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Handling of Oxalate by the Rat Kidney*

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Abstract. Renal transport of ^{14}C-oxalate was studied in the rat by clearance and micropuncture techniques. The ultrafilterability of oxalate was 0.98 ± 0.02 ($n = 7$). Fractional clearance of oxalate was significantly above unity in antidiuresis and volume expansion: mean 1.24 ± 0.04 ($n = 115$). Pyrazinamide ($1.1 \cdot 10^{-3}$ mol/kg BW) and probenecid ($0.35 \cdot 10^{-3}$ mol/kg BW) had no significant effect on oxalate clearance. P-aminohippurate ($1.45 \cdot 10^{-3}$ mol/kg BW) and urate ($0.48 \cdot 10^{-3}$ mol/kg BW) depressed the fractional clearance of oxalate significantly from 116 to 91 and from 125 to 90%, respectively. Excess excretion of ^{14}C-oxalate over ^{3}H-inulin was invariably demonstrable in peritubular microperfusion experiments ($n = 5$) and in microinfusions underneath the kidney capsule ($n = 4$). Together with the first 50% of ^{3}H-inulin 58 $\pm$ 2% of the total ^{14}C-oxalate were excreted in the peritubular microperfusions, and 64 $\pm$ 3% in the subcapsular microinfusions. In tubular microinfusion experiments ($n = 36$) urinary ^{14}C-oxalate recovery was almost complete after early proximal microinfusion (93 $\pm$ 4%) and complete after late proximal microinfusion (102 $\pm$ 4%). In continuous microperfusion experiments of proximal tubules ($n = 42$) a small but highly significant outflux of ^{14}C-oxalate of 7% per mm perfusion distance was found. The data suggest that oxalate is freely filterable at the glomerular site. A small but significant amount of oxalate is reabsorbed in the proximal nephron. Most likely at the same site and in the pars recta oxalate is secreted and tubular load increased to 124% of filtered load. This amount is excreted in final urine. The secretion of oxalate is inhibited by organic acids which are known to be secreted by the proximal tubule and the pars recta.

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Key words: Oxalate – Wistar rat – Microperfusion – Microinfusion – Organic acid secretion.

Introduction

In sharp contrast to its importance as one of the most frequent constituents of urinary calculi [22] very little is known about the renal handling of oxalate. Whereas there is little disagreement about the amount of oxalate excreted in urine [7,13,15], considerable controversy exists about the plasma concentration of oxalate [14,16] and hence the oxalate clearance [23,25]. Reported values for plasma oxalate concentration vary from 10^{-6} to 10^{-4} mol/l [14,16] depending on the method used. In the present investigation no attempt was made to measure oxalate concentration chemically. Instead, isotope techniques (^{14}C-oxalate) were used in all experiments, since no significant metabolisation of ^{14}C-oxalate was detected in pilot studies.

Methods

Male Wistar rats (Ivanovas, D-7964 Kisslegg) 180–330 g BW were used in this study. After an overnight fasting period the animals were anesthetized by 120 mg Inactin (Byk Gulden, D-775 Konstanz) per kg BW. In pilot experiments (7 rats) the ultrafilterability of oxalate was measured by an in vitro technique [11]. The ratio of oxalate concentration in ultrafiltrate over that in plasma was 0.98 $\pm$ 0.02. This indicates complete ultrafilterability of oxalate and thus no correction for ultrafilterability was made in the clearance studies. In another pilot study (4 rats) it was examined whether ^{14}C-oxalate is degraded in the Wistar rat. Using a precipitation technique [1] in the presence of excess concentrations of oxalate ($> 2 \cdot 10^{-3}$ mol/l) samples of plasma, urine, and infusate were analyzed 2 h after the beginning of a ^{14}C-oxalate infusion.

In the infusates 93 $\pm$ 2%, in the urines 92 $\pm$ 3%, and in the plasmas 94 $\pm$ 2% of total ^{14}C-activity was found in the Ca-oxalate precipitate.

The present study includes 4 groups of animals and a total of 33 rats. The first group consisted of 17 rats prepared for clearance

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Table 1. Clearance data. $\dot{v}$ = urinary flow rate, C_{Ox} = oxalate clearance, C_{In} = inulin clearance, C_{Ox}/C_{In} = fractional oxalate clearance

	$\dot{v}$	C_{Ox}	C_{In}	C_{Ox}/C_{In}
	(µl/min · 100 g BW)			
Mean ± S.E.M.	8.7 ± 4.3	1064 ± 59	864 ± 36	1.24 ± 0.04
Extreme values	1.4 – 74	557 – 1467	538 – 1119	0.99 – 1.62
n, rats (observations)	17 (113)	17 (113)	17 (113)	17 (115)

experiments. The animals were placed on a heated table to maintain body temperature at 37.5° C. The surgical preparation included: 1 tracheal cannula, 3 catheters in the right jugular vein, 1 catheter in the right femoral artery, and a bladder catheter. All animals were infused with Ringer's solution at a rate varying between 50 and 300 µl/min. Low and high infusion rates were used to obtain oxalate clearances in antidiuresis and diuresis. After a prime of 2 µCi ^{14}C-oxalate and 10 µCi ^{3}H-inulin (^{14}C-oxalate, CFA 84, 77 Ci/mol, ^{3}H-inulin, TRA 324, 610 Ci/mol, Amersham Radiochemical Centre Ltd., Amersham, Buckinghamshire HP7 9LL, GB) in 0.2 ml of Ringer's solution twice as much was infused continuously over the next 2 h. In the experiments in which the effect of organic acids on oxalate clearance was tested the first hour served as control period and the second as experimental period. Pyrazinamide ($1.1 \cdot 10^{-3}$ mol/kg BW), probenecid ($0.35 \cdot 10^{-3}$ mol/kg BW), and p-aminohippurate ($1.45 \cdot 10^{-3}$ mol/kg BW) were administered i.v. in 2 ml of isotonic saline solution titrated to pH 7.4. Half of the dose was given as a prime over a 10-min period and the rest was continuously infused over the following hour. These doses of pyrazinamide, probenecid, and p-aminohippurate have been shown to act uricosuric in the rat [17]. Urate was infused in an isotonic solution (10^{-2} mol/l, titrated to pH 7.4) instead of the Ringer's solution at 200 µl/min during the second hour of the experiments. Usually clearance periods lasted for 12 min, after which a blood sample of 40 µl was withdrawn for analysis of ^{14}C and ^{3}H activity. In the urate infusion studies 2 additional 200 µl blood samples were taken for analysis of plasma urate concentration.

The second group consisted of 7 animals. The rats of this and the following groups were prepared for micropuncture as described previously [19]. The preparation included a ureteral catheter for sampling of ipsilateral urine and a bladder catheter for sampling of contralateral urine. Peritubular capillaries were microperfused ($n = 5$) at 40 nl/min using a solution containing 40 mCi ^{14}C-oxalate and 200 mCi ^{3}H-inulin per liter isotonic saline. The same solution was used in the subcapsular microinfusion experiments ($n = 4$). In both types of experiments tracer excretion in ipsilateral and contralateral urine was monitored for a period of 30 min after each 2 min of microperfusion. The excess excretion of ^{14}C-oxalate was quantified as the amount of total ^{14}C-oxalate excreted ipsilaterally together with the first 50 % of inulin.

In the third group 36 tubular microinfusions were carried out in 7 rats. At a rate of 8 nl/min the microinfusate was delivered continuously for 4 – 10 min into the tubule fluid of proximal convolutions under free flow conditions. The microinfusate consisted of isotonic saline to which 2 – 200 mCi ^{14}C-oxalate and 10 – 1000 mCi ^{3}H-inulin per liter were added. The fractional urinary recovery of ^{14}C-oxalate was calculated as follows:

$$\frac{(\Sigma^{14}C_i - \Sigma^{14}C_e) \cdot {}^{3}H_{inf}}{(\Sigma^{3}H_i - \Sigma^{3}H_e) \cdot {}^{14}C_{inf}}.$$

In this formula $\Sigma^{14}C_i$ and $\Sigma^{14}C_e$, and $\Sigma^{3}H_i$ and $\Sigma^{3}H_e$ represent the sum of ^{14}C activity and ^{3}H activity in ipsilateral and contralateral urine respectively. $^{3}H_{inf}/^{14}C_{inf}$ is the tracer ratio in the microinfusate. The site of proximal microinfusion was determined by Lissamine green passage times (0.03 ml, 100 g/l): "Early" corresponds to 1 – 3, "middle" to 4 – 7, and "late" to 8 – 10 s after dye appearance in the vascular stars.

In the last series of 9 rats 42 microperfusions of proximal convoluted tubules were carried out using the continuous microperfusion technique [20]. The microperfusate was delivered at 20 nl/min and consisted of a saline solution (110 mmol/l) to which 80 mmol/l mannitol and 40 mCi ^{14}C-oxalate and 200 mCi ^{3}H-inulin per liter were added. The length of the microperfused segments was measured by the latex dissection technique [19].

Radioactivity was counted in a Intertechnique SL 30 LSC. Readysolv HP (Beckman Instruments A-1190 Wien) scintillation solution was used for all samples. Quench correction and tracer discrimination in each sample was carried out automatically by a programmed standard curve obtained from standards prepared identically to the samples.

Statistical analysis was carried out using standard formula. Paired and unpaired *t*-test were used as appropriate. Data are presented as mean ± S.E.M.

Results

The clearance data are presented in Tables 1 and 2 and in Figure 1. As is apparent from Table 1 oxalate clearance exceeds that of inulin significantly ($P < 0.02$) and fractional clearance of oxalate is clearly above unity ($P < 0.001$). Due to variation in the infusion rate urinary flow rate varies considerably from 1 – 74 µl/min · 100 g BW. Fractional clearance of oxalate, though significantly above unity over the whole range of diuresis, shows a small inverse correlation to urinary flow rate. At high urinary flow rates net secretion of oxalate is decreased to some 10 % whereas in antidiuresis net secretion is above 30 % of the filtered load. This is shown in Figure 1 where the single data are presented. The distance of the regression line to the line of identity is small at low U/P_{In} values, corresponding to high urinary flow rates, and larger at high U/P_{In} values.

As is apparent from Table 2 pyrazinamide and probenecid have no significant effect on oxalate clearance whereas p-aminohippurate and urate depress oxalate clearance significantly. In the urate infusion experiments plasma urate concentration increases from a control value of 58 ± 10 µmol/l to 589 ± 150 µmol/l during the experimental period. The depression of

Table 2. Effect of drugs on fractional clearance of oxalate. C = control period, E = experimental period of each animal

	Drug			
	Pyrazinamide	Probenecid	p-Aminohippurate	Urate
Dose (μmol/kg BW)	1.10	0.35	1.45	0.48
C	1.32 ± 0.02 (4)	1.35 ± 0.04 (13)	1.04 ± 0.02 (9)	1.27 ± 0.10 (6)
E	1.59 ± 0.16 (3)	1.09 ± 0.01 (4)	0.95 ± 0.03 (5)	0.87 ± 0.02 (7)
C	1.22 ± 0.11 (4)	1.25 ± 0.03 (5)	0.99 ± 0.02 (4)	1.35 ± 0.10 (4)
E	1.21 ± 0.09 (4)	1.14 ± 0.03 (4)	0.89 ± 0.03 (10)	1.03 ± 0.03 (4)
C	1.30 ± 0.03 (4)	1.17 ± 0.05 (6)	1.17 ± 0.05 (4)	1.12 ± 0.09 (6)
E	1.34 ± 0.03 (5)	1.10 ± 0.03 (5)	0.96 ± 0.03 (5)	0.81 ± 0.03 (6)
C			1.20 ± 0.06 (6)	
E			0.82 ± 0.04 (8)	
C			1.38 ± 0.09 (4)	
E			0.95 ± 0.02 (11)	
C	1.28 ± 0.03	1.26 ± 0.05	1.16 ± 0.07	1.25 ± 0.07
E	1.38 ± 0.11	1.11 ± 0.02	0.91 ± 0.03	0.90 ± 0.07
P	< 0.3	< 0.2	< 0.02	< 0.001

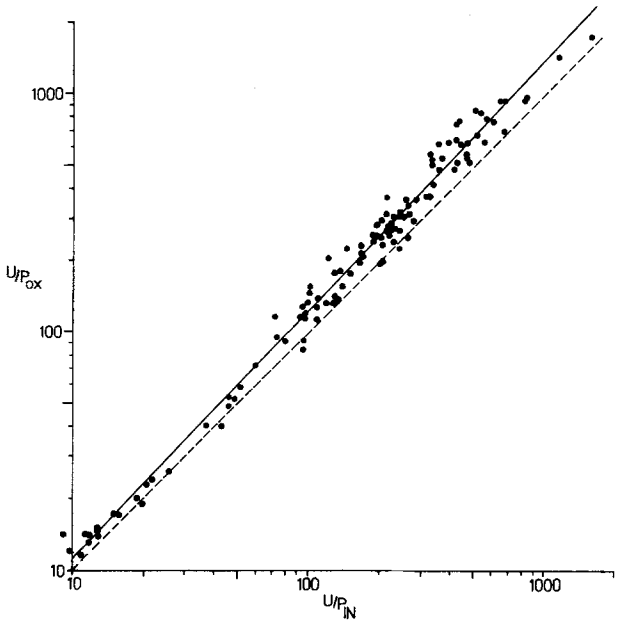

Fig. 1. Clearance data: U/P_{Ox} (i.e. oxalate concentration in urine over that in plasma) is plotted logarithmically versus U/P_{In} (i.e. inulin concentration in urine over that in plasma). The dashed line is the line of identity. The solid line is the linear regression: $U/P_{ox} = 0.969 \cdot U/P_{In}^{1.047}$, $r = 0.993$, $n = 115$

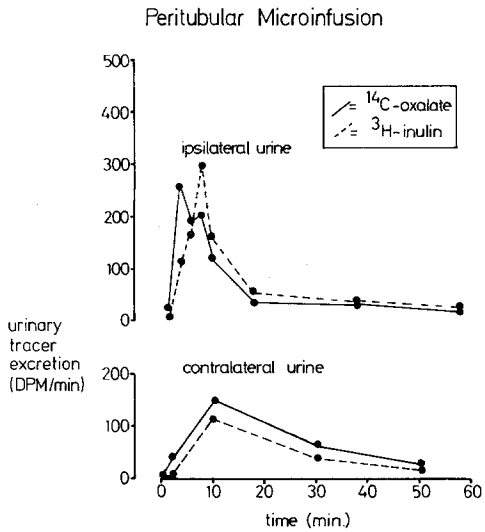

Fig. 2. Microperfusion of peritubular capillaries. The excretion of ^{14}C and ^{3}H label is plotted versus time for ipsilateral and controlateral urine. In ipsilateral urine a significant fraction of total ^{14}C is excreted ahead of ^{3}H

oxalate clearance by the latter two substances unmasks net reabsorption of oxalate of 10 and 9% of the filtered load, respectively.

In all peritubular microperfusion experiments and in all subcapsular microinfusions an excess excretion of ^{14}C-oxalate over ^{3}H-inulin is apparent. A typical experiment is shown in Figure 2. A significant fraction of total ^{14}C-oxalate is excreted ipsilaterally prior to ^{3}H-inulin. The data of this series are summarized in Table 3. A mean of 58 and 64% of total ^{14}C-oxalate is excreted together with the first 50% of ^{3}H-inulin.

The data of 36 microinfusions are shown in Table 4. Urinary recovery of ^{14}C-oxalate is 93% for microinfusions into early proximal tubules and 102% for micro-

Table 3. Peritubular capillary microinfusion and subcapsular microinfusion. Data are presented as percent of total ^{14}C-oxalate recovered in ipsilateral urine together with the first 50% of ^{3}H-inulin. Data are tested against 50%

	Peritubular capillary microinfusion	Subcapsular microinfusion
Mean ± S.E.M.	57.8 ± 2.2 (%)	63.7 ± 2.6 (%)
n, rats (observations)	4 (5)	3 (4)
P	< 0.01	< 0.005

Table 4. Microinfusion data. Fractional urinary recovery of ^{14}C-oxalate

	Site of proximal microinfusion		
	early	middle	late
Fractional urinary recovery	0.93 ± 0.04	0.99 ± 0.04	1.02 ± 0.04
n, rats (observations)	5 (10)	6 (13)	6 (13)

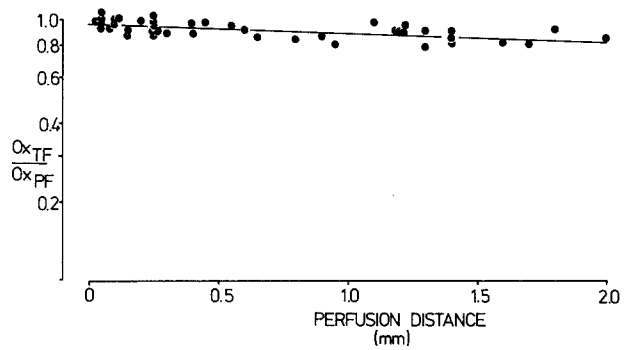

Fig. 3. Microperfusion experiments of proximal convoluted tubules. The oxalate concentration at the puncture site (Ox_{TF}) over that in the perfusate (Ox_{PF}) is plotted semilogarithmically versus the length of the perfused segment. The solid line is the least square regression function: $\log Ox_{TF}/Ox_{PF} = -0.017 - 0.034 \cdot$ perfusion distance, $r = 0.622$, $n = 42$

infusions into late proximal tubules. Though indicative of a limited outflux of ^{14}C-oxalate in the proximal tubule, recoveries for the two sites are statistically not different. In the last series of 42 microperfusions ^{14}C-oxalate outflux was measured in the absence of net water flux. As is apparent from Figure 3, outflux of ^{14}C-oxalate amounts to 7% per mm perfused length, a value significantly different from zero ($P < 0.001$).

Discussion

The systemic use of labelled oxalate to investigate renal oxalate transport is feasible only if oxalate is not degraded nor metabolized over the duration of the experiment. For the guinea pig this has been shown previously [2]. For the rat, however, it was reported that some 10% of the injected amount of ^{14}C-oxalate were metabolized to $^{14}CO_2$ within several days. This moiety was attributed to intestinal bacterial breakdown of ^{14}C-oxalate since it was not seen if the gut was sterilized by the use of antibiotics [5]. In our pilot experiments we were able to show that there is no significant breakdown of ^{14}C-oxalate within 2 h. This documents that in the rat oxalate is an end product of C-2 metabolism.

The present study reports a net secretion of oxalate of some 24% of the filtered load. In man a net reabsorption of oxalate was reported previously [25]. More recent investigations, however, demonstrate net secretion of oxalate for man [15,23] and dog [6]. Thus, at present, most evidence favors the view that oxalate clearances below those of inulin are the result of erroneously high plasma oxalate concentrations as determined by the chemical methods [16].

The data on the inhibition of oxalate secretion by organic acids known to be secreted proximally, are controversial [6,15]. In man probenecid had no effect whereas in dog probenecid, caronamid (a compound similar to probenecid), and p-aminohippurate acted antioxaluric [6]. These differences might reflect species differences in the affinities of different organic anions to the transport system. In our study p-aminohippurate and urate had a significant effect whereas pyrazinamide and probenecid had little or no effect. The failure to demonstrate a significant effect of these later two compounds is not surprising since the rat is not very sensitive to these drugs [4].

From our clearance studies at varying urinary flow rates it becomes apparent that oxalate secretion is dependent on urinary flow rate, i.e. net fractional secretion is more marked in antidiuresis than under volume expansion. This is in some contradiction to previous studies in the dog where under mannitol diuresis oxalate net secretion increased [6]. On the other hand, our experimental design is different: We used volume expansion with Ringer's solution and not mannitol to increase urinary flow rate. Under volume expansion secretion of oxalate might be diminished since it is known that in this condition salt and water reabsorption by the proximal tubule are reduced [9]. Furthermore, it was shown recently that the secretion of p-aminohippurate is functionally interrelated to isotonic volume reabsorption [12].

Using the techniques of peritubular microperfusion and subcapsular microinfusion, secretion of oxalate is

demonstrated at the tubular level. The excess excretion of ^{14}C-oxalate in these experiments cannot be ascribed to an artifact because the same treatment of the data from the tubular microinfusions shows no excess excretion of oxalate ($47 \pm 2\%$ of total oxalate excreted together with the first 50% of inulin ($n = 36$). The rate of secretion is higher in subcapsular microinfusions, probably due to prolonged contact of ^{14}C-oxalate with the secreting epithelium in these experiments. In peritubular microperfusions, on the other hand, the tracer solution is rapidly removed from the secretory site by the blood stream. Excess excretion as determined by this method is indicative of mere unidirectional influx of tracer which might be caused either by passive leakage of the tubule or by a secretory mechanism. In case of oxalate, however, due to the small backleak (v.i.), this excess excretion can be taken as evidence for a secretory process for oxalate. The nephron site of oxalate secretion is most likely the proximal tubule and the pars recta since secretion is inhibited by urate and p-aminohippurate. Both organic anions are secreted at this nephron site [8, 10]. A similar conclusion was drawn from a previous investigation using the stop flow technique [6]. In this study a secretory peak for oxalate was found in the same samples showing a secretory peak for p-aminohippurate. The finding of a proximal secretory site for oxalate gives a ready explanation for the clinical finding that patients suffering from primary hyperoxaluria show oxalate precipitates already in the proximal tubule [24].

From the oxalate clearance experiments with urate and p-aminohippurate infusion it is clear that there must exist some reabsorption of oxalate. According to the tubular microinfusion and microperfusion experiments this reabsorption is localized in the proximal tubule. The outflux of ^{14}C-oxalate is 7% per mm perfused length in the microperfusion series. This corresponds to a permeability of 0.4 mm/s, a value which is twice as high as that for mannitol [3], almost equal to that for p-aminohippurate [21], and below that for urate [18, 21]. It has to be considered, however, that in the present study, due to the specific activity of ^{14}C-oxalate, chemical oxalate concentration in tubule fluid ranged from 30–1000 µmol/l (series 3). This range is clearly above recent best estimates of plasma oxalate concentration [15, 23]. Thus, it is conceivable that a possible reabsorptive mechanism for oxalate would be completely saturated at these "high" concentrations. On the other hand, no dependence of oxalate outflux on oxalate concentration in the microinfusate is observed over the whole range of concentrations used in this study. Furthermore, under maximal suppression of oxalate secretion by $3.6 \cdot 10^{-3}$ mol/kg BW of p-aminohippurate fractional clearance of oxalate never falls below 0.7. This value corresponds reasonably well to a 69% recovery extrapolated from the microperfusion data with the assumption of a tubule length of 5 mm. Oxalate reabsorption, therefore, most likely reflects a passive permeability of the nephron to this small compound (MW 90).

From the present study we conclude that renal oxalate excretion is characterized by free glomerular filtration, secretion in the proximal nephron and, in addition, a limited reabsorption in the proximal tubule. Thus, the amount of oxalate excreted in final urine clearly exceeds the amount filtered. Since with isotope techniques net secretion of oxalate is now established for the dog [6] and man [15, 16, 23] it is reasonable to assume that in these species renal excretion of oxalate follows a qualitatively similar pattern.

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