29. Estimation of Blood Creatinine

By G. E. Delory and J. Jacklin, *From the British Postgraduate Medical School, London, W. 12*

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Creatinine is usually estimated by the method of Folin & Wu [1919], which depends on a comparison with a standard of the colour produced when a deproteinized blood filtrate is treated with alkaline picrate. It is well recognized that the accuracy of the method is severely limited by the fact that the colour produced with the normal range of creatinine is not very much greater than that of the alkaline picrate itself.

The purpose of this paper is to show how the colour of the blank may be corrected for by a simple algebraic calculation, considerably greater accuracy being thus attained.

Beer's law, on which colorimetry is based, states that light in passing through a coloured medium is absorbed in direct proportion to the concentration of coloured substance. Thus the concentration of coloured substance is inversely proportional to the observed depth in a Duboscq colorimeter. In the case of creatinine, the estimation cannot be accurately made because of the large blank colour due to the excess of the alkaline picrate reagent used. The following calculation, applied to the colorimetric readings obtained in the usual way, obviates this difficulty. Let C_S , C_T and C_B be the concentrations of the standard, test and blank, and R_S , R_T and R_B be the corresponding colorimetric readings. Then

$$C_S \propto \frac{1}{R_S} - \frac{1}{R_B} \quad \text{and} \quad C_T \propto \frac{1}{R_T} - \frac{1}{R_B},$$

when by division

$$\frac{C_T}{C_S} = \frac{1/R_T - 1/R_B}{1/R_S - 1/R_B}.$$

Hence

$$\frac{C_T}{C_S} = \frac{(R_B - R_T)/R_T R_B}{(R_B - R_S)/R_B R_S}.$$

And

$$C_T = \frac{R_B - R_T}{R_B - R_S} \times \frac{R_S}{R_T} \times C_S.$$

Table 1 compares the results obtained by means of this calculation with those resulting from the usual calculation, i.e. $C_T = R_S/R_T \times C_S$, when solutions of known creatinine content were used. Table 2 gives a comparison for recoveries from whole blood, while Table 3 gives the results obtained on blood samples sent to the laboratory for routine examination.

Table 1. *Colorimetric analysis of standard creatinine solutions*

Stated conc. mg./100 ml.	(1) By usual calc.	(2) By proposed calc.	Stated conc. mg./100 ml.	(1) By usual calc.	(2) By proposed calc.
	mg./100 ml.	mg./100 ml.		mg./100 ml.	mg./100 ml.
2.5	1.28	2.55	12.5	3.46	12.70
5.0	1.80	5.00	15.0	4.00	15.20
7.5	2.40	7.80	17.5	4.50	17.50
10.0	3.00	10.60	20.0	5.10	20.50

The comparisons were made in a Duboscq colorimeter; matching was facilitated by the use of an Ilford blue-green spectral light filter [King & Haslewood, 1937].

Table 2. *Recovery of creatinine added to blood*

mg./100 ml. added to blood	mg./100 ml. recovered	
	By usual calc.	By proposed calc.
2.5	1.3	2.6
5.0	1.8	5.0
10.0	3.1	9.9
12.5	3.5	12.5

Table 3. *Creatinine concentrations of hospital blood samples by two methods of calculation*

mg./100 ml.		mg./100 ml.	
By usual calc.	By proposed calc.	By usual calc.	By proposed calc.
0.8	0.9	2.0	6.6
1.2	1.2	2.2	6.3
1.3	1.5	2.3	6.5
1.3	1.6	2.6	6.6
1.6	3.3	3.9	14.2

It would of course be unnecessary to correct for the blank if it were possible to have a standard which exactly matched each test, but this is impracticable in a routine method. This point is well illustrated in Table 3. The standard used was equivalent to 1 mg. of creatinine per 100 ml. of blood. When the blood concentration is near this figure the agreement between the results obtained by the two methods of calculation is good. But as the concentration of the test departs from that of the standard, the results obtained by the ordinary calculation become increasingly inaccurate. With the proposed calculation only one standard is needed, and the figures shown in Tables 1 and 2 show an accuracy previously unattained in creatinine estimations. A further advantage of this method is that the tedious purification of the picric acid need not be carried out. It may be objected that since the substance estimated by the picric acid methods may not actually be creatinine, increased accuracy in measurement is pointless; but the chromogenic substance, even if not creatinine, is of considerable importance in clinical biochemistry, and an accurate estimation is extremely useful.

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REFERENCES

- Folin, O. & Wu, H. [1919]. *J. biol. Chem.* **38**, 81.
 King, E. J. & Haslewood, G. A. D. [1937]. *J. Soc. chem. Ind., Lond.*, **56**, 97.