<td>0.07±0.24* (n=12)</td>
</tr>
</tbody>
</table>

Values are means ± SE. CON, normal control; HYP, rats made hyperoxaluric by administration of ethylene glycol; UNI, unilaterally nephrectomized control rats; HRF, unilaterally nephrectomized rats fed ethylene glycol; MA, metabolic acidosis reference group; ND, not determined; A⁻, anions; Anion gap, serum [Na⁺]–(serum [CO₃²⁻] + serum [Cl⁻]). Kidney weights are presented as the weight of the single remaining kidney per kilogram body weight. Unmeasured anions in the urine were calculated as the difference between the number of milliequivalents of the measured cations (Na⁺ + K⁺ + Ca²⁺ + Mg²⁺ + NH₄⁺) and the number of milliequivalents of measured anions (Cl⁻ + phosphate) as previously described (8, 41). *P < 0.05 vs. CON. †P < 0.05 vs. UNI.
oxalate concentrations, and renal oxalate clearances are all within the normal limits for MA rats (20, 21, 23). To our knowledge, this is the first report of oxalate handling in acidotic rats.

Assessment of Renal Function

Serum creatinine (Fig. 6A) and creatinine clearance (Fig. 6B) were similar among all groups examined with the exception of the HRF group. Consistent with previous investigations of this animal model (20, 23), serum creatinine was over twofold higher in HRF rats than in all other groups and, consequently, creatinine clearance was reduced by \( \frac{1}{2} \) in the HRF compared with all other groups. Total acid excretion (Fig. 6B) was not different among CON, HYP, UNI, and HRF rats but was higher in MA rats. * \( P < 0.05 \) vs. CON. † \( P < 0.05 \) vs. UNI.

Urine volumes were similar among CON, HYP, and UNI rats after 2 wk on treatment (data not shown). In contrast, urine volume in the HRF group after 2 wk was nearly threefold higher than in any other group. After 4 wk on their respective treatments, 24-h urine output was significantly higher in HYP rats than in CON rats (Table 2). Urine output in HRF rats at 4 wk followed a similar trend to that observed at 2 wk, with volumes being \( \sim \) 2.5-fold higher in HRF than in CON, HYP, and UNI rats. Urine volumes in the MA rats were similar to those in CON rats (Table 2).

DISCUSSION

EG-induced hyperoxaluria models have been employed in numerous studies of calcium oxalate nephrolithiasis, and much of our current knowledge base in experimental hyperoxaluria and calcium oxalate kidney stone disease is based on this model (20, 21, 27, 28, 34, 35, 47). Like any experimental model, EG-induced hyperoxaluria has advantages and disadvantages. EG is inexpensive and simple to deliver in drinking water, where it is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase/aldehyde dehydrogenase to glycolic acid. Glycolic acid is oxidized to glyoxylic acid, which, in turn, is further oxidized to oxalic acid by glycolate oxidase (13, 31) or lactate dehydrogenase (26), thus promoting hyperoxaluria. There has been some concern, however, that this model also initiates a metabolic acidosis that may confound the interpretation of studies using this oxalate precursor (4, 5, 11). This notion undoubtedly arises from the fact that ingestion of large doses of EG by humans or animals does, indeed, induce metabolic acidosis (25). For example, there are many reports of metabolic acidosis following ingestion of sweet-tasting anti-
freeze (primarily EG) by household pets and of humans intentionally imbibing antifreeze (15, 25). Remarkably, the proposal that EG-induced hyperoxaluria models are complicated by the presence of EG-induced metabolic acidosis has not been experimentally evaluated before the present report.

Metabolic acidosis is most simply defined as a decline in systemic pH produced primarily by a reduction in systemic bicarbonate concentrations (4). A metabolic acidosis induced by the ingestion of nonvolatile acids or acid precursors, like EG, is usually associated with an increase in the anion gap due to the presence of organic anions, principally glycolate (15, 32, 44), generated by EG metabolism. Additionally, metabolic acidosis may be associated with alterations in urine chemistry that reflect biochemical/physiological responses to the increased acid load, such as decreases in urinary pH (17, 38) and urinary citrate excretion (1, 16) and increases in urinary calcium excretion (6), urinary ammonium excretion (6, 17), and phosphate excretion (6). We have evaluated these parameters in several models to test the hypothesis that EG consumption produces acidosis at dosages commonly employed to induce hyperoxaluria (20, 21) and nephrolithiasis in rats (27, 28, 35).

**Two-Kidney Hyperoxaluria Model**

Rats consuming 0.75% EG in their drinking water did not develop any signs of metabolic acidosis after 4 wk. Thus arterial pH, bicarbonate concentrations, and anion gap of these (HYP) rats were not significantly different from two-kidney controls (CON). Urinary pH, titratable acid, and the urinary excretion of citrate, calcium, ammonium, and phosphate were similar in both groups, which further supports the conclusion...
that this frequently employed regimen does not produce metabolic acidosis. In contrast, rats in the commonly employed NH₄Cl ingestion model of metabolic acidosis (MA group) did exhibit all of the hallmarks of acidosis: decreased arterial pH and serum HCO₃⁻ concentrations, together with lower urinary pH and citrate excretion but elevated urinary ammonium and phosphate excretion, which engenders increases in both titratable acid and total acid excretion.

Most likely, acidosis does not develop in the HYP rat model because the EG is delivered at lower dose over a greater time period compared with situations that arise in a clinical environment where accidental or intentional ingestion of antifreeze results in an acutely high dose. Only EG, glycolate, and oxalate accumulate in appreciable quantities in blood and/or urine (7, 14, 44) following EG ingestion. Because glycolate oxidase (GO) is one of the rate-limiting enzymes in the metabolism of EG (14, 44), high doses of EG (>2,500 mg/kg body wt), particularly when given as an oral bolus, cause the saturation-dependent accumulation of glycolic acid in the plasma (7, 32, 44), with metabolic acidosis ensuing (7, 36). Metabolic acidosis probably never emerges in this model of hyperoxaluria because glycolate oxidase never becomes saturated; hence plasma levels of glycolate in this time frame do not rise significantly. The fact that the anion gap in HYP rats was not significantly different from controls further suggests that glycolate (or other anionic metabolites of EG) does not significantly accumulate in this animal model.

One-Kidney Hyperoxaluria Model

Unilateral nephrectomy (UNI group) did not produce metabolic acidosis as arterial pH, serum HCO₃⁻ concentration, and the anion gap were not significantly different from CON rats. Furthermore, there was no significant increase in total acid excretion, reduction in urinary pH, or decreased excretion of citrate in the UNI group, as would be anticipated in acidosis, further suggesting that reduced renal mass per se does not lead to metabolic acidosis. (Ammonium excretion in UNI rats was depressed, which is contrary to expectations of enhanced NH₄⁺ production in acidosis but consistent with the impaired ammonia excretion that accompanies loss of renal mass.)

In contrast, while nephrectomized rats (HRF) given 0.75% EG in their drinking water for 4 wk did not exhibit frank metabolic acidosis, there were some signs that they may be developing an acidic state. Thus although arterial pH and HCO₃⁻ concentrations were not significantly different from either CON or UNI controls, the HRF rats did exhibit a slightly larger anion gap and had a higher urinary phosphate excretion, a lower urinary pH, and an increase in titratable acid. Ammonium excretion in HRF rats was not significantly different from UNI rats, and, as noted above, both were actually lower than in the CON group. It should be noted that the changes in urinary chemistry suggestive of acidosis in HRF rats are quantitatively minor compared with the MA group.

Renal Function and Oxalate Handling in Models

Of the five models examined in this study, only nephrectomized rats ingesting EG (HRF) exhibited renal failure as judged by a significant fall in creatinine clearance and a significant elevation of serum creatinine concentration. This finding is consistent with earlier studies of the HRF model and suggests that oxalate load imposed on nephrectomized rats is a contributing factor in promoting renal failure (20). Indeed, this experimental model was developed to mimic oxalate-related disease states like primary hyperoxaluria with renal insufficiency caused by chronic hyperoxaluria (20). The fact that the HRF model exhibits some characteristics suggestive of a nascent metabolic acidosis is not surprising because renal failure itself causes increased anion gap metabolic acidosis (4) and metabolic acidosis has been observed in patients with primary hyperoxaluria (40).

A novel finding of this study is the observation that metabolic acidosis is not associated with any significant alterations in oxalate homeostasis. In 2001, Bushinsky et al. (6) reported that urinary oxalate excretion was significantly reduced in genetically hypercalciuric stone-forming rats given 0.5–1.5% NH₄Cl for periods of 4–14 wk and, by way of explanation, he suggested that metabolic acidosis alters oxalate metabolism. In our study, which included urine and serum oxalate measurements, as well as an assessment of renal clearance of oxalate in Sprague-Dawley rats, we find that mean urinary excretion of oxalate is somewhat, but not significantly, lower than in controls. Furthermore, all values for each of the parameters examined were within the normal ranges that we have established in our laboratory for Sprague-Dawley rats (20, 21, 23). Thus we conclude from this study that oxalate homeostasis is not influenced by metabolic acidosis. It is, however, quiet possible that GHS rats exhibit unusual metabolic patterns because of their extensive inbreeding.
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