XLII. THE DETERMINATION OF UREA IN 0·1 cc. OF BLOOD BY MICROTITRATION.

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Both in the laboratory and in the clinic determinations of blood-urea at short intervals of time are often required, and it is difficult to make them because of the amount of blood needed.

By means of the method of microtitration described in the preceding paper it is possible to overcome this difficulty.

The method to be described is a modification of the Van Slyke method, depending on conversion of the urea into ammonia by means of urease and subsequent aeration of the ammonia into acid and titration of the excess of acid.

The following stock reagents are needed: $N/30$ solutions of H_2SO_4 and NaOH (and if uraemic blood is to be expected $N/10$ solutions); a 0·005 % solution of methyl red[1]; octyl alcohol; saturated K_2CO_3 solution; a 10 % solution of a urease preparation (freshly prepared each day) in water, or, better, in a buffer solution at p_H 7 (Sørensen phosphate buffer p_H 7 diluted 20 times)[2].

The determination is carried out in the following way:

0·1 cc. of blood is measured out in a test-tube in which have previously been placed 0·5 cc. of water and a trace of powdered sodium oxalate. The pipette is rinsed twice with the water and 0·1 cc. of the urease solution is added. The tube is closed, and after the lapse of the usual time (10 mins. at 40°, 30 mins. or more at room temperature) 0·5–1 cc. of saturated K_2CO_3 and a drop of octyl alcohol are added, and the tube is now ready for aeration.

The titration tubes, about 12 × 1·6 cm., which must be flat-bottomed and of resistant glass, are in the meantime prepared as follows: 0·1 cc. $N/30$ H_2SO_4 is measured from a microburette into a titration tube, 4 cc. of water (best redistilled from acidified distilled water), a drop of octyl alcohol, and 0·1 cc. of the indicator solution are added. If a microburette is not used 1 cc. of $N/300$ H_2SO_4 (diluted fresh every day from the $N/30$ solution with

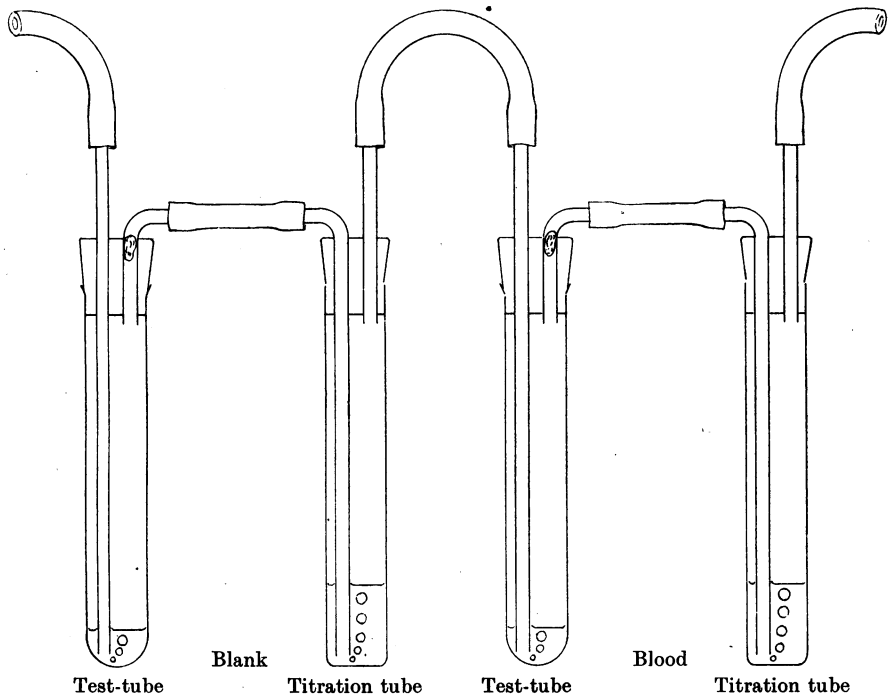
[1] Instead of methyl red a 0·01 % bromocresol purple solution may conveniently be used as indicator—it gives a sharper end point, but it involves the necessity of using solutions absolutely free from carbon dioxide.

[2] 3·9 cc. of a 0·9078 % KH_2PO_4 solution + 6·1 cc. of a 1·1876 % Na_2HPO_4 solution diluted to 200 cc.

a weak indicator solution) is measured out with an accurate pipette and 3 cc. water and octyl alcohol are added. The test-tubes are then connected as shown in the figure, a loose plug of cotton-wool being placed in the tubes between test-tubes and titration tubes, and a current of air, freed from ammonia and carbon dioxide by means of H_2SO_4 and soda-lime, is passed through them.

The time required for aeration depends of course on the rate of the air current, but I have found it possible to drive over all ammonia in 30–45 mins. One or two blank experiments must be run at the same time.

After disconnection the contents of the titration tubes are titrated by means of the microburette with $N/30$ NaOH, as described in the preceding



paper. The difference between a blank and a blood-tube multiplied by the titre factor indicates how many mg. urea are contained in 100 cc. of blood.

Example:

$$100 \text{ mm.}^3 \ H_2SO_4 = 97{\cdot}3 \text{ mm.}^3 \ NaOH$$

$$\text{Titre factor} = \frac{100}{97{\cdot}3} = 1{\cdot}028.$$

Blank = 95·1
Blood = 69·7

$$25{\cdot}4 \times 1{\cdot}028 = 26{\cdot}1 \text{ mg. urea per 100 cc. blood.}$$

This method has in my hands given a mean error of ± 0·7 mg. urea on a single determination, but since it is easy to obtain duplicate analyses it allows of a determination with an accuracy of about 0·5 mg.

As examples I shall give the following experiments:

1. Urea solutions of known concentration:

Urea, mg. per 100 cc.	Found
28·95	29·4
	28·5
	28·2
115·8	115·5
144·75	144·9

2. Blood urea:

	Time	mg. per 100 cc.
Fasting	9.30	21·2
		21·2
	10.00	21·0
		21·3
2 eggs eaten	10.15	
	10.50	21·5
		21·0
	11.25	21·5
		21·2
	12.10	25·8
		25·3

3. Blood urea:

	Time	mg. per 100 cc.
	1.45	18·8
		19·7
20 g. urea taken in water	1·52	
	2.10	36·8
		37·3
	2·42	46·2
		47·6
	3·25	56·0
		56·3