CIII. A COLORIMETRIC METHOD FOR THE DIRECT ESTIMATION OF AMMONIA IN URINE.

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For the estimation of ammonia in minute quantities the reagent almost universally adopted has been the well-known Nessler reagent either in its original form or as Folin's modification [Folin and Denis, 1916]. The use of Nessler's reagent in physiological work is attended by several difficulties, these being avoided, for the most part successfully, by ridding the reacting solutions of undesirable substances. This necessarily lengthens the process.

Tarugi and Lenci [1912] in describing some colour reactions stated that phenol added in excess to an amine or imine reacts with sodium hypochlorite to give a blue coloration. The reaction was first described by Berthelot [1859] being re-discovered by Cotton [1874] and then, as cited above, by Tarugi and Lenci. These last authors state that the reaction with ammonia is more delicate than Nessler's and they suggest its being used as the basis of a quantitative method.

The mechanism of the reaction they describe is as follows. The amine or imine in the presence of sodium hypochlorite reacts with phenol to give first *p*-nitrosophenol which in turn reacts with the excess of phenol to give *p*-benzoquinonoxyphenylimine which they state has a blue colour in solution.

Thomas [1912, 1913] was the first to work out a quantitative method for the estimation of ammonia based on the above reaction and later he applied his procedure to the detection of ammonia in the cerebrospinal fluid. A method similar to that of Thomas with slight differences of technique was later described by Foxwell [1916].

In attempting to apply the reaction to the estimation of ammonia in urine the chief difficulty encountered was the tendency of urea to react with the alkaline hypochlorite. The procedure of Thomas or of Foxwell is not applicable since in alkaline solution on heating urea breaks down to give among other products ammonia which then reacts and vitiates the results. If however the reaction is performed in the cold and the excess of phenol considerably increased the above reaction does not take place.

Of the other constituents of urine only amino acids give the reaction, and, under the conditions found suitable to eliminate the action of urea, of those tested only α-alanine and glycocoll give any reaction. The action of α-alanine

is negligible whilst glycocoll in solutions containing the same amount of amino nitrogen as an ammonia solution gives less than 5 % of the colour given by the ammonia solution.

Sodium hypochlorite solution. The intensity of the blue colour obtained depends on the concentration and amount of hypochlorite solution used. Since however this solution is not very stable it is found advisable to use for comparison in estimations a solution which contains a known amount of ammonia nitrogen per cc. The same hypochlorite being used for standard and unknown, its concentration may vary within limits. Under these conditions the strength may vary for the first week after preparation but thereafter it is reasonably constant for some months. Should the hypochlorite solution become very alkaline it should be rejected, as increase of alkali tends to impart to the colour generally obtained a green tint. A suitable concentration has been found by diluting one volume of the ordinary freshly-prepared commercial sodium hypochlorite solution with an equal volume of distilled water. The undiluted solution may be preserved in a cool dark place in a well-stoppered bottle.

Standard ammonium sulphate solution. A solution containing 0·1 mg. ammonia nitrogen per cc. is prepared by dissolving 0·4716 g. pure dry ammonium sulphate in one litre of distilled water. The pure salt may be prepared from the usual laboratory specimens as described by Folin and Denis [1916].

Method.

The following procedure was finally adopted: 20 cc. urine are diluted to 100 cc. with distilled water. Into a clean beaker of about 50 cc. capacity are weighed out 4·5 g. pure phenol crystals. To this are added 5 cc. of the well-mixed diluted urine and the phenol crystals allowed to be completely moistened. 20 cc. of the sodium hypochlorite solution are carefully added and the beaker agitated gently. The colour is allowed to develop for five minutes and the beaker then washed out with distilled water into a 250 cc. measuring flask containing about 100 cc. distilled water. The solution is then diluted up to the mark and mixed. 5 cc. of the standard ammonium sulphate solution are treated in a similar manner. The colour of the unknown is compared in a Duboscq colorimeter with that of the standard. From the comparison the ammonia nitrogen in the urine may be calculated. If the reading of the unknown be x when the standard is set at 20 for equal intensity of colour then

$$\frac{x}{20} = \frac{0{\cdot}5}{\text{mg. } NH_3N \text{ in 1 cc. urine}}$$

and this $\times 1000$ gives the number of mg. NH_3 nitrogen per litre of urine.

Should the amount of ammonia nitrogen be unusually small or unusually large the dilution of the colour should be arranged to give a convenient reading on the colorimeter.

To indicate the degree of accuracy of the method the results obtained on a series of urines compared with those obtained by the Folin macro-aeration method are shown below.

	Folin macro-method	New method
1. Normal urine	0·50	0·51
2. ,,	0·38	0·35
3. ,,	0·44	0·40
4. ,,	0·56	0·54
5. ,,	0·08	0·06
6. Diabetic urine	1·28	1·25
7. Nephritic urine	0·23	0·28
8. ,,	1·26	1·31
9. Cat urine	0·48	0·53
10. Dog urine	0·67	0·71

The following tests were carried out on solutions of ammonium sulphate, by a science student, Miss Small, who had no previous experience of the method:

Original solution mg. per cc.	Folin macro-method	New method
·025	·027	·024
·05	·05	·05
·06	·06	·06
·07	·07	·07
·08	·08	·08
·09	·09	·09
·15	·16	·16
·20	·23	·20
·25	·27	·25
·30	·31	·30

SUMMARY.

A new and rapid method is described for the estimation of ammonia in urine colorimetrically.

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REFERENCES.

Berthelot (1859). *Repertoire de Chimie Appliqué*, p. 254.
Cotton (1874). *Bull. Soc. Chim.*
Folin and Denis (1916). *J. Biol. Chem.* **26**, 497.
Foxwell (1916). *Gas World*, 64.
Tarugi and Lenci (1912). *Boll. Chim. Farm.* **50**, 907.
Thomas (1912). *Bull. Soc. Chim.* **11**, 796.
—— (1913). *Bull. Soc. Chim.* **13**, 398.