THE DETERMINATION OF UREA IN URINE BY THE UREASE METHOD.

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(Received for publication, October 18, 1915.)

Marshall's¹ urease method, in the modification proposed by Van Slyke and Cullen,² is apparently the most accurate method available for the determination of urea. The method has, however, not yet attained the maximal degree of accuracy to be expected from volumetric processes in general. From the published figures of Van Slyke and Cullen, their modification appears to yield duplicates agreeing within about 1 per cent. Provided the removal of ammonia from the digestion mixture is assumed to be complete, the error must, in all probability, depend upon one of two factors. Either (1) the enzymatic reaction is not absolutely quantitative, or (2) traces of ammonia escape through the acid used for receiving it. As the result of an investigation of this point, the writer has found that, of these two possible sources of error, only the latter is present to a detectable extent. The aim of this paper is to present a further modification of the method which eliminates the above mentioned disturbance.

The greater degree of accuracy attainable by the modification to be described, as compared with those previously suggested, depends mainly upon four points.

1. By increasing the volume of fluid from which the ammonia is to be removed, and at the same time decreasing the concentration of potassium carbonate, it is possible so to regulate the rate of removal of the ammonia that every trace of it is held by the acid in the receiver (at the expense, of course, of a certain amount of time). When this part of the process is conducted as described below, it is possible to collect 7 mg. of ammonia nitrogen quanti-

² Van Slyke, D. D., and Cullen, G. E., ibid., 1914, xix, 211.
tatively in an amount of $\frac{x}{\text{HCl}}$ which is hardly more than sufficient to neutralize it.

2. The aeration tube, after once being closed, is not again opened before the completion of the determination. Any chance of loss of ammonia is thereby eliminated.

3. The back titration is made with $\frac{x}{\text{NaOH}}$ instead of $\frac{x}{\text{HCl}}$, using as the indicator methyl red, which is sensitive to 0.05 cc. $\frac{x}{\text{NaOH}}$.

4. The minimum amount of urea recommended for the determination is the equivalent of approximately 25 cc. $\frac{x}{\text{acid}}$, the maximum about twice that amount. The error due to the titration itself is therefore only 0.1 to 0.2 per cent.

As the result of the above changes in technique, the figures obtained with pure urea solutions, by this method, agree with those obtained by the Kjeldahl method within 0.1 to 0.2 per cent. Duplicates on urine also agree within 0.1 to 0.2 per cent.

The Enzyme Solution.

Any satisfactory urease preparation can, of course, be used, provided it is standardized. In this work aqueous soy bean extracts, prepared as described below, have been employed.

Extract 25 gm. of powdered soy beans for one hour with 250 cc. of distilled water, shaking at intervals. Add 25 cc. of $\frac{x}{\text{HCl}}$, and let stand 5 minutes. Filter with suction. To the filtrate add 5 cc. of a solution made by dissolving 70 gm. Na$_2$HPO$_4.12$H$_2$O and 27 gm. KH$_2$PO$_4$ in 100 cc. of water. Keep in a cold place.

Blanks must be run on the extract at intervals of several days. In this laboratory, where a refrigerator kept at -1$^\circ$ or -2$^\circ$C. is available, extracts keep, without preservative, for as long as four weeks with but little loss of activity, and usually with no marked increase in ammonia content.

Method.

By means of an Ostwald pipette transfer to a large test-tube (preferably heavy walled) an amount of urine containing from 3

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2 Methyl red was used for the micro-titration of ammonia by Pregl, F., Abderhalden's Handb. d. biochem. Arbeitsmethoden, 1912, v, 1346.

to 6.5 mg. of urea nitrogen. Dilute with distilled water to a out
3 cc. Add 2 drops of kerosene and 2 cc. of the urease extract.
Insert the rubber stopper bearing the aeration apparatus, and let
stand for 15 minutes. Now add, by means of a pipette, the tip
of which can be inserted into the air-inlet tube, 5 cc. of a carbon-
ate-oxalate solution.\(^5\) Aerate slowly for 5 minutes, then rapidly
for 1 hour, collecting the ammonia in 25 cc. \(\frac{N}{5}\) HCl contained in
a narrow necked bottle of about 120 cc. capacity. Titrate the
excess of acid with \(\frac{N}{10}\) NaOH, using 2 or 3 drops of a 0.05 per
cent alcoholic solution of methyl red. The end-point is the dis-
appearance of the pink color.

The \(\frac{N}{5}\) HCl and \(\frac{N}{10}\) NaOH solutions used for the urea determina-
tions recorded in this paper were carefully standardized against
\(\frac{N}{5}\) acid and alkali. Of the \(\frac{N}{10}\) solutions, the HCl was standardized
by the AgCl method, the NaOH by means of pure oxalic acid.
The two \(\frac{N}{10}\) solutions were checked against each other with excel-
 lent agreement, and were used in the Kjeldahl determination re-
ported below. Calibrated glassware was used throughout.

**Urea Solution.**

A solution of Kahlbaum's urea was analyzed by the Kjeldahl
method, and found to contain 1.743 mg. of nitrogen per cc. The
following results were obtained with this solution by the urease
method described above.

<table>
<thead>
<tr>
<th>Urea solution</th>
<th>(\frac{N}{10}) HCl neutralized</th>
<th>Urea N.</th>
<th>Urea N per cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>2.0</td>
<td>24.86</td>
<td>3.483</td>
<td>1.741</td>
</tr>
<tr>
<td>3.0</td>
<td>37.31</td>
<td>5.227</td>
<td>1.742</td>
</tr>
<tr>
<td>4.0</td>
<td>49.68</td>
<td>6.964</td>
<td>1.740</td>
</tr>
</tbody>
</table>

\(^5\) This solution is prepared as follows: Dissolve 500 gm. \(K_2CO_3\) in 500
cc. of water, with the aid of a little heat. Add 10 cc. of a 30 per cent solu-
tion of potassium oxalate. Filter if necessary, and let cool before using.
Below are given the results of duplicate determinations obtained from consecutive samples of urine in the course of routine work.

<table>
<thead>
<tr>
<th>Urine No.</th>
<th>Urea + ammonia nitrogen per 24 hrs.</th>
<th>Urine No.</th>
<th>Urea + ammonia nitrogen per 24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td></td>
<td>gm.</td>
</tr>
<tr>
<td>1</td>
<td>1.364</td>
<td>5</td>
<td>1.150</td>
</tr>
<tr>
<td></td>
<td>1.362</td>
<td></td>
<td>1.149</td>
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<tr>
<td>2</td>
<td>1.325</td>
<td>6</td>
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<td></td>
<td>1.326</td>
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<td>1.149</td>
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<tr>
<td>3</td>
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<td>7</td>
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<td></td>
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<td>1.081</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.458</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For many purposes, it is true, the additional accuracy obtained by the above method, at the cost of a certain amount of time (but not of attention), is unnecessary. It must be admitted, however, that in some instances the greatest possible accuracy is essential, and in such cases the comparatively slight extra time required is negligible.
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