NEW TITRATION METHOD FOR THE DETERMINATION OF URIC ACID IN URINE.*

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Determinations of pure uric acid have been made by permanganate titrations for many years. The presence of other substances with uric acid in body fluids, which give permanganate values, has necessitated the separation of uric acid before the titration could be carried out. Such separations have never been perfect. Whether thrown down as such by treatment with stronger acids or precipitated as urates, there has always been a part of the uric acid left in solution. Hopkins\(^1\) came nearest the desired separation with his method of precipitation of ammonium urate. Probably the modification of Folin and Shaffer\(^2\) has proved most useful of all, but it is distinctly imperfect since precipitation is incomplete even after 48 hours.

The quantitative precipitation of uric acid by adding a soluble zinc salt and then making the solution alkaline with sodium carbonate has been described in an earlier paper.\(^3\) At that time it was mentioned that Ganassini\(^4\) had described a qualitative test for uric acid based upon a similar precipitation. It later was

* Preliminary reports of this work were presented before the 1916 and 1917 annual meetings of the Society of Biological Chemists. Cf. Morris, J. L., \textit{J. Biol. Chem.}, 1917, xxix, p. xiii; 1918, xxxiii, p. xxi.


\(^3\) Morris, J. Biol. Chem., 1916, xxv, 205.


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discovered that Bellocq\textsuperscript{5} knew of this reaction in 1900 and used it for determining uric acid. His method involved dissolving the precipitate in hydrochloric acid and then weighing that portion of the uric acid precipitated by hydrochloric acid. It is easy to demonstrate that all the uric acid is not precipitated by such treatment. Kashiwabara\textsuperscript{6} described a similar procedure for separating uric acid, then removed the zinc as sulfide before the final precipitation with hydrochloric acid. Both these methods are long and can give, at best, only approximate results. The fact, demonstrated by the writer,\textsuperscript{3} that uric acid can be completely and instantly precipitated as the zinc compound seemed to warrant efforts to combine this means of separation with a satisfactory method of determination. A combination with the colorimetric procedure of Folin and Denis\textsuperscript{7} was attempted but many difficulties were encountered. Among these the most serious was fading due to the presence of the zinc ion. The long used permanganate titration was then applied with more promising results.

A form of analysis which resulted from the combination of zinc precipitation and permanganate titration has been described before.\textsuperscript{8} Two preliminary precipitations for the removal of interfering substances were required before the uric acid could be separated and, though the end-point of the permanganate titration in acetic acid solution was recognizable within a few per cent, it was not entirely satisfactory. An attempt was made to improve the method by using an iodine end-point. The oxidation was carried out by using an excess of permanganate and this was followed with a larger excess of potassium iodide. It was then possible to titrate with standard thiosulfate. This method made use of the more dependable starch iodide end-point but at the cost of more time-consuming work and did not entirely eliminate the error due to spontaneous reduction of permanganate. The double titration was modified in many ways and finally was combined into one titration in which the oxidation takes place in a solution alkaline with sodium bicarbonate.\textsuperscript{8}

\textsuperscript{5} Bellocq, A., \textit{J. pharm. et chim.}, 1900, xii, 103.
\textsuperscript{6} Kashiwabara, M., \textit{Z. physiol. Chem.}, 1913, lxxiv, 223.
\textsuperscript{7} Folin, O., and Denis, W., \textit{J. Biol. Chem.}, 1912-13, xiii, 469.
\textsuperscript{8} Morris, \textit{J. Biol. Chem.}, 1917, xxix, p. xiii; 1918, xxxiii, p. xxi.
The use of bicarbonate was valuable for it controlled the hydrogen ion concentration so well that a mild selective oxidation took place by which all the uric acid was decomposed before any iodine was set free from the iodide. The blue starch iodide end-point could thus be used directly in the permanganate titrations. On the same principle it seemed that probably many of the substances which interfere with permanganate titrations in acid solution would be less easily oxidized than iodides and thus not interfere with the titration in sodium bicarbonate. The following substances were added to test titrations without any apparent effect: allantoin, caffeine, dextrose, hippuric acid. Phenols affected the end-point, causing a slow fading of the blue color, but earlier work had shown that the zinc precipitation separated uric acid quantitatively from phenols. The results from the few substances tested suggested that possibly the preliminary precipitations of interfering substances were not necessary when oxidizing in the presence of sodium bicarbonate, and investigation showed that such was substantially the case. This made it possible again to shorten the method. The final procedure, described in this paper, requires 30 to 40 minutes for the complete determination. It has proved entirely satisfactory for urine. For the much smaller quantities of uric acid in blood it gives good results also but its usefulness is limited, as is that of all other methods, by the imperfect procedures for removing proteins without at the same time carrying down other substances. When applied to the filtrates from the usual 0.01 N acetic acid precipitation the results agree with those obtained with the colorimetric method.

The steps by which the writer developed the volumetric method here described are given in detail, not only because of his belief that some light has been thrown upon the oxidation of uric acid under varying conditions but in order also to show that the idea of a titration method for determining uric acid has been consistently followed by him for more than 2 years. Curtman and Lehrman have published a volumetric method for uric acid in which the underlying principles are substantially the same as those previously set forth by the writer.

Determination of Uric Acid in Urine

Method.

Reagents.

Preparation of Standard Potassium Permanganate.—A stock 0.1 N solution is made up in the usual manner (3.166 gm. per liter) and kept in a tightly stoppered amber colored bottle. After the usual preliminary period, during which the value decreases somewhat, the value remains as nearly constant as its use in the preparation of 0.002 N solutions requires. When the dilute solution is needed, 10 cc. of the stock solution are measured with a pipette into a 500 cc. volumetric flask and made up to the mark. The resulting 0.002 N solution may then be standardized against a standard uric acid-phosphate solution10 by a titration such as that described later in this paper for the uric acid separated from urine. Three different 0.1 N stock solutions have upon dilution given values of 0.140, 0.143, and 0.139 mg. per 1 cc. of the 0.002 N solutions. If it is considered more convenient, the stock permanganate solution may be standardized in one of the usual ways and its equivalent value in uric acid calculated on the basis of 1 cc. of 0.1 N permanganate being equal to 7.50 mg.1 or 7.22 mg.11 of uric acid. According to our experience with the titration in bicarbonate solution 1 cc. of 0.1 N permanganate is equivalent to 7.32 mg. of uric acid. The dilute permanganate solution keeps its value for a relatively short time, but in no case have we found it to vary appreciably during a day's work.

Other Reagents Required.—Saturated disodium phosphate solution; 10 per cent zinc chloride solution; 10 per cent calcium chloride solution; 10 per cent potassium iodide solution; 20 per cent sodium carbonate solution; 0.5 per cent soluble starch solution (starch will serve but soluble starch gives a better end-point); solid sodium bicarbonate.

Determination.

The procedure as now used is as follows: Pipette 5 cc. of urine into a 50 cc. or 100 cc. centrifuge tube and add 3 cc. of 10 per

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cent zinc chloride. Add 25 cc. of water, mix with a stirring rod, put in a small piece of litmus paper, and add enough 20 per cent sodium carbonate, with stirring, to turn the litmus entirely blue (not the violet shade of neutrality or weak alkalinity). A heavy precipitate of zinc carbonate, zinc phosphate, and zinc urate separates from a clear liquid. Now centrifugalize for 2 or 3 minutes; carefully pour off the liquid, discarding it. The precipitate is sufficiently packed down to remain in the tube. Dissolve this in dilute hydrochloric acid, with stirring. To the acid solution add 15 cc. of saturated disodium phosphate and, if necessary, a few drops more of dilute hydrochloric acid to dissolve a precipitate of zinc phosphate. Finally pour in 2 cc. of 3 per cent uranium acetate and, while stirring, again make the contents of the tube alkaline with sodium carbonate. Centrifugalize for 2 or 3 minutes and pour clear liquid carefully into a 300 to 500 cc. Erlenmeyer flask for titration. In the presence of the large excess of phosphates none of the uric acid will precipitate, while the zinc and uranium have carried down all other substances which would have given a value in the permanganate titration.

Acidify the alkaline liquid containing the uric acid with hydrochloric acid and add 25 cc. of 10 per cent calcium chloride. Then add excess of solid sodium bicarbonate. Calcium phosphate will precipitate when the acid reaction has been neutralized. An excess of sodium bicarbonate beyond this point is desirable. To the very weakly alkaline solution so prepared for titration add 1 cc. of 10 per cent potassium iodide, 3 cc. of 0.5 per cent soluble starch, and enough distilled water to make the entire volume within a few cc. of 250 cc. Now titrate with 0.002 N potassium permanganate until a blue shade spreads throughout the liquid. The color of the drop of permanganate as it hits the surface changes from pink to blue and assists in judging the approach of the end-point. Comparison of the flask from time to time with another flask containing the duplicate awaiting titration or a blank arranged for the purpose has been found to assist in arriving at the end-point. The number of cc. of standard permanganate run in from the burette needs a correction for a blank value corresponding to the amount of free iodine necessary in 250 cc. to show a perceptible blue color. This can easily be
determined for the standard being used by titrating a blank containing 1 cc. of 10 per cent potassium iodide, 3 cc. of 0.5 per cent starch, and 250 cc. of water. It has had a value of 0.8 cc. of permanganate in our experience. Subtracting this figure from the observed reading gives the actual number of cc. of 0.002 N permanganate required for the uric acid present. Multiplying this by the value of each cc. of the permanganate gives the amount of uric acid present in 5 cc. of urine.

**TABLE I.**

Comparative Uric Acid Estimations.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>gm. per 24 hrs.</td>
<td>gm. per 24 hrs.</td>
<td>gm. per 24 hrs.</td>
</tr>
<tr>
<td>F. H.*</td>
<td>0.592</td>
<td>0.592</td>
<td>0.527</td>
</tr>
<tr>
<td></td>
<td>0.586</td>
<td>0.592</td>
<td></td>
</tr>
<tr>
<td>M. S.*</td>
<td>0.505</td>
<td>0.493</td>
<td>0.464</td>
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<td></td>
<td>0.513</td>
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<td></td>
</tr>
<tr>
<td>Dr. C.†</td>
<td>0.597</td>
<td>0.573</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.577</td>
<td>0.577</td>
<td></td>
</tr>
<tr>
<td>Mrs. C.‡</td>
<td>0.270</td>
<td>0.318</td>
<td>0.231</td>
</tr>
<tr>
<td></td>
<td>0.323</td>
<td>0.357</td>
<td>0.279</td>
</tr>
</tbody>
</table>

* Normal.
† Nephritis.
‡ Toxic vomiting of pregnancy.
|| Pregnancy.

**DISCUSSION.**

It will be noted from the typical results recorded in Table I that the agreement between duplicates of the zinc precipitation-alkaline oxidation procedure is as good as those with the colorimetric method. In normal urines the values with both are practically identical. There are some irregularities in the figures given for the pathological cases. These irregularities are present in most of the pathological urines so far examined. Attempts have been made to locate the cause for this in the proposed method by the addition of such substances as might possibly be
present. The absence of any effect which can be attributed to any of the substances tried (Table II) leaves the difficulty unsolved. The figures given for the usual volumetric method (ammonium urate precipitation-acid oxidation) show that all results with that method are decidedly low.

Table II lists a few substances that might be present in urine and might, because of their nature, be suspected of affecting the titration values. The variation in titration values, with the exception of phenols, is no more in their presence than is found between separate titrations of pure solutions. Phenol gives a value in the titration and the end-point is a fading one, indicating the continued oxidation of phenol. This substance, of all those tried, most closely resembles uric acid in the selective oxidation process used. Fortunately the separation of uric acid from phenols by precipitation of the former as the zinc salt precludes the presence of phenols in the final titration.

**SUMMARY.**

A volumetric method for the determination of small amounts of uric acid has been developed and described in detail for urine. It is based upon the instantaneous and complete precipitation of uric acid as the zinc salt and a single direct titration with permanganate in a solution alkaline with sodium bicarbonate. The end-point used is the blue starch iodide color and, because of the

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**TABLE II.**

**Selective Oxidation of Uric Acid by Potassium Permanganate in Solution Made Alkaline with Sodium Bicarbonate.**

<table>
<thead>
<tr>
<th>Substance added</th>
<th>Amount of 0.002 N KMnO₄ required for uric acid</th>
<th>Amount of 0.002 N KMnO₄ required for substance + uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>Caffeine</td>
<td>7.0</td>
<td>7.1</td>
</tr>
<tr>
<td>Allantoin</td>
<td>7.1</td>
<td>7.0</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>7.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>7.1</td>
<td>7.2</td>
</tr>
<tr>
<td>Phenol</td>
<td>7.0</td>
<td>7.8+*</td>
</tr>
</tbody>
</table>

* End-point indefinite and fading.
Determination of Uric Acid in Urine

mild nature of the oxidation and the excess iodide present, there is no fading to confuse the end-point.

The results on the usual blood filtrate obtained by precipitating proteins with dilute acetic acid agree with those obtained with the colorimetric method. Because of the usual difficulties which attend this preliminary precipitation of blood proteins, no detailed report has been made here of results of this application of the method. Attempts are still being made to find a precipitant of proteins which will make it possible to obtain from blood entirely reliable results with this sensitive volumetric method.
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