Comparison of Carbonate and Uricase-Carbonate Methods for the Determination of Uric Acid in Serum

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A colorimetric carbonate procedure for the determination of uric acid has been modified to include incubation of serum with uricase to destroy uric acid. Residual nonurate chromogens are subtracted from total chromogens to obtain the concentration of "true" uric acid. Result obtained by the carbonate and the uricase-carbonate methods were in good agreement. The mean concentration of nonurate chromogens in serum is approximately 2% of the true uric acid values. Recovery of uric acid added to serum is essentially quantitative. Formaldehyde markedly inhibits uricase activity and interferes with recoveries. The uricase-carbonate method is applicable to hemolytic serum and to serums from patients with uremia or those receiving high doses of salicylate in which excess concentrations of nonurate chromogens may be encountered.

Comparison of enzymatic and colorimetric methods of analysis for uric acid has revealed the presence of variable amounts of nonurate chromogens in serum and urine (1–6). The reported concentrations of these chromogens, expressed as uric acid, are of the order of 0.1–2.0 mg./100 ml. of serum. Even greater values may be expected in the serum of patients receiving high maintenance doses of salicylate (7).

Efforts to eliminate interfering reducing substances have included prealkalinization of serum (8, 9); alkaline incubation of protein-free filtrates (10); and incubation of untreated serum for 90 min. at 56° (11). The latter technic was claimed to permit specific estimation of uric acid by a carbonate procedure without treatment by uricase. It has been suggested that a major portion of the nonurate chromogens is ascorbic acid (11, 12). One objective of the present study was to evaluate further the specificity of the carbonate method for the determination of uric acid in serum.

Methods

The carbonate method for uric acid was performed exactly as described in Standard Methods of Clinical Chemistry (10). This method

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includes a 10-min. incubation in alkaline solution to destroy nonurate chromogens prior to addition of phosphotungstic acid. The following procedure is a modification designed to measure chromogenic substances before and after incubation of serum with the enzyme uricase.

Reagents

All reagents and standard solutions should be prepared with chlorine-free distilled water (13).

Borate buffer, 0.05 M, pH 9.8 Transfer 0.620 gm. H$_3$BO$_3$, 0.610 gm. NaCl, and 8.2 ml. of 1.0 N NaOH to a 200-ml. volumetric flask. Dissolve the contents in water, dilute to the mark, and mix thoroughly. When this buffer is mixed with an equal volume of serum, the resulting pH is approximately 9.2 at 25°.

Full strength tungstic acid To 700 ml. of water add 100 ml. of 10% (w/v) Na$_2$WO$_4$ · 2H$_2$O, and 0.1 ml. of 85% H$_3$PO$_4$; mix, then add, with mixing, 100 ml. of 0.67 N H$_2$SO$_4$. This reagent is stable at room temperature (14).

Buffered uricase To 5.3 ml. of borate buffer add 0.1 ml. of well-mixed purified uricase preparation and mix thoroughly. This solution is stable up to 1 month at 4°. The enzyme used in this study was purified uricase with a minimum activity of 0.9 U./ml., Code UP (Worthington Biochemical Corp.).

Sodium carbonate (10% w/v) and phosphotungstic acid (Folin-Denis, diluted 1:10) The same as those used for the standard carbonate method (10).

Procedure

Transfer 1.0 ml. of serum to 2 tubes marked “test” and “control.” Add 1.0 ml. of buffered uricase to the test and 1.0 ml. of borate buffer to the control. Mix and incubate in a 37° water bath for at least 45 min. To each tube add 8.0 ml. of full-strength tungstic acid, mix by inversion, and centrifuge. Transfer 5.0 ml. of clear supernatant solution to corresponding tubes or cuvets and 5.0 ml. of water to a third tube for a blank. To each tube add 1.0 ml. of 10% sodium carbonate, mix, add 1.0 ml. of dilute phosphotungstic acid, remix, and let stand 30 min. Measure the absorbance of the test and control within the next 20 min. against the blank in a spectrophotometer at a wave length of 700 m$\text{\mu}$.

Standards and calibration procedure for this method are the same as those used for the standard carbonate procedure (10). Values for the test and control, expressed as uric acid in mg./100 ml. of serum, are obtained by reference to the standards. The value for the “control” represents total chromogens and for the “test” nonurate chromogens.
The true uric acid concentration of the serum is considered to be: \textit{total chromogens minus nonurate chromogens}.

\textbf{Results and Discussion}

Under the conditions described, it was observed that enzymatic conversion of uric acid in serum was completed in 10 min. and 45 min. at concentrations of 6 and 15 mg./100 ml. respectively. Longer periods of incubation had no effect on the final results. The minimum time of incubation for a given uricase preparation should always be determined on serum with a high concentration of uric acid.

The purified uricase used in this study produced negligible blanks. To 0.5 ml. of buffered uricase is added 4.5 ml. of water, 1 ml. of 10% sodium carbonate and 1 ml. of dilute phosphotungstic acid. The absorbance is measured at 30 min. against a blank in which buffer is substituted for buffered uricase. If the final absorbance exceeds 0.01, it is advisable to include a determination of the uricase blank with each series and subtract it from the reading obtained on the “test” containing serum and uricase.

A stock standard solution containing 100 mg. of uric acid in 100 ml. of 0.1% lithium carbonate solution was prepared. Amounts equivalent to 0–10 mg. of uric acid per 100 ml. of serum were added to aliquots of pooled serum. Results of analyses by the uricase-carbonate method are shown in Table 1. Recoveries ranged from 96 to 102% of theoretical.

Standard solutions to be used with a uricase procedure should contain no formalin preservative. The inhibition of uricase activity by formalin has been suggested by others (15, 16) but may not be generally appreciated. The widely used standard described by Folin (17) contains formalin and is not satisfactory for recovery studies. Neutral formalin solution, containing 2% formaldehyde, was added to aliquots of pooled serum to provide concentrations ranging from 0 to 0.16% formaldehyde. Results obtained with the uricase-carbonate method after 45 min. of incubation are shown in Fig. 1. Marked inhibition of uricase

<table>
<thead>
<tr>
<th>Added (mg.)</th>
<th>Found (mg./100 ml.)</th>
<th>Expected (mg./100 ml.)</th>
<th>% recovery</th>
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<tbody>
<tr>
<td>0</td>
<td>3.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td>5.5</td>
<td>102</td>
</tr>
<tr>
<td>4</td>
<td>7.2</td>
<td>7.5</td>
<td>96</td>
</tr>
<tr>
<td>6</td>
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<td>99</td>
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<td>8</td>
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<td>98</td>
</tr>
<tr>
<td>10</td>
<td>13.1</td>
<td>13.5</td>
<td>97</td>
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</table>
activity was observed with formaldehyde concentrations as low as 0.02%.

Pooled serum, stored at 4° for 1 week, showed no significant changes in true uric acid concentration. Similar stability was observed at 25°; however, occasional bacterial contamination occurred which resulted in marked decrease in the uric acid content of serum stored at this temperature. Storage of buffered uricase preparation at 4° for periods up to 1 month resulted in no apparent loss of activity and no increase in uricase blank values.

Serums from 53 patients were analyzed by the uricase-carbonate method as described. Values for total chromogens ranged from 2.4 to 12.6 mg./100 ml. and for true uric acid from 2.4 to 12.5 mg./100 ml. Values for nonurate chromogens ranged from 0 to 0.4 mg./100 ml., with a mean value of 0.10 mg./100 ml. Forty-six of the 53 values were 0.1 mg./100 ml. or less. Regression analysis shown in Fig. 2 demonstrates excellent agreement between true uric acid and total chromogen concentration over the entire range of values studied.

Direct comparison of the uricase method with the standard carbonate method was made on 44 serums. The mean value obtained by the carbonate method was 0.08 mg./100 ml. greater than that obtained for total chromogens by the uricase method and 0.16 mg./100 ml. greater than that obtained for true uric acid by the uricase method. Results are summarized in Table 2. It is concluded that the carbonate method for uric acid in serum is sufficiently specific for routine use, provided that alkaline incubation is included to destroy reducing substances other than
uric acid. The 45-min. incubation of serum near pH 9 in the uricase method serves a similar function, and further alkaline incubation of the filtrate is unnecessary.

Combined enzymatic-colorimetric methods for the determination of true uric acid, based on the difference between total chromogens and nonurate chromogens, have been described by others for whole blood (18, 19), plasma or serum (1, 2, 5, 20–22), and for urine (5, 15, 23). During the course of this study, a technical bulletin appeared which describes a uricase-carbonate method similar to the one presented here (24). In contrast to the carbonate procedure, results by the uricase-carbonate method are not affected by moderate hemolysis. The method may be applied to serum from individuals receiving large doses of sali-

Fig. 2. Total chromogens and true uric acid in serum. Equation shown is that of best straight line through points.

Table 2. Total Chromogens and Nonurate Chromogens in Serum

<table>
<thead>
<tr>
<th>Determination</th>
<th>Method</th>
<th>No.</th>
<th>Range (mg./100 ml.)</th>
<th>Mean</th>
</tr>
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<tr>
<td>Total chromogens</td>
<td>Uricase</td>
<td>53</td>
<td>2.4 - 12.6</td>
<td>6.00</td>
</tr>
<tr>
<td>True uric acid</td>
<td>Uricase</td>
<td>53</td>
<td>2.4 - 12.5</td>
<td>5.90</td>
</tr>
<tr>
<td>Nonurate chromogens</td>
<td>Uricase</td>
<td>53</td>
<td>0 - 0.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Carbonate</td>
<td>44</td>
<td>2.5 - 11.4</td>
<td>5.25</td>
</tr>
<tr>
<td>Total chromogens</td>
<td>Uricase</td>
<td>44</td>
<td>2.4 - 11.6</td>
<td>5.17</td>
</tr>
<tr>
<td>True uric acid</td>
<td>Uricase</td>
<td>44</td>
<td>2.4 - 11.3</td>
<td>5.09</td>
</tr>
</tbody>
</table>
cylates for therapeutic purposes. Patients with uremia also tend to show higher values for nonurate chromogens (20, 25).

Provided certain modifications are made to correct for the relative absence of protein, the uricase-carbonate method may also be applied to the determination of uric acid in urine. Borate buffer, pH 9.2, is used. This is prepared with 0.620 gm. H₃BO₃, 0.610 gm. NaCl, and 5.35 ml. of 1.0 N NaOH diluted to 200 ml. with water. Urine is diluted 1:20 with water, and 1 ml. aliquots used for the determination. One milliliter of buffer is added to the control, and 1 ml. of buffered uricase, pH 9.2, is added to the test. After 1 hr. of incubation at 37°, 3 ml. of water is added to each tube, followed by 1 ml. of 10% sodium carbonate and 1 ml. of dilute phosphotungstic acid as described for serum. Final results are multiplied by 10 to correct for the dilution factors. If the concentration of uric acid exceeds 100 mg./100 ml., the determination should be repeated on more dilute urine. Application of this modified procedure to random specimens demonstrated that nonurate chromogens are present in urine in highly significant but variable concentrations. In contrast to serum, these chromogens are not effectively destroyed by the period of alkaline incubation.

Normal values adopted for uric acid in serum as determined by either the carbonate method or the uricase-carbonate method are: males, 3–7 mg./100 ml.; and females, 2–6 mg./100 ml. These values are in agreement with 9 recent reports in which uricase, cyanide, silicate, and carbonate methods were employed on serum from 776 males and 533 females (1, 2, 5, 9, 10, 26–29). Additional surveys in which uricase methods were applied to serum from over 900 normal males resulted in similar conclusions, i.e., the 95% range for serum uric acid in normal adult males is 3–7 mg./100 ml. (30, 31). A bimodal distribution has been reported in a survey of executives, of whom 16% had serum uric acid values exceeding 7 mg./100 ml. (32). The hyperuricemic effect of alcohol (33) and small doses of salicylate (7) should also be noted.

References