Oxalate Is Overestimated in Alkaline Urines Collected during Administration of Bicarbonate with No Specimen pH Adjustment

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We compared measurements of daily urine oxalate excretion in urines collected at the prevailing urine pH with measurements of urine oxalate excretion in urines collected into 20 mL of 6 mol/L HCl. We studied eight healthy adults fed constant diets. Urines were collected during control conditions and, in each subject, during the administration of NaCl, KCl, NaHCO\textsubscript{3}, or KHCO\textsubscript{3}, 90 mmol/day. Daily urine oxalate excretion calculated for collections made in acid averaged 271 (SD 79) \textmu mol/day and did not vary with any of the salt supplements. When urines were collected at ambient urine pH (average 5.94, SD 0.23) during control conditions, and during the administration of NaCl or KCl, urine oxalate excretion averaged 263 (SD 88) \textmu mol/day, a value not different from that for collections in acid. However, when urine was collected with no pH adjustment during NaHCO\textsubscript{3} or KHCO\textsubscript{3} administration (average pH 6.90, SD 0.14), apparent urine oxalate excretion averaged 398 (SD 132) \textmu mol/day, significantly (P < 0.025) exceeding the mean observed when urines were collected in acid. Moreover, the percentage increase in apparent oxalate excretion increased with urinary pH. These observations reinforce recommendations that urine specimens for measurement of oxalate be collected in acid to avoid the increase in apparent oxalate content that occurs during collection of alkaline urines. This increase presumably results from the well-known in vitro nonenzymatic conversion of ascorbate to oxalate.

\textbf{Additional Keyphrases:} calculous disease \hspace{1em} variation, source of

Currently, ~80\% of the kidney stones examined in the United States are composed in part of calcium oxalate (J).

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Received June 2, 1989; accepted July 5, 1989.

\textbf{CLINICAL CHEMISTRY, Vol. 35, No. 10, 1989 2107}
6 mol/L HCl. On day four of each period, the subjects collected 24-h urine specimens preserved with thymol and phenylmercuric nitrate and kept under a layer of mineral oil. None of the urines was refrigerated during collection. The subjects carried on their usual activities. Nene was given vitamin supplements.

We also reviewed measurements of urinary oxalate excretion in six-day urine pools (prepared by combining 2% of the volume of 24-h urine specimens preserved with thymol and phenylmercuric nitrate and collected under mineral oil) from four additional subjects during control conditions and during the administration of NaHCO$_3$, 60 mmol/day, and during KClO$_3$, 60 mmol/day, in a previously reported study (8).

Urinary pH was measured with a Radiometer pH meter in samples anaerobically aspirated from beneath the layer of mineral oil. Aliquots of the urine were frozen at -20 °C for subsequent batch analysis. Daily urine volumes were measured in graduated cylinders. Urinary oxalate concentrations were measured by the method of Hodgkinson and Williams (9). All urines from any one subject were analyzed in a single assay. Analytical recoveries of sodium oxalate added to urine during 25 separate assays averaged 91% (SD 9%). Urinary creatinine was measured with an AutoAnalyzer (Technicon Instruments, Tarrytown, NY).

**Statistics.** Results are presented as individual or group means ± SD. For statistical comparisons we used Student's paired t-test. Graphs were prepared and correlations were calculated by use of the Cricket Graph program (Cricket Software, Inc., Malvern, PA) on a MacIntosh SE computer (Apple Computer, Inc., Cupertino, CA).

**Results**

As shown in Table 1, the group mean urinary oxalate excretion rate for the six urines from each subject, collected and preserved in acid, was 271 ± 79 μmol/day. The individual mean coefficients of variation (CVs) averaged 6.4%.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Oxalate excretion (mmol/day)</th>
<th>pH</th>
<th>Oxalate excretion (mmol/day)</th>
<th>Administration of NaHCO$_3$ or KClO$_3$ (n = 2 each)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.282 ± 0.023</td>
<td>6.28 ± 0.13</td>
<td>0.349 ± 0.030</td>
<td>6.90 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>0.242 ± 0.013</td>
<td>5.75 ± 0.07</td>
<td>0.240 ± 0.021</td>
<td>6.93 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>0.263 ± 0.007</td>
<td>5.54 ± 0.33</td>
<td>0.214 ± 0.017</td>
<td>6.98 ± 0.14</td>
</tr>
<tr>
<td>4</td>
<td>0.261 ± 0.015</td>
<td>6.09 ± 0.13</td>
<td>0.284 ± 0.015</td>
<td>6.85 ± 0.14</td>
</tr>
<tr>
<td>5</td>
<td>0.210 ± 0.009</td>
<td>6.09 ± 0.14</td>
<td>0.189 ± 0.025</td>
<td>7.10 ± 0.14</td>
</tr>
<tr>
<td>6</td>
<td>0.250 ± 0.018</td>
<td>5.96 ± 0.29</td>
<td>0.237 ± 0.025</td>
<td>6.88 ± 0.14</td>
</tr>
<tr>
<td>7</td>
<td>0.458 ± 0.042</td>
<td>5.81 ± 0.23</td>
<td>0.436 ± 0.068</td>
<td>6.60 ± 0.14</td>
</tr>
<tr>
<td>8</td>
<td>0.213 ± 0.017</td>
<td>6.00 ± 0.08</td>
<td>0.177 ± 0.009</td>
<td>6.95 ± 0.14</td>
</tr>
<tr>
<td>Group mean</td>
<td>0.271 ± 0.079</td>
<td>5.94 ± 0.23</td>
<td>0.263 ± 0.088</td>
<td>6.90 ± 0.14</td>
</tr>
</tbody>
</table>

During control period only (no NaCl or KCl administered), n = 1 each.
During NaHCO$_3$ and during KClO$_3$, n = 2 each.
value for the six urines collected in acid exceeded 100% of the latter value during the administration of NaHCO₃ or KHCO₃ and increased progressively as the urine pH exceeded 6.5.

Moreover, as shown in Figure 2, when the data for all 12 subjects were evaluated, the percentage increase in urinary oxalate during administration of NaHCO₃ or KHCO₃ above the value observed during the administration of NaCl or KCl and (or) control conditions was significantly related to the mean urinary pH produced during the administration of NaHCO₃ or KHCO₃. These percentage increases in urinary oxalate in urines collected with no pH adjustment during NaHCO₃ and KHCO₃ administration were not related to estimates of individual dietary ascorbic acid or oxalate contents among the subjects. Furthermore, although individual urinary oxalate excretion rates during the administration of NaCl or KCl and (or) control conditions were correlated to body weight (Figure 3), the percentage increases in apparent oxalate excretion during NaHCO₃ or KHCO₃ administration were not related to either basal oxalate excretion or body weight.

**Discussion**

The results of the present study confirm, for subjects fed constant diets, previous studies demonstrating that apparent urinary oxalate excretion is falsely increased when some urine specimens are either collected at the spontaneous (and presumably alkaline) urine pH (5) or urine is experimentally alkalinized in vitro (3, 6, 7). This effect appears to be the consequence of nonenzymatic conversion of urinary ascorbate to oxalate, because several studies have demonstrated nearly stoichiometric decrements in urinary ascorbate and increments in urinary oxalate in such alkaline urines (7).

The careful study of Mazzachi et al. (6) demonstrated that holding the pH of the urine, to which ascorbic acid had been added to give concentrations of 1 to 15 mmol/L, at pH 5.0 or pH 6.0 had no significant effect on urinary oxalate concentrations as determined by a chromatographic technique after oxalate was separated from urine by precipitation with calcium chloride. By contrast, at pH 7.0, those studies demonstrated that apparent urinary oxalate concentration increased about 15% per millimole increase in urinary ascorbate. Such conversion of ascorbate to oxalate was rapid, occurring within a minute at pH 9.0. Although not tested, presumably the conversion of ascorbate to oxalate would occur more slowly at a urinary pH >6.0 but <9.0.

The method we used for determining urinary oxalate involves increasing the pH of the sample to 7.0 for the initial precipitation of oxalate with calcium. We have considered whether this brief period of alkalinization affected the results. It appears unlikely that this procedural step permits significant conversion of normal urinary ascorbate concentrations to oxalate, because measured urinary oxalate excretion rates were not different in urines collected in acid (pH 7.2) in comparison with urines collected at ambient pH in the absence of bicarbonate loading (pH 6.0). It seems more likely that the increases in urinary oxalate in urines collected at ambient pH during bicarbonate loading (pH 6.9) occurred in these more alkaline urines as the urines were being collected.

We did not experimentally vary ascorbate intake, so it may be that, when urinary ascorbate concentrations are
high, as may occur among patients taking ascorbic acid, other techniques such as the addition of NaNO₂ or FeCl₃ and Na₂EDTA (7, 9, 10) would be required to prevent conversion of ascorbate to oxalate when analytical procedures require even brief alkalinization of urine samples.

We have also considered our observations in relation to measurements of urinary oxalate excretion, also using the method of Hodgkinson and Williams (9), among patients with nephrolithiasis eating ad libitum before and during the administration of potassium citrate, 60 mEq/day (11, 12). In those studies, the urinary pH averaged ~6.0 before treatment and ~6.7 while the subjects took potassium citrate, pH increments comparable with those observed in the present study. Despite the more alkaline urines, oxalate excretion, which averaged ~350 μmol/day, either did not change (11) or increased by only ~10% (12). We have no explanation for these discrepant observations.

We conclude that urines for the measurement of oxalate excretion must be collected in acid to prevent artifactual increase in urinary oxalate concentrations as a result of the in vitro nonenzymatic conversion of ascorbate to oxalate when the urinary pH exceeds 6.0.

Supported in part by USPHS grants RR00058 and DK15089. We acknowledge the expert assistance of the nursing and dietary staffs of the Medical College of Wisconsin Clinical Research Center in the performance of these studies.

References

Extending the Detection Limit of the TDx Fluorescence Polarization Immunoassay for Benzoylecgonine in Urine

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This modified calibration method decreases from 300 to 27 μg/L the limit of detection for the cocaine metabolite (hydrolysis product), benzoylecgonine, by the Abbott Laboratories TDx fluorescence polarization immunoassay. For this determination we used 30 controls prepared from a single urine pool known to be negative for cocaine metabolite. Assay of 80 controls prepared from different patients' urine samples yielded a limit of detection of 44 μg/L. To test these limits of detection, we analyzed 90 patients' urine samples known to be negative for cocaine metabolite and 74 patients' samples known to be positive for cocaine metabolite, using the TDx with our revised calibration. Results for two of the known negative samples and 96% of the samples containing cocaine in the 50 to 100 μg/L range fell above the 44 μg/L limit. The TDx showed excellent calibration stability. For 28 days during the test, the instrument was not recalibrated. During this period the day-to-day analysis of 50 μg/L controls produced a mean TDx response of 0.485 (SD 0.007) with a coefficient of variation of 1.5%.

Additional Keyphrases: abused drugs · cutoff values

For some time we have been using EMIT DAU reagents (Syva Co., Palo Alto, CA) for cocaine metabolite with a Hitachi 705 (Boehringer Mannheim, Indianapolis, IN) automatic analyzer. Our cutoff limit for positive detection is 50 μg/L, one-sixth that recommended by Syva. The confirmation rate by full-scan gas chromatography/mass spectrometry (GC/MS) has exceeded 96%. Most of the samples that were not confirmed had interfering mass spectral peaks; thus, although not truly negative, they did not meet our criteria for full-scan confirmation. The cocaine metabolite assay for the TDx (Abbott Labs., N. Chicago, IL), an automatic analyzer that makes use of the fluorescence polarization immunoassay technique, has been evaluated previously by Poklis (1). An improvement in detection cutoff for the TDx would permit this instrument to be used to detect cocaine metabolite at concentrations already achieved with the Hitachi 705.