A MICRO METHOD FOR THE ESTIMATION OF AMMONIA IN BLOOD AND IN ORGANIC FLUIDS.

By K. L. GAD-ANDRESEN.

(From the Laboratory of Zoophysiology, University of Copenhagen, Copenhagen, Denmark.)

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In an earlier publication1 dealing with a micro method for the estimation of urea in blood, I also described a micro method for the estimation of ammonia in blood and in organic secretions. For the determination of the ammonia two analyses are made; in the first the total urea plus ammonia is determined; and in the second the urea alone after the ammonia has been removed by evaporating the blood in vacuum. The difference between the two analyses gives the ammonia content.

In the urea method itself the blood proteins are precipitated with 0.01 N acetic acid to which sodium acetate is added as a buffer. The precipitate is filtered off and the urea in the filtrate is estimated by means of its decomposition with hypobromite. The nitrogen produced is measured in Krogh's micro respirometer and calculated with the aid of Krogh's formula in terms of c.mm. When this figure is multiplied by 1.256 a correction for the weight of nitrogen is obtained in mg. and can, if desired, be found in terms of urea. For the purpose of this determination the micro respirometer is provided with ellipsoid bottles of 10 to 15 cc. capacity, fitted with ground glass stoppers on which are sealed small containers for the hypobromite. The determinations require from 0.10 to 0.15 cc. of blood and the error is ±0.5 mg. per 100 cc. Since the value for the ammonia by this method is obtained as the difference between two such estimations, both subject to error, the possible error on the ammonia estimation itself is relatively high.

1 Gad-Andresen, K. L., Biochem. Z., 1919, xcix.
As it is well known from the work of Henriques and Christiansen, the amount of ammonia in blood is very small and is probably constant at about the value 0.25 mg. per 100 cc., a figure of about the same magnitude as the error in the determination. Estimations by this method, therefore, give positive results only when the ammonia content is abnormally high. The method was worked out at the time because I felt that my urea method would be more accurate if the urea and ammonia were analyzed separately. But when Henriques and Christiansen's work appeared, it seemed that further study in the interest of the accuracy of the urea method was unnecessary; the ammonia correction of 0.25 mg. per 100 cc. could simply be subtracted from the total found in order to get the true urea value.

A series of ammonia estimations were, however, made in part on human and in part on ox blood. The highest value found was 0.8 mg. per 100 cc. but as a rule none was found, indicating the amount present to be less than 0.5 mg. per 100 cc.—in good agreement with the results of Henriques and Christiansen with their macro method.

If 0.1 to 0.15 cc. of blood be used the method can indicate only, whether the ammonia content is about normal or not. Greater accuracy might be obtained with larger amounts of blood but in its present form the method can only be used with small amounts of blood. Were larger quantities of blood used, correspondingly larger quantities of fluid would be required for coagulating the blood and washing the precipitate, the size of containers must needs be increased, and hence the change in the manometer would be reduced. The requirements for an exact method are evidently that the amounts used for analysis be large and the value for the container as small as possible.

The method to be described meets these requirements. The amount of blood used is 1 cc. and the volume of the container is reduced to about 10 cc. The ammonia is estimated directly. After addition of borate (Sörensen), the blood is evaporated to dryness in the apparatus presently to be described. The ammonia

2 Henriques, V., and Christiansen, E., Biochem. Z., 1917, lxxviii, 165; lxxx, 297.
volatilized is driven by means of an air current over into the bottle of the micro respiration apparatus which contains weak sulfuric acid. It is thereupon estimated in the same way as in the urea method described above, the ammonia being decomposed by hypobromite, and the volume of nitrogen is determined.

The evaporating apparatus is very simple. It consists of a glass tube 25 cm. × 10 mm. provided with a bulb, as shown in Fig. 1, in which the blood and borate are mixed. The air current which is introduced at the other end through a small bent glass tube and rubber stopper first passes through a wash bottle containing concentrated sulfuric acid, which not only frees it from ammonia but also dries it and thus greatly increases the rate at which the blood is evaporated. The other end of the evaporating apparatus is drawn out into a narrow tube and bent as shown, so that it will dip under the surface of the sulfuric acid in the analysis bottle. During evaporation the apparatus is placed in a thermostat at about 25°. A temperature exceeding 30° must be avoided because, as it is well known, urea is then converted into ammonia.

A hypobromite solution of constant composition must be used, controlled, and corrected for by a Kjeldahl determination, as Krogh\textsuperscript{4,5} showed for urea determinations. Her formula is used for

\footnotesize
this purpose, the solution containing 1 cc. of bromine to 100 cc. of 2 N sodium hydroxide. The ratio between the nitrogen as determined by a Kjeldahl estimation and as found by the present method gives the factor for correction.

In the present instance the factor was found as follows:

An ammonium sulfate solution containing 0.4715 gm. per 100 cc. of water yielded 0.101 gm. of nitrogen. A series of estimations with the micro respirometer on the same solution gave an average value of 0.0919 gm. of nitrogen per 100 cc. and the correction was, therefore, \( \frac{0.101}{0.0919} = 1.09 \).

The estimations with the micro respirometer were made under precisely the same conditions as those for blood. 0.1 cc. of the ammonium sulfate solution was measured into the analysis bottle in which had been placed 0.5 cc. of 0.2 N sulfuric acid and 1.9 cc. of water, making in all 2.5 cc. of fluid of the same acidity as under the conditions of blood ammonia analysis. The procedure was precisely as described in the urea method.

The ammonia estimations on blood are done as follows: 1 cc. of blood is accurately measured out and placed in the bulb of the apparatus, into which has been previously put 0.1 cc. of borate (9 cc. of borate + 1 cc. of NaOH). The stopper is inserted and the tube rotated so that blood and borax are thoroughly mixed. The apparatus is then tilted so that the blood flows towards the end of the tube where the air current enters. Care must be taken not to get any of the blood into the narrow connecting tube because when the air current is opened it may then be carried over into the acid and ruin the determination. The blood is distributed over the entire surface of the tube and the latter is then placed in the water bath and connected up with the bottle of the micro respiration apparatus, in which has been placed 0.5 cc. of 0.2 N H_2SO_4. The air current is cautiously opened and regulated so as to prevent the possibility of the material splashing out of the bottle. Evaporation to dryness is complete in about 30 minutes and the ammonia is estimated in the usual way in the micro respiration apparatus.

In order to facilitate the calculation of the evolved quantity of nitrogen it is to be noted that the same quantity of fluid is to be used in the analysis reservoir each time. This can be made so that the reservoir is tared and then, when the analysis is finished, weighs up with distilled water to a certain weight; for instance,
2.5 gm. This weighing must be made as correct as possible and must not differ at the utmost more than 0.10 gm. If care is taken that the volume of the analysis reservoir is not altered, the calculation of the analysis result is very simple, as the c.mm. of evolved nitrogen is multiplied by the proportion:

$$\frac{1.256 \times 1.09 \times 10^2}{V \times 10^6}$$

in which \( V \) signifies the quantity of blood used for the determination.

Analysis of ammonia both in normal blood and in the blood to which definite amounts of ammonia, as ammonium sulfate, have been added, are given in Table I.

### TABLE I.
**Estimations on Blood to Which Ammonia Has Been Added, Calculated as Ammonia Nitrogen per 100 Cc.**

Double estimation directly on blood.

<table>
<thead>
<tr>
<th>Blood</th>
<th>Ammonia nitrogen per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.41 - 0.44</td>
</tr>
<tr>
<td>2</td>
<td>0.51 - 0.46</td>
</tr>
<tr>
<td>3</td>
<td>0.41 - 0.38</td>
</tr>
<tr>
<td>4</td>
<td>0.39 - 0.39</td>
</tr>
</tbody>
</table>

Blood 4 added 3.43 mg. of ammonia nitrogen per 100 cc.

<table>
<thead>
<tr>
<th>Blood</th>
<th>Ammonia nitrogen per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found.</td>
</tr>
<tr>
<td></td>
<td>mg.</td>
</tr>
<tr>
<td>8</td>
<td>3.86 - 3.84</td>
</tr>
</tbody>
</table>

Blood 4 added 5.36 mg. of ammonia nitrogen per 100 cc.

<table>
<thead>
<tr>
<th>Ammonia nitrogen per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found.</td>
</tr>
<tr>
<td>mg.</td>
</tr>
<tr>
<td>5.78 - 5.81</td>
</tr>
</tbody>
</table>
The results show an average error of 0.03 mg. per 100 cc., but an error of 0.05 mg. may be taken as the limit.

Secretions as well as blood may be analyzed by this method, but it cannot be used for tissues, because, as I have shown in an earlier paper, there is a rapid conversion of urea into ammonia after death. It may be prevented if the tissue is at once placed in alcohol cooled to $-20^\circ$, but the employment of the alcohol will prevent an exact estimation with the micro respirometer.

I am obliged to Professor A. Krogh, the chief of the Zoophysiological Laboratory of Copenhagen for his kind attention to my work.

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