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ORIGINAL PAPER

Partitioning of ^{14}C-oxalate excretion in rats during a persistent oxalate challenge

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Abstract This study was done to resolve published discrepancies in oxalate excretion between humans and rats and to characterize oxalate partitioning in rats during persistent severe hyperoxaluria, such as that seen in many bariatric patients. Osmotic minipumps dispensing 360 μmole/day KOx + 3.9 ± 0.14 μCi/day ^{14}C-oxalate were implanted subcutaneously. All excreta were collected. Rats were killed on day 13 and carcasses were dissected, ground, dissolved in HCl, and subjected to scintillation counting, and 92.1 ± 3.9% of total oxalate administered was recovered. This was partitioned among the skin complex (38.2 ± 7.7%), carcass complex (24.5 ± 5.9%), and excreta (29.5 ± 1.9%). The distribution of oxalate in the skin and carcass complexes led us to infer that only 29.5 ± 1.9% of the administered oxalate entered the circulation. Of the circulated oxalate, 98.4 ± 0.4% was excreted (total urine 78.9 ± 1.7%; raw feces 21.0 ± 1.7%). Thus, most oxalate that does enter the circulation is promptly excreted in rats, as in humans. Consequently, even after a large, persistent oxalate challenge, very little oxalate had accumulated in the internal organs, muscle, and skeleton. Unlike humans, however, rats excrete a significant fraction of oxalate in the feces.

Keywords Urolithiasis · Oxalate · Kidney stone · Nephrocalcinosis · Rat · Minipump

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Introduction

Oxalate is a terminal metabolite in mammals because they lack the oxalate-oxidase or oxalyl-CoA decarboxylase complexes needed to break it down [1]. They must excrete or sequester oxalate to maintain homeostasis. Previous human studies on urinary oxalate excretion used radiolabeled intravenous infusions smaller than the typical daily urinary excretion and reported 90–100% recovery of oxalate [2–6]. An oxalate to creatinine clearance ratio of 2 and distribution volume of 1.5-fold the extracellular space led investigators to hypothesize both renal secretion and extrarenal clearance of some oxalate [4, 7]. Some results depended on unproven assumptions, incomplete collections, and/or indirect oxalate measurements. Previous rat studies consistently failed to account for all administered oxalate and reported significant fecal oxalate excretion. Radiolabeled doses smaller than the typical daily excretion were administered acutely by intraperitoneal injection [8, 9] or persistently over 4 days by subcutaneous minipump [10]. Forty-three to seventy-nine percent of the oxalate was recovered in the urine and feces in a ratio of about 4:1, and oxalate clearance ratios and distribution volumes were like those of humans [10, 11]. Neither rats nor humans excrete appreciable ^{14}C-oxalate (^{14}C-Ox) as $^{14}C–CO_2$ [8, 12, 13]. To determine if protocol or species differences account for different oxalate recoveries in rat and human studies, we recovered and localized as much oxalate as possible from rats treated with 360 μmole of potassium oxalate (KOx)/day for 13 days. The entire carcass was processed and all fluids and tissues were identically processed, thus allowing direct sample comparisons.

This minipump protocol causes persistent high levels of hyperoxaluria and deposition of CaOx nephrocalcinosis in the medullary collecting ducts [14, 15], reminiscent of the most severe hyperoxaluria cases seen in patients following

old style bariatric surgery [16]. Bariatric surgery, especially of the gastric bypass type, effects long-term weight loss more reliably than other weight reduction therapies [17, 18], thus it is not surprising that the number of procedures has increased fivefold since 2000 [18]. While the CaOx nephrocalcinosis and renal failure seen previously are not reported in current bariatric patients, elevated oxalate excretion is common [19] and daily excretions over 100 mg/day are reported in Roux-en-Y bypass patients [20]. As bariatric surgery becomes ever more popular for reducing morbid obesity and its attendant comorbidities [17, 18], the number of patients with persistent moderate to severe hyperoxaluria will probably increase. Due to our interest in such pathophysiology, we chose our established high dose protocol for the present study.

Methods

Application of osmotic minipumps

Male Sprague Dawley rats ($n = 6$, 250–275 g) were obtained from Harlan (Indianapolis, IN) and maintained under standard conditions until pump implantation, after which they were housed in metabolic cages. Food and water were freely available. The NIH Guide for the Care and Use of Laboratory Animals was adhered to at all times.

Minipumps were filled with 2 ml of a working solution containing phosphate buffered saline (PBS) vehicle, 1.5 M potassium oxalate (360 µmole KOx in 240 µl PBS/day) and ^{14}C-oxalic acid (^{14}C-Ox, 3.9 ± 0.14 µCi/day; spa. 8 mCi/mmole, Sigma, St. Louis, MO). The tracer oxalate was radiolabeled on both carbons. The daily radioactivity administered by each pump was calculated by averaging the dpm/240 µl in the filling solution and the dpm/240 µl recovered from the spent pump. Multiplying this by the number of days the pump was in use gave the total radioactivity administered by that pump. Adding the values for both pumps yielded the total radioactivity administered to each rat. The oxalate-oxidase assay (Trinity, Brae Ireland) was used per manufacturer's directions to confirm that the KOx concentration in the stock solution was 1.5 M. The total oxalate dose (Admin-Ox, µmole) was found by summing the µmole of KOx and ^{14}C-Ox administered.

Pump priming and pump implantation/replacement surgeries were as previously described [14, 15]. The fluid around the pump and the saline wash were collected at pump replacement. Rats were killed on day 13 by anesthesia overdose.

Collection of excreta and tissues

Urine was collected at 25°C in vessels containing 50 µl of 0.02% sodium azide plus sufficient 12 N HCl to produce a concentration of 1 N in an average sample. Before storage the final concentration of HCl was adjusted to 1 N. Urine oxalate concentrations were determined using the oxalate-oxidase assay in samples adjusted to pH 5–7.

Raw urine in the collection vessel, feces, and food crumbs at the bottom of the collection funnel were collected daily and stored at −80°C. Respired air was not collected, as rats and their intestinal flora metabolize only minute amounts of oxalate to CO_2 [8, 12]. At death the skin, internal organs, and carcass were divided as shown in Table 1 and stored at −80 C.

Determination of radioactivity

All samples were processed as recommended by Beckman Technical Service (Fullerton, CA). Solid excreta and tissues were ground to a fine powder by mortar and pestle under lqN_2. All tissues and excreta were ground in their entirety and thoroughly mixed before being aliquoted. Table 1 shows the subdivisions of the pump filling solutions, excreta, skin complex, and carcass complex, as well as the size of the aliquots and their number per tissue or day.

Aliquot sizes were adjusted to keep counting efficiency at ≥80%. Aliquots were cooked in 2.4 ml of 1 N HCl at 70°C for 2 h at 100 rpm. After cooling 15 ml of scintillation fluid (ReadyScint, Beckman) was added and vials sat overnight to let autofluorescence dissipate. Samples were counted on a Packard Tri-Carb 2000 with QuantaSmart software for 5 m. Counts were corrected for chemical and color quench and expressed as dpm using the counter's internal programs. Conversion of dpm to µmole was calculated as follows [(dpm per organ or g of tissue)/total dpm administered to each rat)] × (µmole KOx + µmole ^{14}C-Ox administered to each rat). The amount of oxalate in the three major compartments of the carcass shell were estimated using standard values: skeleton = 9% of body weight, skeletal muscle = 40% of body weight, plasma = 3.5 ml/100 g body weight.

Statistical analysis

Analysis of variance (ANOVA) was used to determine differences in excretion among rats (SPSS, Chicago, IL). Data are presented as mean ± standard error of the mean (SEM).

Results

Tissue processing

The hot-acid protocol dissolves both tissue and CaOx crystals. To confirm that it does not oxidize oxalate to CO_2,

Table 1 Subdivisions of rat carcasses for liquid scintillation counting

Compartment	Subdivisions	Number of aliquots	Size of aliquots	Average weight or volume
Pump Solutions	Filling solution	5/pump	10 µl	2.05 ml
	Recovered pump solution	5/pump	10 µl	0.352 ± 0.036 ml
Excreta	Urine	3/day	200 µl	26.2 ± 2.8 ml/day
	Feces	3/day	50 mg	5.2 ± 0.2 g/day
	Urine soaked crumbs of food (crumbs)	3/day	50 mg	1.7 ± 0.1 g/day
Skin complex	Pump pocket skin: pump portal	≥5	200 mg	9.1 ± 1.5 g
	Pump pocket skin: pump reservoir	5	200 mg	7.3 ± 0.8 g
	Nonpocket skin: rump	3	200 mg	1.9 ± 0.4 g
	Nonpocket skin: chest	3	200 mg	1.6 ± 0.3 g
	Nonpocket skin: remaining skin	10	200 mg	22.3 ± 2.2 g
	Fur	3	200 mg	2.3 ± 0.1 g
	Accumulated fluid and pocket washes	3/pump removal	50 µl	9.9 ± 2.4 ml
Carcass complex	Alimentary canal: organs	3/region	200 mg	8.3 ± 0.4 g
	Alimentary canal: contents	3/region	50 mg	10.4 ± 0.9 g
	Internal organs, eyes, brain	3/organ	200 mg	24.2 ± 0.7 g
	Carcass: plasma	3	200 µl	9.7 ± 0.1 ml
	Carcass: muscle	3	200 mg	112.3 ± 2.2 g
	Carcass: bone	3	200 mg	25.3 ± 0.5 g
	Carcass: remaining shell (taken while spinning in lqN_2)	10	200 mg	146.1 ± 4.9 g

^{14}C-oxalate was added to liver samples either before or after heating ($n = 5$/treatment). The amounts of radioactivity in the two sets of vials were identical (^{14}C-oxalate added pre-heating, 20,120 ± 498 dpm; ^{14}C-oxalate added post heating, 20,096 ± 224 dpm, $p = 0.9$). Thus, ^{14}C-oxalate clearly does not oxidize to CO_2 during processing.

To process the remaining shell of the carcass, all of it was ground to a fine powder and well mixed in lqN_2. We tested two protocols for determining the dpm/g. First, the ground remaining shell was divided into 10 aliquots which were mixed well by grinding. Five 200 mg aliquots/aliquot were processed and counted (248,096 ± 21,220 dpm/g). Second, the ground remaining shell was continuously mixed in lqN_2 and ten 200 mg subaliquots were taken, processed and counted (261,275 ± 18,301). There was no difference between protocols in the dpm/g ($p = 0.6$); we chose the second protocol to process the rest of the remaining shells.

To insure that a sufficient number of aliquots had been taken from the ground tissues, intra- and inter-assay coefficients of variation were determined for the remaining skin, the remaining carcass shell and liver. The intra-assay coefficient of variation for the remaining skin (6 runs, 5 replicates/run) was 27.2% and the inter-assay coefficient of variation was 9.8%. For the remaining shell of the carcass (5 runs, 10 replicates/run) the intra-assay coefficient of variation was 13.9% and the inter-assay coefficient of variation was 5.0%. For the liver (10 runs, 3 replicates/run) the intra-assay coefficient of variation was 6.6% and the inter-assay coefficient of variation was 3.8%. It is expected that the inter-assay coefficient of variation will be larger than the intra-assay coefficient of variation. Here the values are reversed which reflects the difficulty of getting tissues and CaOx crystals ground to truly homogenous powders. The relatively small inter-assay coefficients of variation indicate that sufficient aliquots were analyzed in each assay to accurately determine the dpm/mg of each sample.

Oxalate assay

To confirm that scintillation counting and the oxalate-oxidase assay give the same results, we used both methods to determine oxalate excretion in the raw urine (urine in the collection vessels under the cage). There was no difference ($p = 0.2$) in urinary oxalate excretion between the two assays (^{14}C-Ox 74.0 ± 3.5 µmole/day, 962.1 ± 105.9 µmole total; oxalate-oxidase assay 68.4 ± 3.0 µmole/day, 889.7 ± 73.3 µmole total). Moreover, the assays yielded nearly identical patterns of excretion (Fig. 1).

Recovery of administered oxalate

Overall recovery of the Admin-Ox (4,686 ± 0.2 µmole over 13 days) was 92.1 ± 3.9%. In four of the rats, 97.6 ± 3.1% of the Admin-Ox was recovered, but only 81.2 ± 0.2% in the first two processed. We could not

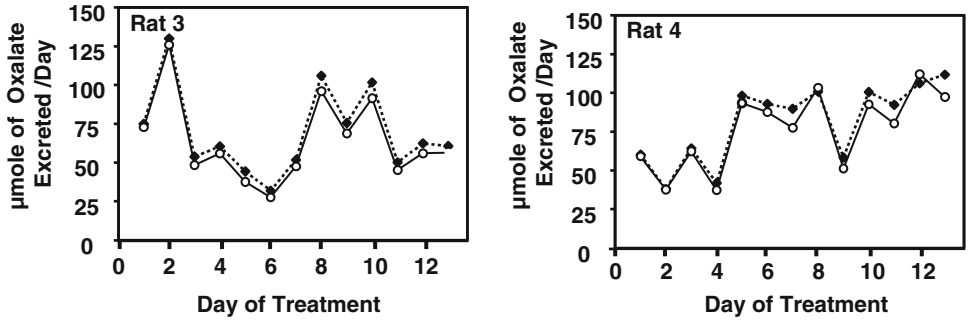

Fig. 1 Comparison of the percentage of the daily dose of oxalate excreted in the raw urine as determined by liquid scintillation counting (*open circles, solid line*, µmole oxalate/day) and by the oxalate-oxidase assay (*solid squares, dashed lines*, µmole oxalate/day). Results are from two rats processed in separate runs. Osmotic minipumps releasing 360 µmole KOx + 3.9 ± 0.14 µCi ^{14}C-Ox/day were implanted subcutaneously on day 0 and day 6

explain the discrepancy and so left the low recovery rats in the analysis. We used Admin-Ox as our reference because it is independent of collection, dissection, and tissue processing.

Figure 2 shows the partitioning of administered oxalate; the legend presents the means ± SEM's of the absolute content of oxalate for each subdivision of the major sites of oxalate partitioning. In the skin complex 38.2 ± 7.7% of Admin-Ox was recovered (skin, fur, fluid surrounding the pump + pocket washes), 24.9 ± 6.0% of Admin-Ox was recovered in the carcass complex (internal organs, alimentary tract + contents, remaining carcass shell (skeleton, skeletal muscle, blood)), and 29.1 ± 2.0% of the Admin-Ox was recovered in the excreta (urine, feces, food crumbs).

Partitioning of oxalate in the skin complex

The fluid surrounding the pump + pocket washes contained surprisingly little oxalate (0.75 ± 0.2 Admin-Ox). Trace amounts were recovered from the washed fur (0.009 ± 0.002% Admin-Ox), which may have been due to urinary or fecal contamination.

Table 2 lists oxalate concentrations in several skin regions. The skin over the pump port contained 78.6 ± 8.7% of the total skin complex oxalate (29.1 ± 5.9% Admin-Ox). H&E slides of this skin revealed many birefringent crystals at the border of the dermis and hypodermis and in the walls of the vasculature. Oxalate concentrations fell sharply outside this area. Even the skin over the pump reservoir, while also bathed in the fluid around the pump, accounted for only 11.8 ± 6.4% of the total skin complex oxalate (6.3 ± 3.8% Admin-Ox). While all of the nonpocket skin accounted for only 7.1 ± 4.3% of the total skin complex oxalate (2.1 ± 1.1% Admin-Ox), the concentration in regions containing areas next to the pump pocket and port was almost 30-fold higher than in two remote regions, the chest and rump. From the localization of the skin complex oxalate, we infer that almost all of it accumulated without ever entering the circulation.

Partitioning of oxalate in the carcass complex

The remaining shell contained 98.0 ± 0.25% of the carcass complex oxalate (24.5 ± 5.9% Admin-Ox). However, its major subdivisions accounted for less than 1% of Admin-Ox, as follows: skeleton, 33.4 ± 3.9 µmole (0.71 ± 0.08% Admin-Ox); skeletal muscle, 5.2 ± 0.3 µmole (0.02 ± 0.001% Admin-Ox); plasma, 4.69 ± 0.39 µmole (0.1 ± 0.008% Admin-Ox). We could not quantify the oxalate in the erythrocytes due to hemoglobin's intense color quench. The internal organs together accounted for 0.866 ± 0.28% of the carcass oxalate (0.23 ± 0.1% Admin-Ox). The tissues and contents of the gastrointestinal tract accounted for 1.1 ± 0.25% of the carcass oxalate (0.22 ± 0.04% Admin-Ox). Tissues and contents of the stomach through the jejunum showed low oxalate concentrations, with markedly increased concentrations in the contents from the ileum through the distal colon. Table 3 shows the oxalate distribution in the gastrointestinal tract.

Figure 3 presents the oxalate accumulations in the skin complex and remaining carcass shell. Together these accounted for 62.6 ± 2.3% (2,936 ± 109 µmole) of Admin-Ox. From the minimal oxalate in the skeleton, skeletal muscle, and plasma, we infer that almost all of the carcass shell oxalate accumulated near the pump site without ever entering the circulation. If this is true, then most (but not all!) of the oxalate in the carcass shell can be classified, along with that accumulated in the skin complex, as noncirculated. Circ-Ox, i.e., the total oxalate that ever entered the vascular circulation, was 29.5 ± 2.0% (1,383 ± 92 µmole) of Admin-Ox by subtraction and further inference. The oxalate accumulations in the skin complex and remaining carcass shell varied between the highest and lowest rats by 3.7- and 5.2-fold, respectively, but the sum of oxalate in the skin complex and remaining carcass shell varied by only

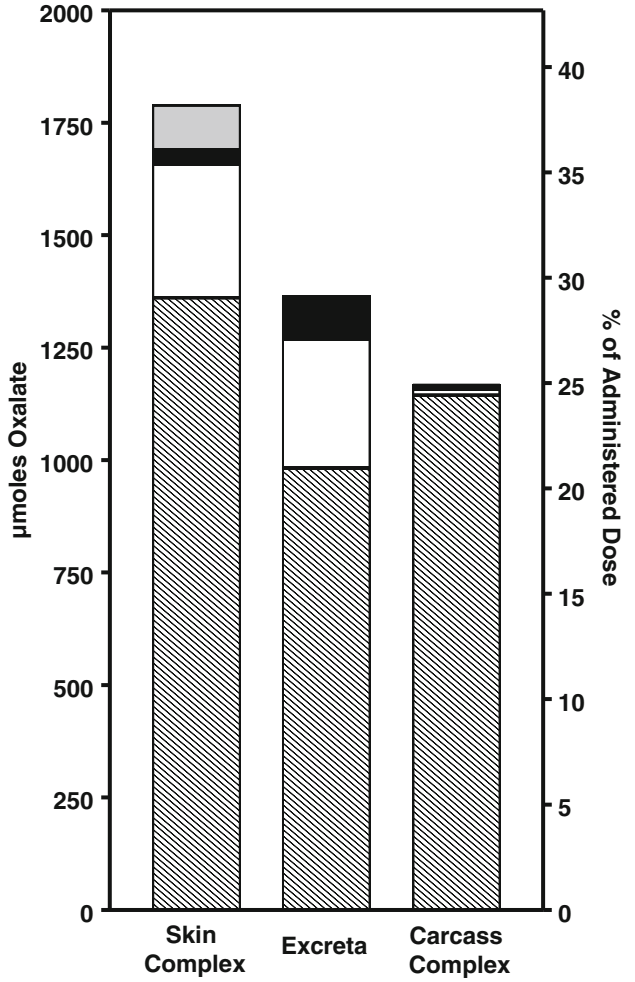

Fig. 2 Recovery and partitioning of administered oxalate (4,686 ± 0.2 µmole of oxalate over 13 days). Overall, 92.1 ± 3.9% of the administered dose was recovered and partitioned as follows: (1) *Skin Complex*: *striped* = skin over pump port, 1,363 ± 276 µmole; *white* = skin over pump reservoir, 293 ± 179 µmole; *black* = fluid surrounding pump + pocket washes, 35 ± 9 µmole; *gray* = nonpocket skin, 97 ± 54 µmole; *stippled* = fur, 0.42 ± 0.09 µmole; (2) *Excreta*: *striped* = raw urine, 984 ± 96 µmole; *white* = feces, 279 ± 10 µmole; *black* = urine soaked food crumbs, 99 ± 6 µmole; (3) *Carcass Complex: striped* = remaining shell, 1,146 ± 276 µmole; *white* = internal organs, 11.0 ± 5.0 µmole; *black* = GI tract and contents, 10.2 ± 1.9 µmole. Data are presented as mean ± standard error of the mean

Table 2 Concentration of oxalate in the skin

	µmole/g wet weight
Total skin	40.3 ± 7.3
Pocket skin	87.9 ± 10.3
Pocket skin: pump port	144.8 ± 8.6
Pocket skin: pump reservoir	31.4 ± 18.0
Nonpocket skin	3.9 ± 2.1
Nonpocket skin: rump + chest	0.12 ± 0.02
Nonpocket skin: remaining skin	4.4 ± 2.4

Table 3 Distribution of oxalate in the alimentary canal tissues and contents

	Tissue µmole/g wet weight	Contents µmole/g wet weight
Stomach	0.08 ± 0.001	0.08 ± 0.03
Duodenum[a]		0.14 ± 0.02
Jejunum	0.09 ± 0.01	0.13 ± 0.03
Ileum	0.09 ± 0.01	0.66 ± 0.21
Cecum	0.08 ± 0.02	1.14 ± 0.28
Proximal colon	0.14 ± 0.03	1.16 ± 0.30
Distal colon	0.17 ± 0.04	2.14 ± 0.47
Clean feces[b]	–	2.83 ± 0.59

[a] Tissue + contents

[b] Feces removed from the anus, and thus free of urinary oxalate contamination, at death on day 13

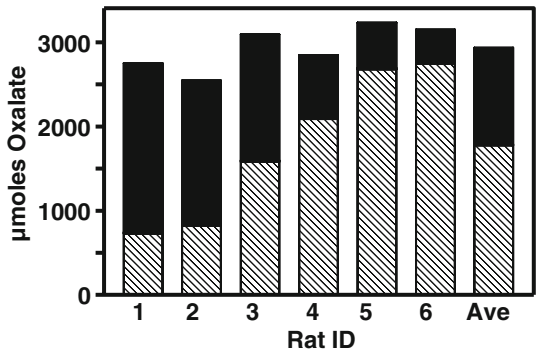

Fig. 3 Micromole of administered oxalate in the remnant shell (*black*) and skin complex (*crosshatch*) and inferred not to have entered the circulation

1.3-fold, suggesting that it should be considered an unitary, noncirculated pool.

Partitioning of oxalate in the excreta

Oxalate in the raw urine accounted for only 71.5 ± 2.2% of total excreted oxalate (21.0 ± 2.1% Admin-Ox). This standard measure of oxalate excretion understates the true total. First, it omits fecal excretion, which accounted for 21.0 ± 1.7% of excreted oxalate (5.9 ± 0.2% Admin-Ox). Second, it omits urine contaminating both the food crumbs (7.4 ± 0.6% of excreted oxalate, 2.1 ± 0.1% Admin-Ox) and the feces. The ^{14}C-labeling enabled direct measurement of urinary oxalate contaminating the food crumbs (traditional assays cannot distinguish this from intrinsic oxalate). The sum of this contaminating oxalate plus that in the raw urine is termed total urinary oxalate (TotUrine), which accounted for 78.9 ± 1.7% of excreted oxalate (23.1 ± 2.1% Admin-Ox).

TotUrine does not include the oxalate in urine contaminating the feces, as it was hard to obtain clean feces. The final concentration of oxalate in clean feces was estimated to be 2.8 ± 0.6 μmole/g, based on harvesting a fecal pellet from the anus at death. The oxalate concentration in raw feces collected on day 13 was 1.5-fold higher, 4.4 ± 0.6 μmole/g. The ratio of oxalate in the raw verses the clean feces ranged from 0.36 to 0.84 depending on individual defecation habits. The higher oxalate concentration in raw feces probably resulted from a combination of urine contamination and fecal drying. We used the raw feces values in the present study, understanding that this slightly overestimates fecal oxalate excretion.

If the excreted oxalate is expressed as a percentage of the circulated oxalate then it appears that like humans, rats do promptly excrete most of a load of oxalate. However, unlike humans the excretion is partitioned between the urine and feces. In these rats 98.4 ± 0.4% of the circulated oxalate was excreted in the TotUrine (77.7 ± 1.9% of the circulated oxalate) and raw feces (20.7 ± 1.7% of the circulated oxalate).

Discussion

The available oxalate-oxidase colorimetric assay kit has been used by many labs, including ours, to determine urinary oxalate excretion in rats. However, it was designed and validated to measure oxalate in human urine. In this study, we show that if rat urine is collected under acidified conditions and adjusted to pH 5–7, the assay kit gives results statistically identical to those obtained by liquid scintillation counting of ^{14}C-Ox excreted in the urine. Scintillation counting will not measure urinary oxalate that was produced by the liver and erythrocytes, arising from the degradation of ascorbate or absorbed by the diet [21]. In our rats this amounts to 5–10 μmole of oxalate per day [14, 15] and thus scintillation counting should have under reported raw urinary oxalate by this amount. However, because of the dose of oxalate delivered by the pumps was extremely large as compared to normal raw urinary excretion, this expected difference was not detected. Additionally, because oxalate excreted fecally or contaminating food crumbs cannot be measured by the assay kit, it systematically under measures urinary and total oxalate excretion. It is likely the under measurement can be safely ignored for many purposes, since the fecal and food crumb oxalate does not vary among rats (although the crumb oxalate can vary with diet and style of metabolic cage).

In the skin, carcass, or excreta, 92.1% of the oxalate dispensed by the pumps was accounted for. No doubt some portion of the unrecovered oxalate is attributable to losses incurred during daily cage washings, dissection, and mathematical "reassembly" of carcasses. Previous rat studies reporting lower recoveries did less extensive dissection, processed different tissues differently, or processed only selected tissues [8–10].

In the skin, fluid around the minipump, and fur, 38.2% of Admin-Ox was recovered. Because almost all of this oxalate accumulated in the skin over or close to the pump port or pump pocket, we infer that it diffused directly into the skin rather than passing through the circulatory system. The small amount of oxalate recovered from distant regions of the skin (chest, flank) may have arrived via the circulation, as only skin associated with the pump pocket was in contact with fluid from the pump. The dynamics of the oxalate depot near the pump are unknown. Would lower oxalate doses result in a lower proportion of Admin-Ox in the skin over the pump port? Or is formation of an epidermal depot of CaOx crystals an inherent characteristic of subcutaneous oxalate administration? It should be noted that the other article based on using subcutaneous minipumps reported recovering only 50% of the administered oxalate, most of which was in the excreta [10].

In the remaining carcass shell, internal organs and gastrointestinal tract, 29.5% of Admin-Ox was recovered. The remaining shell contained 98.0% of this, i.e., only 2.0% was recovered in the internal organs and gastrointestinal tract. As expected from Ussing chamber data on oxalate transport [22] and the distribution of the oxalate transporter SLC26a6 in the intestine [23], oxalate concentration in the contents began to increase in the ileum and continued to increase through the cecum and large intestine, most likely due to water reabsorption, one of the colon's major functions. There are oxalate transporters in the colon (SLC26a3), however, and Ussing chamber data suggest the colon can secrete oxalate into its lumen under hyperoxalemic conditions [23, 24]. This may have occurred in our experiments, as ion exchange chromatography has shown that rats treated with 360 μmole of KOx/day for 13 days have plasma oxalate concentrations at least double those of control rats (Marengo, data not shown; measurements by the Ion Analysis and Hyperoxaluria Laboratory of Wake Forest School of Medicine (Winston-Salem, NC).

The three major subdivisions of the remaining carcass shell, the skeleton, skeletal muscle, and plasma, accounted for 0.83% of Admin-Ox. The bone [10, 25–27] and possibly the erythrocytes [5] accumulate oxalate, so this relatively low accumulation in still growing rats was unexpected. Calcium oxalate crystals accumulate throughout the bone, especially in the epiphysis and metaphysis [25, 26]. Deposits have also been reported in the mononuclear phagocytes and multinucleated giant cells in the marrow [26, 27]. The factors driving oxalate deposition in the several bone compartments are not yet known.

Except for separately analyzed aliquots of the plasma, skeleton, and skeletal muscle, the remaining carcass shell was processed as an unit. Thus, there can be no direct evidence that the recovered oxalate was concentrated near the pump. Absent definitive data, however, this seems a reasonable inference due to the minimal oxalate found in the plasma, skeleton, and skeletal muscle. Unlike the thickened skin over the pump port, the body wall next to the pump port and pocket has never appeared abnormal. Thus, unfortunately, we paid it no individual attention during dissection.

Osmotic minipumps can be implanted in the peritoneal cavity, which would eliminate the concentrated accumulation of oxalate in the skin. However, there is no guarantee that similar deposits would not form in the inner body wall and/or internal organs and tissues next to the pump. Pumps implanted in the peritoneal cavity would essentially bathe the abdominal organs, including the kidneys, in oxalate. This delivery mode would also require using the far less soluble sodium oxalate to avoid potassium induced cardiac depolarization (unpublished observations).

The present minipump model avoids several limitations of other models which provide oxalate via i.p. injection, oral administration, or oral administration of precursors, e.g., sudden, brief oxalate surges, variable oxalate absorption from the gut, variable metabolism of precursors to oxalate by the liver, or the production of extraneous and potentially nephrotoxic metabolites. However, like the other delivery methods mentioned, the minipump cannot introduce a precisely predictable oxalate dose into the circulation. Overall, our data strongly suggest that reported differences in oxalate recovery between rats and humans largely reflect different oxalate delivery.

In the urine and feces, 29.1% of Admin-Ox was excreted. Previous studies using doses smaller than the usual daily urinary oxalate excretion reported recoveries from the urine and feces of 43 and 79% from rats receiving an acute intraperitoneal injection of oxalate [8, 9] or 50% from rats treated with oxalate by subcutaneous minipump for 4 days [10]. Our results matched fairly well when expressed as percentage of Admin-Ox (lower end) or of Circ-Ox (upper end), even though we administered a daily oxalate dose 144-fold greater for three times as long. The elimination ratio between TotUrine and fecal oxalate was about 4:1, which also tracks previous results [8–10].

Human and rat studies have always differed in the fraction of administered oxalate recovered. Several human studies using intravenous bolus or "equilibration" infusions of oxalate into the peripheral circulation or renal artery reported ~100% of infused oxalate excreted in the urine [2–4, 6]. Rats have excreted a significant amount of oxalate fecally in all studies, even with oxalate doses not expected to induce hyperoxalemia [8–10]. Unlike rats, hyperoxalemic dialysis patients apparently excrete little oxalate in their feces [13], although this needs to be confirmed.

Given that only a small fraction of Admin-Ox was accounted for in the internal organs, gastrointestinal tract, skin not associated with the pump pocket, skeleton, skeletal muscle, and plasma, we must conclude that a very large proportion of an oxalate dose that enters the circulation is promptly excreted in the urine and feces. The urinary plus fecal oxalate excretion accounts for 98.4% of Circ-Ox in the present study, even without creative data manipulation, e.g., removing from the data set the two rats with lesser Admin-Ox recovery or adjusting Circ-Ox for each rat's recovery.

The question, of course, is whether humans (who have not been shown to excrete a significant fraction of their plasma oxalate in their feces) can nonetheless excrete a large, persistent oxalate challenge through their kidneys The answer is probably yes, given that patients with primary hyperoxaluria only show clinically evident damage to the skeleton, peripheral nerves, and cardiovascular system after a period of years. Thus, our data also support the hypothesis that patients with severe hyperoxaluria only accumulate oxalate in their internal organs and tissues fairly slowly, resulting in an extended opportunity to reduce oxalate excretion before damage can accumulate in nonrenal tissues.

In conclusion, we have characterized the partitioning of a persistent, very high dose, subcutaneously delivered KOx challenge in rats and inferred that most of the oxalate never entered the circulation (more experiments would be needed to confirm this). The rest of the recovered oxalate, which we inferred had entered the circulation, was almost all (98.4%) excreted in the urine (77.7%) and feces (20.7%). Based on these data and the published literature, it appears that rats are like humans in promptly excreting most of an oxalate load but unlike humans in partitioning about a fifth of their oxalate excretion to the feces.

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