THE GASOMETRIC DETERMINATION OF UREA IN URINE.

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Several months ago in a communication describing a gasometric method for the determination of total nitrogen, mention was made of the fact that when carried out in vacuo the reaction between sodium hypobromite and urea results in the liberation of the theoretical volume of nitrogen. The necessary vacuum is readily obtainable with the Van Slyke apparatus for determining the carbon dioxide content of blood plasma. Some effort was spent at the time in attempting to utilize the reaction for a blood urea method but it became evident that the error introduced by other nitrogenous constituents of blood was greater than permissible.

Recently Youngburg has shown that the urease method for urinary urea may be somewhat simplified by first removing the ammonium salts and then carrying out the urease decomposition and aeration in the manner described by Van Slyke and Cullen. The ammonium salts are removed by shaking the urine with permutit. The idea immediately suggested itself that here was a place to apply the hypobromite reaction. In the older hypobromite methods the results were unsatisfactory because the reaction (using pure urea solutions) was found to give less than the theoretical amount of nitrogen even when allowed to continue for hours, and in addition ammonium salts and other urinary constituents also yielded nitrogen when subjected to the action of hypobromite. With the aid of permutit the ammonium salts

may now be eliminated as sources of error. It remained, therefore, to determine the error which might be introduced by other substances present in urine.

Assuming for the moment that an average 24 hour specimen of urine contains 15 gm. of urea nitrogen, 0.300 gm. of creatinine nitrogen, and 0.250 gm. uric acid nitrogen, it is evident that if all the nitrogen of the two latter constituents was liberated quantitatively the error introduced would be about 3.7 per cent. In order to determine the actual quantities liberated these two compounds were subjected to the action of hypobromite. It was found that creatinine yielded about one-seventh of its nitrogen while uric acid yielded about one-half. In the case of the latter, however, the evolution of nitrogen takes place slowly and in consequence, by limiting the reaction time to that required for the urea reaction to go to completion, the error need not exceed about 0.3 per cent.

Conceivably, hippuric acid and amino-acids might be sources of error. However, the first does not yield any nitrogen with hypobromite. Glycocoll yields about 3 per cent of its N. Inasmuch as the amount of amino-acids present in urine is very small to begin with and provided that other amino-acids conduct themselves similarly to glycocoll, it is evident that the error from this direction is negligible. Creatine, which is

<table>
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<tr>
<td>mg.</td>
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<tr>
<td>7.60</td>
<td>7.48</td>
<td>7.53</td>
</tr>
<tr>
<td>7.52</td>
<td>7.55</td>
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<td>6.40</td>
</tr>
<tr>
<td>6.89</td>
<td>6.89</td>
<td>5.18</td>
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<td>6.66</td>
<td>6.71</td>
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</tr>
<tr>
<td>4.72</td>
<td>4.72</td>
<td>6.73</td>
</tr>
<tr>
<td>14.4*</td>
<td>14.5</td>
<td>4.72</td>
</tr>
<tr>
<td>11.0*</td>
<td>10.9</td>
<td>14.6</td>
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</table>

* Dog urine.

sometimes a constituent of urine, liberates 2 of its 3 nitrogen atoms under the conditions of the experiment. Since it is usually absent or present only in small amounts this source of nitrogen may in general be neglected. Allantoin conducts itself very similarly to uric acid.

Table I contains some results obtained by the hypobromite method, and the urease method. In the case of the latter, determinations were made by Youngburg’s modification as well as by the usual procedure of Van Slyke and Cullen.

In view of the general satisfaction given by the urease method the present method may seem superfluous. It is fitting, therefore, that its advantages be stated. They are: (1) Rapidity. Starting with a sample of urine the urea content may be known in 10 minutes. (2) Standard solutions are not employed. (3) Reagents necessary are simple and easily prepared. (4) There is practically no opportunity in the procedure for things to go awry as there are opportunities in the urease method. For example, the keeping qualities of dilute standard solutions and of urease solutions are not matters of concern. Neither is there any question of foaming nor about how long and at what rate to aerate.

Procedure.

25 cc. of diluted urine (diluted in the ratio of 1:10) are shaken with 4 gm. of permutit for 4 minutes. The mixture is then centrifuged or filtered. 1 cc. of the NH₃-free urine is introduced into the Van Slyke CO₂ apparatus, the last portion being rinsed in with 1 cc. of water followed by 1 cc. of sodium hypobromite solution. (This is made by mixing equal volumes of two solutions, one containing 12.5 gm. of sodium bromide and 12.5 gm. of bromide per 100 cc. and the other 28 gm. of sodium hydroxide per 100 cc.) The mercury is lowered to the 50 cc. mark and the apparatus is then shaken vigorously for about half a minute. The aqueous solution is collected in the proper chamber below the lower stop-cock, mercury is admitted to the 50 cc. chamber, and after adjusting the pressure the volume of nitrogen is read. Correction is then made for the dissolved air con-
tained in the diluted urine, the rinse water, and the hypobromite solution.\(^5\)

It may be assumed with reasonable accuracy that the solubility of air in the diluted urine is the same as in pure water. For temperatures between 15 and 30\(\text{°C.}\) and a pressure of 1 atmosphere the solubilities are as given in Table II. The volumes are those which the gas would occupy at 760 mm. and the temperature in question and may, therefore, be subtracted from the gas volume as read. Determinations of the air dissolved in the hypobromite solution showed that between 15 and 20\(\text{°C.}\) this amounts to 0.006 cc. and between 21 and 25\(°\) to 0.005 cc.

### Table II

Cc. of Air Measured at 760 mm. of Mercury and the Temperature in Question per 1 Cc. of Water.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Volume</th>
<th>Temperature</th>
<th>Volume</th>
<th>Temperature</th>
<th>Volume</th>
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</thead>
<tbody>
<tr>
<td>15</td>
<td>0.0216</td>
<td>21</td>
<td>0.0198</td>
<td>27</td>
<td>0.0184</td>
</tr>
<tr>
<td>16</td>
<td>0.0212</td>
<td>22</td>
<td>0.0196</td>
<td>28</td>
<td>0.0182</td>
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<tr>
<td>17</td>
<td>0.0209</td>
<td>23</td>
<td>0.0193</td>
<td>29</td>
<td>0.0180</td>
</tr>
<tr>
<td>18</td>
<td>0.0206</td>
<td>24</td>
<td>0.0191</td>
<td>30</td>
<td>0.0178</td>
</tr>
<tr>
<td>19</td>
<td>0.0203</td>
<td>25</td>
<td>0.0188</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.0201</td>
<td>26</td>
<td>0.0186</td>
<td></td>
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</table>

The corrected volume is then reduced to standard conditions (0\(°\) and 760 mm. mercury) by means of the following formula.

\[
V_o = V \frac{P_o - h}{(1 + 0.00367 t) 760}
\]

where

- \(V\) = gas volume as measured.
- \(P_o\) = corrected barometric pressure.
- \(h\) = aqueous tension.
- \(t\) = temperature at which gas was measured.

To facilitate the calculation it will be found advantageous to refer to almost any of the compilations of physical and chemical data for useful gas reduction tables.

Once the volume of nitrogen measured at 0\(°\) and 760 mm. of Hg has been determined the weight is determined by mul-

\(^5\) The correction for the air content of the diluted urine and rinse water can be eliminated by extracting the two in the apparatus and expelling the extracted gases before adding the hypobromite.
tiplying by 0.0012507, the weight of 1 cc. of nitrogen. Taking
the dilution and amount of sample into consideration the amount
of urea nitrogen per 1 cc. of urine is easily found.

Some consideration was given to the adaptability of the hypobromite reaction to the determination of ammonium salts in
urine as well as to the determination of urea. Theoretically
there are no difficulties. The difference between the quantities
of nitrogen liberated by diluted whole urine and by urine treated
with permutit represents ammonia N and that only. The diffi-
culty is in the measurement of the two quantities with sufficient
accuracy to make it possible to express the ammonia N concen-
tration with at least two significant figures. The ordinary Van
Slyke apparatus cannot be read without a possible error of at
least 0.003 of a cc. in the opinion of the writer. In addition
no protection is provided against slight differences between the
temperatures of the gas itself and the air registered by the ther-
rometer close by. Consequently since the ammonia N is usually
less than one-tenth of the sum of the ammonia and urea nitrogen
and therefore occupies less than one-tenth of a cc. in the appa-
tratus, its volume cannot be expressed accurately in thousandths
of a cc. as is necessary if the result is to be expressible with two
significant figures. If an approximate relation between the urea
N and ammonia N is desired this can be very readily obtained.

SUMMARY.

A method for determining urea in urine is described which
is both brief and accurate. Ammonium salts are removed by
treating the urine with permutit and the ammonium-free solu-
tion is then subjected to the action of sodium hypobromite in
the vacuum obtained with a Van Slyke CO₂ apparatus. Nitrogen
is liberated quantitatively from the urea but to an entirely
negligible extent from other urinary constituents.

Comparative analyses obtained with the urease and hypo-
bromite procedures demonstrate the accuracy of the method.