Comparison of the Archibald–Kern and Stransky Colorimetric Procedure and the Praetorius Enzymatic Procedure for the Determination of Uric Acid

Carl Alper and Joseph Seitchik

The establishment and acceptance of an analytic procedure as a method to be relied upon with complete confidence demands: (1) specificity of the method, i.e. minimal interference by other compounds; (2) quantitative recovery of the specific substance when it is added to the medium in which it is usually determined; and (3) reproducibility of results within reasonable limits on replicate samples.

The colorimetric methods for the determination of uric acid depend upon the mild oxidation of uric acid in an alkaline or acid medium by molybdate-free phosphotungstic acid (1, 2), arsenotungstic acid (3, 4), or ferricyanide (5, 6, 7). The latter method has been used in combination with the specific enzyme uricase. Among the sources of error associated with these procedures are the inhibition of the uric acid reaction by some of the blood constituents and the precipitation of uric acid with the serum protein in the preparation of a protein-free filtrate, which would yield results below those obtained by uricase. The presence of other substances in blood filtrates which may also reduce the nonspecific oxidizing agents...
and the occurrence of turbidity due to instability of reagents would yield results greater than those obtained by uricase. Lous and Sylvest (8) have demonstrated that most of the results of 188 samples of serum determined by the Folin procedure and compared with results of the enzymatic procedure are less than those obtained by uricase. On the other hand, of 233 samples of serum assayed by the titrimetric method and compared with the enzymatic method, all of the results are greater than those obtained by the uricase method. Indeed, it is interesting to note that, as chemical technics are perfected, the average figures of blood uric acid levels have become greater, whereas the normal range of values has become more limited.

Since there are interfering substances such as ascorbic acid, glucose, amino acids, and glutathione normally present in the blood and urine which can be readily oxidized, the analytic problem is twofold: (1) the selection of an oxidizing agent in the proper medium with an oxidation-reduction potential which will just satisfy the demands for the oxidation of uric acid and (2) the selection of a system for the development of color which will be specific stoichiometrically for the products of oxidation of uric acid, the unused oxidizing agent, or the reduced form of the oxidizing agent. When this has been accomplished, one can then evaluate the over-all qualitative and quantitative specificity of the method for uric acid.

It would be desirable also to compare this method with a procedure of known specificity, one which may not lend itself to the routine clinical chemistry laboratory. The problem is resolved then in the use of the Archibald modification (10) of the Kern and Stransky (11) method for the assay of uric acid in blood and a comparison of results obtained by this method with those obtained by the enzymatic method of Praetorius (12, 13).

METHODS

A detailed account of the colorimetric procedure for the determination of uric acid is described by Archibald (10) in an accompanying paper in this issue of CLINICAL CHEMISTRY. The enzymatic procedure was described by Praetorius (12, 13).

RESULTS

Spectral Characteristics

In photometric analysis the percentage of light transmitted through a colored solution is related to the concentration of the light-absorbing
material. Figure 1 demonstrates that the blue color which develops from
the reduction of phosphotungstic acid by uric acid follows Beer's law.
The color formation is a function of time and the readings on the photom-
eter should be taken at exactly 15-minute intervals after the addition
of the special uric acid reagent. The absorbance measurements are obtained
on a spectrophotometer with light of wave length of 700 m, or with the
aid of a 660 red filter in a photoelectric colorimeter.

Reliability of the Method

The specificity of the colorimetric method is demonstrated by the
quantitative recovery of uric acid added to serum. The results depicted
in Table 1 illustrate that the excellent recovery of uric acid by this pro-
cedure is of the same order of accuracy as that observed for the enzymatic
procedure.

![Standard curve of serum uric acid.](image)

**Table 1. Determination of Uric Acid**

<table>
<thead>
<tr>
<th>Serum Conc. (µg./ml.)</th>
<th>Archibald data (µg./ml.)</th>
<th>Per cent recovery</th>
<th>Serum Conc. (µg./ml.)</th>
<th>Proctorius data (µg./ml.)</th>
<th>Per cent recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47.2</td>
<td>. .</td>
<td>0</td>
<td>52.6</td>
<td>. .</td>
</tr>
<tr>
<td>5</td>
<td>52.2</td>
<td>100</td>
<td>8.1</td>
<td>60.2</td>
<td>99.1</td>
</tr>
<tr>
<td>10</td>
<td>57.2</td>
<td>100</td>
<td>13.25</td>
<td>65.8</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>61.6</td>
<td>99.3</td>
<td>27.3</td>
<td>81.0</td>
<td>101</td>
</tr>
<tr>
<td>20</td>
<td>66.0</td>
<td>101</td>
<td>37.7</td>
<td>90.0</td>
<td>99.6</td>
</tr>
<tr>
<td>25</td>
<td>71.9</td>
<td>99.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A comparison of results obtained by both procedures on the same sample of serum in sixty-one instances demonstrates that the Archibald colorimetric procedure yields results which are 96.1 ± 1.4 per cent of the result obtained by the specific enzymatic procedure. The actual range is 94.3 per cent to 98.0 per cent of the enzymatic procedure, which is less than two standard deviations of the mean percentage.

Duplicate analysis of serum uric acid is demonstrated most satisfactorily by a scattergram (Fig. 2), which illustrates linear correlation in 109 duplicate determinations. The determination of the standard deviation for duplicate analyses yields a value of 0.1 according to the formula of Youden (14).

\[
\text{S.D.} = \frac{1}{2n} \times d^2
\]

where \(d\) is the differences between duplicates and \(n\) is the number of degrees of freedom.

![Fig. 2. Duplicate analysis of serum uric acid.](image-url)
Normal Distribution of Values

A study of the distribution of values on 143 samples of serum from blood donors to the American Red Cross demonstrates a bimodal distribution (Fig. 3). This would indicate the presence of two distinct population groups based on a parameter which is sex-linked. The mean value for a male group of 95 samples is $5.4 \pm 0.82$ mg./100 ml. of serum; whereas the mean value for a female group of 48 samples is $4.0 \pm 0.72$ mg./100 ml. serum. A determination of the significance of the difference between the two means (15) presented in Table 2 yields a value for $t$ which demonstrates that the mean values represent results derived from two distinct population groups.

![Distribution of values of serum uric acid](image)

**Table 2. Determination of Uric Acid**

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations</td>
<td>95</td>
<td>48</td>
</tr>
<tr>
<td>Mean</td>
<td>$5.4 \mu g/ml. \pm 0.82$</td>
<td>$4.0 \mu g/ml. \pm 0.72$</td>
</tr>
<tr>
<td>$t$</td>
<td>$\bar{X}<em>{males} - \bar{X}</em>{females}$</td>
<td>$S.E. \text{ of difference}$</td>
</tr>
<tr>
<td>$t$</td>
<td>9.9</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Uric Acid Level in Serum (Apparentiy Normal Individuals)

<table>
<thead>
<tr>
<th>Author</th>
<th>Method and Reference</th>
<th>Sex of Subject</th>
<th>No. observed</th>
<th>Mean value (µg./mL)</th>
<th>Range (µg./mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brøchner-Mortensen (7)</td>
<td>(7)</td>
<td>M</td>
<td>33</td>
<td>7.6</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>37</td>
<td>6.4</td>
<td>..</td>
</tr>
<tr>
<td>Natelson and Kaser (18)</td>
<td>Brown (2)</td>
<td>M + F</td>
<td>51</td>
<td>4.3</td>
<td>2.4-5.9</td>
</tr>
<tr>
<td>Gjørup, Poulsen, and Praetorius (16)</td>
<td>Praetorius (13)</td>
<td>M</td>
<td>157</td>
<td>5.04</td>
<td>2.6-7.5</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>143</td>
<td>3.84</td>
<td>2.0-5.7</td>
<td></td>
</tr>
<tr>
<td>Alper and Seitchik</td>
<td>Archibald (10)</td>
<td>M</td>
<td>95</td>
<td>5.4</td>
<td>3.8-7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>45</td>
<td>4.0</td>
<td>2.6-5.4</td>
</tr>
</tbody>
</table>

Although the sex difference in plasma or serum uric acid concentration has been noted previously (7, 16, 17), in general it has been overlooked, as evidenced by the values reported in Natelson and Kaser (18). The values reported in Table 3 agree well with results based upon the enzymatic procedure.

DISCUSSION

From a clinical point of view, the determination of serum uric acid with precision and accuracy is desirable. There is no question that an enzymatic procedure would fulfill these requirements. On the other hand, the enzymatic procedure, either as devised by Praetorius (12, 13) or as modified by Feichtmeir and Wrenn (17), requires the use of an ultraviolet spectrophotometer. Such equipment is not always available in the clinical chemistry laboratory. Consequently, it is desirable to provide a colorimetric procedure which approximates or very closely approximates the specific enzymatic procedure.

The colorimetric method devised by Archibald (10) has fulfilled all of the requirements set forth for the establishment of the reliability of an analytical procedure. It is specific in respect to recovery, lack of interference by other reducing substances found in serum in usual quantities, and in comparison with the specific enzymatic procedure. In addition the method is rapid and easily carried out.

The objections raised by Feichtmeir and Wrenn (17) to the Archibald modification of the Kern and Stransky procedure can be overcome if the procedure as described is followed exactly. Feichtmeir (18) in adapting the Archibald procedure did not alkalinate the plasma prior to precipitation of protein, prepared a more acid serum filtrate, and used a different proportion of reagents to develop color. Alkalization of the serum may be expected to destroy interfering substances such as ascorbic acid or sulfhydryl groups. The acidity of the serum or plasma filtrate and the proportions of reagents will affect color formation.
These results are significant in that they provide proof of the adequacy of a procedure which can find universal use in clinical chemistry.

Great emphasis should be placed on the difference between values for males and females. It is interesting to note that the blood levels of uric acid in pregnancy fall within the range for males. At this point we offer no explanation for this fact. It is rather difficult to assign limits for normal range of values. We agree with Sjørup, Poulsen, and Praetorius (16) that this is an arbitrarily defined limitation and further concur that two times the standard deviation of a single observation which should include 95.5 per cent of all observations would make desirable limits.

SUMMARY

The Archibald modification of the Kern and Stransky procedure for uric acid has been studied and its efficacy for routine use in clinical chemistry evaluated. Mean values and the range of values for men and women have been determined.

REFERENCES

1. Folin, O., J. Biol. Chem. 101, 111 (1933); 106, 311 (1934).
19. Feichtmeir, T. V. Personal communication.