Positive Interference of Catecholamine Metabolites with Quantitation of Urinary Uric Acid by the Direct Acid Ferric-Reduction Method

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We report the effect of major catecholamine metabolites such as homovanillic acid and vanilmandelic acid, normally present in urine, on quantitation of urinary uric acid by the direct acid ferric-reduction procedure [(Am. J. Clin. Pathol. 60, 691 (1973)]. When aqueous uric acid standards containing a mixture of these two metabolites, in concentrations comparable to those in a normal human 24-h urine, were added to Fe³⁺-phenanthroline or Fe³⁺-2,4,6-tripyridyl-s-triazine reaction mixture, the resulting absorbances at 505 or 593 nm considerably exceeded those of standards without such additions. Moreover, quantitation of urinary uric acid by the direct acid ferric-reduction procedure gave values that significantly exceeded those determined by the uricase method. Interference by the two metabolites was eliminated by extracting urine specimens with ethyl acetate before quantitation. Spectral scans revealed that homovanilic acid or vanilmandelic acid alone could also effectively reduce Fe³⁺-chelator complex and that the effect of these compounds on the analytical reaction was additive.

Additional Keyphrases: ethyl acetate extraction • uric acid by the uricase method

Quantitation of uric acid by the direct acid ferric-reduction (DAFR) procedure depends on reduction by uric acid of a Fe³⁺-phenanthroline complex to its ferrous form, which absorbs maximally at 505 nm (1). This procedure was modified by Morin (2), who measured the absorbance at 593 nm as 2,4,6-tripyridyl-s-triazine (Fe³⁺-TPTZ) complex, which was reduced by uric acid to its Fe²⁺-TPTZ form. Nelson and Batra (3) adopted this method to a single-channel continuous-flow analyzer to speed quantitation of urinary uric acid. However, the suitability of the procedure for measuring uric acid in urine remained inconclusive, because only 20 urine specimens were evaluated. In addition, possible interference from substances in the urine was not studied.

It can be anticipated that any reducing substance present in the urine might interfere with the assay, including catechols, because the phenolic group can function as a reducing agent. In a preliminary study, we demonstrated that a compound containing a catechol nucleus or its O-methylated counterpart, such as homovanillic acid (HVA) or vanilmandelic acid (VMA), exhibited significant reductive capability at a concentration of 50 mg/L (unpublished data).

Here we report evidence indicating that quantitation of urinary uric acid by the DAFR procedure can lead to erroneous results owing to the presence of catecholamine metabolites, HVA and VMA, that normally are present in urine. Accordingly, we cannot recommend quantitation of urinary uric acid by this procedure.

Materials and Methods

VMA was a product of Calbiochem, Los Angeles, CA 90064. Uric acid, HVA, 1,10-phenanthroline, and TPTZ were purchased from Sigma Chemical Co., St. Louis, MO 63178. Aqueous uric acid standard (1.00 g/L) was prepared according to the method of Nelson and Batra (3), but with thymol diluent replaced by de-ionized water. All reagents required for the manual uric acid determination, with Fe³⁺-phenanthroline as redox indicator, were prepared as described by Nelson and Batra (3). If Fe³⁺-TPTZ was used as the redox indicator, all reagents were prepared according to the method described by Morin (2). The 3-mL reaction mixture included: 2.5 mL of buffer; 0.4 mL of redox indicator (chelator/Fe³⁺ = 3/1 by vol), and 0.1 mL of urine or standard. Absorbances at 505 or 593 nm were measured 10 min after urine or standard was added.

Uric acid in samples, with and without the pre-extraction with ethyl acetate, and in standards, with and without the addition of HVA and VMA, were assayed manually by the procedure of either Morin (2) or Nelson and Batra (3). For comparison, we also measured uric acid in some of the above urine samples by a uricase (EC 1.7.3.3) method (4). Absorbances were measured with a Model DU spectrophotometer (Beckman Instruments Co., Fullerton, CA 92634) equipped with a Model 2000 absorbance recorder (Gilford Instrument Laboratories, Inc., Oberlin, OH 44074).

Spectral scans were done on a 0.1-mL aliquot of aqueous uric acid standard (50 mg/L), uric acid-free HVA or VMA mixture (10 and 5 mg/L, respectively), and aqueous uric acid standard (50 mg/L) containing HVA and VMA (10 and 5 mg/L, respectively) added to buffered Fe³⁺-phenanthroline reaction mixture to give a total volume of 3.0 mL. Absorbances were recorded automatically vs. a reagent blank, with a Model 25 spectrophotometer (Beckman Instruments Co., Fullerton, CA 92634) having a scanning speed of 100 nm/min.

Urine samples used for this study were obtained from two groups of individuals. Group I samples were obtained from 10 apparently healthy volunteers. Group II samples were se-

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Received Oct. 25, 1978; accepted Feb. 13, 1979.

Nonstandard abbreviations used: DAFR, direct acid ferric-reduction; Fe³⁺-TPTZ, Fe³⁺-2,4,6-tripyridyl-s-triazine; HVA, homovanillic acid; and VMA, vanilmandelic acid (4-hydroxy-3-methoxymandelic acid).
Results and Discussion

Effect of HVA and VMA or uric acid calibration curves. According to LaBrosse et al. (6), the major urinary catecholamine metabolites are HVA, VMA, and metanephrine, but the amount of metanephrine in normal urine is only 10% of the amount of either HVA or VMA. To determine whether or not the reductive capability of these compounds would affect the DAFR process, aqueous uric acid standards ranging in concentration from 20 to 80 mg/L were supplemented with a mixture of HVA and VMA in a concentration comparable to that to be expected in a normal human 24-h urine (10 and 5 mg/L, respectively).

When calibration curves were compared, we found that the absorbances at 505 nm (Fe³⁺-phenanthroline as redox indicator; Figure 1, I, line B) or 593 nm (Fe³⁺-TPTZ as redox indicator; Figure 1, II, line B) for these standards were substantially higher than those obtained for the HVA- and VMA-free counterparts (Figure 1, I and II, line A). Further, because the absorbances at 505 nm or 593 nm contributed by the added HVA and VMA were independent of the uric acid concentration, the effect of these compounds on the increment in absorbances was additive.

Interference by catecholamine metabolites on urinary uric acid measurement. The concentrations of catecholamine metabolites in normal and pathological urines are considerably higher than catecholamine concentrations in the blood (7, 8). Therefore, one could anticipate that catecholamine metabolites would interfere with urinary uric acid quantitation by the DAFR procedure. We tested this hypothesis on a group of 10 healthy volunteers (Figure 2, Group I) and 20 individuals suspected of excreting increased amounts of catecholamine metabolites (Figure 2, Group II). In both cases, urinary uric acid values (mean ± SD) as determined by the DAFR procedure were significantly different from those concurrently quantitated by the uricase method (Group I, 414 ± 116 mg/24 h by DAFR vs. 347 ± 107 mg/24 h by uricase; Group II, 524 ± 241 mg/24 h by DAFR vs. 377 ± 151 mg/24 h by uricase; p < 0.001 in both cases). We attributed this discrepancy to the presence of interfering substances in the DAFR procedure.

The falsely high uric acid values determined by the DAFR procedure were, owing to the presence of interfering substances, decreased (Figure 2) when urinary HVA and VMA were removed by extraction with ethyl acetate. The values (mean ± SD) after ethyl acetate extraction were comparable to those measured by the uricase method (Group I, 351 ± 100 mg/24 h by DAFR vs. 347 ± 117 mg/24 h by uricase; Group II,
Nature of the interference by catecholamine metabolites with the measurement of urinary uric acid by the DAFR method. To explore the nature of the absorbance-increment effect of HVA and VMA, it was necessary to determine whether or not there is an actual interaction between uric acid and these catecholamine metabolites. We performed a spectral scan (Figure 3). When a 0.1-mL aliquot of uric acid standard (50 mg/L) was added to buffered Fe³⁺-phenanthroline reaction mixture, a maximum absorption peak at 505 nm was observed (curve B). However, if a 0.1-mL aliquot of the same uric acid standard was supplemented with a mixture of HVA and VMA (10 and 5 mg/L, respectively), and then added to buffered Fe³⁺-phenanthroline reaction mixture, the spectral pattern did not change; however, the absorbance at 505 nm was augmented (curve C).

Further, when a 0.1-mL aliquot of a mixture of HVA and VMA (10 and 5 mg/L, respectively) was added to the same reaction mixture, a similar spectrum was obtained and a maximum absorption peak at 505 nm was also observed (curve A).

These data demonstrate that HVA and VMA can also effectively reduce Fe³⁺-phenanthroline complex. Moreover, the absorbance at 505 nm ascribable to the presence of HVA and VMA in the uric acid standard was equal to the sum of the individual absorbances of the above components measured separately (A = 0.23 and 0.13, respectively). This further suggests that the effect of HVA and VMA on the increment of absorbance is additive. Hence, the possibility that HVA and VMA interact with uric acid can be ruled out.

We gratefully acknowledge the editorial assistance of H. Griscom, Ph.D., during the preparation of this manuscript.

References