XLV. THE ESTIMATION OF AMMONIA AND UREA IN BLOOD AND URINE.

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The principles underlying the chemical changes involved in the method here described are exactly those of the well-known Folin macro aeration method. But the objections to the latter are that the apparatus is very cumbersome, and unless extremely well set up is likely to give too low results.

The apparatus I here propose is that designed by Cannan and Sulzer [1924] for the estimation of alcohol in blood. The great advantage of the apparatus is that it is very compact and easy to handle. The estimation takes less time than in the ordinary aeration apparatus; the loss of ammonia and errors due to rubber connections are cut down to a minimum. This form of apparatus can be quite easily used by students in class. The great advantage in time and ease with which the whole operation is carried out makes it desirable that the details of the procedure should be described.

Method for Ammonia in Urine.

In the outer vessel (Fig. 1) place 20 cc. urine. To the inner vessel add 20 cc. $N/10$ H_2SO_4, a few drops of methyl red and enough distilled water to cover the end of the spiral. Insert the inner part in position, then make connections at X with a sulphuric acid washer and at Y with a pump. Raise the cork and deliver into the bottom of the jacket 5 g. anhydrous Na_2CO_3 from a narrow test-tube. Press the cork in tightly, place the apparatus in a bath at 40° and aerate with a very brisk current of air for 20 to 30 minutes. After this time disconnect from the pump and washer, remove the inner vessel and wash this well on the outside. Then, holding the apparatus over a funnel placed in a flask, remove the inner part, transfer the contents to the flask and wash all the parts very thoroughly. Then titrate the contents of this flask with $N/20$ NaOH. The accuracy of the method has been tested by using a standard ammonium sulphate solution (1 cc. = 1 mg. N) and aerating for 30 minutes; the following results were obtained.

mg. N taken	mg. N estimated
10	9·87
10	9·87
10	9·94
10	10·00
	Mean 9·92

The results for the ammonia in urine have been checked by the recently described colorimetric method of Orr [1924].

Aeration method. mg. ammonia N per 100 cc.	Colorimetric. mg. ammonia N per 100 cc.
55·0	56·3
24·2	24·6
16·18	16·18
39·9	41·0
17·5	17·5

Method for Urea in Urine.

The quantities used are 5 cc. of urine (of known ammonia content), 15 cc. of active urease solution prepared by the method of Folin and Wu, and 5 cc. of sodium pyrophosphate solution. The inner vessel contains 10 cc. $N/2$ H_2SO_4, distilled water and methyl red, as in the case of the ammonia determination. The digestion by the urease is allowed to proceed in a bath at 40° for 20 minutes. Then the sodium carbonate is added and the air current passed for half-an-hour. The rest of the estimation is carried out as described before, $N/10$ alkali being used in the titration of excess sulphuric acid.

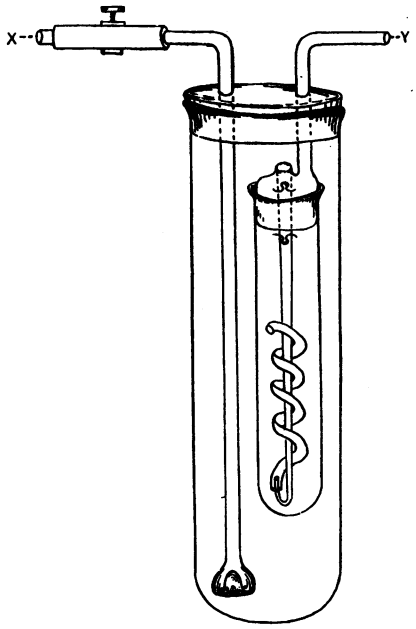

Fig. 1.

These quantities and times have been tested on a standard urea solution of about the same concentration as urine (1 cc. = 10 mg. N).

mg. urea N taken	mg. urea N estimated
100	98·2
100	97·5
100	98·2
100	98·4

Method for Ammonia and Urea in Blood.

A fairly accurate estimation of the urea in blood can be made in a similar way. This is carried out in a smaller form of the apparatus, in which the jacket is 3 cm. in diameter and the inner vessel correspondingly smaller. The volume of blood taken should not be less than 0·6 cc. The proteins are first precipitated by the method of Folin and Wu [1919], and 5 cc. of the clear liquid, which is equivalent to 0·5 cc. blood, are used for the estimation.

I have used 2 cc. $N/100$ H_2SO_4 in the inner vessel to catch the ammonia and titrated the excess acid with $N/200$ NaOH from a burette graduated to 0·02 cc.

For two estimations on a sample of cat's blood I obtained the following values.

Equivalent of blood taken cc.	$N/100$ H_2SO_4 neutralised cc.	mg. urea N per 100 cc. blood
0·5	0·68	19
0·5	0·62	16

The error in this method amounts to not more than ± 4 mg. urea nitrogen per 100 cc. blood.

Although a method for the estimation of urea in 0·2 cc. of blood has been described by Thomas [1924], using $N/400$ acid and alkali, I have been unable to get consistently good results when employing it. This is not difficult to understand, for, if the proteins are precipitated by MacLean's method [1919], the filtrate obtained could not represent more than $\frac{5}{6}$ of the original blood taken, which for a normal blood, containing between 15 and 20 mg. urea nitrogen per 100 cc. blood, corresponds to 0·03 mg. of urea nitrogen. Working with so small a quantity of urea nitrogen, the doubtful quantitative precision of $N/400$ acid and alkali, contained probably in soda glass vessels, to my mind tends to make the method somewhat unreliable.

The extension of Orr's method for the Estimation of Ammonia to the Estimation of Urea in Blood and Urine.

Orr [1924] has recently described a colorimetric method for the estimation of ammonia in urine. I have found the results obtained in this way to agree very closely with those obtained by the aeration method. The estimation is very rapid and, although there are inherent objections to colorimetric methods, it seemed worth while to extend the method to the estimation of urea, since it is usual to have to estimate ammonia and urea in the same sample of urine.

The application of the principles of the colorimetric method to urea estimation was tested, using standard solutions of urea. The details were as follows:

10 cc. of standard urea solution (containing 10 mg. nitrogen per cc.) were pipetted into a 100 cc. graduated flask; 20 cc. of the active urease solution and 10 cc. of pyrophosphate solution were added and the whole made up to

100 cc. The contents of the flask were rapidly transferred to a small conical flask which was very tightly corked and then placed in a bath at 40° for half-an-hour. The flask was then cooled under the tap, the cork raised, and 10 cc. of the solution rapidly pipetted into a 100 cc. flask containing approximately 90 cc. of distilled water; the volume was adjusted to the mark.

The estimation was then carried out with 5 cc. of this solution exactly as described by Orr and using the standard ammonium sulphate (1 cc. = 1 mg. N) for comparison.

This method seems to estimate only 94 % of the total urea and as this figure was very constant with the standard urea solutions, and the values for urea in urine by this method were lower than in the aeration method, it is proposed to make a correction for this in stating results. This low value is no doubt due to inactivation of the urease.

The hydrolysed urea solutions are very easily compared; there is no turbidity due to the enzyme solution and no alteration in the tint of the solution.

If soya-bean meal alone is used the comparison is not so easily made and several times when using the meal I have obtained results much higher than the theoretical value.

Estimation of Urea in Urine Colorimetrically.

10 cc. urine, 20 cc. urease solution, 10 cc. pyrophosphate solution are run into a 100 cc. flask and made up to the mark. This is transferred to a conical flask, corked well and digested in a bath at 40° for half-an-hour. After cooling, 10 cc. of this solution are taken and diluted to 100 cc., as described before. 5 cc. are used for the estimation in the usual way.

Example.

Ammonia N aeration. mg. per 100 cc.	Ammonia N colorimetric. mg. per 100 cc.	Urea N aeration. mg. per 100 cc.	Urea N colorimetric. mg. per 100 cc.	Corrected value for colorimetric. mg. per 100 cc.
55·0	56·3	990	941·5	1000
39·6	41·0	1031	960·0	1021

Estimation of Urea in Blood.

Though the details have been worked out for 0·2 cc. of blood, larger quantities can be used, probably with better results, but for clinical purposes it seems desirable to have a method suitable to this small quantity. If a systematic blood analysis is being made by the Folin-Wu procedure then the estimation is carried out on 2 cc. of the protein-free filtrate.

Method for 0·2 cc. of blood. Place in a small centrifuge tube of about 7 cc. capacity 4·6 cc. of distilled water, run the blood into this from a MacLean 0·2 cc. pipette, washing the pipette out in the usual way. When the blood is laked add 0·6 cc. of 10 % sodium tungstate and then 0·6 cc. of $\frac{2}{3}$ N H_2SO_4 (giving a total volume of 6 cc.). Mix and shake till the typical chocolate colour is obtained and heat gently. Centrifuge the tube till the precipitate

is well settled. Then transfer by means of a pipette 5 cc. of the supernatant liquid, which represents $\frac{5}{6}$ of 0·2 cc. of blood, into the outer jacket of the small aeration apparatus described above, add 2 cc. urease extract and 0·5 cc. of pyrophosphate solution. In the inner vessel place 2 cc. of distilled water, two drops of $N/10$ H_2SO_4 and 2 g. of pure phenol. Place the apparatus in a bath at 40°, connect to a pump in the usual way and leave to digest for 10 minutes. Then disconnect, run in 5 cc. saturated Na_2CO_3 solution, replace the apparatus in the bath and aerate for 20 minutes.

Take two 25 cc. graduated tubes; to one which is to be used as the standard add 2 g. of phenol, 4 cc. of distilled water and then 2 cc. of standard ammonium sulphate solution containing 20 mg. nitrogen per 100 cc. Into the other tube empty the contents of the inner tube of the aeration apparatus, wash this tube and spiral with 2 cc. of water and add the washings to the graduated tube. Then to each of the tubes add 5 cc. of sodium hypochlorite, gradually and with shaking, allow to stand for 5 minutes, make each up to the 25 cc. mark and compare at once in a colorimeter with the standard at 30 mm. or 20 mm. as preferred. A stronger standard will sometimes be needed or alternatively the unknown solution can be diluted.

Results. Standard urea solution containing 20 mg. urea N per 100 cc.

Reading for standard ammonium sulphate solution: 20 mg. N per 100 cc. mm.	Reading for the urea solution mm.	Strength of urea solution calculated: mg. per 100 cc.
30	28·7	20·9
30	30·6	19·6
30	31·0	19·0
	Mean	19·8

Results for Blood. A. Using 2 cc. Folin-Wu filtrate ≡ 0·2 cc. blood.

	Standard reading mm.	Blood reading mm.	mg. urea N per 100 cc.
A_1	20	26·6	15
A_2	30	39·6	15
B_1	30	29·6	20

B. Using 0·2 cc. of same blood as used in A_1 and A_2 and precipitating as described.

Standard reading mm.	Blood reading mm.	mg. urea N per 100 cc.
20	29·6	16
30	45·2	15

The colour of these solutions is a clear blue, slightly greener than that obtained with the urine standard, and it is, I think, much more suitable for colorimetric comparison than the yellow of Nesslerised solutions of the same ammonia content. It does not seem possible to use the protein-free filtrate directly in this estimation, for if this solution is digested and then run on to the phenol a green coloration develops on the subsequent addition of the hypochlorite solution.

SUMMARY.

1. Ammonia and urea in urine and blood are estimated by aeration in a simple form of apparatus.

2. Urea in urine is estimated colorimetrically.

3. Urea is estimated in 0·2 cc. blood by combination of the aeration and colorimetric methods.

REFERENCES.

Cannan and Sulzer (1924). *Heart*, **11**, 148.
Folin and Wu (1919). *J. Biol. Chem.* **38**, 81.
MacLean (1919). *Biochem. J.* **13**, 135.
Orr (1924). *Biochem. J.* **18**, 806.
Thomas (1924). Communicated to Biochem. Soc.