High Incidence of Significant Urinary Ascorbic Acid Concentrations in a West Coast Population—Implications for Routine Urinalysis

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Examination of 4379 routine urinalysis specimens with dipsticks sensitive to ascorbic acid showed that 22.8% were positive specimens. The mean vitamin C concentration in this population was 2120 μmol/L. There was a high rate of false-negative dipstick results for hemoglobin in patients with vitamin C in the urine. The highest false-negative rates were observed in urine samples containing <50 erythrocytes per high-power field. In further experiments when volunteers consumed supplemental oral USP vitamin C at doses of 100, 250, 500, and 1000 mg or vitamin C-containing fruits, even the lowest doses of oral vitamin C or juice resulted in sufficient urinary vitamin C to produce false-negative dipstick results in hemoglobin and glucose testing. To prevent potentially dangerous false-negative results, screening urinalysis protocols relying only on dipstick testing should include a check for urinary vitamin C or use a dipstick that is not subject to vitamin C interference.

Vitamin C (ascorbic acid) is increasingly ingested as a nonprescribed dietary supplement. Dipstick tests for urinary glucose and hemoglobin that involve peroxidase–redox indicator systems are known to be subject to interference by ascorbic acid. To date, however, no systematic studies have been published of the incidence or clinical significance of this interaction in a typical outpatient ambulatory urinalysis population. Several dipstick manufacturers now claim relative resistance to vitamin C interference in hemoglobin and glucose determinations.

As a result of frequently noting erythrocytes in microscopic urinalysis despite a negative dipstick screen result for hemoglobin, we undertook to determine the incidence and mean concentration of urinary ascorbic acid in our routine urinalysis population. We also studied volunteer laboratory workers taking 100–1000 mg of supplemental USP vitamin C or one of three amounts of fruit juices to determine whether the urinary concentrations of vitamin C produced were sufficient to interfere with dipstick determinations of glucose and hemoglobin. Finally, we tested three of the most frequently used urinalysis dipsticks as well as one prototype dipstick to assess the degree of vitamin C interference in glucose and hemoglobin reactions, especially within the range of ascorbate concentrations found in our urinalysis population.

Materials and Methods

Ascorbic acid assay. Ascorbic acid in the urine was quantified either with a manual or an automated method. The manual method consisted of the oxidation of ascorbate by 2,6-dichlorophenolindophenol, with subsequent hydrolysis and formation of an osazone by combination with 2,4-dinitrophenylhydrazine, which rearranged to a stable reddish-brown product that was measured photometrically (1). The automated method consisted of the reduction of ferric ion by ascorbate and the formation of a colored complex between the resulting ferrous iron and Ferrozine. The color was measured photometrically with a Cobas-Bio centrifugal analyzer (Roche Diagnostics, Nutley, NJ) (2). A comparison of these two methods by an in-house method-comparison program in which scatterplot, least-squares regression, Deming’s method regression, sign test, and Wilcoxon’s test were used found no difference between methods.

Urine assays for vitamin C. We processed 4379 serial routine urine specimens submitted for analysis. Before microscopic analysis, all specimens were screened with two dipsticks: the Multistix-10SG (Ames Division, Miles Canada Inc., Etobicoke, Ontario, M9W 1G6) and the Rapignost Basic-Screen Plus (Behringwerke, Marburg, F.R.G.). Samples that tested positive for vitamin C with the Rapignost dipstick subsequently were analyzed for vitamin C by either the manual or the automated method—either within the same working day, or after storage at 4 °C until quantification of vitamin C by assay by the automated method, or after being frozen for later analysis by the manual method. All frozen specimens were analyzed within 60 min of thawing. Stability studies performed on control specimens stored and handled in this fashion revealed no significant deterioration in vitamin C content.

Vitamin C interference with hemoglobin detection. We subsequently quantified the concentration of vitamin C in 129 of the 159 patients who had hematuria present on microscopic examination and who were positive for vitamin C by dipstick examination. The incidence of positive dipstick test results for hemoglobin in these urine samples was compared with the incidence of positive dipstick test results for hemoglobin in samples displaying hematuria but containing no ascorbate. For these comparisons, the degree of microscopic hematuria was stratified according to the following values for erythrocytes per high-power field (RBCs/HPF): 4–6, 6–10, 10–50, and >50.

Oral vitamin C and juice supplementation study. To investigate the various concentrations of urinary vitamin C resulting from oral supplementation with vita-
min C, five volunteers took daily supplemental vitamin C at successive doses of 100, 250, 500, and 1000 mg. Each dose of vitamin C was taken for seven consecutive days, starting with the lowest doses. The supplement was taken at the same time each morning, and urine was collected on days 3–7. On collection days, each volunteer provided as many urinary specimens as possible during the working day. Specimens were collected in standard sterile urinary containers, refrigerated at 4 °C immediately after collection, and analyzed within the same working day.

To investigate the effect of different vitamin C-containing fruit juices on the urinary concentrations of ascorbate, five volunteers consumed 180-mL glasses of a fruit juice of their choice—orange, apple, grape, or cranberry—on the following weekly schedules: breakfast glass; breakfast and lunch glasses; and breakfast, lunch, and supper glasses. Each volunteer consumed the same kind of juice for the entire study. Juice drinking started on day 1 and urinary specimens were collected on days 3–7. These specimens were collected and analyzed in the same manner as for the supplemental oral vitamin C part of the study.

Throughout the seven-day supplementation studies, volunteers did not consume any other exogenous vitamin C or multivitamin preparations.

Interference in glucose and hemoglobin reactions. To check for interference in glucose and hemoglobin reactions, we tested four dipsticks: Multistix-10SG, Rapignost Basic-Screen Plus, Chemstrip 10 (Boehringer Mannheim Canada Ltd., Laval, Quebec, H7V 3Z9), and an experimental formulation, Multistix (Ames 8SG-100S; Miles Canada Inc.). Each dipstick test is semiquantitative for glucose reactions and is calibrated from 2.85 to 114.0 mmol/L. Although the package inserts for all stripes state that the dipsticks are subject to vitamin C interference with glucose reactions, in other advertising Chemstrip claims that their glucose pad exhibits virtually no ascorbic acid interference.

In the experimental design, glucose in concentrations of 0, 2.86, 5.7, 11.4, 22.8, or 45.6 mmol/L was added to urine specimens containing ascorbic acid at 0, 500, 1000, 2000, 4000, or 10 000 μmol/L. Each urine specimen was tested twice and all color reactions for reagent strips were read by the same medical technologist to ensure consistency. All determinations were performed within 1 h of glucose addition.

Potential ascorbate interference with the hemoglobin reaction was studied by using the same four dipsticks. Both Ames dipsticks have five color blocks, corresponding to negative, trace, +1, +2, and +3 quantities of blood. Chemstrip 10 has four color blocks, corresponding to negative, 10, 50, and 150 erythrocytes per microliter. Rapignost has a negative block and six color blocks corresponding to 10, 50, and 300 erythrocytes per microliter and 1+, 2+, and 3+ hemoglobin reactions. Whereas the literature accompanying Rapignost and Multistix states that ascorbic acid may interfere with the dipstick hemoglobin reaction, the Chemstrip 10 insert states that the presence of vitamin C does not affect this test. For the experimental design, urine specimens were contrived to contain hemoglobin at 0, 0.15, 0.3, 0.6, 1, 2, 4, 6, or 10 mg/L and ascorbic acid at 0, 500, 1000, 2000, 4000, or 10 000 μmol/L. These urine specimens were tested and strips were read as in the glucose-ascorbate experiment.

Results

Urinary Ascorbate Concentrations

Of 4379 routine urine specimens screened with the Rapignost dipstick vitamin C pad, 997 (22.8%) tested positive. The mean urinary vitamin C concentration was determined in 557 of these 997 specimens by either the manual or the automated method and was 2120 μmol/L (range 405–19 350 μmol/L). Figure 1 shows the distribution of values within this range of urinary ascorbate concentrations. The Rapignost information insert claims that the vitamin C dipstick test pad first shows positive results at an ascorbate concentration of 570 μmol/L (100 mg/L). Our own sensitivity testing showed that trace reactions with the vitamin C dipstick test pad appeared at 284 μmol/L (50 mg/L), whereas consistently positive reactions were noted at >398 μmol/L (70 mg/L). For the purpose of this study, any quantitatively analyzed ascorbate value <400 μmol/L that gave a positive dipstick-pad reaction was considered to represent a false-positive result. The study population gave 14 such false-positive reactions, for an overall false-positive rate of 2.5% for the Rapignost vitamin C dipstick pad.

Ascorbate Interference with the Detection of Hemoglobin

Of the 4379 urinalysis specimens, 561 demonstrated hematuria on microscopic examination. Of these 561 patients, 407 had no vitamin C detected in the urine on dipstick testing, and 154 had vitamin C present. Quantitative analysis of the vitamin C concentration in 129 of these 154 patients revealed a mean urinary vitamin C concentration of 2173 μmol/L (range 458–9602 μmol/L).

![Fig. 1. Distribution of urinary ascorbate concentrations in the 557 patients' specimens assayed](CLINICAL CHEMISTRY, Vol. 38, No. 3, 1992 427)
Of the 407 patients with hematuria and no vitamin C in the urine, 43 tested negative for hemoglobin with Multistix. Of the 154 patients with hematuria and vitamin C in the urine, 109 had false-negative results for hemoglobin with Multistix, a significant difference ($P = 0.001$).

The incidence of false-negative results for hemoglobin detection was directly related to the degree of hematuria present on microscopic exam (Table 1). With the ascorbate-positive urine specimens, false-negative rates ranged from >99% in specimens containing 4–6 RBCs/HPF to 5% for specimens with >50 RBCs/HPF. Even when these specimens contained >10 RBCs/HPF, the false-negative rate was high (44%). In the ascorbate-positive specimens, a significant degree of hematuria (>50 RBCs/HPF) was required to produce a low false-negative rate. In specimens with microscopic hematuria that were negative for vitamin C on dipstick testing, the sensitivity of the dipstick to hemoglobin improved once the urine contained >10 RBCs/HPF; in this group, a value >50 RBCs/HPF was associated with 0% false-negative reactions for hemoglobin detection.

**Oral Vitamin C and Juice Supplementation**

Figure 2 shows the relationship between the daily dose of ascorbic acid supplementation and the urinary ascorbate concentrations observed: with the 100-mg oral dose, the mean urinary ascorbate value was 889 μmol/L (range 125–3676 μmol/L); with the 250-mg oral dose, the mean urinary ascorbate value was 1765 μmol/L (range 153–5889 μmol/L); with the 500-mg oral dose, the mean urinary ascorbate value was 3544 μmol/L (range 106–12800 μmol/L); and with the 1000-mg oral dose, the mean urinary value was 3565 μmol/L (range 129–16970 μmol/L). As Figure 2 illustrates, a wide range of urinary vitamin C concentrations was associated with each individual's oral vitamin C intake. Volunteers also demonstrated considerable intra-individual variation at each vitamin C dose. Even at the lowest supplemental oral dose of 100 mg a day, every volunteer produced at least one urinary ascorbate value >1000 μmol/L.

The results of the supplemental juice intake are illustrated in Figure 3: with one glass of juice daily, the mean urinary ascorbate value was 556 μmol/L (range 62–2293 μmol/L); with two glasses of juice, the mean urinary ascorbate value was 804 μmol/L (range 72–4129 μmol/L); and with three glasses of juice, the mean urinary ascorbate value was 903 μmol/L (range 52–3585 μmol/L). As for the oral vitamin C supplements, a wide range of values (with considerable intra-individual variation) of urinary ascorbate concentrations was noted at each level of juice consumption. When taking three glasses of juice daily, every volunteer produced at least one value for urinary ascorbate >1200 μmol/L except for the individual drinking cranberry juice, whose ascorbate values were consistently lower.

**Interference in Glucose Reactions**

The 36 test urine specimens assayed were grouped by ascorbic acid and glucose concentrations (Table 2). With no ascorbic acid present, each strip performed as specified. When ascorbic acid at 500 or 1000 μmol/L was added, the reagent strips reacted about as expected. At 2000 μmol/L for ascorbic acid, both types of Multistix and the Rapignost strips showed a shift to weaker reactions, whereas at >4000 μmol/L these three strips showed abolition of reactions. The Chemstrip 10 showed

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**Table 1. Incidence of False-Negative Multistix Dipstick Results for Hemoglobin in Patients' Urine Specimens with or without Vitamin C**

<table>
<thead>
<tr>
<th>RBCs/HPF</th>
<th>Vitamin C negative</th>
<th>Vitamin C positive</th>
</tr>
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<tr>
<td>4–6</td>
<td>23/108 (21)</td>
<td>65/66 (99)</td>
</tr>
<tr>
<td>6–10</td>
<td>18/100 (18)</td>
<td>25/28 (93)</td>
</tr>
<tr>
<td>10–50</td>
<td>2/121 (1.6)</td>
<td>17/39 (44)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>0/78 (0)</td>
<td>1/21 (5)</td>
</tr>
</tbody>
</table>

Note: Number of erythrocytes per high-power field in microscopically examined urine.
<table>
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<tr>
<th>Glucose concn, mmol/L</th>
<th>0</th>
<th>500</th>
<th>1000</th>
<th>2000</th>
<th>4000</th>
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<td>Multistix-10SG</td>
<td>5.6</td>
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<td>Neg</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td>22.4</td>
<td>28</td>
<td>14</td>
<td>14</td>
<td>14</td>
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<th>Hemoglobin concn, mg/L</th>
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<th>500</th>
<th>1000</th>
<th>2000</th>
<th>4000</th>
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<tr>
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**Discussion**

As the public has become more involved with healthcare issues, interest in alternative treatments such as vitamin therapy has increased dramatically. For instance, more than half the patients attending cancer clinics are also reportedly participating in some form of alternative therapy, which usually includes vitamin supplements (3). In particular, the opinions of Linus Pauling have done much to promote self-medication with vitamin C (4). Our study was initially prompted by the frequent observation of dipstick tests that were negative for hemoglobin despite the consistent observation of erythrocytes in microscopic analysis of the same specimens.

Although aware of the public’s interest in vitamin supplementation, we were still surprised to discover the relatively high proportion of our routine urinalysis population (22.8%) who demonstrated substantial amounts of urinary vitamin C. In 1986, Zweig and Jackson (5) stated that “interference in routine urinalysis of outpatients may be more common than is generally recognized because ascorbic acid is so commonly taken,” but no formal study had verified their hypothesis. Published information on the frequency of signifi-
cant urinary ascorbic acid concentrations in the general population is scanty. Informal studies performed several years ago by a dipstick manufacturer suggested a prevalence of <5% for urinary ascorbate concentrations of ≥2280 μmol/L (Ames Division, Miles Canada Inc., personal communication). After surveying a Scandinavian population, Berg (6) stated that ascorbate concentrations of 600–1200 μmol/L were found in 3–20% of urine specimens but that higher concentrations were very rare. Besides documenting a substantial incidence of urinary ascorbate, our study also shows that, within the population we surveyed, concentrations >2280 μmol/L are not uncommon.

Previous workers documented the interference of ascorbic acid with the dipstick indicator substances for hemoglobin (6, 7). Our data confirmed that this was a problem in our routine urinalysis population. As in other studies, our data show that this phenomenon was quantitative, related to the number of erythrocytes present. With >50 RBCs/HPF, false-negative results tended to disappear regardless of the ascorbic acid concentration. Previous studies of the performance characteristics of individual dipsticks documented false-negative rates for hemoglobin detection as great as 30% (8, 9). Because most of these investigations did not include testing for urinary vitamin C, we speculate that unsuspected urinary ascorbate may have been responsible for some of these false-negative results. Any future investigations of individual dipsticks regarding hemoglobin or glucose testing should include measuring vitamin C in the test urine specimens.

Although there has been considerable debate regarding the extent of investigation appropriate for patients with hematuria, several recent studies indicated that hematuria of any degree should be followed up in older patients because it may indicate any of several treatable abnormalities (10–12). Unsuspected vitamin C interference with dipstick hemoglobin testing could have serious consequences in this population. Our study of an actual outpatient urinalysis population demonstrates how significant this interaction can be. Within the population of 4379 patients, 109 of the 407 ascorbate-positive urine specimens with hematuria would have been missed had a microscopic examination not followed dipstick testing. Moreover, of the 561 patients with hematuria, 462 (or 82%) had <50 RBCs/HPF, the cutoff for substantial ascorbate interference with hemoglobin detection. False-negative dipstick results for glucose are also undesirable. In the unusual case of a diabetic subject using urinary glucose testing for therapeutic monitoring at home, the difference between a negative reaction and even a weak positive reaction may affect disease management.

It is interesting to speculate on the mode of production of the positive urinary ascorbate values observed in 22.8% of our population. Although the juice-supplementation study did show that fruit juices could produce urinary ascorbate concentrations in the range of those detected in our routine urinalysis population, the over-all values in our survey population were generally higher. Unless patients were consuming at least three glasses of juice per day, the results indicate that most individuals consume vitamin C tablets. In addition, examination of the urinary ascorbate concentrations produced by the USP oral vitamin C supplements suggests that the majority of patients were consuming ≥250 mg of vitamin C daily, consistent with the larger doses advocated by Pauling (4). A survey of the mean and median doses of vitamin C consumed by the actual population who take extra daily vitamin C would be of interest.

In an attempt to improve the performance of dipsticks in the presence of ascorbic acid, manufacturers have developed various strategies. Recent studies show that the incorporation of oxidants into the reagent strip diminishes the interference in glucose reaction by rapidly oxidizing the ascorbic acid to dehydroascorbate acid. For example, mercuric acetate will abolish the ascorbic acid interference, with little effect on the activity of the glucose oxidase or peroxidase present (7). The Chemstrip 10 uses an iodate-impregnated mesh layer to resist ascorbic acid interference. The mechanism by which the experimental Multistix formulation is protected from ascorbate interference with hemoglobin detection is currently proprietary information. In our tests, the Chemstrip 10 appeared to be the most resistant to ascorbic acid interference with both glucose and hemoglobin determinations. The experimental Multistix formulation appeared to be less sensitive than the Multistix-10SG to ascorbate interference for hemoglobin but not for glucose.

The high incidence of urinary vitamin C concentrations documented in this study, coupled with the performance characteristics of the strips tested, has several implications for routine urinalysis. The past few years have seen a tendency to attempt to reduce costs by streamlining laboratory procedures. Numerous authors have proposed that, if the results of macroscopic urinary examination and dipstick analysis are normal, one probably need not proceed with the microscopic examination (8, 9, 13, 14). However, our study demonstrates that if such a policy is adopted and urinary ascorbate content is not considered, potentially dangerous false-negative results for hemoglobin and glucose may be reported. Some laboratories have attempted to circumvent this problem by using dipsticks such as the Chemstrip 10 that appear to be relatively resistant to vitamin C. Another approach would be to screen with a strip with a vitamin C indicator pad, such as the Rapignost Basic-Screen Plus. Subsequently, specimens that test positive for vitamin C could be reported as possibly invalid for hemoglobin and glucose determinations because of the presence of urinary vitamin C, or a subsequent microscopic urinalysis could be performed on all specimens that had tested positive for ascorbate.

All of the dipsticks evaluated in this study were supplied by the manufacturers free of charge except for the Ames Multistix-10SG strips, which were purchased.
Low Bias in Assayed Values of Lipoprotein Antigens—Lipoprotein(a) and Apolipoproteins A-I and B—in Midday Postprandial Blood Specimens Compared with Morning Fasting Specimens

Kenneth Emancipator

Two-hour postprandial specimens have a $-14\%$ proportional bias for lipoprotein(a) (Lp(a)), a $-0.035$ g/L systematic bias for apolipoprotein (apo) A-I, and a $-9\%$ proportional bias for apo B, compared with values in 12-h fasting specimens. Although a physiological hemodilution appears to account for a portion of these biases, other major factors must be implicated for Lp(a) and apo B. Even after dilutional effects are controlled for, assayed values of Lp(a) are 11–13% lower, and assayed values of apo B are 8–9% lower, in postprandial specimens than in fasting specimens. Therefore, the time of collection of a blood sample relative to the last meal can significantly affect assayed values of lipoprotein antigens. Further studies are needed to determine whether these observations result from a physiological sequestering of lipoproteins in the postprandial state or from negative interferences affecting the assays of lipoprotein antigens.

Additional Keyphrases: sample collection • variation, source of

One previous report (1) stated that there is no significant difference between fasting and postprandial serum values for apolipoprotein (apo) A-I and apo B, whereas another report (2) stated that both of these analytes are lower during the postprandial period. Both studies involved only a few subjects (10 and 8, respectively), and statistical analysis was limited to paired sample t-tests. Here, I report the relationship between fasting and postprandial apo A-I, apo B, and lipoprotein(a) [Lp(a)] measured in a larger number of subjects.

Materials and Methods

Fasting and postprandial blood samples were obtained from healthy human donors, all of whom were laboratory employees. Donors were instructed to fast overnight for at least 12 h. After the fasting specimen was obtained, the subjects consumed a lipid-rich breakfast of their choice from the Clinical Center cafeteria. Each subject consumed at least (a) two eggs, (b) one egg and three strips of bacon, (c) one egg and three breakfast sausages, or (d) one egg and an order (~100–200 g) of fried potatoes. Individuals were allowed to consume larger meals if this was their habit. The postprandial specimen was obtained 2 h after completion of the meal.

Blood samples were drawn into 7-mL Vacutainer® Tubes (Becton Dickinson, Rutherford, NJ 07070) containing no additive ("red top"). Samples were allowed to clot for 30–60 min and then were centrifuged at 2000 × g for 15 min. On the same day that the samples were collected, sera were assayed for apo A-I and apo B (both apo B-100 and apo B-48 are recognized by the latter assay) with the Array® nephelometer (Beckman Instru-