LXXXIX. UREA ESTIMATIONS ON SMALL QUANTITIES OF BLOOD.

By JOCELYN PATTERSON.

From the Charing Cross Hospital Medical School, London.

(Received June 11th, 1925.)

MUCH attention has been given recently to attempts to reduce the amount of blood required for blood urea estimations to considerably less than the volume ordinarily taken in the well-known titration method of Marshall, Cullen and Van Slyke, for, where extended clinical work has demanded a number of estimations on the same individual, it becomes an impracticable proposition to obtain large blood samples each time.

The methods of Hindmarsh and Priestley [1924], Rehberg [1925, 1] and Murray [1925] deviate from the classical method either in employing colorimetric means or special apparatus, whilst that of Thomas [1924] represents an adaptation of the old form to one-fifteenth of the ordinary amount of blood, by use of more dilute standard solutions of acid and alkali. With respect to the latter, however, it has been found [Murray, 1925]—and my own experience confirms the finding—that it fails to give consistent results. There would still seem, therefore, to be a place for a method following the lines of the long-established method of estimating blood urea, which overcomes in great measure the chief sources of possible error involved in the modification devised by Thomas.

In making clinical blood urea estimations, I was led to attempt to adapt the ordinary titration method to cases where only 0.2–0.5 cc. of blood were available, on somewhat similar lines to those described by the last-mentioned author, but could not produce such satisfactory results as he has done even when using a larger quantity of material. Not only did there enter the fallacy of titrating with dilute standard solutions in comparatively large volume [see Rehberg, 1925, 2], but the use of methyl red as indicator also presented difficulties unless due care were taken to perform the final titration in complete absence of CO_2 .

While experimenting with different indicators, my attention was attracted to the suggestion to use a combination of methyl red and methylene blue [de Wesselow, 1924], and I had no difficulty in finding a mixture of the two which gave rise to none of the difficulties encountered in the use of methyl red itself. The combined indicator, made up as stated below, is extremely sensitive to small additions of $N/100$ alkali over the slight range of p_H in which it changes from purple to green, and when once just completely green shows no signs of giving a return colour. Using a microburette with a capillary jet coated on its exterior with a thin film of paraffin wax, each drop can be reduced to a volume of 0.014 cc., sufficient to produce quite a perceptible change

in the indicator. The greatest possible error in the titration, apart from errors in the calibration or reading of the burette, is represented by this figure, equivalent to slightly over 2 mg. per 100 cc. of blood, where 0.2 cc. are employed for the actual estimation.

The technique incorporating the use of this indicator in order to work upon small quantities of blood and titrate in small volume is as follows.

1 cc. of 0.6 % potassium dihydrogen phosphate is placed in a flat-bottomed, stout glass tube and the blood sample (0.2–0.5 cc.) added to it from a capillary pipette, the latter being afterwards washed out with a portion of the phosphate solution. 0.2 g. of a well-mixed sample of finely ground soya bean meal is accurately weighed out and added to the contents of the tube, which are then incubated at 40° for 15 minutes. The ammonium salt is decomposed by the addition of 4 cc. of saturated potassium carbonate solution and 2 g. of solid potassium carbonate, a few drops of caprylic alcohol are then added, and the tube attached for aeration to sulphuric acid and titration tubes in the usual way. The titration tube is of resistance glass, 6" $\times$ $\frac{5}{8}$ ", and contains 5 cc. of *N*/100 sulphuric acid if 0.5 cc. of blood is available for analysis, 2 cc. diluted with 2 cc. of distilled water, if the estimation is being done on 0.2 cc. of blood, with 3–4 drops of an indicator made up of 100 cc. of 0.02 % methyl red and 30 cc. of 0.1 % methylene blue.

Aeration requires about 1 hour if conducted at a rate which will ensure with certainty that the ammonia will be completely absorbed by the small volume of acid used. *N*/100 sodium hydroxide added from a 5 cc. microburette (a 2 cc. burette, graduated to 0.02 cc., when 0.2 cc. of blood are taken) is used in the final titration.

The usual blank determination is performed in the same way as above, and its value subtracted from the blood values.

For 0.5 cc. blood (blood reading – blank reading) cc. $\times$ 60 = mg. urea per 100 cc. blood.

For 0.2 cc. blood (blood reading – blank reading) cc. $\times$ 150 = mg. urea per 100 cc. blood.

In this way the figures obtained with 0.5 cc. of blood all agree within 1 mg. per 100 cc. of blood with the values given by the ordinary method on 3 cc. (using the mixed indicator in place of methyl red for the latter). Typical figures obtained by use of 0.2 cc. of blood and an ordinary make of 2 cc. microburette are:

1. Standard urea solution.

Urea mg. per 100 cc.	Found
20.0	18.0
	19.5
	19.5
39.5	36.0
	37.5
	40.5
	40.5
98.7	94.5
	93.0
	94.5
	96.0

2. Blood urea.

Urea mg. per 100 cc.	Found
26.7 (by ordinary standard method)	27.0
43.0 " "	40.5
61.5 " "	60.0

Such figures may be taken as sufficiently accurate for most clinical purposes.

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