XX. THE ESTIMATION OF BLOOD SUGAR.

BY ERIC PONDER AND LAURENCE HOWIE.

From the Department of Physiology, University of Edinburgh.

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THE method devised by Folin and Wu in 1918 and revised by them in 1920 [1918, 1920] has afforded a means of estimating blood sugar with a high degree of consistency and accuracy. To obviate the one objection to this method—namely, the necessity of using a larger quantity of blood than is readily obtained from a finger prick—Mackenzie Wallis and Gallagher [1920] have devised a micro-method, based partly on the work of I. Bang [1914], but chiefly on the method of Folin and Wu. That their method is convenient in the respect that small quantities of blood are required, is beyond doubt: their technique, however, introduces several very undesirable features, besides being somewhat lengthy. In their method the small quantity of blood required is weighed on absorbent paper on a torsion balance: the glucose is then extracted from the paper by distilled water, the proteins precipitated by tungstic acid, and the sugar in the filtrate estimated by the method of Folin and Wu.

The use of the torsion balance is an unfortunate feature of this method, for it almost necessarily introduces an initial error of considerable magnitude, owing to the evaporation from the absorbent paper. We have performed a series of weighings to demonstrate the extent of this error as follows: the subject was bled directly on to a piece of absorbent paper of known weight, hanging on the torsion balance, and the weight of the blood obtained by rapid weighing, the weighing being completed within ten seconds after the withdrawal of the blood from the finger. The piece of paper with the absorbed blood was then left hanging on the balance, and repeated weighings made at the end of one, two, three, and four minutes. The progressive loss of weight, which was greatest during the first minute, was found to be fairly constant for all samples of blood. That this loss by evaporation should occur is unavoidable, and only to be expected: when it occurs to the extent of several milligrams in a sample of only about 150 mg. weight, the percentage error is too great to be disregarded.

We give a table of the results obtained with five weighings—these five being representative of a large number made (the balance used was an instrument by Hartmann and Braun).

Weight of blood	Loss % occurring in			
	1 min.	2 mins.	3 mins.	4 mins.
163	1.2	1.8	3.0	3.0
163	1.8	2.4	2.4	3.7
165	1.8	3.0	3.6	3.6
165	1.8	2.4	3.0	3.6
160	1.9	2.5	3.1	3.7
Mean	1.7	2.4	3.0	3.5

It will be seen that if the interval between the withdrawal of the blood from the finger of a patient and the completion be as long as one minute an error of nearly 2 % is introduced, whereas if the interval be longer, a greater error will be present. This is unsatisfactory. Mackenzie Wallis and Gallagher prefer this method to the use of small sized pipettes of doubtful accuracy; since, however, it is an easy matter to calibrate a pipette to deliver 0.2 cc. with an error of as little as 0.5 %, we have preferred the use of such to the use of the torsion balance. The use of pipettes further greatly simplifies the technique, and at the same time avoids an obvious fallacy in the micro-method of these authors, the assumption that all the glucose in the blood is extracted from the absorbent paper by distilled water; it also avoids the inconvenient lengthening of the time required for the estimation, which this extraction entails.

It may be pointed out that the error introduced by the use of the torsion balance (tending to give too high a percentage), may be compensated by an incomplete extraction of the glucose from the absorbent paper, the results finally obtained perhaps agreeing with results given by the method of Folin and Wu. To rely on this occurring is, however, most undesirable, for this compensation may not regularly occur.

We suggest the use of the following simple micro-method, which is essentially the method of Folin and Wu applied to small quantities.

Special apparatus, etc. required:

1. The solutions required for the precipitation of proteins and the estimation of sugar in blood by the method of Folin and Wu.
2. Small boiling tubes, about 7 × 70 mm. The necks of these tubes are drawn out so as to form a tube of about 10 mm. long and 3 mm. in diameter. The tubes must be able to contain 1.2 cc., but not more than 1.5 cc.
3. A micro-filtration apparatus. A small funnel about 1 inch in diameter, is passed through a rubber stopper in a wide, flat-bottomed tube, carrying a side tube which may be attached to a filter pump. The funnel delivers the filtrate into a small tube, about 10 × 30 mm. which is contained inside the tube above described. Over the filter is a cover, to protect the blood from evaporation during filtration. Slight suction only is used, applied not constantly, but intermittently, and only occasionally; the papers used with the filter are "extra-hardened." This filter will produce 0.6 cc. of filtrate from the amount of blood used.

4. Pipettes, accurately calibrated by weight, to deliver 0.4 cc. and 0.2 cc. These may be operated by teats.

The estimation is done as follows:

1. Blood is drawn from the finger into a 0.2 cc. pipette. The contents of the pipette are added to 1 cc. of distilled water: the pipette is then twice filled with water, and these volumes, carrying with them the 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 then added: the tube is allowed to stand for five minutes at least, its contents being occasionally shaken gently.

2. The contents of the tube are placed in the filter; after about two minutes' filtration through hardened paper under slight pressure, sufficient filtrate will be obtained. It is important to keep the filter covered during filtration.

3. To a small boiling tube, as above described, is added 0.4 cc. of a 0.01 % glucose solution; while to a similar tube is added 0.4 cc. of the filtrate. To each is added 0.4 cc. of the alkaline copper tartrate solution. The contents are mixed by stirring. The narrow tube-like necks of the boiling tubes are sealed rapidly in a blow pipe flame, care being taken not to heat the contents of the tube. The air space left will be small if the tubes are of the proper size. The tubes are immersed in a boiling water bath for six minutes.

4. At the end of this time, the temperature of the bath is lowered to about 80°. The necks of the tubes are cut off, and 0.4 cc. of the phenol reagent are added to each tube; this requires care lest the contents of the tubes be expelled. The tubes are allowed to cool. Each is emptied into a tube containing 3.8 cc. of water, thus making the volume of the contents of this tube up to 5 cc.

5. The contents of these tubes are matched in the colorimeter in the usual way; and the percentage of glucose present in the blood calculated.

This method has in our hands given results closely comparable with the results given by the method of Folin and Wu. The following table shows the results obtained in ten specimens of blood examined (a) by the method of Folin and Wu, and (b) by the method above described.

Specimen	% of sugar	
	Folin and Wu	Micro-method
1	0.121	0.123
2	0.103	0.102
3	0.093	0.094
4	0.055	0.054
5	0.148	0.148
6	0.125	0.122
7	0.150	0.151
8	0.145	0.142
9	0.127	0.127
10	0.138	0.139

It should be noted that these are not all samples of normal blood under the usual conditions, since certain of them were taken after prolonged fasting, while others were taken after excessive carbohydrate intake.

It is claimed for this modification that (1) the amount of blood required may readily be obtained from the finger, (2) that this amount of blood is accurately known, and that none of it is lost by manipulation, (3) that re-oxidation during boiling is reduced to a minimum, (4) that the colour finally obtained in the "blood" tube is very similar to that in the "standard," and (5) that the results obtained are practically identical with those given by the method of Folin and Wu.

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