THE RENAL CLEARANCE OF OXALATE IN NORMAL SUBJETS AND PATIENTS WITH PRIMARY HYPEROXALURIA

H. E. WILLIAMS, GLORIA A. JOHNSON AND L. H. SMITH, JR

Department of Medicine, University of California, and Medical Services, San Francisco General Hospital

(Received 18 March 1971)

SUMMARY

1. The renal clearance of oxalate was studied in six normal subjects and in two patients with primary hyperoxaluria utilizing a constant infusion of $[^{14}\text{C}]$ oxalic acid.
2. The $[^{14}\text{C}]$ oxalate clearance in normal subjects was between 101 and 217 ml/min with a range in the ratio of $[^{14}\text{C}]$ oxalate clearance to creatinine clearance of 1.33-2.09.
3. The oxalate clearance in two patients with primary hyperoxaluria was within the range found in the normal subjects.
4. This study does not confirm the previous report of a low oxalate/creatinine clearance ratio in man nor the finding of an elevated oxalate clearance in patients with primary hyperoxaluria.
5. Estimates of serum oxalate concentration based on these clearance values suggest that the serum oxalate concentration in normal subjects is less than 100 $\mu$g/100 ml.

The diagnosis and pathogenesis of primary hyperoxaluria has been well delineated in several laboratories (Hockaday, Clayton, Frederick & Smith, 1964; Williams & Smith, 1968a, b; Wyngaarden & Elder, 1966; Hodgkinson & Zarembski, 1968). Despite these extensive investigations relatively little information is available about the renal clearance of oxalate in man. Cattell, Spencer, Taylor & Watts (1962) found that the renal clearance of infused sodium $[^{14}\text{C}]$ oxalate was 68 ml/min in the dog (mean of twenty-five observations on six dogs) with average ratio of oxalate clearance to inulin clearance and/or exogenous creatinine clearance of 1.28. In contrast with these studies Zarembski & Hodgkinson (1963) using a fluorimetric method for the determination of oxalate found that oxalate clearance in normal subjects varied between 3.4 and 5.0 ml/min. In addition they noted an elevated renal clearance of oxalate in seven patients with primary hyperoxaluria. These discrepant results suggested a difference between the renal handling of oxalate in man and in the dog. We have infused $[^{14}\text{C}]$ oxalic acid, and determined the renal clearance of $[^{14}\text{C}]$ oxalate in normal...
subjects and in two patients with primary hyperoxaluria. The results of this investigation are reported in this paper.

MATERIALS AND METHODS

Six normal healthy male subjects (volunteer laboratory personnel) between 24 and 36 years old, and two patients with primary hyperoxaluria were studied. One patient, a 30-year-old white man, had type I or glycolic aciduria; the other, a 35-year-old black man, had type II or L-glyceric aciduria (Williams & Smith, 1968b). The subjects were informed of the nature of the investigation and all gave informed consent before the study. In addition, the protocol for the study and the patient consent forms were approved by an ad hoc faculty Committee on Human Experimentation. The subjects had fasted for 8 h before the study was begun, and the patients received no medications for 2 days before the study.

$^{14}$C Oxalic acid was obtained from New England Nuclear Corporation and purified by chromatography on Dowex 1(X8, acetate form) (Hockaday, Frederick, Clayton & Smith, 1965); the final specific radioactivity was 2.0 μCi/μmol. The oxalic acid was then diluted in normal saline to a final concentration of 782 000 c.p.m./ml and sterilized by passage through a millipore filter. On the day of study, each patient drank approx. 1–2 litres of water to establish a urine flow rate of approx. 10 ml/min. A loading dose of $^{14}$C oxalic acid (10 μCi) was administered rapidly intravenously, and constant infusion (via a Harvard infusion pump) of $^{14}$C oxalic acid was started simultaneously at a rate of 0.2 ml/min or 156 000 c.p.m./min. Assuming that $^{14}$C oxalic acid is evenly distributed throughout the extracellular fluid, it was calculated (Cattell et al., 1962) that the amount of infused oxalate raised the concentration of oxalate in the serum by approx. 0.003 mg/100 ml. Urine samples were collected by spontaneous voiding at 20 min intervals; blood samples were collected in heparinized tubes at the midpoint of each 20 min period. The total infusion period was 2–3 h; the total dose of $^{14}$C oxalic acid administered to each subject was approx. 25 μCi; the estimated total body absorbed dose of radiation being 0.00021 rad.

Urine samples were treated as follows: 0.025 ml samples of each urine sample were counted in triplicate in 1.975 ml of ethanol (anhydrous) and 10 ml of Liquifluor (New England Nuclear Corporation) in a Packard Tri-Carb liquid-scintillation spectrometer at a counting efficiency of 69%. Plasma samples were collected by centrifugation and treated as follows: 0.45 ml of plasma was added to 0.05 ml of 30% (v/v) perchloric acid, the perchlorate was removed by precipitation with 10% (w/v) KOH at 4°C, and duplicate 0.1 ml samples of the clear supernatant were counted in 1.9 ml of ethanol (anhydrous) and Liquifluor as described above. The renal clearance of oxalate was determined by the standard formula ($U × V)/P$ where $U =$ urinary $^{14}$C oxalate excretion (c.p.m./ml), $V =$ urine flow rate (ml/min), and $P =$ plasma $^{14}$C oxalate concentration (c.p.m./ml) using the mean values for each sample. The simultaneous creatinine clearance was calculated for all specimens by the method of Edwards & Whyte (1958). The clearance values were not corrected to a standard body surface area.

The validity of the count clearances was established by adding carrier oxalate (200 μmol) to 20 ml portions of urine samples and to 5 ml portions of plasma samples collected during the infusion studies. Calcium oxalate was precipitated and determined by the method of Hockaday et al. (1965) in these samples. All of the $^{14}$C measured in these samples before the oxalate precipitation was recovered in the separated oxalate. As noted in previous studies (Cattell
Renal clearance of oxalate

et al., 1962; Zarembski & Hodgkinson, 1963, 1965), there is no appreciable binding of oxalate to serum proteins in man.

RESULTS

The \([^{14}C]oxalate\) and endogenous creatinine clearances in six normal subjects and two patients with primary hyperoxaluria are given in Table 1. The mean oxalate clearance in the normal subjects was 169 ml/min with a range of 101–217. The oxalate/creatinine clearance ratio in these subjects was between 1·33 and 2·09 with a mean of 1·64. The oxalate clearance values and

<table>
<thead>
<tr>
<th>Subject</th>
<th>Clearance (ml/min)</th>
<th>(\frac{[^{14}C]Oxalate}{Creatinine})</th>
<th>(\frac{[^{14}C]Oxalate\ clearance}{Creatinine\ clearance})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>152</td>
<td>108</td>
<td>1·41</td>
</tr>
<tr>
<td>2</td>
<td>162</td>
<td>93</td>
<td>1·74</td>
</tr>
<tr>
<td>3</td>
<td>167</td>
<td>80</td>
<td>2·09</td>
</tr>
<tr>
<td>4</td>
<td>101</td>
<td>51</td>
<td>1·98</td>
</tr>
<tr>
<td>5</td>
<td>215</td>
<td>162</td>
<td>1·33</td>
</tr>
<tr>
<td>6</td>
<td>217</td>
<td>122</td>
<td>1·78</td>
</tr>
<tr>
<td>Mean</td>
<td>169</td>
<td>103</td>
<td>1·64</td>
</tr>
<tr>
<td>Patients with primary hyperoxaluria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>212</td>
<td>120</td>
<td>1·77</td>
</tr>
<tr>
<td>Type II</td>
<td>150</td>
<td>100</td>
<td>1·50</td>
</tr>
</tbody>
</table>

Table 2. Changes in oxalate and creatinine clearances and urine flow rate in a normal subject

<table>
<thead>
<tr>
<th>Period (20 min)</th>
<th>Clearance (ml/min)</th>
<th>(\frac{[^{14}C]Oxalate}{Creatinine})</th>
<th>(\frac{[^{14}C]Oxalate\ clearance}{Creatinine\ clearance})</th>
<th>Urine flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>240</td>
<td>169</td>
<td>1·42</td>
<td>14·8</td>
</tr>
<tr>
<td>2</td>
<td>195</td>
<td>107</td>
<td>1·82</td>
<td>13·0</td>
</tr>
<tr>
<td>3</td>
<td>260</td>
<td>171</td>
<td>1·52</td>
<td>10·8</td>
</tr>
<tr>
<td>4</td>
<td>180</td>
<td>153</td>
<td>1·18</td>
<td>4·2</td>
</tr>
<tr>
<td>5</td>
<td>225</td>
<td>146</td>
<td>1·54</td>
<td>9·6</td>
</tr>
<tr>
<td>6</td>
<td>197</td>
<td>164</td>
<td>1·20</td>
<td>12·5</td>
</tr>
<tr>
<td>7</td>
<td>210</td>
<td>226</td>
<td>0·93</td>
<td>13·7</td>
</tr>
</tbody>
</table>

\* \(\frac{[^{14}C]Oxalate}{Creatinine}\) = ratio of oxalate clearance to endogenous creatinine clearance.

the oxalate/creatinine clearance ratios for both patients with primary hyperoxaluria were within the normal range (212 ml/min and 1·77 for the patient with type I and 150 ml/min and 1·50 for the patient with type II, respectively).

Table 2 shows the values obtained during \([^{14}C]oxalic acid\) infusion in one normal subject.
Oxalate clearance rates varied within a small range (180–260 ml/min) and showed no correlation with the changes in urine flow rate (4.2–14.8 ml/min). Similarly no significant correlation between urine pH (5.8–7.0) and [14C]oxalate clearance could be demonstrated in three subjects in whom it was measured.

An estimation of the serum concentration of oxalate can be made from these values for [14C]oxalate clearance. Assuming an average daily oxalate excretion of 40 mg, the mean excretion of oxalate/min would be 0.0278 mg. By using a mean oxalate clearance of 169 ml/min, the calculated plasma concentration of oxalate would be 16.5 μg/100 ml of plasma.

DISCUSSION

Calcium oxalate is a major constituent of nearly two-thirds of all renal stones (Prien & Prien, 1968). In most patients with recurrent calcium oxalate nephrolithiasis the urinary excretion of oxalate is within the normal range of 20–60 mg/24 h. Hyperoxaluria accounts for the propensity to form stones in a small percentage of patients. This increased excretion of oxalate is related to increased ingestion of oxalate or its precursors (Zarembski & Hodgkinson, 1967), to pyridoxine deficiency (Gershoff, 1964), or to two well-defined inherited enzyme deficiency states, primary hyperoxaluria types I and II (Williams & Smith, 1968a). Despite the frequency of this clinical problem and the large amount of information concerning the metabolism of oxalate and its precursors, little is known about the mechanisms of renal handling of oxalate in man.

Cattell et al. (1962) studied the mechanisms of renal oxalate excretion in fifteen dogs by using infusion of sodium [14C]oxalate to determine oxalate clearance; the mean renal clearance of oxalate was 68 ml/min with a ratio of oxalate clearance to inulin clearance and/or exogenous creatinine clearance of 1.28. This finding, together with the results of stop–flow analyses and the effects of caronamide and probenecid administration, suggested that oxalate excretion is effected by glomerular filtration, tubular secretion, and passive tubular reabsorption. In contrast with these studies in dogs, the mean renal clearance of oxalate in six normal adults was 3.9 ml/min with an oxalate/creatinine clearance ratio of 0.034; a fluorimetric method being used for the oxalate determinations (Zarembski & Hodgkinson, 1963). These results suggested that excretion of oxalate in man occurs primarily by glomerular filtration and active tubular reabsorption. In contrast with these studies in dogs, the mean renal clearance of oxalate in six normal adults was 3.9 ml/min with an oxalate/creatinine clearance ratio of 0.034; a fluorimetric method being used for the oxalate determinations (Zarembski & Hodgkinson, 1963). These results suggested that excretion of oxalate in man occurs primarily by glomerular filtration and active tubular reabsorption. In the seven patients with primary hyperoxaluria, oxalate clearance varied between 12.4 and 42.6 ml/min. This finding was interpreted as demonstrating a specific defect in the tubular reabsorption of oxalate in patients with primary hyperoxaluria in addition to the known increased endogenous production of oxalate in these patients. The difference in oxalate clearance in man (Zarembski & Hodgkinson, 1963) and the dog (Cattell et al., 1962) was thought to indicate a difference in the mechanisms of renal handling of oxalate in the two species.

In the current study using methods similar to those used by Cattell et al. (1962) in the dog, the mean oxalate/creatinine clearance ratio of 1.64 in normal human subjects corresponds closely to that found in the dog and suggests that tubular secretion in man plays an important role in the renal handling of oxalate. The difference between these findings and those of Zarembski & Hodgkinson (1963) can only be explained at this time by differences in the two methods. The finding in the present study of normal oxalate clearance in two patients with primary hyperoxaluria similarly differs from the findings of Zarembski & Hodgkinson (1963) in their six patients and obviates the need to consider more than one metabolic defect in
Renal clearance of oxalate

patients with this pair of inborn errors of metabolism. As noted by Zarembski & Hodgkinson (1963), it would not be possible to explain hyperoxaluria and oxalosis simply on the basis of an increased clearance of oxalate in the absence of increased endogenous production, which is a well-documented finding in both types of primary hyperoxaluria (Williams & Smith, 1968a).

It has been possible to estimate the concentration of oxalate in serum in man to be approx. 16 µg/100 ml of plasma on the basis of the present results. This value is considerably lower than other reported values of serum oxalate in man (Hodgkinson & Zarembski, 1968; Barber & Gallimore, 1940; Barrett, 1943; Crawhall & Watts, 1961; Pernet & Pernet, 1965; Cochran, Hodgkinson, Zarembski & Anderson, 1968) obtained by other methods. It is consistent with the results of preliminary studies in our laboratory with isotope-dilution and gas chromatographic methods. These show the oxalate concentration in serum to be less than 100 µg/100 ml in normal subjects.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr R. Curtis Morris of the University of California, San Francisco, for his assistance in the performance of the clearance studies in these subjects. The work was supported by U.S. Public Health Service Grant AM 09406.

REFERENCES

