CXLVIII. A COLORIMETRIC METHOD FOR THE DIRECT DETERMINATION OF UREA IN URINE.

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SINCE Fosse [1914] introduced a gravimetric method for the estimation of urea in urine based on the precipitation of urea as dioxanthylurea, no attempt has been made to apply the principle to meet the requirements of the routine laboratory analyst who has many estimations to perform in a limited time. The purpose of this paper is to present a method suitable for use in the routine laboratory.

The tedious process of collecting, washing and weighing the dioxanthylurea has been replaced by the separation of the precipitate by centrifuging; the nitrogen content of the substance is determined by a colorimetric estimation of the ammonia produced in a micro-Kjeldahl digestion, which has been facilitated by the introduction of a modified digestion reagent containing selenium, instead of copper, as catalyst [Lauro, 1931], and perchloric acid instead of sulphuric acid as the oxidising agent. The advantages of perchloric acid are mentioned in a recent paper by King [1932], who uses this acid for the estimation of total phosphorus in blood. The whole process may be carried out in the same tube. The difficulty of sedimentation of the light flocculent precipitate of dioxanthylurea has been overcome by precipitating the dioxanthylurea in the presence of a very fine suspension of barium sulphate, when the two precipitates centrifuge down together leaving a clear supernatant liquid which may be safely decanted.

Proteins and interfering substances are removed from the urine by adsorption on "colloidal iron" which is produced in the liquid by the interaction of dilute ferric chloride and sodium bicarbonate solutions.

METHOD.

Reagents.

- (1) 1 % crystalline ferric chloride in water.
- (2) 0.5 % sodium bicarbonate in water.
- (3) Barium reagent. Dissolve 10 g. of sodium chloride and 2 g. of sodium sulphate in 90 cc. of distilled water. Add this solution to 400 cc. of glacial

acetic acid, and to the mixture add 1.2 g. of barium chloride dissolved in 10 cc. of water.

(4) 2.5 % solution of xanthydrol (Schering-Kahlbaum) in glacial acetic acid. This solution should be kept in a small well-stoppered bottle and prepared every few days.

(5) Digestion mixture:

- 100 cc. of 60 % perchloric acid;
- 40 cc. of phosphoric acid (sp. gr. 1.75).
- 20 cc. of 3 % sodium selenate solution;
- 0.5 cc. of 10 % sodium sulphate solution.
- 130 cc. of water.

(6) Nessler reagent [Folin and Wu, 1919].

(7) Ammonium sulphate solution. Dissolve 0.1179 g. of pure ammonium sulphate in water. Preserve from moulds with two drops of strong sulphuric acid and dilute to 1 litre.

Procedure. Measure 1 cc. (or 2 cc. if the urea content is very low) of urine from an Ostwald pipette into a 100 cc. graduated flask, add 2 cc. of 1 % ferric chloride and, while rotating the liquid, 10 cc. of 0.5 % sodium bicarbonate solution. Dilute to the mark, mix and filter. Transfer a suitable amount of filtrate (1–5 cc. depending upon the urea content) with an Ostwald pipette to a 4"×1" hard glass round-bottomed centrifuge-tube¹ of 35–40 cc. capacity. Table I has proved useful in choosing the volume of filtrate required, but it

Table I.

Specific gravity of specimen	cc. of filtrate required
1030–1025	1
1025–1020	1.5
1020–1015	2
1015–1010	3
1010–1005	5
Below 1005	Use 2 cc. of urine for filtration

must be borne in mind that other factors beside the urea content affect the specific gravity of a urine.

Dilute the filtrate in the centrifuge-tube to 5 cc. with water, add 5 cc. of well mixed barium reagent and 1 cc. of xanthydrol solution. Mix the liquid, and allow the tube to stand in a bath of ice-cold water for 20 minutes. Mix the liquid by rotating the tube; wash down the sides of the tube with 1 cc. acetone, which is allowed to float on the surface. Centrifuge for five minutes and decant the supernatant liquid, placing the inverted tube on a sheet of filter-paper for two minutes to drain. Remove the excess liquid from the inside of the tube by wiping with filter-paper.

Add 1 cc. of digestion mixture and a piece of porous pot. Heat the tube gently on a sand-bath in a fume chamber, covering the mouth of the tube with a watch-

¹ Supplied by C. Dixon and Co., 27 Devonshire Street, W.C. 1.

glass when heavy white fumes appear and the liquid darkens. Twenty seconds after the brown-coloured liquid becomes colourless remove the tube from the sand-bath. Add 20 cc. of water after the acid has cooled for a few minutes. Cool to room temperature and add 7.5 cc. of Nessler reagent while twirling the liquid in the tube. After mixing, centrifuge for three minutes to remove the precipitate of barium sulphate. Compare the crystal clear supernatant liquid with the standard which is prepared at the same time as follows. Place 5 cc. of barium reagent and 5 cc. of water *etc.* in a 4"×1" tube, and centrifuge, drain and digest as described for the unknown. After heating, add 15 cc. of water and 5 cc. of standard ammonium sulphate solution before adding 7.5 cc. of Nessler reagent.

The use of a thick blue glass light filter for the comparison in the colorimeter is recommended, since by this means the difficulty always associated with the matching of yellow or brownish-yellow colours is eliminated; the red tints which result from the use of the blue glass being easily compared.

Calculation (if the standard is set at 20 mm.):

$$\frac{53.6}{\text{Colorimetric reading} \times \text{number of cc. filtrate used}} = \text{g. of urea per 100 cc. of urine.}$$

Table II, which contains results taken at random from many analyses, gives the experimental values found by the analysis of standard urea solutions.

Table II.

Concentration of solution taken in g. per 100 cc. %	Experimental result in g. per 100 cc. %	Percentage error
4.2	4.23	+1
3.0	3.05	+1.5
2.8	2.73	-2.5
2.0	1.98	-1
1.5	1.48	-1
1.0	0.98	-2
0.75	0.73	-2.5
		Mean error 1.6

The recovery of urea added to urine is shown in Table III.

Table III.

% urea in urine	% urea added	Total urea %	% urea found
0.81	1.0	1.81	1.82
0.65	1.0	1.65	1.67
0.85	1.5	2.35	2.32
4.21	0.75	4.96	5.00
0.29	1.5	1.79	1.75
1.58	1.0	2.58	2.55

The close agreement between the xanthidrol and urease methods when applied to a number of specimens of urine is shown in Table IV. The direct titrimetric method of Cole [1931] was employed for the estimation of urea by the urease method.

Table IV. *Comparison of results obtained by urease and xanthydrol methods.*

Result by xanthydrol method (g./100 cc.)	Result by urease method (g./100 cc.)
1.55	1.56
1.19	1.18
0.54	0.55
1.53	1.48
0.57	0.56
1.57	1.55
2.31	2.27
2.80	2.81
3.15	3.08
0.72	0.71
2.76	2.78
1.38	1.35

DISCUSSION.

Twelve urines were examined in order to demonstrate the absence of adsorption of any nitrogen-containing substance on the barium sulphate. To illustrate this, thirty times the amounts of reagents and urine filtrate normally taken were used, xanthydrol not being added. After the precipitate of barium sulphate was spun down and subjected to the usual treatment, no appreciable colour developed when the liquid was treated with Nessler reagent.

The digestion is free from difficulty, as owing to the small volume of liquid which has to be removed by boiling, bumping is practically unknown. The end of the digestion is clearly defined, the liquid changing from brown to colourless in a few seconds.

It is unnecessary to wash the precipitate of dioxanthylurea as the amount of urea-free liquid left in the tube after the treatment described is of the order of 0.1 cc. If desired, the tubes may be calibrated at 30 cc. and the contents of the tube diluted to this mark, but this is not essential.

The ferric chloride and sodium bicarbonate solutions when used in the amounts stated will remove up to 2.5 % of protein from urine. That no adsorption of urea takes place during the precipitation was shown by treating a mixture of 1 cc. of 0.6 % urea solution and $\frac{1}{3}$ cc. of plasma (containing 40 mg./100 cc. of urea) with the protein precipitants before diluting to 100 cc. The urea content of the filtrate was found to be 0.0061 %.

We are at present engaged in applying the principle to the determination of urea in blood-filtrates.

SUMMARY.

A method is described for the routine estimation of urea in urine based on the formation of dioxanthylurea. Interfering substances are removed from the urine with ferric chloride and sodium bicarbonate, and the urea is precipitated from the filtrate by xanthydrol. The precipitate is centrifuged in the presence of barium sulphate and its nitrogen content determined colorimetrically after a micro-Kjeldahl digestion.

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