LXXXVI. A MICRO-METHOD FOR THE COLORIMETRIC DETERMINATION OF UREA IN BLOOD.

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THE method is based on the gravimetric method of Fosse, Robyn and François [1914] in which urea is precipitated as dixanthylurea from deproteinised blood-serum by the addition of an alcoholic solution of xanthhydrol. Fosse's method has been carried out on a micro-scale by Nicloux and Welter [1921], and was found to give reliable results for quantities of urea as small as 0·05 mg.

In the following method the dixanthylurea precipitate was prepared according to Nicloux and Welter, but, instead of being weighed, it was dissolved in sulphuric acid (50 % by volume) in which xanthhydrol and its compounds dissolve, producing an intense yellow colour. This colour reaction is of an intensity which makes it possible to detect differences of 0·14 mg. of dixanthylurea, or in terms of urea, 0·02 mg. per 100 cc. 50 % sulphuric acid.

METHOD.

0·5–1·0 cc. of blood is deproteinised by the method of Folin-Wu.

To 1 vol. of blood add 7 vols. of distilled water, 1 vol. of 10 % sodium tungstate and 1 vol. of 2/3 *N* sulphuric acid. Shake and filter.

Place 1 cc. of the filtrate (= 0·1 cc. blood) in a centrifuge tube.

Add 1 cc. of glacial acetic acid, and 0·2 cc. of a solution of 5 % xanthhydrol in methyl alcohol.

Allow this to stand for 5 minutes. Then stir it with a glass rod, and allow it to settle again for half to one hour, or centrifuge for 20–30 minutes.

(When the quantity of urea is small, 20 mg. per 100 cc. of blood or less, the longer time is necessary to ensure complete precipitation.)

Filter off the precipitate by suction through a small Gooch crucible packed with asbestos.

Wash alternately three times with 2 cc. of methyl alcohol and distilled water, preferably saturated with dixanthylurea.

During the washing with methyl alcohol detach the pump until the methyl alcohol has almost drained through, to allow of complete solution of the excess xanthhydrol.

Test the third methyl alcohol washing with 50 % sulphuric acid. If no yellow colour appears, the washing is complete.

Wash out the flask and place a small receiving tube inside so as to catch the succeeding filtrate. Pour about 5 cc. of 50 % sulphuric acid into the crucible.

When the precipitate is dissolved, attach the pump, and draw the yellow

solution into the receiving tube. Wash with a further 4 cc. of sulphuric acid. Remove the tube and make up to 10 cc. in a graduated test-tube. Compare in a colorimeter with a standard solution.

The precipitate can be filtered, washed, and dissolved in sulphuric acid in about 10 minutes.

Preparation of standard solution. Dilute 0·4 cc. of 0·01 % urea solution to 1 cc. with distilled water. The same procedure is then adopted as in the case of blood-filtrate, *i.e.* 1 cc. urea solution, 1 cc. glacial acetic acid, 0·2 cc. 5 % methyl alcohol solution of xanthhydrol. The precipitate is centrifuged, filtered, washed and dissolved in 10 cc. of 50 % sulphuric acid.

This standard corresponds to 0·04 mg. urea in the 10 cc. of acid used. The standard retains its colour indefinitely.

In cases in which the blood-urea is markedly increased, the solution may be diluted with 50 % sulphuric acid until the colour is near that of the standard.

Calculation. $\frac{10}{x} \times 40 =$ mg. urea per 100 cc. blood; where standard is set at 10 mm. and $x =$ length of column of solution of unknown concentration.

When the unknown solution has been diluted, $\frac{y}{x} \times 40 =$ mg. urea per 100 cc. blood; where $y =$ no. of cc. to which unknown solution has been diluted.

Some results obtained by this method are given below:

(*a*) for solutions of known urea concentration;

(*b*) blood-urea values obtained by this method, compared with results obtained by the urease-aeration method of Marshall [1913].

(*a*) *Urea in mg. per* 100 *cc. solution.*

Theoretical	Xanthhydrol colorimetric method
45	46
55	54
25	25
30	30
35	33
80	81
100	99

(*b*) *Urea in mg. per* 100 *cc. blood.*

	Urease method	Xanthhydrol colorimetric method
Rabbit	40	39·6
Sheep	44·8	45
Human blood, from cases of renal inefficiency	46	46
	70	69
	71	71
	52	50·6
	58	59
Human blood (normal)	36	35
	30	29·3
	29·5	30
	28	29

REFERENCES.

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