CLXXXV. THE COLORIMETRIC DETERMINATION OF URIC ACID IN URINE.

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THE colorimetric method of estimating uric acid in urine has not received attention in the same way as the method for estimating uric acid in blood. Although one of the difficulties of the blood-uric acid determination, namely the development of turbidity in the final solution used for colorimetric comparison, has been entirely overcome, the urine-uric acid method has not been studied, so far as is known, with a view to overcoming this difficulty of cloudy solutions. The Folin method of estimating uric acid in urine [1923] invariably gives turbid final solutions. It has been found always necessary to centrifuge before making the colour comparison. This procedure is satisfactory, except that frequently the turbidity is not completely removed, and even where clear solutions are obtained a fresh clouding often develops while the comparison is being made. Accordingly, the effect of substituting the improved uric acid reagents in Folin's method for urine-uric acid has been investigated and the conditions of reaction modified so that the final solutions are perfectly clear.

Briefly, Folin's method depends upon the reduction of phospho-18-tungstic acid by uric acid in the presence of a large excess of sodium carbonate. Sodium cyanide is also present and the reaction is allowed to proceed at room temperature.

EXPERIMENTAL.

The following reagents were employed.

- (1) Uric acid reagent of Folin and Marenzi [1929].
- (2) Uric acid standard solution of Folin [1930] containing 1 mg. uric acid per cc. This was diluted ten times with water alone, to prepare the working standard when required.
- (3) Cyanide-urea reagent of Folin [1930].
- (4) 15 % aqueous sodium cyanide.
- (5) Acid silver lactate reagent prepared by the method of Folin [1922]. The solution was always filtered immediately before use.
- (6) Sodium carbonate solution containing 20 g. anhydrous sodium carbonate per 100 cc. solution. This is referred to as "20 % sodium carbonate." Suitable dilutions were made to prepare other strengths, *e.g.* "10 % sodium carbonate" (1 : 1 dilution).

The influence of the concentration of sodium carbonate upon colour development and formation of turbidity.

5 cc. portions of dilute standard uric acid solution (each containing 0.5 mg. uric acid) were transferred to each of a series of eleven 100 cc. graduated flasks. To each was added 20 cc. sodium carbonate solution of different concentrations as indicated below. 2 cc. 15 % sodium cyanide were then added to each flask, followed by 5 cc. uric acid reagent. The contents of the flasks were mixed and, after standing for 5 minutes at room temperature, diluted to 100 cc. with water. The turbid solutions were then quickly centrifuged and the colours compared in the colorimeter, using the first solution as a standard set at 20 mm.

Table I.

% concentration of sodium carbonate used	20.0	17.5	15.0	12.5	10.0	7.5	5.0	2.5	2.0	1.5	1.0
Colorimeter reading in mm.	20.0	19.0	18.3	16.6	15.5	14.7	12.8	11.5	11.6	19.9	44.7
Precipitate formation	++	++	++	+	+	±	-	-	-	-	--

It is seen that a reduction in the concentration of sodium carbonate used from 20 % to 2.5 % causes a steady increase in the amount of colour developed. However, the formation of precipitate is only just avoided at the 5 % concentration.

The experiment was then repeated exactly, except that 5 cc. cyanide-urea reagent were substituted for the 2 cc. 15 % aqueous sodium cyanide. The results of this series are below.

Table II.

% concentration of sodium carbonate used	20.0	17.5	15.0	12.5	10.0	7.5	5.0	2.5	2.0	1.5	1.0
Colorimeter reading in mm.	20.0	18.3	17.4	16.3	15.5	14.3	12.6	10.6	10.9	12.7	26.7
Precipitate formation	Trace	Trace	Trace	Trace	-	-	-	-	-	-	-

Again, the colour developed was progressively greater as the concentration of sodium carbonate used was decreased from 20 % to 2.5 %. The formation of precipitate was very slight in the first four solutions, and entirely avoided in the 10 % concentration and below.

Application of the new technique to urine.

As a result, it was proposed at first to use 20 cc. 2.5 % sodium carbonate and 5 cc. cyanide-urea reagent in the uric acid determination as, under these conditions, no precipitate formed in the final solution, and colour development was at a maximum. This modified technique was applied to urine and the results compared with those obtained by Folin's original method.

Two portions of 1 cc. urine were transferred to each of two centrifuge tubes and 3 cc. water followed by 3 cc. acid silver lactate added to each. After mixing and standing in the dark for a few minutes, the tubes were centrifuged and

the supernatant liquid was discarded. One precipitate was dissolved in 2 cc. 15 % sodium cyanide and transferred with 5 cc. water to a graduated 100 cc. flask. The colour was then developed, using 20 cc. 20 % sodium carbonate, and matched against a standard prepared from 5 cc. dilute uric acid standard solution, after centrifuging to remove the precipitate. The result was calculated in grams uric acid per 100 cc. urine. The second silver precipitate was dissolved in 5 cc. cyanide-urea reagent and treated in a similar way except that 2.5 % sodium carbonate was used. The colour comparison was made with an appropriate standard. The type of result obtained is shown in Table III.

Table III.

Specimen	Folin method (15 % NaCN, 20 % sod. carb.)	New technique (Cyanide-urea, 2.5 % sod. carb.)
1	0.064	0.058
2	0.053	0.045
3	0.074	0.061

It is seen that the new method yielded results much below those of Folin's original method, this being apparently due to the reduction in the concentration of sodium carbonate used. Hence, 10 % sodium carbonate was chosen as a possible suitable concentration of alkali. It had already been found that this solution, in combination with the cyanide-urea reagent, yielded the desired colours without turbidity, but at the same time maintained the reaction mixture very strongly alkaline.

Using a number of urine samples, a comparison was made as above between the original Folin method and the new method, using cyanide-urea and 10 % sodium carbonate. Typical results are shown below.

Table IV.

Specimen	Folin's method (15 % NaCN, 20 % sod. carb.)	New technique (Cyanide-urea, 10 % sod. carb.)
1	0.044	0.043
2	0.062	0.063
3	0.051	0.053
4	0.119	0.123
5	0.068	0.067
6	0.051	0.052
7	0.044	0.045
8	0.033	0.033
9	0.074	0.076
10	0.060	0.060
Average	0.0606	0.0615

Agreement between the two methods is here seen to be quite close. In no case was any turbidity produced except when using urines containing less than 0.025 % uric acid. In such a case it is considered that the uric acid reagent is in great excess of the amount needed to react with the uric acid, and this excess is decomposed by the alkali, giving a turbidity. This difficulty was easily avoided by using more than 1 cc. urine in the first treatment with acid silver lactate.

Within limits the new technique was found to give a good degree of proportionality between the uric acid content and colour intensity. This was shown by estimating the uric acid by the new technique in different quantities of the same urine, calculating each estimation to g. per 100 cc.

Table V.

cc. urine taken	...	...	...	...	...	1.0	1.5	2.0
Colorimeter reading when standard set at 15 mm.						27.4	17.9	13.8
% uric acid in the urine	...	...	...	...	...	0.027	0.028	0.027

It is recommended that uric acid be estimated in urine by the following method.

Transfer 1 cc. urine (or more if the uric acid content is low) to a centrifuge-tube. Add 3 cc. water and 3 cc. acid silver lactate solution. Mix, stand in the dark for a few minutes, then centrifuge. Discard the supernatant liquid. Dissolve the precipitate in 5 cc. cyanide-urea reagent and transfer quantitatively to a 100 cc. graduated flask, using successively two portions of 10 cc. 10 % sodium carbonate solution and finally 5 cc. water.

Prepare a standard by transferring to a 100 cc. graduated flask 5 cc. uric acid standard solution (containing 0.5 mg. uric acid), 5 cc. cyanide-urea reagent and 20 cc. 10 % sodium carbonate solution.

To each flask add 5 cc. uric acid reagent, mix and allow to stand 5 minutes. Dilute to volume, mix and compare in the colorimeter.

SUMMARY.

A modification of Folin's method of estimation of uric acid in urine is described. It is easily carried out in routine practice, and yields results strictly comparable with those obtained by Folin's original technique. It is free from the objection of turbidity in the final colours.

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