THE DIRECT COLORIMETRIC DETERMINATION OF UREA IN BLOOD AND URINE

By S. B. BARKER

(From the Department of Physiology, College of Medicine, University of Tennessee, Memphis)

(Received for publication, November 22, 1943)

Methods for the determination of urea in biological fluids have passed through an interesting cycle (cf. Peters and Van Slyke (3) pp. 539-544). The earliest procedures were strictly chemical ones, involving precipitation of the urea as an insoluble mercury complex or decomposition of the urea by means of heat or hypobromite. The use of the highly specific enzyme urease occurring in the jack and soy bean, introduced in 1913 by Marshall, has been the foundation of all generally accepted procedures since that time, although heat and hypobromite are still occasionally employed clinically as being more convenient than urease. The most widely used modification is that in which the ammonia formed by enzymatic action is aerated into acid and titrated or nesslerized. Many attempts have been made to work out a simpler procedure with direct nesslerization of the urease-treated filtrate in order to avoid aeration; however, turbidity too often materializes to warrant the wide acceptance of direct nesslerization.

Fosse introduced xanthydrol as a reagent for precipitating urea, but this method has not gained wide usage, principally because of its lack of dependability. In 1942, Ormsby reported a technique for the direct colorimetric determination of urea itself, using diacetyl monoxime, a reagent employed by Fearon for the estimation of citrulline (2). Thus, the determination of urea has turned away from enzymatic methods and back to more strictly chemical ones. This change certainly involves a decrease in specificity, but at the same time a tremendous increase in convenience. Urease methods will remain the standards of reference, but need not be required for many ordinary purposes. The present paper describes a further modification of the diacetyl monoxime procedure, rendering it more uniform and convenient.

EXPERIMENTAL

Reagents—

Sulfuric acid. A 50 per cent by volume solution. This should be prepared by the cautious addition of 500 ml. of c.p. sulfuric acid to 500 ml. of distilled water in a Pyrex vessel. The solution should be thoroughly cooled after being mixed, transferred to a liter volumetric flask or to a graduated cylinder, and made to volume. This elaborate procedure eliminates gross

Downloaded from http://www.jbc.org/ by guest on October 27, 2017
variabilities resulting from the volatilization of water from the hot mixture as the acid is being added.

Diacetyl monoxime. 3 per cent aqueous solution. This solution can be kept indefinitely if stored in a refrigerator when not in use. It is even stable for 4 weeks at room temperatures of 30–40°, after which time there are indications of changes in the solution.

Potassium persulfate. 1 per cent aqueous solution. Samples of this reagent have been kept in the refrigerator for as long as 8 weeks, and one solution for 6 weeks at elevated room temperatures, with no indication of deterioration. However, it would seem wise to renew the solution every 4 to 6 weeks, and to store it, when not in use, in a refrigerator.

Urea standards. A convenient stock standard is one which contains exactly 1 mg. of urea N per ml., obtained by dissolving 1.0717 gm. of desiccator-dried c.p. urea in distilled water and making the solution to 500 ml. A few drops of toluene or chloroform should be used as a preservative. This stock solution can be diluted to whatever concentration may be desired.

Protein Precipitation—The routine Folin-Wu tungstate or Somogyi's zinc procedures can be used (cf. (3) p. 65). If the latter is to be employed, the modification in which an acid zinc sulfate solution is used (4) is recommended. Trichloroacetic acid does not give satisfactory results.

Procedure

If the blood urea level is completely unknown, 1.0 ml. of a 1:10 protein-free filtrate is used plus 1.0 ml. of distilled water; if the level is known to be less than 75 mg. of urea N per 100 ml. of blood, 2.0 ml. of filtrate are used, without the addition of water. In urea clearance tests, the greater accuracy of the procedure with 2.0 ml. is highly desirable.

The 2.0 ml. of urea-containing solution are placed in a Klett-Summerson colorimeter tube, followed by 0.25 ml. of the 3 per cent diacetyl monoxime solution. 4 ml. of the 50 per cent sulfuric acid are added, and the contents thoroughly mixed.

The tubes are placed in a suitable rack, and put into vigorously boiling water for 10 minutes. Glass marbles are used to cover the open ends of the tubes while they are being heated. At the end of the 10 minute heating period, 0.25 ml. of the 1 per cent persulfate solution is added to each tube not later than 5 minutes after the tubes are taken from the bath. Each tube should be shaken immediately after the persulfate is added to mix the contents thoroughly and quickly. The tubes are then left at room temperature.
The calorimeter reading on each tube should be made 15 minutes after the addition of persulfate. In this laboratory, the readings are made with a Klett-Summerson photoelectric calorimeter (5), with the Klett No. 42 blue filter. If the reading is higher than 500, representing more than 150 $\gamma$ of urea N, the determination can be saved by adding 5.0 ml. of distilled water, mixing thoroughly, and reading. Such a dilution tested on standard solutions, urines, and uremic blood filtrates containing up to 250 $\gamma$ has given, within 5 per cent, the same intensity of color as that produced by using one-half of the original aliquot. By this simple expedient, the range of the method can be extended by two-thirds, to 250 mg. per cent of urea N.

The procedure has been successfully applied to urine, without any necessity for the preliminary removal of ammonia with permutit. A convenient dilution for normal urines has been found to be 1:200 if 2.0 ml. are to be used, or 1:100 with 1.0 ml. If unusually dilute or concentrated urines are encountered, these dilutions must, of course, be altered correspondingly.

Results with Method—Fig. 1 shows the relationship obtained in this laboratory between micrograms of urea N and calorimeter readings (after the blank value for the reagent is subtracted). It will be noted that a straight line is obtained below 40 $\gamma$, enabling one to use a simple proportionality calculation for this lower portion. If amounts higher than 40 $\gamma$ are encountered, the curve must be used. When the aliquot is 1.0 ml. of a 1:10 filtrate, the dilutions are such that the micrograms of urea N in the 1.0 ml. are also the milligrams of urea N per 100 ml. of blood. When 2.0 ml. of filtrate are taken, the value in micrograms must be divided by 2 in order to give mg. per cent. Amounts present in filtrates representing dilutions other than 1:10, in aliquots other than 1.0 or 2.0 ml., or in diluted urines can easily be calculated.
It is now generally recognized that any colorimetric procedure is more accurate if a photoelectric colorimeter is used instead of visual comparison of the colors; this is especially true for yellows. However, many readings of blood filtrate and urine colors have been performed in this laboratory with a visual colorimeter containing a blue filter. Provided that not less than 20 μ of urea N are present in the sample taken, clinically satisfactory results have been obtained. This necessitates the use of 2.0 ml. of filtrate for normal blood.

**Proof of Method**—The customary comparison and recovery tests have been conducted with this procedure. Table I summarizes the results obtained with human blood. As the reference, a well standardized urease procedure was used, in which the ammonia formed from the urea by the enzyme was diffused over into acid in a Conway unit and determined by nesslerization (1). Most of the results shown were obtained on the Folin-Wu tungstate filtrate; as can be seen from Table I, good agreement was obtained between the two procedures applied to filtrates covering a wide range of values. Furthermore, 5.0 to 100.0 mg. per cent of urea N added to various blood samples before protein precipitation were quantitatively recovered. The Somogyi zinc precipitation was also employed in many instances, and in general proved to be satisfactory. However, in several cases of very low urea levels, the zinc filtrate gave distinctly lower values than the tungstate, as indicated by the use of urease. No recoveries of added urea were made in these instances, and the cause of the low results is not known.

Table II shows that equally satisfactory results can be obtained with human urines. The values obtained with this diacetyl monoxime technique agreed well with those obtained when urease was used, and the recoveries were excellent. Inasmuch as ammonia does not react with the diacetyl reagent, urines do not need to be treated with permutit, but can be diluted and run directly. The pigment should be adequately disposed of by the 1:200 dilution; if not, permutit can be used for decolorization.

No attempt has been made in this study to check all the substances which might yield color with this procedure. Such complete lists are available for other procedures with a diacetyl monoxime reagent (cf. Ormsby (2)). It was thought quite adequate to determine the effects of only those substances likely to be encountered in blood and urine. Uric acid, creatine, creatinine, and several amino acids (glycine, alanine, glutamic acid, arginine, lysine, asparagine), all in amounts equivalent to 20 mg. of N per 100 ml. of blood, gave no color themselves and did not interfere with the color intensity of 20 mg. per cent of urea N. Neither ammonia equivalent to 50 mg. of N per 100 ml. of blood nor glucose (500 mg. per cent) reacted or interfered.
Allantoin, on the other hand, gave about 70 per cent as much color as urea when calculated on the basis of the two ureido nitrogens (a logical procedure, since the essential difference between allantoin, which reacts, and uric acid, which does not, is the presence in the former of the opened ureido chain). This is of no concern for human blood or urine. However, in the analysis of blood or urine from animals with a high uricolytic index, such as the dog, the colorimetric values should represent urea plus allantoin.

From Tables II and III, it can be seen that the diacetyl monoxime method

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Urea N</th>
<th>Added</th>
<th>Recovered</th>
<th>Urea N</th>
<th>Added</th>
<th>Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>per cent</td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>1</td>
<td>5.2</td>
<td>10.0</td>
<td>96</td>
<td>4.5</td>
<td>15.0</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>10.0</td>
<td>98</td>
<td>5.2</td>
<td>10.0</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
<td></td>
<td></td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.1</td>
<td></td>
<td></td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.2</td>
<td>15.0</td>
<td>95</td>
<td>6.8</td>
<td>15.0</td>
<td>99</td>
</tr>
<tr>
<td>6</td>
<td>7.6</td>
<td></td>
<td></td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.1</td>
<td>10.0</td>
<td>98</td>
<td>9.2</td>
<td>10.0</td>
<td>97</td>
</tr>
<tr>
<td>8</td>
<td>10.1</td>
<td></td>
<td></td>
<td>9.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10.7</td>
<td>15.0</td>
<td>102</td>
<td>10.7</td>
<td>15.0</td>
<td>92</td>
</tr>
<tr>
<td>10</td>
<td>11.1</td>
<td>100.0</td>
<td>98</td>
<td>10.6</td>
<td>10.0</td>
<td>85</td>
</tr>
<tr>
<td>11</td>
<td>11.2</td>
<td>10.0</td>
<td>98</td>
<td>11.4</td>
<td>10.0</td>
<td>95</td>
</tr>
<tr>
<td>12</td>
<td>12.2</td>
<td>10.0</td>
<td>93</td>
<td>13.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>14.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>16.1</td>
<td>5.0</td>
<td>105</td>
<td>15.2</td>
<td>5.0</td>
<td>106</td>
</tr>
<tr>
<td>15</td>
<td>17.3</td>
<td>30.0</td>
<td>95</td>
<td>14.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>17.8</td>
<td>10.0</td>
<td>97</td>
<td>18.1</td>
<td>10.0</td>
<td>95</td>
</tr>
<tr>
<td>17</td>
<td>18.8</td>
<td>10.0</td>
<td>100</td>
<td>18.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>21.4</td>
<td>50.0</td>
<td>102</td>
<td>21.2</td>
<td>50.0</td>
<td>99</td>
</tr>
<tr>
<td>19</td>
<td>33.2</td>
<td></td>
<td></td>
<td>32.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>45.5</td>
<td></td>
<td></td>
<td>45.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>50.5</td>
<td></td>
<td></td>
<td>50.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>51.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>51.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>54.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>57.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>62.2</td>
<td>50.0</td>
<td>100</td>
<td>61.7</td>
<td>50.0</td>
<td>98</td>
</tr>
<tr>
<td>27</td>
<td>71.1</td>
<td></td>
<td></td>
<td>69.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>76.0</td>
<td></td>
<td></td>
<td>77.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>116.0</td>
<td></td>
<td></td>
<td>116.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>316.0</td>
<td>25.0</td>
<td>97</td>
<td>315.5</td>
<td>25.0</td>
<td>96</td>
</tr>
</tbody>
</table>

Determination of Urea in Human Blood

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Folin-Wu filtrate</th>
<th>Semogyi filtrate</th>
<th>Urea N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea N</td>
<td>Added</td>
<td>Recovered</td>
</tr>
<tr>
<td></td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>1</td>
<td>5.2</td>
<td>10.0</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>10.0</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.2</td>
<td>15.0</td>
<td>95</td>
</tr>
<tr>
<td>6</td>
<td>7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.1</td>
<td>10.0</td>
<td>98</td>
</tr>
<tr>
<td>8</td>
<td>10.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10.7</td>
<td>15.0</td>
<td>102</td>
</tr>
<tr>
<td>10</td>
<td>11.1</td>
<td>100.0</td>
<td>98</td>
</tr>
<tr>
<td>11</td>
<td>11.2</td>
<td>10.0</td>
<td>98</td>
</tr>
<tr>
<td>12</td>
<td>12.2</td>
<td>10.0</td>
<td>93</td>
</tr>
<tr>
<td>13</td>
<td>14.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>16.1</td>
<td>5.0</td>
<td>105</td>
</tr>
<tr>
<td>15</td>
<td>17.3</td>
<td>30.0</td>
<td>95</td>
</tr>
<tr>
<td>16</td>
<td>17.8</td>
<td>10.0</td>
<td>97</td>
</tr>
<tr>
<td>17</td>
<td>18.8</td>
<td>10.0</td>
<td>100</td>
</tr>
<tr>
<td>18</td>
<td>21.4</td>
<td>50.0</td>
<td>102</td>
</tr>
<tr>
<td>19</td>
<td>33.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>45.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>50.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>51.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>51.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>54.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>57.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>62.2</td>
<td>50.0</td>
<td>100</td>
</tr>
<tr>
<td>27</td>
<td>71.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>76.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>116.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>316.0</td>
<td>25.0</td>
<td>97</td>
</tr>
</tbody>
</table>
applied to dog blood and urine has been found to give consistently more "urea N" than the urease technique. In the case of the urines, the differences are small (about 5 per cent), and may well represent allantoin. However, on the tungstate filtrates this difference was 1 to 6 mg. of "urea N" per 100 ml. of blood, averaging 3.7 mg. per cent, as compared with the average true urea N of 12.4 mg. per cent (about 30 per cent). When this value is multiplied by the factor 8.3 (color from 1.00 mg. of urea N = color from 1.47 mg. of allantoin ureido N = 8.30 mg. of allantoin), an average of 31 mg. per cent of allantoin is obtained. This is so high as to cast considerable doubt on interpretation of the difference as allantoin.

It will be noted from Table III that zinc filtrates usually gave values for dog blood lower than did tungstate, but still considerably above the true (urease) values. In following this up, we precipitated blood proteins by a variety of other reagents, but without finding any improved filtrate. Even the use of plasma instead of whole blood did not alter the results. The only procedure which was found to yield values close to the true ones involved the treatment of the Somogyi filtrate with permutit. In the experiments listed in the lower portion of Table III, 7.0 ml. of zinc filtrate were shaken 5 to 10 minutes with 1 gm. of permutit and filtered. The

\[\text{Table II}\\
\text{Determination of Urea in Human and Dog Urine}\\
\]

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Human</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diacetyl monoxime method</td>
<td>Urea method</td>
</tr>
<tr>
<td></td>
<td>Added</td>
<td>Recovered</td>
</tr>
<tr>
<td></td>
<td>mg. per 100 ml.</td>
<td>mg. per 100 ml.</td>
</tr>
<tr>
<td>1</td>
<td>105</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>275</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>374</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>431</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>438</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>463</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>480</td>
<td>200</td>
</tr>
<tr>
<td>8</td>
<td>485</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>513</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>501</td>
<td>50</td>
</tr>
<tr>
<td>11</td>
<td>587</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>589</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>725</td>
<td></td>
</tr>
</tbody>
</table>

\[2\text{ Although the permutit used was labeled "Prepared according to Professor Otto Folin," it was found necessary to wash it several times with distilled water to remove a fine, slowly settling material. The remaining permutit was oven-dried for use.}\]
zinc filtrate values, previously 15 to 45 per cent too high, were brought down to −5 to +8 per cent of the urease figures by this procedure. That this decrease was not due to the removal of urea is supported by the

**Table III**

*Determination of Urea in Dog Blood*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Diacetyl monoxime method</th>
<th>Urea N</th>
<th>Added</th>
<th>Recovered</th>
<th>Urea N</th>
<th>Added</th>
<th>Recovered</th>
<th>Permuted value</th>
<th>Urea N</th>
<th>Added</th>
<th>Recovered</th>
<th>Permuted value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Folin-Wu filtrate</td>
<td></td>
<td></td>
<td></td>
<td>Somogyi filtrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>per cent</td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>per cent</td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td>mg. per cent</td>
<td></td>
<td></td>
<td>mg. per cent</td>
</tr>
<tr>
<td>1</td>
<td>11.8</td>
<td>15.0</td>
<td>106</td>
<td>11.6</td>
<td>15.0</td>
<td>98</td>
<td>8.9</td>
<td>11.5</td>
<td>12.2</td>
<td>10.4</td>
<td>14.0</td>
<td>13.0</td>
</tr>
<tr>
<td>2</td>
<td>12.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14.2*</td>
<td>15.0</td>
<td>103</td>
<td>13.2</td>
<td>15.0</td>
<td>101</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15.3</td>
<td>10.0</td>
<td>98</td>
<td>14.3</td>
<td>15.0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>15.5*</td>
<td>15.0</td>
<td>101</td>
<td>14.1</td>
<td>15.0</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>15.6</td>
<td>10.0</td>
<td>97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16.5</td>
<td>15.0</td>
<td>97</td>
<td>15.6</td>
<td>10.0</td>
<td>99</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>9</td>
<td>17.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>18.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>18.8</td>
<td>10.0</td>
<td>95</td>
<td>16.9</td>
<td>10.0</td>
<td>97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>19.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>21.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average... | 16.2                      | 100      | 15.3    | 99        | 12.5      |           |           |                |        |       |          |                |           |           |                |           |

|            |                         | 9.8      | 9.8     | 8.7       | 9.2      | 9.5      | 9.4       | 8.9          | 9.4    | 11.2  | 11.0     | 10.4          | 10.1      | 10.0       | 10.1          | 10.0       |
|            |                         | 11.5     | 11.7    | 11.1      | 11.6     | 11.0     | 11.1      | 11.7          | 11.0   | 11.2  | 11.1     | 10.4          | 10.1      | 10.1       | 10.1          | 10.1       |
|            |                         | 11.8*    | 11.6    | 9.4       | 13.0     | 11.2     | 11.0      | 9.7          | 11.2   | 12.3  | 12.1     | 12.1          | 12.1      | 12.1       | 12.1          | 12.1       |
|            |                         | 13.1     | 13.0    |           |          | 10.5     | 10.4      | 9.7          | 11.0   | 12.1  | 12.1     | 12.1          | 12.1      | 12.1       | 12.1          | 12.1       |
|            |                         | 14.2*    | 13.2    |           |          | 10.5     | 10.4      | 10.1         | 10.1   |       |          |                |           |           |                |           |
|            |                         | 15.5*    | 14.3    |           |          | 10.1     | 10.1      | 9.7          | 11.0   | 11.2  | 11.1     | 12.1          | 12.1      | 12.1       | 12.1          | 12.1       |
|            |                         | 16.5*    | 14.1    |           |          | 9.7      | 10.0      | 9.7          | 11.0   |       |          |                |           |           |                |           |
|            |                         | 16.5     | 15.6    |           |          | 13.1     | 12.1      | 10.1         | 10.1   |       |          |                |           |           |                |           |
|            |                         | 22.0     | 21.2    |           |          | 15.7     | 15.0      | 15.7         | 15.0   |       |          |                |           |           |                |           |
|            |                         | 22.2     | 21.8    |           |          | 18.0     | 16.8      | 18.0         | 16.8   |       |          |                |           |           |                |           |

**Average...** | 15.3                      | 14.6      | 11.6    | 11.3      |           |           |           |                |        |       |          |                |           |           |                |           |

* These values are duplicated in the upper portion of the table.

quantitative recovery of urea added to the filtrate before treatment with permuted.

Spectrophotometric study, with a Beckman quartz spectrophotometer, was undertaken in an attempt to obtain further information on the source of the extra color yielded by filtrates of dog blood. As can be seen from
Figs. 2 and 3, the absorption curves of human and dog urines and of human blood filtrates correspond very closely to the standard urea curves. In contrast, the dog blood filtrates show considerable deviation from pure urea in the region of 400 to 450 m\(\mu\) (and also from 500 to 550 m\(\mu\), although this region is not transmitted by the blue filter used), especially marked when the Folin Wu filtrate is used. Curves for allantoin and citrulline are included, the latter taken from Ormsby (2). The absorption characteristics of the allantoin color so closely resemble those of the color with urea as to

![TRANSMISSION](http://www.jbc.org/)

*Fig. 2. Absorption characteristics of the diacetyl monoxime color with human urine and blood filtrates.*

make even qualitative identification in a mixture impossible; this combines well with the reason previously advanced for not considering allantoin responsible for much of the difference between colorimetric and urease values actually found with dog blood. If citrulline were present, considerable flattening of the typical absorption peak at 476 m\(\mu\) would be expected. Since this was not found with the filtrates, citrulline can probably be ruled out. Study of the permutit-treated zinc filtrates revealed only a non-specifically decreased absorption all along the curve.
DISCUSSION

Analytical Precautions—Although the procedure has been simplified to the point of uniform use of 2.0 ml. of aqueous urea solution and 4.0 ml. of 50 per cent sulfuric acid, 1.0 ml. of filtrate can be used with 5.0 ml. of 40 per cent acid. Logically, this relationship could be carried out to 4.0 ml. of filtrate and 2.0 ml. of concentrated sulfuric acid, if very dilute urea solutions were to be encountered, or to give a deep color for visual colorimetry. This can be done, but the results are extremely erratic, because the addition to water of sulfuric acid any stronger than 50 per cent evolves so much heat that the 10 minute heating period at 100° cannot be judged with any accuracy. Cooling could probably be resorted to, but was considered to introduce an unjustified complication.

As indicated under “Procedure,” the period of heating at 100° should be 10 minutes. ½ minute on either side of this does not significantly alter the results; longer heating produces an undesirable darkening. As Ormsby noted, the tubes must be kept out of any direct sunlight during and after

Fig. 3. Absorption characteristics of the diacetyl monoxime color with dog urine and blood filtrates.
the heating. Such exposure causes a premature development of the color, followed by a rapid fading. In fact, the determination is best carried out in a part of the laboratory definitely removed from windows, to avoid even indirect glare. There is, however, no advantage in keeping the tubes in complete darkness.

The addition of the persulfate to the hot solution is advantageous in that maximum color development is achieved within 15 minutes for all amounts of urea. If the tubes are thoroughly cooled before the addition of persulfate, the time course of color development varies widely, according to the amount of urea present. It has been found unnecessary to control the actual temperature of the tubes at the time of persulfate addition, provided this is done within 5 minutes after removal from the water bath. The tubes ordinarily are still warm at the end of the 15 minutes, when the colors are to be read. If there appears to be any particular reason for desiring cool solutions, the tubes may be placed in cool tap water during the 12 to 14 minute interval without altering the results.

After the maximum color development is reached, fading becomes noticeable to the extent of about 0.3 γ per 5 minutes, the same in blood filtrates and urines as in standard solutions. The percentage error caused by this fading depends, of course, on the total amount of urea N present. With 20 γ, from 2.0 ml. of a 1:10 filtrate of normal blood, 0.3 γ represents an error of -1.5 per cent. It will readily be seen that an extra 15 minute delay in reading the colors would produce less than 5 per cent error. If the blood urea N level is elevated, the error will be correspondingly less. The number of determinations that can be performed in one series with only a 5 per cent error due to fading thus depends upon the analyst’s ability to add persulfate to all tubes within the 5 minute limit and opportunity to read all colors between 15 and 30 minutes later. The maximum number will usually be found to be about twenty single analyses, or ten in duplicate, when a photoelectric colorimeter is available. If a visual colorimeter must be used, the readings are slower, but this is compensated for by the fact that the standards will fade along with the unknowns. For the most accurate results with this procedure, a definite routine should be worked out for the entire process from the time of addition of the acid to the reading of the colors. If the steps involved are varied too widely, the results obtained can be expected to be erratic.

Preservatives—If blood filtrates or urines are not to be analyzed promptly after they have been obtained, they should be stored in a refrigerator after the addition of a drop of toluene or chloroform. Thymol interferes with the reaction, and must not be used.

The advantages of a direct colorimetric method for the determination of urea are apparent to anyone who has used urease procedures, especially
when only small amounts of blood are available. The present method differs from that devised by Ormsby in three principal ways: (1) the use of a non-volatile acid instead of concentrated HCl, (2) a need for only 1.0 or 2.0 ml. of filtrate for each determination, instead of 3.0, and (3) the addition of persulfate to the hot solution to produce quick and uniform color development from all amounts of urea tested. It should also be noted that the range of the method, 10 to 150 mg. per cent of urea N, broad as it is, can be extended to 250 mg. per cent simply by the addition of 5.0 ml. of distilled water to the final solution. This covers practically all values encountered.

SUMMARY

1. A direct colorimetric procedure has been described for the quantitative determination of urea in blood and urine. The urea is condensed with diacetyl monoxime in the presence of sulfuric acid, and the resulting color enhanced by the addition of potassium persulfate.

2. 10 to 150 γ of urea N can be determined, corresponding to 10 to 150 mg. of urea N per 100 ml. of blood. By a supplementary dilution, this range can be extended to 250 mg. per cent if necessary.

3. The method has been found to give the same results on human blood filtrates and urines as those by urease. The colorimetric results on dog urine also are very close to the urease values, but, in the case of dog blood, this procedure indicates 1 to 6 mg. per cent more “urea N” than the urease method shows to be present. This discrepancy can largely be removed by treating the Somogyi zinc filtrate with permutit. This modification is entirely empirical, since the extra color cannot be attributed to any specific substance.

The author wishes to acknowledge gratefully the work of Miss Hortense S. Louckes on the charts.

BIBLIOGRAPHY

THE DIRECT COLORIMETRIC DETERMINATION OF UREA IN BLOOD AND URINE
S. B. Barker


Access the most updated version of this article at http://www.jbc.org/content/152/2/453.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/152/2/453.citation.full.html#ref-list-1