CLXXVIII. ESTIMATION OF URIC ACID BY BENEDICT'S METHOD.

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BENEDICT's very useful and delicate method for the estimation of uric acid in the blood [1922, 1, 2] depends on the appearance of a blue colour in a Folin-Wu blood filtrate, on adding to it appropriate amounts of 5 % sodium cyanide solution and his special arsenic phosphotungstic acid reagent. In a blank determination, however, a blue colour also appears, which, as Benedict has pointed out, varies in intensity with different samples of sodium cyanide. To overcome this difficulty Benedict adds a trace of ammonia to the cyanide solution, whilst Folin [1922], pointing out that strongly ammoniacal solutions do not yield the maximum colour and that they show a greater tendency to produce turbidity, uses a two to three weeks old solution of 15 % sodium cyanide in 0.1 *N* sodium hydroxide. Benedict supposes that the efficacy of this latter solution is due to the spontaneous formation of ammonia in cyanide solutions. Neither of the above methods, however, completely solves the difficulty, for some colour still persists in a blank determination, with the result that, using certain samples of sodium cyanide, the colorimetric reading over a wide range is by no means proportional to the amount of uric acid present. Concerning this Benedict writes [1922, p. 192]: "Using 0.02 mg. of uric acid as standard, results are satisfactorily exact when the unknown reads between 10 and 24 mm., when the standard is set at 15 mm.... With 2 mg. of uric acid results may be about 10 % too high." And again [1922, pp. 192-3]: "...the colour from a blank is negligible inside the limits above mentioned, since it is present both in standard and unknown." It is obvious that this last statement is only absolutely true when standard and unknown have the same intensity of colour; it becomes less and less true as the disproportion between standard and unknown becomes more marked and the colour produced in a blank determination increases.

The object of the present paper is to show (1) that it is, from a purely mathematical standpoint, essential to take into account the colour given by the blank controls, and (2) that if this is done, the method gives consistently accurate results over a wide range of concentration.

Let three solutions *C* (containing 0.4 mg. of uric acid per 100 cc.), *B* (containing 0.2 mg. of uric acid per 100 cc.) and *A* (a blank determination) be of the same colour with colorimetric readings of *x*, *y* and *a* mm. respectively.

Then since the amount of colour-producing substance is inversely proportional to the reading (*i.e.* the depth of columns of identical colour)

$$\left(\frac{1}{x} - \frac{1}{a}\right) = 2 \left(\frac{1}{y} - \frac{1}{a}\right).$$

i.e. $y = \frac{2ax}{a+x}, \text{ or } a = \frac{xy}{2x-y}.$

Thus if any two of the three depths x , y and a are known the third may be deduced.

Supposing when $x = 10$, $a = 35$ (as with the specimen of sodium cyanide we are now using), then

$$y = 15.6;$$

thus solution B , containing half the amount of uric acid of solution C , reads not 20, the theoretical value if the colour in the blank is regarded as negligible (because it is present in both standard and unknown, within the limits mentioned by Benedict), but 15.6. This is shown to be true experimentally when solutions C and B are read against one another (Table I).

Table I.

	Solution C (0.4 mg. %)	Solution B (0.2 mg. %)
Theoretical (no correction for blank)	10	20
Theoretical (correction for blank)	10	15.6
Exp. 1	10	15.7
" 2	10	15.5
" 3	10	15.4
" 4	10	15.5
" 5	10	15.6

In a similar way if solution B contains an unknown quantity of uric acid, say Q mg. %, then if the standard solution C contains 0.4 mg. %.

$$\left(\frac{1}{x} - \frac{1}{a}\right) = \frac{0.4}{Q} \left(\frac{1}{y} - \frac{1}{a}\right),$$

i.e. $Q = \frac{0.4 \times x(a-y)}{y(a-x)}.$

With the specimen of sodium cyanide we are using and $x = 10$, $a = 35$,

$$\therefore Q = \frac{0.4(350-10y)}{25y} = \frac{140-4y}{25y}.$$

The results shown in Table II prove that in pure uric acid solutions the intensity of colour produced is directly proportional to the amount of uric acid present when Benedict's reagents are employed and *correction is made for the colour in a blank determination.*

Taking Benedict's range of accuracy, *i.e.* readings between 10 and 24 mm. when the standard (x) reads 15 mm., and taking a as 52.5 mm., then when the unknown solution

(a) reads 24 mm. Benedict's result = 0.25 mg. %, corrected result = 0.19 mg. %, *i.e.* error approximately 30 %,

(b) reads 10 mm. Benedict's result = 0.6 mg. %, corrected result = 0.68 mg. %, *i.e.* error approximately 12 %.

Since none of the figures in the third column (*i.e.* corrected for blank colour) differs from the true amount present by more than ± 3 % (*i.e.* not greater than colorimeter error), the advantage of the correction is evident.

Table II.

	Uric acid in mg. % in pure solution	Amount given by direct relation, <i>i.e.</i> $\frac{0.4x}{y}$	Amount given when corrected for blank colour
1	0.800	0.699	0.816
2	0.600	0.537	0.592
3	0.533	0.485	0.519
4	0.300	0.327	0.297
5	0.266	0.306	0.269
6	0.200	0.258	0.201
7	0.133	0.211	0.135

The above results are specimens of many repeated experiments.

It should be added that we have been unable either by Benedict's or Folin's method of reducing the colour in the blank determination to reduce the value of "*a*" below 60 ($x = 10$), with the specimens of sodium cyanide used. It is therefore necessary to determine the value of "*a*"¹ for the particular reagents employed, and correct the final results accordingly.

It will be seen from the above discussion and Table II that the uncorrected results are too high when the amount of uric acid present is less than 0.4 mg. % (*i.e.* the amount of uric acid in the standard solution), and too low when the amount of uric acid in the unknown solution is greater than that in the standard. The correction probably serves, at any rate in part, to explain why the Benedict method gave apparently higher results than the Folin-Wu method, since as Benedict himself writes [1922, 1, p. 203], "the difference between the two methods tends to disappear or is reversed as soon as the uric acid by the Folin-Wu method approaches a figure of 3.5 to 4.0 mg."

REFERENCES.

- Benedict (1922, 1). *J. Biol. Chem.* 51, 187.
 — (1922, 2). *J. Biol. Chem.* 54, 233.
 Folin (1922). *J. Biol. Chem.* 54, 153.

¹ This can be done in less than 10 minutes, and may be repeated each week.