Routine Micromethod for Determination of Oxalic Acid in Urine by Atomic Absorption Spectrophotometry

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The method of Gitelson et al. was modified to a micro-scale procedure and adapted for atomic absorption spectrophotometry. Oxalic acid in urine is precipitated with excess of calcium ions at pH 5. The excess of calcium present in the supernate and the total calcium added and present in the urine at pH 2 are determined by atomic absorption spectrophotometry. The oxalic acid present in the precipitate is calculated indirectly from the difference between the two determinations of calcium. This method is useful for routine determination of urinary oxalic acid.

In recent years there has been an increasing demand for the determination of oxalic acid in urine, especially in urology and nephrology departments in hospitals, during the treatment of patients who are forming calcium oxalate stones.

A review of current methods for determining oxalic acid in biological material appeared in 1970 (1). In the same year Gitelson et al. (2) described determination of oxalic acid in urine, based on its precipitation with excess calcium chloride and determination of this excess by titration. This method gives good results, but it requires large-scale instrumentation and many operations and is thus not well suited for routine work.

I have modified this method to a small-scale procedure and adapted it for atomic absorption spectrophotometry. The present method permits routine determinations at a rate of 30 samples per day, by simple manual operations, with no loss in accuracy, precision, and sensitivity as compared to the method of Gitelson et al.

Materials and Methods

Principle of the Method

Oxalates are precipitated from the urine with excess calcium ions at pH 5. The calcium in the precipitate is determined indirectly by atomic absorption spectrophotometry, subtracting the excess calcium measured in the supernate from the total calcium present and added to the urine in a similar sample at pH 2. By multiplying this figure by the appropriate factor, the oxalic acid of the precipitate is deduced.

There is no interference from phosphorus as coprecipitate if, before the pH is adjusted to pH 5, it is adjusted to pH 8, as also noted by Gitelson et al. (2) and by Archer et al. (3). Urinary phosphorus also does not interfere in the atomic absorption spectrophotometry if, as in the usual determination of calcium with such instruments, the samples are diluted with lanthanum chloride.

Instrumentation

We used a Perkin-Elmer Atomic Absorption Spectrophotometer, Model 290 B, but any other similar instrument may be used.

Reagents

1. Hydrochloric acid, 5 mol/liter. Dilute one volume of concd. HCl with one volume of water.
2. Ammonium hydroxide, 3 mol/liter. Dilute concd. NH₄OH with water to a final concentration of 105 g/liter.
3. Acetic acid, 6 mol/liter. Dilute glacial acetic acid with water to a final concentration of 360 g/liter.
4. Calcium chloride reagent. Dissolve 2 g of CaCl₂.6H₂O in 100 ml of water. This calcium is sufficient for the precipitation of as much as 53 mg of oxalic acid per 100 ml of urine under the conditions of the analysis.
5. Oxalic acid standard. This contains the equivalent of 30 mg of anhydrous oxalic acid per liter and is prepared by dissolving 47 mg of sodium oxalate in 1 liter of water.
6. Lanthanum chloride diluent. This contains 2 g of lanthanum chloride per 100 ml of a 20-fold dilution of concd. hydrochloric acid.

Procedure

Gitelson et al. recommend that the urine be filtered before any other treatment, a step we found to be unnecessary and in fact undesirable, because it may cause important loss of oxalates. Our procedure is as follows:

Pipet two 5-ml samples of urine from a well-mixed 24-h collection into each of two test tubes, marked "test sample" and "control sample."

Adjust the pH of both to 2 with a few drops of hydrochloric acid (reag. 1).
Table 1. Oxalic Acid, Calcium, and Phosphorus Excretion in Some Cases of Oxaluria.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Urine vol, ml/24 h</th>
<th>Oxalic acid, mg/24 h</th>
<th>Ca, mg/24 h</th>
<th>P, mg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A</td>
<td>3245</td>
<td>250</td>
<td>150</td>
<td>510</td>
</tr>
<tr>
<td>B</td>
<td>2200</td>
<td>220</td>
<td>85</td>
<td>350</td>
</tr>
<tr>
<td>C</td>
<td>1650</td>
<td>200</td>
<td>120</td>
<td>395</td>
</tr>
<tr>
<td>2. A</td>
<td>950</td>
<td>154</td>
<td>50</td>
<td>390</td>
</tr>
<tr>
<td>B</td>
<td>1000</td>
<td>50</td>
<td>90</td>
<td>600</td>
</tr>
<tr>
<td>3. A</td>
<td>2100</td>
<td>73</td>
<td>150</td>
<td>310</td>
</tr>
<tr>
<td>B</td>
<td>2300</td>
<td>6</td>
<td>94</td>
<td>775</td>
</tr>
<tr>
<td>4. A</td>
<td>2500</td>
<td>54</td>
<td>50</td>
<td>495</td>
</tr>
<tr>
<td>B</td>
<td>1500</td>
<td>8</td>
<td>90</td>
<td>765</td>
</tr>
</tbody>
</table>

* A, B, and C are specimens collected on different days from the same person.

Readjust the “test sample” to pH 8 with a few drops of ammonium hydroxide (reag. 2). Readjust the pH of contents of the same tube to pH 5 with acetic acid (reag. 3), drop by drop.

Prepare two standard oxalate tubes (reag. 5) in a similar manner, one at pH 2 and one at pH 5, as “control” and “sample.”

Place the two series of tubes in boiling water, add to each 0.5 ml of calcium chloride (reag. 4), mix, and continue heating for an additional 10 min.

Cool the tubes in a refrigerator for at least an hour and centrifuge (3000 rpm, 10 min). Oxalates are quantitatively precipitated at pH 5 (test sample) but not at pH 2 (control sample).

Assay the supernate for calcium by atomic absorption spectrophotometry. If a Perkin-Elmer Model 290 B spectrophotometer is used, a 20-fold dilution with reagent 6 is necessary.

After setting the zero with diluted lanthanum chloride, set a 20-fold dilution (reag. 6) of a standard calcium (10 mg/dl) at the 50 division of the scale. The difference in readings between control and sample, multiplied by 2/10, gives the milligrams of calcium precipitated from 100 ml of urine. The following formula gives the 24-h excretion of oxalic acid: Anhydrous oxalic acid, mg/24 h = (CR − TR) × 2/10 × v × 2.25 = (CR − TR) × (0.45) × v, where CR is the control reading and TR is the test reading. 0.45 is the conversion factor for calcium to oxalic acid, and v is the urine volume in deciliters.

Results

Analytical Variables

Accuracy and precision. Recovery tests were conducted on urine samples at two ranges of oxalic acid excretion, the normal range (0–40 mg/24 h) for 20 cases, and the pathological range (100–200 mg/24 h) for 10 cases. Oxalate added was, for the normal range, 30 mg/24 h, and for the pathological range, 50 mg/24-h urine. Percentage recovery for the normal-range specimens was 95% (85–102%) and for the pathological range 97% (92–105%). I found no need to add an internal standard as suggested by Gitelson et al. (2).

Duplicate determinations were conducted on 10 urine samples of normal oxalate concentration and on 10 pathological samples. These duplicate results agreed within ±4 mg/24 h for the normal range, and ±2.5 mg for the pathological range, calculated from the formula s = R × 0.88 (4).

Interferences. Because phosphates are the principal interfering salts (by possible coprecipitation with oxalates) and because phosphorus is the principal interferant in calcium determination by atomic absorption spectrophotometry, 0.4 to 0.7 g of potassium dihydrogen phosphate per liter was added to 10 samples of urine containing various amounts of oxalic acid (20 to 200 mg/24 h). The standard deviation for all these samples was in the same range as that previously mentioned.

Normal Values

For 30 adult subjects of both sexes, who were on their normal diet, I found the mean excretion of oxalic acid to be 16 mg/24 h (range 0–40 mg, SD ±7.5 mg).

Some Pathological Values

Table 1 shows results for a few pathological cases. Case 1 is an idiopathic oxaluria, 200–300 mg of oxalic acid being excreted per 24 h on three different days. The excretion was independent of urine volume, calcium excretion (83–150 mg), and phosphorus excretion (83–510 mg). In contrast, in nonprimary oxaluria, as in cases 2, 3, and 4 (Table 1), oxalic acid excretion was generally correlated with phosphorus excretion, and also with phosphate intake, in accordance with the findings of Fleish et al. (5).

References