Non-Urate Chromogens in Body Fluids

Wendell T. Caraway*

Compounds which interfere in the analytic determination of uric acid are referred to as "non-urate chromogens." Ascorbic acid, when added to serum, causes a significant increase in apparent uric acid levels as determined by a carbonate-phosphotungstate method. The ratio of apparent urate to ascorbic acid is 1:3, a value in close agreement with that obtained for the ratio of labile non-urate chromogens to ascorbic acid in fresh plasma. Ascorbic acid, as well as most of the non-urate chromogens, can be eliminated by mild alkaline treatment prior to adding phosphotungstic acid. These results indicate that the major non-urate chromogen in the average serum is ascorbic acid. The nature of other non-urate chromogens in body fluids is reviewed.

COLORIMETRIC METHODS for the determination of uric acid usually depend upon the reduction of phosphotungstate by urate at pH 9–10 to produce a blue color. One criticism of these methods is the reported lack of specificity. Reducing substances other than uric acid which may produce color are commonly referred to as "non-urate chromogens" (1). Typical examples are listed in Table 1 (2–4).

The compounds shown in Column A of Table 1 have been reported to reduce phosphotungstate under certain conditions. Interference from these compounds is most likely associated with traces of phosphomolybdate in the reagent, with induced oxidation in the presence of cyanide or with color obtained by heating the reaction mixture. In agreement with Eichorn and Rutenberg (4), the present author has been unable to demonstrate any significant color production by these compounds in the carbonate-phosphotungstate methods (5, 6), either when used alone or in the presence of uric acid.

Of the compounds shown in Column B of Table 1, glutathione and ergothioneine occur in the blood only in the red cells and require no further consideration when nonhemolytic serum or plasma is used. Ascorbic acid, with an average concentration of 1 mg/100 ml in fresh

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Table 1. Non-Urate Chromogens

<table>
<thead>
<tr>
<th>Column A</th>
<th>Column B</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>Gluthione</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Ergothioneine</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>Polyphenols</td>
</tr>
<tr>
<td>Cystine</td>
<td>Aminophenols</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1-Methyluric acid</td>
</tr>
<tr>
<td></td>
<td>3-Methyluric acid</td>
</tr>
<tr>
<td></td>
<td>1,3-Dimethyluric acid</td>
</tr>
<tr>
<td></td>
<td>Gentisic acid</td>
</tr>
</tbody>
</table>

plasma, produces significant color if not destroyed prior to addition of phosphotungstic acid (7). Certain polyphenols and aminophenols are capable of reducing phosphotungstic acid (8), but little information is available regarding potential interference with uric acid determinations.

The methyl-substituted uric acids shown are metabolites of caffeine and theophylline. These produce falsely elevated results for uric acid by colorimetric methods applied directly to urine (2, 3), but their concentrations in plasma apparently have not been studied. Theobromine is metabolized to methylxanthines and 7-methyluric acid, which do not reduce phosphotungstate (9).

Gentisic acid, a metabolite of salicylate, also has been reported to interfere with the colorimetric determination of uric acid in urine (10). Relatively high concentrations appear in the urine of patients receiving salicylate, but the concentrations in plasma are reported to be negligible. Gentisic acid may be removed from either serum or urine, after acidification, by extracting with ethyl ether. Other products of salicylate metabolism are acyl and phenolic glucuronides (11), which are presumably nonchromogenic; however, high concentrations of uricase-resistant chromogens have been reported in the serum of gouty patients receiving high maintenance doses of salicylate (12). The nature of these substances is unknown.

Most of the non-urate chromogens in serum can be eliminated by preliminary oxidation by oxygen in mildly alkaline solutions. Archibald (13) mixed serum for 1 min with dilute sodium hydroxide prior to precipitation of proteins. Results obtained for uric acid with this technic have been shown to agree closely with those obtained by spectrophotometric uricase methods (14, 15). The present author proposed a 10-min incubation of the protein-free filtrate with sodium carbonate to destroy ascorbic acid and other labile reducing substances (5). This procedure was shown to produce results in excellent agreement with a
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Labile non-urate chromogens are also destroyed by heating serum at 56° for 90 min (4) and by storing serum in a refrigerator for 3 days (4) or in the frozen state for 3 weeks (17). These observations suggest that ascorbic acid is the major non-urate chromogen usually found in fresh serum inasmuch as ascorbic acid is readily destroyed under similar conditions. This assumption is supported by direct comparison of ascorbic acid and labile non-urate chromogen concentrations in plasma.

Methods

Uric acid was determined on plasma or serum by a carbonate-phosphotungstate method which included a 10-min alkaline incubation period prior to addition of phosphotungstic acid (5). Total chromogen was measured by adding phosphotungstic acid prior to adding carbonate. The order of addition of reagents was shown to have no effect on the final absorbance obtained for standard solutions of uric acid. The difference between the values obtained for total chromogen and uric acid was considered to be alkali-labile non-urate chromogens (ALNUC) expressed in terms of uric acid. This value represents a relatively small difference between much larger values and is estimated to have a precision (coefficient of variation) of approximately ±15%.

Ascorbic acid was measured by the dichlorophenolindophenol method of Owen and Iggo (18) as described by Henry (19). For comparative purposes, protein precipitation for both uric acid and ascorbic acid were completed within 10 min of each other, and subsequent analyses were started without delay.

Results and Discussion

In preliminary studies, ALNUC was determined on 27 samples of fresh plasma obtained, for the most part, about 2 hr postprandially on healthy laboratory personnel (Table 2). The mean value and the range of values coincide fairly well with the reported normal range for ascorbic acid. In a second study, serum from hospitalized patients was

<table>
<thead>
<tr>
<th>Specimens (No.)</th>
<th>Non-urate chromogens (mg/100ml)</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh plasma</td>
<td>27</td>
<td>1.2 ± 0.7</td>
<td>0.2-2.9</td>
</tr>
<tr>
<td>Separated serum</td>
<td>21</td>
<td>0.8 ± 0.4</td>
<td>0.0-1.7</td>
</tr>
<tr>
<td>(6 hr at 25°)</td>
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separated from the clot and kept at room temperature for 6 hr prior to analysis. The results (Table 2) show that significant interference still exists—ie, values for uric acid averaged 0.8 mg/100 ml higher when the period of alkaline incubation was omitted.

Fresh plasma, obtained from 21 hospitalized patients, was next analyzed for both ALNUC and ascorbic acid. Similar analyses were performed on pooled serum to which ascorbic acid had been added immediately prior to precipitation of proteins. Results are shown in Fig 1. The straight line drawn through the open circles represents results obtained on the pooled serum with added ascorbic acid. One milligram of ascorbic acid was found to produce color equivalent to 1.32 mg of ALNUC (expressed as uric acid) when measured by the methods described. Results obtained on specimens of fresh plasma show a similar correlation. The mean value for plasma ascorbic acid in this series was 1.00 mg/100 ml and for ALNUC 1.34 mg/100 ml. This provides a mean ratio of 1.34 (SD ± 0.20) almost identical with that for

Fig 1. Comparison of ascorbic acid and non-urate chromogen concentrations in plasma. Solid dots: fresh plasma. Open circles: ascorbic acid added to serum.
ascorbic acid added to serum. These observations indicate that the major alkali-labile chromogen in the average serum is ascorbic acid.

In this series, chromogens resistant to both alkali and uricase also were determined and were found to have a mean value of 0.17 mg/100 ml (range 0.08-0.29), in agreement with earlier observations (16). None of these specimens was from patients with uremia. Not shown in Fig 1 are results from 1 patient with uremia (urea N 150 mg/100 ml) who was receiving ascorbic acid intravenously at the time blood was obtained. The plasma ascorbic acid in this case was 9.2 mg/100 ml and the ALNUC was 11.7 mg/100 ml. The ratio of values is 1.3 in agreement with the values shown above. The alkali-stable uricase-resistant chromogen in this patient was 0.9 mg/100 ml. Gross and Bolliger (20) have shown that the uricase-resistant chromogens in serum can reach values of 8 mg/100 ml in severe uremia and as high as 15 mg/100 ml in eclampsia of pregnancy. In a more recent study, the ratio of true urate to total chromogens was found to be 0.85 in both acute and chronic renal failure (21).

Ascorbic acid also may interfere in the direct enzymatic spectrophotometric technics for measuring uric acid. The absorption maximum for ascorbic acid is 265 nm, but significant absorption occurs at 292 nm, the wavelength used to monitor the disappearance of uric acid by the action of uricase. Since ascorbic acid readily undergoes oxidation by oxygen at the pH employed (9.2), it becomes necessary to include a serum or sample blank to correct for these spontaneous changes (22, 23). Serum or assay fluids should not be used in the reference cell because of their relative instability. This correction is especially significant for cerebrospinal fluids where the concentration of uric acid is low, and higher fluid concentrations are employed (24). Similarly, for enzymatic technics in which the uric acid is measured by the difference in uricase-treated and untreated specimens, both specimens must be incubated under exactly the same conditions of time, temperature, and pH to equalize loss of alkali-labile chromogens.

Hemolytic serum contains glutathione and ergothioneine from the red cells. Of the two, ergothioneine produces considerably more color per milligram than glutathione in the carbonate-phosphotungstate methods. Neither compound is destroyed by the alkaline incubation technic used in this study. In low concentrations (2-4 mg/100 ml) 1 mg of ergothioneine was observed to reduce phosphotungstate to approximately the same extent as did 1 mg of uric acid.

Colorimetric methods appear to be sufficiently specific for the routine determination of uric acid in serum, provided that the serum, protein-
free filtrate, or dialysate is treated with alkali to destroy ascorbic acid prior to color development. Results may be falsely elevated in the serum from patients with uremia or eclampsia and possibly in those receiving high maintenance doses of salicylate. In urine, however, up to 15% of the total chromogen may be resistant to both alkali and uricase (1); hence, for accurate determinations, an enzymatic technic is indicated.

References