XL. THE ERROR DUE TO IMPURE PICRIC ACID IN CREATININE ESTIMATIONS.

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IN the estimation of creatinine in very small concentrations, such as are met with in blood filtrates, by comparing the colours produced on the addition of alkali to the solution to be estimated and to a solution of known creatinine content, both saturated with picric acid, the purity of the picric acid used is an important factor. As picric acid can never be obtained without a perceptible amount of impurity, and is liable to deteriorate on keeping, it was considered that it might be better to work with picric acid admittedly impure and to estimate and allow for the impurity. No mention of the correction for the picric acid described in this paper having been found in a fairly careful search through the literature of the estimation, it was thought good to publish it, as, if it is found sound, it avoids any special purification of the picric acid, and either a great multiplication of unstable standards on the one hand, or lengthy calibrations of a few of them on the other.

In rough estimations of creatinine an assumption commonly made is, that the concentrations of the unknown and the standard are inversely proportional to the lengths of the columns in the colorimeter required for a match. That is to say, if

s is the concentration of the standard solution;

u " " unknown " ;

r is the reading of the colorimeter with the standard set at 10;

that

$$s/u = r/10 \text{ and hence } u = 10s/r.$$

Unless, however, the standard and the unknown are of nearly equal concentrations this assumption is only roughly true, as Hunter and Campbell [1917] and others have shown. The fault appears to lie chiefly, if not entirely, in the picric acid. All workers agree that the picric acid used in these determinations should be specially purified, and Folin and Doisy [1917] have given a somewhat elaborate method of purifying it. The most careful purification will not, however, make the above equation true, and the purest picric acid is liable to become impure if kept in solution. Hunter and Campbell have proposed to overcome the difficulty by reading the standard solutions against a large number of solutions of known creatinine content, and plotting the readings in a curve which is subsequently used in determining unknown

solutions. For accuracy this procedure would require repetition with each fresh lot of picric acid brought into use, and even with one lot if in use for more than a week or two, to make sure that it had not changed.

It was thought that a simple correction might be introduced into the above equation, on the assumption that the picric acid used contains a small amount of some substance which gives a red colour with alkali equivalent to so many milligrams of creatinine per 100 cc. If this amount is called p , and s and u are expressed in milligrams of creatinine per 100 cc., then the equation will run:

$$\frac{s+p}{u+p} = \frac{r}{10} \text{ and hence } u = \frac{10s}{r} + \frac{p(10-r)}{r}.$$

The value of p can be estimated for any sample of picric acid by reading two solutions of known creatinine concentration against each other, each being made up with the picric acid in question. Using the value of p thus found, standards made with this picric acid can be used for the determination of any unknown solutions which will give matchable colours. The purer the picric acid the smaller will be the value of p , but even with the purest picric acid p will not be so small that it can be neglected, except for a small range of unknowns which are nearly of the same concentration as the standard used.

In estimating the value of p for any lot of picric acid it is necessary to choose the concentrations of the two solutions to be compared suitably, or the error of the estimation will be very large. If the concentrations of the two solutions are called s and c , s being set at 10^1 , then the value of p is given by

$$\frac{s+p}{c+p} = \frac{r}{10}; \text{ hence } p = \frac{10s-cr}{r-10}.$$

The concentrations s and c should be chosen so that:

(1) they will give readings as far apart as is consistent with fairly matchable colours, and

(2) they shall be as small as is consistent with colours deep enough to read.

It was found that concentrations of 0 and between 0.2 and 0.3 mg. per 100 cc. met the above requirements.

To check the above reasoning a lot of saturated picric acid solution was made up from some "pure" picric acid, once re-crystallised, and its p estimated as follows.

A solution was made containing 0.2023 mg. of creatinine in 100 cc. of this saturated picric acid. 10 cc. of this and 10 cc. of the saturated picric acid itself were then taken and to each 0.5 cc. of 10 % sodium hydroxide solution was added, and the resulting colours read after standing ten minutes. The readings were as 10 : 19.5, which gives

$$\frac{0.2023+p}{0+p} = \frac{19.5}{10}; \text{ hence } p = 0.21.$$

Standards containing 0, 0.25, 0.5 and 1.0 mg. of creatinine per 100 cc., made up with this lot of picric acid, had been read against each other and against other solutions of known creatinine content, and the readings observed

¹ It goes without saying that the standard may be set at any other more convenient figures 20, 30, etc. and these figures substituted for 10 in the equation.

and those calculated on the assumption that $p = 0.21$ are shown in the following table.

TABLE.

Concentration of standard	Concentration of the other solution	Reading of the colorimeter	Calculated reading ($p = 0.21$)
1.0	0.25	26.2	26.3
	0.33	23.0	22.4
	0.5	17.0	17.0
	0.67	13.8	13.7
	0.92	11.0	10.7
	1.0	10.0	10.0
	1.68	6.3	6.4
	2.33	5.1	4.7
	2.88	4.1	3.8
0.5	0.25	15.6	15.4
	0.5	10.0	10.0
	1.0	5.9	5.9
0.25	0.0	20.9	21.9
	0.25	10.0	10.0
	0.5	6.4	6.5
	1.0	3.8	3.8
0.0	0.0	10.0	10.0
	0.2	5.1	5.1
	0.25	4.8	4.6

It is suggested that a determination of this p affords a more accurate and useful guide to the purity of a sample of picric acid, than the test proposed by Folin and Doisy. In the latter test the colour of an alkaline picrate made by adding 5 % of 10 % sodium hydroxide solution to a saturated solution of the picric acid is compared with that of the picric acid itself. Their requirements of any sample to be used are that the colour of the picrate should not be more than twice as deep as that of the picric itself. This test is open to the objection that the colour of saturated solutions of picric acid, at the same temperature, varies considerably with different samples.

Two samples of picric acid were compared: (1) a solution which had been made a month previously of some acid re-crystallised from B.D.H. "pure," and (2) a freshly prepared solution of some picric acid purified by the method given by Folin and Doisy. Compared by Folin and Doisy's test the alkaline picrates of these gave readings of (1) 8.3, and (2) 8.6, against the respective acids at 20.

When the colours of the two solutions themselves, without the addition of sodium hydroxide, were compared, they read about 20 : 12.8 (number (2) being the lighter), but the colours were hardly matchable.

The value of p for the two samples, using in each case a 0 and a 0.2023 mg. per 100 cc. solution of creatinine in them, worked out at (1) 0.21, and (2) 0.10 respectively.

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REFERENCES.

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