LXIX. DETERMINATION OF UREA IN BLOOD.

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THE methods for the determination of urea in blood described below have been developed from a procedure described by Kennaway [1920]. The ammonia produced by the action of urease is determined directly and without previous aeration; consequently no special apparatus is required and the possible error due to incomplete removal or loss of ammonia is avoided.

The macro-method has proved most reliable and simple as a routine procedure.

MACRO-ESTIMATION OF UREA IN BLOOD.

Solutions required (1) 10 % sodium tungstate; (2) 0.75 N H_2SO_4 ; (3) urease extract; made by shaking about 1 g. of powdered soya bean with 50 cc. of 0.85 % saline and filtering after 20 minutes.

Method. Measure 5 cc. of blood into a 175 cc. conical flask, followed by 5 cc. of 0.75 N H_2SO_4 and 5 cc. of 10 % sodium tungstate.

Now add 35 cc. water and shake thoroughly. Filter into a 50 cc. measuring cylinder. Place 30 cc. of filtrate into another 175 cc. conical flask and wash out the cylinder with 10 cc. water. Add 3 drops 0.2 % methyl red or chlorophenol red, boil and cool under the tap. With each batch of estimations a blank is run in order to allow for the alkalinity of the enzyme extract. This consists of 40 cc. of water placed in a similar flask with an equal quantity of indicator. Adjust to a reddish yellow colour by means of $N/100$ acid or alkali. Now neutralise the test, first approximately by means of $N/10$ alkali and finally match with the blank by means of $N/100$ alkali. Add 5 cc. of enzyme extract to both blank and test. Incubate for 1 hour at about 50°. Add $N/100$ acid in 10 cc. lots until the solution is definitely acid. Most samples will not require more than 10 cc. The blank receives 10 cc. Boil, cool under the tap and titrate with $N/100$ alkali matching the test against the blank. The volume of alkali run in is subtracted from that required for the blank and the difference multiplied by 10 gives the percentage of urea in the blood.

The sensitivity of the method depends largely upon the sharpness of the end-point. Even with $N/100$ alkali in the presence of blood-salts and bean extract this is surprisingly sharp, provided (a) that the amount of indicator

added is not too great and is the same as that used for the blank, (b) that the minimum quantity of potassium oxalate is used as anticoagulant; 1–2 drops of a 20 % solution are ample for 10 cc. blood.

If these conditions are carefully observed a few drops of the alkali produce an unmistakable change in shade.

MICRO-ESTIMATION OF UREA IN BLOOD.

A 4 in. $\times$ $\frac{1}{2}$ in. test-tube is calibrated to hold 10 cc. Place 8–9 cc. water in the tube and then run in 0.25 cc. of blood, washing the pipette out with the water. Now add 0.25 cc. 0.75 *N* H₂SO₄, and, after mixing, 0.25 cc. 10 % sodium tungstate. Mix again. Wash down the sides with a little water bringing volume almost up to 10 cc. Centrifuge for 5–10 minutes at about 2000 revs. The supernatant fluid must be water-clear. Make up to the 10 cc. mark and drain off into an accurate 10 cc. measure. Read volume—about 8–9 cc.—and transfer to a 50 cc. conical flask washing out the measure with 2 cc. water. Add 1 drop of 0.2 % methyl red from a capillary pipette. Boil, cool. Neutralise, first roughly with *N*/10 NaOH and finally with *N*/500 alkali or acid. Two blanks consisting of 12 cc. water and a drop of indicator are set up and brought to neutrality. The test is matched against them. Now add 0.5 cc. urease solution to each flask and incubate at 50° for $\frac{1}{2}$ hour. After incubation run in *N*/500 acid from a micro-burette until the solution gives a marked red colour. Note the volume. The blanks should each receive 1 cc. of acid. Now boil, cool and titrate with *N*/500 alkali. The number of cc. of acid neutralised by the ammonia multiplied by 300 and divided by the volume of supernatant fluid used represents the urea in mg. %.

Notes. (1) The blood will usually be taken from a finger and delivered direct into the test-tube. If this is not convenient it is absolutely essential that the minimum quantity of oxalate is used.

(2) It is important to use the same amount of indicator in both the test and the blank. If this is done there should be no difficulty with the end-point, at any rate in good daylight. The two blanks are placed on either side of the test during titration and take the place of a comparator.

Table I. *Comparison of macro- and micro-methods.*

	Urea mg. %																			
Macro-method (5 cc. oxalated blood)	68	274	89	30	57	23	116	34	211	35	123	44	37	47	48	59	45	38	24	
	43	13	26	224	69	26	47	33	63	39	49	60	42	65	27	41	173	48	51	
Micro-method (0.2 cc. oxalated blood)	73	256	82	32	53	19	109	30	188	36	134	46	37	43	39	57	36	33	23	
	39	20	29	247	68	27	47	37	62	39	53	58	44	59	25	42	180	50	54	

We do not claim for this method the accuracy obtainable with some of the more elaborate procedures available (see review by Klisiecki [1926]), but suggest that it has the advantage of simplicity. In our hands it has given results which agree fairly well with those obtained on the larger quantities of blood. These comparisons were made without undue attention to detail so

that an opinion could be formed of the order of accuracy that might be expected under routine conditions.

In Table II this method was used to follow the rise in the blood-urea after administration of 15 g. urea.

Table II.

		Subject		
		A.	B.	C.
Blood-urea	Initial	38	30	36
mg. %	$\frac{1}{2}$ hour	54	43	54
	1 hour	—	52	55
	$1\frac{1}{2}$ hours	76	52	55
	2 hours	77	—	50
	$2\frac{1}{2}$ hours	51	51	50

REFERENCES.

- Kennaway (1920). *Brit. J. Exp. Path.* **1**, 135.
 Klisiewicz (1926). *Biochem. Z.* **176**, 490.