

# Extrarenal Clearance of Oxalate Increases With Progression of Renal Failure in the Rat<sup>1</sup>

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## ABSTRACT

Oxalic acid is an end product of metabolism, and no significant degradation of oxalate occurs in mammals. The sole route of oxalate excretion is believed to be via the kidney. The extrarenal clearance of oxalate in control rats ( $N = 16$ ) and in  $\frac{5}{6}$  nephrectomized rats ( $N = 25$ ) with renal insufficiency was investigated. ( $^{14}\text{C}$ )oxalic acid,  $\sim 2 \mu\text{Ci/day}$ , was infused sc by a mini osmotic pump over 4 days. Excretion of  $^{14}\text{C}$  was measured in urine, in feces, and in expired  $\text{CO}_2$ . The  $^{14}\text{C}$  content of kidney, heart, liver, muscle and bone was also determined at the time the animals were killed. Plasma oxalate was determined by an enzymatic method and by an isotopic dilution procedure. Creatinine clearance in the controls was  $1.82 \pm 0.1 \text{ mL/min}$  (mean  $\pm$  SE) compared with  $0.31 \pm 0.04 \text{ mL/min}$  ( $P < 0.0005$ ) in the nephrectomized rats. Plasma oxalate was  $5.6 \pm 0.6 \mu\text{mol/L}$  in controls and  $27.0 \pm 3.9$  (mean  $\pm$  SE;  $N = 24$ ) in nephrectomized animals ( $P < 0.0005$ ). The total  $^{14}\text{C}$  recovered in urine, feces, and  $\text{CO}_2$  combined was similar in both groups. The  $^{14}\text{C}$  excreted in the feces over the 4-day period was  $27.8 \pm 1.5\%$  (of the  $^{14}\text{C}$  recovered) in rats with renal failure and  $6.5 \pm 0.5\%$  in controls ( $P < 0.0005$ ). Percent fecal  $^{14}\text{C}$  excretion in nephrectomized rats was inversely correlated with creatinine clearance ( $r = 0.80$ ;  $P < 0.0001$ ) and directly correlated with plasma oxalate ( $r = 0.66$ ;  $P < 0.001$ ). Bone  $^{14}\text{C}$  was inversely correlated with creatinine clear-

ance in nephrectomized rats ( $r = 0.69$ ;  $P < 0.02$ ;  $N = 11$ ). All of the fecal  $^{14}\text{C}$ ,  $100.2 \pm 0.9\%$  (mean  $\pm$  SE), was in the form of ( $^{14}\text{C}$ )oxalate. It was concluded that fecal oxalate excretion plays an important and increasing role in oxalate balance with the progression of renal failure. Bone oxalate deposition increases with the progression of renal failure and may alter bone metabolism.

**Key Words:** Plasma oxalate, creatinine clearance, oxalate transport, oxalate in bone

Oxalic acid is an end product of metabolism, and no significant degradation of oxalate occurs in mammals (1). The sole route of oxalate excretion is believed to be via the kidney (2). The total body burden of oxalate is greatly increased in patients on maintenance dialysis, and this has been partly ascribed to lack of renal excretion (3). We have recently demonstrated that the rate of oxalate removal in patients on maintenance hemodialysis is significantly greater than the urinary excretion of normal subjects (4), suggesting that oxalate accumulation does not result from lack of oxalate removal on dialysis and must result from increased gastrointestinal absorption and/or endogenous synthesis. The purpose of the study presented here was to investigate whether the route(s) of oxalate excretion are altered in ESRD. An improved understanding of oxalate biosynthesis and excretion in this population is essential if appropriate strategies are to be designed to decrease the body burden of oxalate and its consequences. The rat was chosen as an appropriate model for oxalate balance studies because of the substantial fund of knowledge that exists on oxalate metabolism in the rat (5), the similarities in oxalate metabolism between the rat and humans (6), and the ease of inducing renal failure with the  $\frac{5}{6}$  nephrectomy model.

## MATERIALS AND METHODS

### Materials

Oxalate decarboxylase EC 4.1.1.2 was prepared from the mycelium of *Collybia velutipes* (strain S) as described by Shimazono and Hayaishi (7) and

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purified through their "first" acetone fraction. The lyophilized enzyme was stored at  $-20^{\circ}\text{C}$ . The enzyme is commercially available from Sigma Chemical Co. (St. Louis, MO) and gives similar results (unpublished data). Formate dehydrogenase was obtained from Sigma.  $[\text{U}-^{14}\text{C}]$ oxalic acid, 4.6 to 5.6 mCi/mmol, and Biofluor scintillation fluid were from DuPont, NEN Research Products (Boston, MA). Hionic-Fluor and Soluene-350 were obtained from United Technologies Packard (Downers Grove, IL); Alzet<sup>®</sup> osmotic pumps were from Alza Corporation (Palo Alto, CA); NAD (lithium salt) was from Boehringer Mannheim (Indianapolis, IN); and PM-30 membranes were from Amicon (Danvers, MA). All other chemicals were reagent grade.

### Animals

Male Wistar rats (Hilltop Lab Animals, Inc., Scottsdale, PA), 250 g, were housed in individual metabolic cages and fed a rat chow diet (Purina 5001; Ralston Purina Company, Richmond, IN) *ad libitum*. Animals were allowed a minimum of 1 wk to become acclimated. All animal experimentation was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals. Five-sixth nephrectomy was carried out by surgical ablation of both poles (approximately two-thirds) of the right kidney with the rat under ether anesthesia. The animals were given 1 wk in which to recover from this procedure. A left nephrectomy was then performed. A sham operation was performed in control animals by making a similar abdominal incision as in the  $\frac{5}{6}$  nephrectomized (NPX) rats. Control and NPX rats were housed for periods of from 1 to 5 months. The creatinine clearance of the animals was monitored during this period in order to study animals with varying degrees of renal insufficiency. Sham-operated controls were studied at similar times after surgery as NPX rats.

### Experimental Procedure

To study the excretion of oxalate by control and NPX rats,  $[\text{U}-^{14}\text{C}]$ oxalic acid was infused sc,  $\sim 2 \mu\text{Ci}/\text{day}$  over 4 days, by a mini osmotic pump (Alza Corporation). The exact dose infused was determined from the pump rate, microcuries of  $[\text{U}-^{14}\text{C}]$ oxalate per milliliter, and the number of hours infused. The rats were transferred to metabolic cages that allowed the separate collection of urine, feces, and expired  $\text{CO}_2$  to be made. During the study period, the rats were fed 12 g of powdered rat chow (Purina 5001) daily. Twenty-four-hour urines were collected into urine cups containing 5 mL of 3.5 N HCl. Expired  $\text{CO}_2$  was collected in two linked conical flasks containing 350 mL/flask of 2.5 N NaOH. Room air was pulled through each metabolic cage and through the two flasks containing NaOH. Urine, feces, and expired  $\text{CO}_2$  were collected

every 24 h throughout the 4-day infusion period. Blood samples, 1.0 to 2.0 mL, were withdrawn from the orbital sinus before the beginning of the study and each day of the study for plasma creatinine and  $^{14}\text{C}$  determination. After the 4-day infusion period, the rats were exsanguinated by cardiac puncture after IP administration of pentothal, 60 mg/kg. Plasma  $^{14}\text{C}$  concentration, plasma oxalate, and creatinine were determined on this sample. The heart, liver, kidneys, muscle (of front and hind quarters), and bone (front and hind limbs) were removed from a number of control and NPX rats and stored frozen at  $-20^{\circ}\text{C}$  for subsequent  $^{14}\text{C}$  determination.

### $^{14}\text{C}$ Determination of Urine and $\text{CO}_2$

Urine aliquots, 0.5 mL from 24-h collections, were counted in 5 mL of Biofluor scintillation fluid on a Packard liquid scintillation counter, Model 4530. The disintegrations per minute were determined by quench correction. Plasma samples, 200  $\mu\text{L}$ , were counted in a similar fashion. One-milliliter samples of NaOH containing expired  $^{14}\text{CO}_2$  were counted in 10 mL of Hionic-Fluor.

### Fecal $^{14}\text{C}$ Extraction

Twenty-four-hour fecal collections were extracted in 0.5 N HCl, 1 g/5 mL, vigorously mixed, and allowed to extract for 24 h at room temperature. These were then centrifuged, 1,000 g for 10 min, and the volume of the supernatant was determined. A further two or three extractions of the fecal precipitate in 0.5 N HCl were carried out as described above (with the exception that extractions, other than the first, lasted for about 15 min) to ensure extraction of  $>99\%$  of the fecal  $^{14}\text{C}$ . Aliquots, 0.5 mL, from each extract supernatant were counted in a similar fashion as described for urine above.

### Tissues $^{14}\text{C}$ Extraction

Kidneys and hearts ( $N = 8$  in controls;  $N = 11$  in NPX rats) were minced with a scalpel, transferred to screw-top Nalgene (Nalgene Company, Rochester, NY) centrifuge tubes, and lyophilized for 16 h, and the dry powder was crushed with a spatula. The weights of the wet tissue and lyophilized powder were recorded. Lyophilized kidneys and hearts were defatted with four washes of 1:1 ether:petroleum ether mixture, 2 mL/wash. Washings one to three were allowed to settle for 5 min, and the organic solvent was removed and discarded. After the final wash, the samples were centrifuged (15 min; 7000g) to allow maximum removal of the solvent. The tissue was dispersed in each tube and allowed to dry in the fume hood for about 2.5 h before being lyophilized for 1 h. To extract the  $^{14}\text{C}$ , 6 mL of 0.5 N HCl were

added to each tube containing lyophilized defatted kidney or heart tissue. The tubes were capped, continuously mixed on a rotary mixer for 16 h at room temperature, and centrifuged (20 min; 7000g), and the supernatant was removed and retained. A second extraction was carried out with 4 mL of 0.5 N HCl and treated as described above, and the supernatant was combined with the first acid extract in a 50-mL Pyrex tube (Corning Glass Works, Corning, NY). Previous studies have shown that a third extraction is unnecessary (unpublished data). The volume of the combined supernatants was determined, and duplicate aliquots, 0.5 mL, were counted as previously described for urine.

### Bone $^{14}\text{C}$ Extraction

Bone from the front or hind limbs ( $N = 8$  in controls;  $N = 11$  in NPX rats) was cleaned free of tissue by being wiped with a dry gauze sponge. The bone was lyophilized for a minimum of 1 h, and a section of ~100 mg was broken off and cleaned of any marrow. The bone was defatted with 5 mL of petroleum ether, lyophilized, and ground in a Wiley Mill (A.H. Thomas, Philadelphia, PA) (with a 20-mesh filter). The ground bone was again defatted in petroleum ether, dried, and reground in the Wiley Mill (with a 60-mesh filter). Ground bone, 18 to 20 mg, was extracted in 3.0 mL of 0.5 N HCl in a capped 15-mL polypropylene tube for 16 h with constant mixing. The samples were centrifuged at 500g for 10 min, and the supernatant was removed for counting. Duplicate 1-ml aliquots of the supernatant were counted in 5 mL of Biofluor. A second extraction of the homogenized bone in 0.5 N HCl yielded few counts and was therefore considered unnecessary.  $^{14}\text{C}$  in bone was expressed as disintegrations per minute per gram of bone. The loss of  $^{14}\text{C}$  during defatting was <5% of the total  $^{14}\text{C}$  extracted from kidney, heart, and bone.

### Liver $^{14}\text{C}$ Extraction

The entire liver ( $N = 8$  in controls;  $N = 9$  in NPX rats) was weighed and homogenized (Tissue Homogenizer; Brinkman Inst., Westbury, NY) in 0.5 N HCl—5 mL/g of liver and centrifuged (1000g for 30 min), and the supernatant volume was determined. Quadruplicate 1.0-mL aliquots of the supernatant were counted in 10 mL of Biofluor as previously described. The liver precipitate was further homogenized in 0.5 N HCl, and the supernatant was counted as described above. Further extraction of the liver homogenate was found unnecessary.

### Muscle $^{14}\text{C}$ Extraction

Muscle from front and hind quarters ( $N = 8$  in controls;  $N = 11$  in NPX rats) was weighed and ho-

mogenized in 0.5 N HCl, 5 mL/g of tissue, and 0.5-mL aliquots of the homogenate were counted in 5 mL of Biofluor as previously described. When sections of the same muscle tissue were minced, lyophilized, and digested with Soluene-350 and counted, similar results to the homogenized extract were obtained, demonstrating that tissue digestion was unnecessary.

### Determination of ( $^{14}\text{C}$ )Oxalate in Fecal Extract, Urinary and Plasma Oxalate, and Creatinine

Analysis of the extract supernatants for [ $^{14}\text{C}$ ]oxalate was carried out as previously described (8). A published procedure was used for the determination of urinary oxalate (9). Plasma oxalate was determined by an isotopic dilution procedure (10). When plasma oxalate for NPX rats ( $N = 24$ ) determined by the latter procedure was compared with analysis of plasma from the same animals by our enzymatic method (11), an excellent correlation was observed ( $r = 0.958$ ; data not shown). Plasma and urinary creatinine were determined by the Jaffe reaction (12) on an Abbott Blochromatic Analyzer-100 (Abbott Laboratories, South Pasadena, CA).

### Statistics

Statistical analysis of the results was performed by the  $t$  test and linear regression analysis. All values are mean  $\pm$  SE.

### RESULTS

Rat weight, creatinine clearance, urinary and plasma oxalate, and  $^{14}\text{C}$  excreted in the urine, feces, and  $\text{CO}_2$  of both groups is shown in Table 1. Although rats from both groups were studied at the same age, the weight of the control rats was significantly more than that of the NPX rats ( $P < 0.005$ ). The estimated calcium ingested during the  $^{14}\text{C}$  infusion was  $117 \pm 1.0$  mg/day (mean  $\pm$  SE) in controls and  $100 \pm 6$  mg/day in NPX rats. The estimated oxalate ingested was  $11.72 \pm 0.1$  mg (mean  $\pm$  SE) in controls and  $10.0 \pm 0.6$  mg in NPX rats. The creatinine clearance of the controls was significantly higher than that of the NPX rats ( $P < 0.0005$ ; Table 1). The renal function of the uremic rats did not change over the period of [ $^{14}\text{C}$ ]oxalate infusion; creatinine clearance on day 1,  $0.31 \pm 0.05$  mL/min (mean  $\pm$  SE), was the same as that on day 4,  $0.31 \pm 0.05$  mL/min. Twenty-four-hour urinary oxalate excretion (micromoles per 100 grams body weight) was significantly higher in control rats ( $P < 0.005$ ), whereas plasma oxalate of the NPX rats was almost five times that of the control rats (Table 1). The  $^{14}\text{C}$  recovered in urine, feces, and  $\text{CO}_2$  combined was similar in both groups:  $53 \pm 4\%$  of the infused dose in control rats and  $52 \pm 3\%$  in NPX rats. The urinary  $^{14}\text{C}$  (expressed as a percentage

**TABLE 1. Excretion of <sup>14</sup>C in normal and 5% NPX rats infused with [<sup>14</sup>C]oxalate<sup>a</sup>**

	Normal Rats (N = 16)	5% Nephrectomy (N = 25)	P
Creatinine clearance (mL/min)	1.82 ± 0.13	0.31 ± 0.04	<0.0005
Rat weight (g)	625 ± 29	532 ± 15	<0.005
Urinary <sup>14</sup> C	90.5 ± 0.6	68.7 ± 1.6	<0.0005
Fecal <sup>14</sup> C	6.5 ± 0.5	27.8 ± 1.5	<0.0005
Expired <sup>14</sup> CO <sub>2</sub>	3.0 ± 0.4	3.5 ± 0.4	NS
Urinary oxalate (μmol/100 g body wt/ 24 h)	1.87 ± 0.09	1.54 ± 0.07	<0.005
Plasma oxalate (μmol/L) <sup>b</sup>	5.6 ± 0.6	27.0 ± 3.9	<0.0005

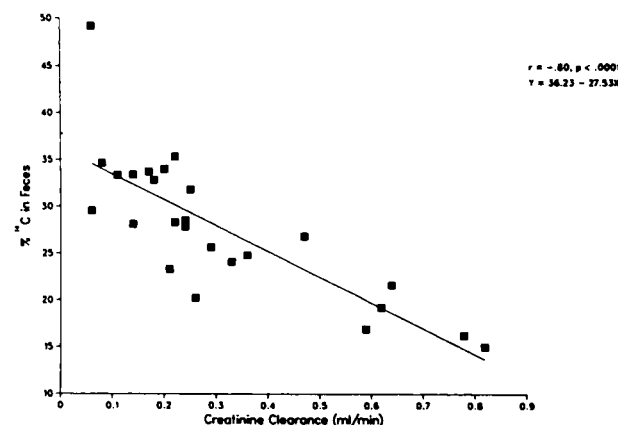
<sup>a</sup> All values are mean ± SE. Rats were infused with [<sup>14</sup>C]oxalate, ~2 μCi/day, over 4 days by mini osmotic pump implanted sc. The <sup>14</sup>C excreted in urine, feces, and expired CO<sub>2</sub> is expressed as percentage of the total <sup>14</sup>C excreted over the 4-day period. Male Wistar rats in both groups were the same age and housed for approximately the same length of time.

<sup>b</sup> Plasma oxalate was determined by an isotopic dilution procedure.

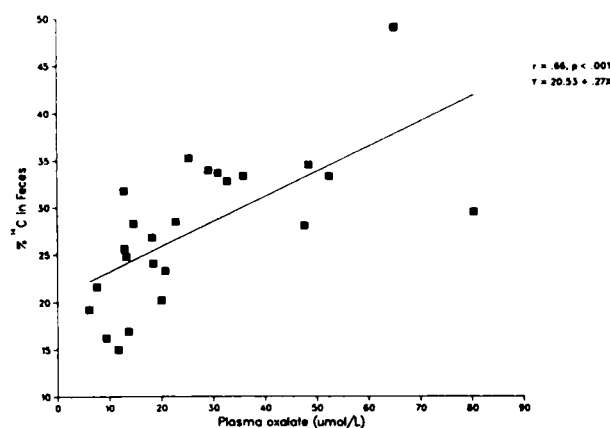
**TABLE 2. Excretion of <sup>14</sup>C in feces, urine, and CO<sub>2</sub> during the 4 days of [<sup>14</sup>C]oxalate infusion in normal and 5% NPX rats<sup>a</sup>**

	Day	Urine	Feces	CO <sub>2</sub>
Control (N = 16)	1	92.7 ± 0.6	4.2 ± 0.5	3.1 ± 0.5
	2	88.0 ± 1.0	8.4 ± 1.0	3.6 ± 0.6
	3	88.7 ± 1.3	8.2 ± 1.4	3.0 ± 0.4
	4	87.6 ± 1.0	9.5 ± 0.8	3.0 ± 0.4
NPX (N = 25)	1	77.7 ± 1.7	16.1 ± 1.8	6.3 ± 0.9
	2	73.3 ± 2.7	22.6 ± 3.0	4.2 ± 0.5
	3	72.1 ± 2.6	24.8 ± 2.7	3.1 ± 0.4
	4	59.4 ± 2.8	38.4 ± 2.8	2.3 ± 0.2

<sup>a</sup> Values are mean ± SE. The <sup>14</sup>C excreted in urine, feces, and expired CO<sub>2</sub> is expressed as a percentage of the total <sup>14</sup>C excreted each day.



**Figure 1. Percent <sup>14</sup>C in feces (values are a percentage of the total <sup>14</sup>C recovered in urine, feces, and CO<sub>2</sub> over 4 days) and creatinine clearance in rats with renal failure infused sc with [<sup>14</sup>C]oxalate, ~2 μCi/day over 4 days.**



**Figure 2. Percent <sup>14</sup>C in feces (values are a percentage of the total <sup>14</sup>C recovered in urine, feces, and CO<sub>2</sub> over 4 days) and plasma oxalate concentrations in rats with renal failure infused sc with [<sup>14</sup>C]oxalate, ~2 μCi/day over 4 days.**

of the total <sup>14</sup>C recovered) was significantly higher in the controls ( $P < 0.0005$ ), whereas the percent fecal <sup>14</sup>C was higher in the NPX rats ( $P < 0.0005$ ). The percent recovered as <sup>14</sup>CO<sub>2</sub> was not different between the two groups (Table 1). <sup>14</sup>C recovered in the feces showed a substantial increase from day 1 to day 4 (Table 2), increasing 123% in controls and 139% in NPX rats. Urinary <sup>14</sup>C and <sup>14</sup>CO<sub>2</sub> decreased over this period in both groups. Enzymatic analysis of the <sup>14</sup>C extracted from feces ( $N = 10$ ) demonstrated that all of the <sup>14</sup>C ( $100.2 \pm 0.9\%$ ) was in the form of [<sup>14</sup>C]oxalate (data not shown). The percent fecal <sup>14</sup>C excretion in NPX rats was inversely correlated with creatinine clearance ( $r = 0.80$ ;  $P < 0.0001$ ; Figure 1) and directly correlated with plasma oxalate ( $r = 0.66$ ;  $P < 0.001$ ; Figure 2). The <sup>14</sup>C extracted from various tissues is shown in Table 3. Because of the higher concentration of [<sup>14</sup>C]oxalate in the plasma of the

**TABLE 3.** <sup>14</sup>C in kidney, heart, liver, muscle, bone, and plasma of normal and 5% NPX rats after (<sup>14</sup>C)oxalate infusion<sup>a</sup>

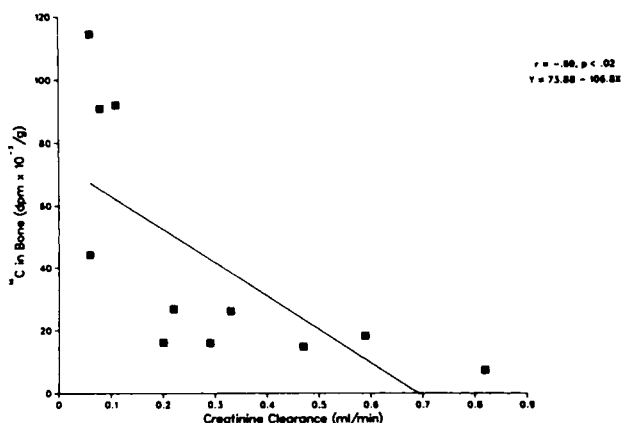
	Controls (dpm)	Tissue/Plasma <sup>14</sup> C Ratio <sup>b</sup>	% Nephrectomy (dpm)	Tissue/Plasma <sup>14</sup> C Ratio <sup>b</sup>
Kidney <sup>c</sup>	12,555 ± 1,709	14.4	26,422 ± 4,535	6.53
Heart <sup>c</sup>	1,318 ± 247	1.5	7,054 ± 1,783	1.7
Liver <sup>d</sup>	1,750 ± 96	2.0	3,130 ± 588	0.77
Muscle <sup>d</sup>	328 ± 116	0.38	1,415 ± 313	0.35
Bone <sup>c</sup>	3,100 ± 194	3.55	45,670 ± 3,829	11.3
Plasma (dpm/mL)	872 ± 80		4,043 ± 947	
Plasma oxalate SA (dpm/μmol)	14.48 ± 0.9		14.45 ± 1.1	

<sup>a</sup> All values are mean ± SE. Tissues and plasma of 8 control and 11 NPX rats were studied, except in liver of NPX rats where livers of 9 rats were studied. Rats were infused with (<sup>14</sup>C)oxalate, ~2.0 μCi/day, over 4 days. Plasma disintegrations per minute and oxalate specificity activity are values at time of death.

<sup>b</sup> Tissue (disintegrations per minute per gram) divided by the plasma disintegrations per minute per milliliter.

<sup>c</sup> Kidney, heart, and bone (disintegrations per minute) are per gram of dry tissue.

<sup>d</sup> Liver and muscle (disintegrations per minute) are per gram of wet tissue.



**Figure 3.** <sup>14</sup>C in bone (disintegrations per minute per gram of dry bone) and creatinine clearance in rats with renal failure infused sc with (<sup>14</sup>C)oxalate, ~2 μCi/day over 4 days. Bone <sup>14</sup>C was determined as described in Materials and Methods after death at the end of a 4-day study.

NPX rats compared with that of the controls, the disintegrations per minute per gram for each tissue was divided by the mean plasma disintegrations per minute per milliliter for each group. This value (tissue/plasma <sup>14</sup>C ratio) reflects the degree to which the tissue <sup>14</sup>C is concentrated compared with the plasma <sup>14</sup>C and allows a valid comparison of the tissue <sup>14</sup>C in both groups. The kidneys of the controls had a <sup>14</sup>C tissue/plasma ratio that was twice that of the NPX rats (14.4 versus 6.53), whereas the liver <sup>14</sup>C of the controls was 2.6-fold that of the liver in the NPX rats (2.0 versus 0.77) (Table 3). The bone <sup>14</sup>C ratio of the NPX rats was 3.2 times higher than in the controls (11.3 versus 3.55; Table 3). The bone <sup>14</sup>C deposition in uremic animals shows an inverse correlation with the creatinine clearance ( $r = 0.69; P < 0.02; N = 11$ ;

Figure 3), whereas plasma oxalate specific activity was virtually identical in both groups (Table 3).

## DISCUSSION

In this study, we demonstrated substantial extrarenal excretion of oxalate in rats with chronic renal failure. Fecal <sup>14</sup>C was found to be all in the form of [<sup>14</sup>C]oxalate, demonstrating that oxalate is transported into the gut without being degraded. Because the total <sup>14</sup>C recovered was similar in control and NPX rats, these studies demonstrate that considerably higher quantities of oxalate are secreted into the gut in NPX rats compared with that in controls. Although we are unable to explain the large increase in fecal <sup>14</sup>C in NPX rats from day 3 to 4 (Table 2), nonetheless, fecal <sup>14</sup>C in NPX rats was approximately three- to fourfold that of control rats on every day of the study (Table 2). Urinary oxalate excretion expressed as micromoles per 100 grams body weight was significantly higher in normal rats compared with NPX rats ( $P < 0.005$ ; Table 1). However, when both mean values were corrected for fecal and CO<sub>2</sub> loss (as determined by <sup>14</sup>CO<sub>2</sub> and fecal <sup>14</sup>C), total oxalate excretion was similar in both groups (control,  $2.07 \pm 0.11$ ; NPX rats,  $2.24 \pm 0.09 \mu\text{mol}/100 \text{ g}/24 \text{ h}$ ). It is of interest that after a 96% reduction in creatinine clearance in NPX rats, urinary oxalate excretion fell by only 40% (data not shown). As renal function decreased, percent excretion of [<sup>14</sup>C]oxalate via the gut increased (Table 1), showing a highly significant inverse correlation with the creatinine clearance ( $r = .80; P < .0001$ ; Figure 1). The percent <sup>14</sup>C excreted in the feces showed a direct correlation with plasma oxalate concentration ( $r = 0.66; P < 0.001$ ; Figure 2). Whether the extrarenal loss of oxalate results from diffusion across a concentration gradient, some ac-

tive transport mechanism induced in chronic renal insufficiency, reduced reabsorption, or a combination of these mechanisms is unknown. Dobson and Finlayson studied oxalate transport from plasma into gut lumen in the rat immediately after nephrectomy (13). They demonstrated a concentration of oxalate in the jejunum three times that of the plasma and equal to plasma concentration in the ileum (13). Watts *et al.* (14) reported extrarenal loss of oxalate in normal human subjects to be 5.7% (of the oxalate removed), which is close to that in normal rats (fecal  $^{14}\text{C}$ , 6.5%; Table 1). In five patients with primary hyperoxaluria who had a mean plasma oxalate of 11.6  $\mu\text{mol/L}$ , compared with 0.94  $\mu\text{mol/L}$  in normal controls, extrarenal loss of oxalate was 9.2% (mean value) (14), supporting an increase in extrarenal loss of oxalate in human subjects with raised plasma concentrations. However, in this latter study, the route of oxalate loss was not identified (14). In patients on maintenance hemodialysis, plasma oxalate concentrations reach a mean value of about 49  $\mu\text{mol/L}$  (4), compared with the 27  $\mu\text{mol/L}$  in rats with renal failure, (Table 1). An extrarenal oxalate loss in hemodialysis patients similar to that found in rats with renal failure would represent a major route of oxalate excretion in renal insufficiency. Furthermore, it would suggest that the oxalate appearance rate of patients on maintenance dialysis is substantially greater than that determined by the oxalate removed on dialysis alone (4). Because oxalate is both absorbed from and secreted into the gut, trapping of oxalate in the gut, as suggested by Dobson and Finlayson (14), offers a possible therapeutic strategy for decreasing the body burden of oxalate in patients with renal failure.

Gut flora of patients with renal failure are reported to degrade oxalate more efficiently than flora from normal subjects (15). Substantial increases in the numbers of oxalate-degrading bacteria have been found in normal human subjects fed a high oxalate diet (16). The greater ability of gut flora from renal failure patients to degrade oxalate may result from the increased loss of oxalate from the plasma into the gut, resulting in greater numbers of these oxalate-degrading bacteria.

After a single iv or ip administration of [ $^{14}\text{C}$ ]oxalate to rats, 94 to 97% of the  $^{14}\text{C}$  was recovered over 3 days between urine, feces, and  $\text{CO}_2$  (17), which is similar to that recovered from human subjects (18). However, when [ $^{14}\text{C}$ ]oxalate was continuously infused by mini osmotic pump in the study presented here, recovery of infused  $^{14}\text{C}$  over 4 days was  $53 \pm 4\%$  in control rats and  $52 \pm 3\%$  in NPX rats. Rat tissues where considerable  $^{14}\text{C}$  was deposited are shown in Table 3. Tissue  $^{14}\text{C}$ , in disintegrations per minute per gram, was divided by plasma  $^{14}\text{C}$  concentrations, in disintegrations per minute per milliliter,

in order to allow direct comparison of tissue  $^{14}\text{C}$  deposits in control and NPX rats. Although the kidney and liver of the control rats had higher  $^{14}\text{C}$  concentrations than those of NPX rats, muscle and heart had similar values relative to plasma  $^{14}\text{C}$  levels (Table 3). However, most of the  $^{14}\text{C}$  was deposited in rat bone with substantially more  $^{14}\text{C}$  in the bone of rats with NPX compared with that of controls ( $P < 0.0005$ ; Table 3). Curtin and King also reported high concentrations of  $^{14}\text{C}$  in normal rat bone after the administration of [ $^{14}\text{C}$ ]oxalate (19). The greater deposition of oxalate in NPX rats did not result from a difference in plasma oxalate specific activity, which was virtually identical in both groups (Table 3), but may have resulted from a greater rate of bone turnover in NPX rats. Whether the oxalate concentration in the bone of NPX rats continues to increase over time is unknown; however, the bone [ $^{14}\text{C}$ ]oxalate in NPX rats was inversely correlated with creatinine clearance ( $r = 0.69$ ;  $P < 0.02$ ; Figure 3).

Crystals of calcium oxalate deposit in the synovium, tendon, cartilage, and bone of patients with primary hyperoxaluria (20–23) where there is excessive hepatic production of oxalate due to an enzyme defect. Calcium oxalate bone deposits have also been seen in patients on maintenance dialysis who develop hyperoxalemia and secondary oxalosis (24–28). From studies on four patients who had been on maintenance hemodialysis from 6 to 11 yr, Reginato *et al.* (28) concluded that calcium oxalate crystalline deposits have a predilection for bone. Calcium oxalate crystal deposits have been associated with arthritis (26) and osteoclastic-like cell infiltrates, causing increased bone resorption (25) in ESRD. In primary hyperoxaluria, crystals are found within mononuclear phagocytes and multinucleated giant cells, where it has been proposed that they induce increased bone resorption (21). In a patient with primary hyperoxaluria, crystalline deposits in periodontal ligament provoked a granulomatous foreign-body reaction that resulted in external root resorption leading to pulp exposure and tooth mobility (29). Hoffman *et al.* (26) suggested that calcium oxalate microcrystalline aggregates can cause or augment cartilage and bone destruction, which may result from increased secretion of collagenase and neutral protease. This is supported by the finding that calcium oxalate crystals markedly stimulated secretion of collagenase and prostaglandin  $\text{E}_2$  when incubated with rabbit synovial fibroblasts in culture (30). Because calcium oxalate crystal deposits in bone mimic radiographic manifestations of osteitis fibrosa cystica (25), calcium oxalate-induced bone disease in uremia may in many instances go undetected. An awareness of its features could result in increased recognition of this bone disease. We believe that the study of calcium oxalate deposition in bone and its

effect on bone metabolism in patients with ESRD warrants further investigation.

In summary, we have demonstrated substantial extrarenal excretion of oxalate via the gut in the rat with chronic renal failure. This excretion is inversely correlated with increased renal insufficiency and directly correlated with plasma oxalate concentration. In the rat with renal failure, oxalate accumulates in bone more rapidly than in normal animals and is inversely correlated with the creatinine clearance.

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