STUDIES ON URIC ACID AND RELATED COMPOUNDS

I. QUANTITATIVE DETERMINATION OF URIC ACID IN BIOLOGICAL FLUIDS

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The available methods of determination of uric acid can be divided into three main groups: (a) the direct determination of the reducing power of uric acid; (b) the coprecipitation of the uric acid or its adsorption on a precipitating material, dissolving the acid, and determination of its reducing power; and (c) quantitative decomposition of uric acid by uricase.

A survey of these methods has been given recently (1). The direct determination of the reducing power of uric acid is not specific at all, and the reaction is not stoichiometric. Therefore, slight variations in the preparation of the oxidizing reagent influence the results considerably. In addition, the non-linear relationship between concentration and optical density makes accurate measurement difficult. The method of precipitation is more specific, but again suffers from lack of a stoichiometric reaction in the final determination. The enzymatic determination is sufficiently specific and accurate (2), but requires exactly controlled conditions and new standards whenever the enzyme preparation is changed. Therefore the uricase method is not easy to handle and is unsuitable for routine work. None of the methods mentioned permits the quantitative analysis of substituted uric acids such as appear in serum and urine after ingestion of xanthines. The only method applicable so far to this problem involves complicated counter-current extractions (3).

The lack of a quick and reliable method for uric acid determination, especially in plasma analysis, led Peters and Van Slyke to make the statement (4) that "values given for uric acid of whole blood, or even plasma, must be accepted with reservations." Likewise uric acid clearance has not yet been satisfactorily measured owing to the lack of "analytical methods of undoubted specificity and accuracy" (4).

It is the purpose of this series of investigations to study quantitatively the clearance of uric acid, the metabolism of xanthines, and the influence of these substances or their metabolites on uric acid excretion. Undoubtedly the most accurate analytical method for uric acid consists of the determination of the optical density at 290 m\(\mu\) (5); however, numerous other metabolites absorb in this region, and, in urine, urochrome as well
as indoxyl derivatives prevent the application of this procedure. It therefore appears necessary to remove uric acid selectively from biological fluids in order to apply the spectrophotometric determination. In the search for insoluble derivatives, our attention was focused on mercuric urate (6). Since the solubility product of this compound is greater than the concentration of mercuric ions, obtained by dissociation of mercuric chloride, it is possible to dissolve the precipitate of mercuric urate in sodium chloride. A method was developed which precipitates uric acid selectively in an acid medium, even in the presence of xanthines and methyl-substituted uric acids, which also form insoluble complexes but are soluble in an excess of the reagent used (see below). Therefore, these substances do not interfere with the determination of uric acid, provided that their concentration in plasma is below 0.2 mg. per ml. and in urine below 1 mg. per ml.

An important prerequisite for the applicability of the procedure is appropriate deproteinization, since part of the uric acid is easily lost by adsorption on the protein precipitate if the details described below are not scrupulously followed. Since procedures requiring the use of heavy metals are precluded, and since our method involves precipitation of mercuric urate in acid medium, it is convenient to carry out deproteinization by an acidic reagent. The method of Neuberg and Strauss (7), which utilizes dilute perchloric acid, serves the purpose admirably.

**Materials—**

Solution 1, 0.1 M mercuric acetate in 5 per cent acetic acid.
Solution 2, 0.5 M sodium chloride in 1 per cent acetic acid.
Solution 3, wash liquid for the precipitate. To 1 liter of 0.01 M mercuric acetate in 1 per cent acetic acid is added a solution of 10 mg. of uric acid in water. The mixture is now heated for about 1 hour to 80-90° to complete precipitation and then left overnight. It is filtered and kept in a closed bottle.
Solution 4, 10 per cent sodium tetraborate, Na₂B₄O₇·10H₂O in 7 per cent NaOH. Other reagents; 6 per cent perchloric acid, 3 per cent sodium tetraborate.

**Method for Determination of Uric Acid in Urine**

Protein-Free Urine—Dilute the urine 50 times. Put 1 ml. of Solution 1 into a centrifuge tube, kept in a gently boiling water bath, and, during 4 minutes, add 4 ml. of the diluted urine dropwise with continuous swirling. Then heat the tube for 10 minutes and cool rapidly in ice water. Centrifuge at 3000 r.p.m. for 10 minutes. The supernatant fluid is now decanted cautiously, the last part being removed with a small pipette, care being taken not to stir up the precipitate. The precipitate is dispersed in 5 ml. of Solution 3 and again centrifuged, and the supernatant fluid is removed
as before. Then 8 ml. of Solution 2 are added and the precipitate is dissolved by shaking. If necessary, filter by gravity. The filtrate is read in a Beckman ultraviolet spectrophotometer at 290 m\(\mu\). The optical density found, when multiplied by 1.55, gives the concentration of uric acid in mg. per ml. of undiluted urine. If the density is too high, Solution 2 is used for further dilution, and the calculation is changed accordingly. If the initial concentration of uric acid is too small, the first dilution is diminished as required.

Uric acid, at pH <4, exhibits its absorption maximum at 285 m\(\mu\). However, since the reagents used, and also possible contaminants which may accompany the precipitate, still absorb at this wave-length, we have selected 290 m\(\mu\) as the region in which the "background absorption" becomes negligible.

Protein-Containing Urine—Dilute the urine five times, add 3 ml. of this dilution to 3 ml. of perchloric acid, and filter off the precipitate by gravity. The filtrate is diluted five times with borate. Put 1 ml. of Solution 1 into a centrifuge tube, kept in a gently boiling water bath, and add 4 ml. of the deproteinized dilution. Continue as before.

Method for Determination of Uric Acid in Plasma

Centrifuge 5 ml. of heparinized (or citrated, but not oxalated) blood for 10 minutes and dilute the plasma 5-fold with water. To 10 ml. of perchloric acid add 10 ml. of the diluted plasma drop by drop, with continuous swirling. Then heat to 60° for 5 minutes, cool in ice water, and filter by gravity. To 10 ml. of filtrate add 2.5 ml. of Solution 4, mix well, and take out 10 ml., to which add 2 ml. of Solution 1. Boil the mixture for 1 minute over an open flame and leave for 5 minutes in a gently boiling water bath. Then centrifuge at 3000 r.p.m. for 10 minutes and remove the supernatant fluid cautiously. The precipitate is dissolved in 8 ml. of Solution 2 and, if necessary, filtered by gravity. The optical density is determined as before and multiplied by 0.155 to convert it into mg. of uric acid per ml. of undiluted plasma.

This procedure permits an accurate determination of a minimal concentration of 20 \(\gamma\) per ml. of undiluted plasma. If lower values have to be measured, more plasma is taken initially, and the whole procedure is carried out with correspondingly larger volumes. The final precipitate is again dissolved in 8 ml., and the factor calculated accordingly.

When the presence of substituted xanthines or their metabolites can be excluded, it is not necessary to precipitate the uric acid from plasma, since we have observed that the deproteinized solution can usually be read directly. In this case, however, a blank value of 0.04 has to be deducted from the optical density. The optical density per microgram of uric acid
per ml. is 0.0645 at 290 m\(\mu\) in 1 per cent acetic acid. Therefore, a final concentration of 2 \(\gamma\) per ml. can be determined with an accuracy of \(\pm 3\) per cent. As shown in Table I, extinction is a linear function of concentration within the range of 1 to 15 \(\gamma\) per ml., the upper limit being set only by the instrument used. Since final readings are taken on a solution of pH \(<4.0\), uric acid (pK = 5.8) is practically all in the form of the free acid.

Table I

Comparison of Uric Acid Determination by Hg and by Folin-Wu Method

<table>
<thead>
<tr>
<th></th>
<th>Hg method</th>
<th>Folin-Wu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original urine, undiluted</td>
<td>0.70</td>
<td>0.58</td>
</tr>
<tr>
<td>&quot; &quot; diluted 2 times</td>
<td>0.35</td>
<td>0.40</td>
</tr>
<tr>
<td>&quot; &quot; 4 &quot;</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>&quot; &quot; 8 &quot;</td>
<td>0.085</td>
<td>0.07</td>
</tr>
<tr>
<td>Original urine &quot; &quot; after addition of 1 mg. per ml. uric acid</td>
<td>0.64</td>
<td>1.66</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The precipitation of uric acid via its mercuric derivative is specific and quantitative. The following substances did not change the uric acid values by more than \(\pm 5\) per cent, if their concentration just before the addition of mercuric acetate did not exceed 20 \(\gamma\) per ml.: 1- and 3-methyl-uric acid, 1,3-dimethyluric acid, xanthine and its methylated derivatives, and all common barbiturates.

It was also found that cysteine or glutathione is not precipitated in the presence of excess mercuric acetate and thus does not interfere with the determination of uric acid.

The procedure is based on the observation that, among all related substances, mercuric urate possesses by far the lowest solubility in excess of mercuric acetate in the presence of 1 per cent acetic acid. As shown in Table I, optical density is a linear function of concentration within the range readable on the instrument. The method has proved itself suitable for routine use in clinical laboratories.

**SUMMARY**

Uric acid can be determined quantitatively in biological fluids, even in the presence of xanthines and substituted uric acids, by selective precipitation as mercuric salt, redissolution in sodium chloride, and measurement of the optical density at 290 m\(\mu\).
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BIBLIOGRAPHY
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