XXVI. THE ESTIMATION OF NON-PROTEIN NITROGEN IN BLOOD.

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THE object of this paper is to describe a rapid micro-method for the estimation of non-protein nitrogen in blood. Several methods have hitherto been described, but, for various reasons, are unsatisfactory. The method described by Cole [1919] is very lengthy, and requires 5 cc. of blood—an amount which cannot be obtained from small animals, and only with some inconvenience from man. The method of Folin and Denis [1916] is even more unsatisfactory. Several cc. of blood are required. The digestion mixture does not appear to be a suitable one, the contents of the incineration tube turning solid before the incineration is complete, and being afterwards quite insoluble in water: this defect is apparently due to an excess of phosphoric acid. Even if this occurrence be avoided, Nesslerisation is very difficult: Cole has observed this, and notes that a cloud rapidly appears. Frequently a brick-red precipitate appears also.

The following method, which may be applied to small quantities of blood, obviates these difficulties, and may be used with success when estimating the non-protein nitrogen in the blood of small animals.

Special apparatus etc. required:

1. The solutions required for the preparation of blood filtrates, as described by Folin [1919].

2. A digestion mixture. To 50 cc. of a 5 % copper sulphate solution add 100 cc. of 85 % phosphoric acid, and 300 cc. of pure concentrated sulphuric acid.

3. A boiling tube, 10×1 cm. The tube need not be of special glass. It is graduated at 3.5 cc. It is provided with a small loose stopper, shaped like a specific gravity bulb, which is introduced neck downwards into the boiling tube, so as to close the orifice.

4. A micro-filtration apparatus, such as is described in the paper dealing with the author's method for the estimation of blood-sugar [Ponder and Howie, 1921]. This is essentially a small filter which filters under slight suction.

5. Small calibrated pipettes, to deliver 0.2, 0.15, 0.5 and 1.5 cc.

6. Standard ammonium sulphate solution, containing 0.4716 g. of the purified salt per litre; Nessler's reagent, as described by Folin or Cole.

The estimation is performed as follows:

1. Blood is drawn from the finger, or from an animal's vein, into a 0.2 cc. pipette. The contents of the pipette are added to 1 cc. of distilled water:

the pipette is twice filled with water, and these volumes, carrying with them all traces of blood from the walls of the pipette, are added to the tube containing the blood and distilled water. 0.2 cc. of sodium tungstate and 0.2 cc. of $2/3 N$ sulphuric acid are added: the tube is allowed to stand for five minutes at least, its contents being occasionally shaken.

2. A filtrate is produced from the contents of the tube by about two minutes filtration in the micro-filter.

3. 0.5 cc. of filtrate is placed in the boiling tube, and to it is added 0.2 cc. of the digestion mixture diluted 1 in 4. The contents are boiled over a micro-burner, very gently. As soon as boiling occurs, the stopper is placed in the mouth of the tube. The boiling is continued for two minutes. The flame should be very low; bumping is thus prevented. At the end of the incineration, the flame is removed, and a few drops of water are added. The tube is allowed to cool, and is then filled to the mark with distilled water.

4. A standard is prepared. Place 3.15 cc. of distilled water in a tube, add 0.15 cc. of standard ammonium sulphate solution, and 0.2 cc. of the diluted digestion mixture.

5. Nesslerise the contents of the boiling tube and the standard simultaneously, by adding 1.5 cc. of Nessler's reagent. There is no difficulty in obtaining a clear yellow solution.

6. The solutions are matched in the colorimeter in the usual way. If the standard be set at 15, the non-protein nitrogen in 100 cc. of blood is 15 divided by the reading, and multiplied by 30.

It has not been possible, for the reasons given above, to compare this method with that of Folin and Denis. The method has however been applied to nitrogenous substances, of known nitrogen content, which require incineration before producing a colour with Nessler's reagent, and has been found accurate and satisfactory, the results obtained by the method and the calculated results agreeing closely.

Some results obtained in the examination of blood of various animals are given in the following table:

Animal	mg. in 100 cc.	Animal	mg. in 100 cc.
Man	36	Rabbit	43
„	25	Frog	93
Cat	67	Lizard	76

In the case of the frog and lizard, the blood was obtained from the heart.

It is claimed for this method that (1) a very small quantity of blood is required, (2) that the technique is simple and rapid, (3) that the final Nesslerisation presents no difficulty, and (4) that the results given are accurate.

REFERENCES.

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