Determination of Uric Acid

An Automated Analysis Based on a Carbonate Method

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An automated method is described for the determination of uric acid by a carbonate method. Since uric acid is separated from proteins by dialysis, the loss of uric acid due to protein precipitation in manual methods is avoided. There is no appreciable interference from salicylates, high levels of blood sugar, or ascorbic acid. The method shows an acceptable correlation with the uricase method.

A variety of methods is available for the colorimetric determination of uric acid. In general, these are based on precipitation of proteins, followed by the reduction in an alkaline medium of phosphotungstate by the uric acid in the protein-free filtrate. Results of colorimetric determinations may at times vary from "true" uric acid values as determined by uricase methods. This is because the colorimetric methods of analysis are subject to both positive and negative errors. Positive errors arise because colorimetric methods are not specific for uric acid and are influenced by nonuric acid reducing substances present in serum. Negative errors are the result of variable losses of uric acid during precipitation of serum proteins prior to color development. The purpose of this paper is to describe an automated colorimetric method for the determination of uric acid, based on a carbonate method. In this method, uric acid is separated from proteins by dialysis, thereby avoiding loss of uric acid incident to precipitation of proteins.

Method

Reagents

The following reagents are used in the procedure.

1. *Saline diluent* Add 10 drops caprilic alcohol to 1000 ml. 0.9% (w/v) sodium chloride.

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2. **Stock Folin-Denis reagent**  Dissolve 100 gm. molybdate-free sodium tungstate (Na₂WO₄ · 2H₂O) in about 800 ml. distilled water in a florence flask. Add 80 ml. 85% phosphoric acid and glass beads. Attach reflux condenser and boil gently for 2 hr. Cool, dilute to 1000 ml. and add 40 gm. lithium sulfate (LiSO₄ · H₂O). Store in refrigerator in an amber bottle.

3. **Dilute Folin-Denis reagent**  Dilute the stock reagent 1:5 with distilled water.

4. **15% sodium carbonate**  Dissolve 150 gm. anhydrous sodium carbonate in distilled water and dilute to 1000 ml. Bottle in polyethylene bottle. The reagent is stable at room temperature.

5. **Stock uric acid standard: 1 mg./ml.**  In a 250-ml. flask dissolve 0.6 gm. of lithium carbonate (Li₂CO₃) in 150 ml. of distilled water. Shake to dissolve and heat for 5 min. on water bath. Filter to remove any insoluble material. Weigh out 1 gm. uric acid and place in a separate 1-L. volumetric flask. Pour the lithium carbonate into the flask containing the uric acid and shake to dissolve. Dilute to volume with distilled water and bottle in brown bottle. Store in refrigerator.

6. **Working uric acid standards**  Prepare dilute standards of 3, 6, 9, and 12 mg./100 ml. by diluting in separate 100-ml. volumetric flasks 3, 6, 9, and 12 ml. of stock standard and diluting to volume with 0.9% saline solution. Working standards are not stable and should be prepared at least weekly and stored under refrigeration.

**Procedure**

The recommended rate of analysis is 40 per hour (providing 20 analyses per hour), with samples and standards separated either by empty spaces or by cups containing saline. The use of spacing between specimens prevents carryover contamination of a specimen of low uric acid value following a specimen of high uric acid value, and insures adequate washout of colorimeter and sample line. A standard Auto-Analyzer* is used equipped with a heated dialyzer unit containing a single set of dialyzer plates and a standard-thickness Cellophane dialysis membrane.

A manifold of plastic tubes and glass coils is constructed to provide the required flow rates and to allow mixing of reagents at proper intervals (Fig. 1). Glass "H" connectors with capillary side arms are used to join reagent streams and dialysate stream, with reagents

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*Technicon Instruments Inc., Chauncey, N. Y.
pumped through the capillary portion of the "H" connection. The serum sample (flow rate, 1.2 ml./min.) is segmented with air (1.2 ml./min.) and diluted with saline diluent (1.2 ml./min.). The uric acid in the diluted specimen is dialyzed into a stream of saline diluent (2.5 ml./min.) segmented with air (1.6 ml./min.). The dialysate then is joined by a stream of sodium carbonate (0.6 ml./min.) and mixed in a short glass coil. The dilute phosphotungstic acid reagent (0.6 ml./min.) then joins the alkaline dialysate stream and is mixed in a short glass coil. The reagent stream passes into a double-length glass coil where color development proceeds and then is fed into a 10-mm. colorimeter cuvet. The developed color is recorded as 660 wavelength and compared with a series of standards run concurrently.

Results

Figure 2 illustrates typical calibration curves relating percentage transmission to concentrations of uric acid. The method has been designed to provide adequate sensitivity by dialysis through a single set of dialyzer plates, and is the method recommended for routine use. Some increase in sensitivity can be obtained by double dialysis, as indicated.
Figure 3 shows a typical strip chart recording of the procedure. This illustrates the stable base line at the beginning and end of the analysis, stability and absence of noise during continuous aspiration of a 6-mg.% standard, and reproducibility of several analyses performed on a single 6-mg.% standard. The figure also illustrates the deflections produced by low to high standards in ascending order, and also absence of contamination of the low 3-mg.% standard following the high 12-mg.% standard.

Ten separate recovery experiments were performed in which various amounts of uric acid were added to pooled serum of low uric acid

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**Fig. 2.** Calibration curves relating uric acid concentration to per cent transmission using both single and double dialysis.

**Fig. 3.** Strip chart recording of procedure illustrating: (1) stable base line at beginning and end of analysis, (2) absence of noise level during continuous aspiration of 6-mg.% standard, (3) reproducibility of several analyses performed on a single 6-mg.% standard, (4) low to high standards in ascending order, (5) absence of contamination with low standard following high standard.
content. Recoveries ranged from 97 to 105%, with the average recovery of 100%. The precision of the method (95% confidence as determined by 20 replicate analyses of a single pooled specimen) was 2.8%, which is slightly better than the precision (about 4.0%) of the manual carbonate method and significantly better than the reproducibility of the uricase method (1).

Analyses performed on serum obtained from 25 men and 25 women yielded the expected variation related to sex. The mean normal established for men was 5.6 mg./100 ml., with a standard deviation of ± 0.9. The mean for women was determined as 4.4 mg./100 ml., with a standard deviation of ± 0.8.

Since the major components known to cause false elevations in uric acid in colorimetric procedures are salicylate, ascorbic acid, and high levels of glucose (2), solutions of these substances were analyzed by this method in order to determine the effect of these substances on the phosphotungstic acid reagent under the conditions of the analysis. Solutions of both salicylic acid and sodium salicylate in concentrations of 100 mg./100 ml. produced a small amount of color equivalent to 0.7 mg./100 ml. of uric acid. Salicylate concentrations of 20 mg./100 ml., more comparable to concentrations likely to be encountered clinically, produced no detectable color, suggesting that moderate levels of salicylate do not cause errors in the method. Ascorbic acid at a level of 1.5 mg./100 ml., comparable to a high normal level of ascorbic acid in serum, produced a small chromogenic effect, equivalent to about 0.1 mg./100 ml. of uric acid. A glucose solution of 500 mg./100 ml. concentration produced a small amount of color, equivalent to about 0.2 mg./100 ml. of uric acid.

A small series of serums was analyzed by both uricase and automated colorimetric methods. On the average, the colorimetric value was about 0.3 mg./100 ml. higher than the uricase result. Maximum variations of the colorimetric method on individual serums ranged from 0.3 mg./100 ml. lower to 1.1 mg./100 ml. higher than the uricase method. The results of this comparison are comparable to those obtained by Feichtmeier and Wrenn (3) using a modification of the Kern and Stransky procedure, and by Henry and associates (1) using a carbonate method.

Discussion

In general, all colorimetric methods are based on the reduction of phosphotungstic to a colored phosphotungstite complex in an alka-
line medium, and the methods differ primarily in the type of alkali used and the details of preparation of the phosphotungstic acid reagent. Some methods also employ cyanide to intensify the color of the phosphotungstite complex. Although a significant increase in sensitivity can be achieved by cyanide, this reagent has serious disadvantages. The chief objections to the use of cyanide are its extreme toxicity, high and variable reagent blanks, the poor reproducibility of standard curves when such reagents are used, and the occasional development of turbidities (1,4). These disadvantages are less serious in an automated system of analysis, and an automated method using cyanide is available from the Technicon Company. However, the toxicity of this material and its instability still remain major disadvantages to its use.

The type of alkali used in the colorimetric method appears to make little difference in the color reaction (1). Sodium carbonate was preferred by Caraway (4) because of its stability and buffering capacity, and this alkali was used by Henry and associates in their modification of the Caraway method (1). The two methods differ primarily in the concentration of phosphotungstic acid and sodium carbonate used. Color development is more rapid in the method of Henry because of the higher final concentration of carbonate. In the automated method, the final pH for color development is 10, as in the Caraway method, but the final concentration of carbonate is approximately that used by Henry, in order to accelerate the rate of color development.

Although colorimetric methods are generally quite precise, it is generally recognized that they are not specific for uric acid. These methods are based on the reduction of phosphotungstate and are influenced by nonuric acid reducing substances present in serum. Most authors indicate that the quantity of nonuric acid substances yielding color in manual methods is equivalent to between 0.3 and 0.5 mg./100 ml. uric acid in normal serums, and comparisons between a uricase method and the automated method also indicate positive errors of this magnitude. However, it has been reported that nonuric acid chromogens may be significantly higher in serums with high levels of glucose, and in patients receiving large quantities of salicylates (2,5). The Archibald modification of the Kern and Stransky procedure manifests a better correlation with the uricase method, probably due primarily to alka-linization of the serum with sodium hydroxide prior to precipitation of proteins (6,7). The high pH probably destroys ascorbic acid and sulfhydryl groups which are capable of reducing phosphotungstic
acid. However, the Archibald modification has also been shown to be influenced by nonurate chromogens in the serums of patients on salicylate therapy (5), and similar interference would also be expected from high levels of glucose. In contrast, the automated method is apparently not influenced by salicylate, and only minimal positive errors are introduced by ascorbic acid and high levels of glucose.

The excellent recoveries of uric acid indicate that the automated method also avoids the loss of uric acid which is unavoidable in manual methods requiring the preparation of a protein-free filtrate. This loss of uric acid varies from 5 to 20% depending on the acidity of the protein precipitating solution. Separation of uric acid by dialysis eliminates the negative error in colorimetric methods and contributes to increased specificity.

Normal mean values for males and females correlate with reported normals for the uricase method. However, the range of normal for the uricase method itself is not firmly established. Reported mean values for males range from 5.0 to 5.9 mg./100 ml., and for females, from 3.75 to 4.3 mg./100 ml., (3,5) and it is difficult to define precisely the upper limit of normal by either colorimetric or enzymatic analysis.

References