CXIV. A METHOD FOR THE ESTIMATION OF GLUCOSE IN BLOOD.

By JOHN ALEXANDER MILROY.

From the J. C. White Biochemical Department, The Queen's University, Belfast.

(Received July 30th, 1925.)

So many excellent methods are available for the estimation of glucose in blood, that the introduction of another one requires some justification. The following method probably yields less precise results than several others; but has the advantage of simplicity, and also enables one to determine the approximate amount of sugar in blood without the aid of a colorimeter.

The method is based on the fact that nitro-anthraquinonesulphonates are reduced, when heated with glucose in alkaline solution, first to the corresponding hydroxylamine derivatives having an intense green colour [Wacker, 1902] and later to a deep red substance, which is probably an amine derivative.

The red-coloured final product of reduction is only sparingly soluble in water, and consequently the method is only applicable to dilute solutions of glucose.

A number of nitro-anthraquinonesulphonic acids are known, the chief of these being the 1·6-, 1·7-, α - and β -acids of Claus [1882] and the 1·5- and 1·8-acids [Schmidt, 1904]. These acids, especially the 1·6-acid, are decomposed more or less readily when heated with caustic alkalis, and more slowly by alkaline carbonates, the NO_2 radical being replaced by OH [Frobenius and Hepp, 1907]. The 1·5-acid was found to be the most resistant to the action of alkali, and was therefore selected for the following method. It can be easily prepared in the way described by Schmidt. The isolation of the compound is greatly facilitated by filtering it off through the fritted glass filter discs devised by Prausnitz. Filtration through asbestos is a much slower process. The crystalline precipitate thus separated was purified by recrystallisation from water, and dried *in vacuo* at room temperature.

All the nitro-anthraquinonesulphonic acids have the power of precipitating proteins, the most effective in this respect being the 1·6-acid, but the nitro-anthraquinonesulphonic acid cannot be used both as a protein precipitant and as a reagent for the reduction colour test, since its concentration must be the same in the standard glucose solutions and in the blood filtrates.

Of the different nitro-anthraquinonesulphonic acids, the 1·6-acid was found to be the most delicate with respect to the glucose reaction, but the 1·5-acid

is to be preferred for quantitative work for the reason already given. 0.1 cc. of 0.01 % glucose, 0.1 cc. of 0.4 % solution of the 1.5-acid, and 1.0 cc. of 50 % potassium carbonate solution were made up to 5 cc. and heated in the water-bath for 8 minutes, a similar blank experiment with the reagents being carried out concurrently. Under these conditions a faint red tint was just perceptible in the glucose test when compared with the control.

In view of the fact that blood contains organic substances other than glucose, such as uric acid and creatinine, which act as reducing agents in alkaline solution, it was important to ascertain whether these substances reduce nitro-anthraquinonesulphonates under the conditions of the method for the estimation of glucose to be given later. The ratio of the reducing power of glucose to that of uric acid, dissolved as lithium urate, was found to be 2.35 : 1. The reducing power of creatinine was only about one-tenth that of glucose. These statements only hold when alkaline carbonates are used. In the presence of caustic alkalis the reducing powers of creatinine and urate are increased. The amounts of uric acid and creatinine found in blood cannot therefore seriously affect the calculated glucose values. When the percentages of uric acid and creatinine in blood are much higher than normal, the glucose values may be corrected on the basis of the foregoing ratios.

An intense green colour which later passes into a deep red is obtained when normal urine is rendered alkaline with sodium or potassium carbonate and heated with a solution of the 1.5-acid.

Technique of method.

The proteins are precipitated from blood by the tungstic acid method [Folin and Wu, 1918], the blood being therefore diluted 1 in 10. The mixture of diluted blood with sodium tungstate and $2N/3$ H_2SO_4 is allowed to stand for 30 minutes prior to filtration. 5 cc. of the protein-free blood filtrate are measured into a graduated test tube of 15 cc. capacity. 1.5, 1.8, 2.1, 2.5 and 3.0 cc. of 0.02 % glucose are then measured with a normal pipette or micro-burette of 5 cc. capacity, graduated in 0.02 cc., into five numbered graduated test tubes each of 15 cc. capacity and 12 mm. bore. These quantities are in a geometrical series having an approximate common ratio of 1.2 and correspond to 0.06, 0.072, 0.084, 0.1 and 0.12 % glucose in the undiluted blood with the protein-free filtrate of which they may be found to yield a colour match. If necessary the series may be extended in either direction; but, owing to the sparing solubility of the red product of reduction, the method in this form is only applicable to samples of blood containing not more than 0.16 % glucose. In cases of hyperglycaemia it is therefore necessary to use a smaller quantity of blood filtrate, *e.g.* 2.5 cc., and to make allowance for this fact in the calculation.

One cc. of 0.4 % aqueous solution of 1.5-nitro-anthraquinonesulphonic acid, and 2 cc. of a solution of potassium carbonate, containing 50 g. per 100 cc. of solution, are then added to the contents of each test-tube. All the

solutions are then diluted up to 10 cc. with distilled water, and mixed thoroughly. At this stage a small quantity of the sparingly soluble potassium salt of the 1·5-acid may separate as a crystalline precipitate if the temperature of the room be low. The test tubes are immersed in boiling water for 8 minutes, then cooled thoroughly in running water, and the volume of each solution made up to 12·5 cc. In many estimations the volume was simply restored to 10 cc. The red tint of the tube containing blood filtrate is then compared with the colours yielded by the glucose solutions. An approximate estimate of the percentage of blood sugar is thus obtained.

1 cc. of 0·4 % solution of 1·5-nitro-anthraquinonesulphonic acid was found to be sufficient for amounts of glucose up to at least 5 cc. of a 0·02 % solution.

The method can be rendered more accurate by using amounts of standard glucose solution in a geometrical series having a smaller common ratio, *e.g.* 1·1. This procedure was adopted after an approximate result had been obtained by the first method. As a rule only one intermediate glucose standard requires to be prepared.

As regards the degree of accuracy of the method one may infer from the limits of sensitiveness of the colour reaction, on which the method is based, that the minimal error in the estimation of blood sugar in a sample of blood containing 0·1 % glucose is about 4 %. The percentage error would be proportionally greater than this for samples of blood containing less than 0·1 % and less for those containing more than this amount.

If a colorimeter be available, the solution derived from the blood filtrate is finally compared in it with the glucose solution which it most nearly matches. As a rule, the results were found to be most accurate when a slightly higher glucose standard was taken for comparison.

It is important to avoid delay in making the colour comparison, especially when dealing with samples of blood containing as much as 0·16 % glucose, since part of the sparingly soluble red product of reduction gradually separates when the solutions are allowed to stand for several hours.

The sugar was estimated in a large number of samples of blood from normal animals and from animals under the influence of drugs or subjected to operative interference. A few of these results are given in the following table. For purposes of comparison the sugar in each sample of blood was also determined by Folin and Wu's method [1920].

Table I.

Source of blood	% of glucose		Condition of animal
	(new method)	(Folin and Wu's method)	
Rabbit	0·178	0·170	Ether anaesthesia
"	0·334	0·330	Ether and cooling of pancreas
Dog	0·134	0·121	Ether anaesthesia
Cat	0·43	0·40	Ether and adrenaline
Man (normal)	0·084	0·076	After 4 hours' fast
Ox	0·102	0·096	Normal
Cat	0·03	0·026	During convulsions after insulin.

As will be seen from the table the maximal difference between the two methods is about 10 % except in cases of hypoglycaemia, in which the discrepancy is greater.

SUMMARY.

1. A colorimetric method for the estimation of glucose in blood is described.
2. The method gives somewhat higher values for glucose than that of Folin and Wu.
3. At least 1 cc. of blood is required for an estimation.

I wish to express my great indebtedness to Dr A. E. Campbell and Dr J. S. Loughridge for carrying out a large number of analyses.

REFERENCES.

- Claus (1882). *Ber. deutsch. chem. Ges.* **15**, 1514.
Folin and Wu (1918). *J. Biol. Chem.* **38**, 106.
—— — (1920). *J. Biol. Chem.* **41**, 367.
Frobenius and Hepp (1907). *Ber. deutsch. chem. Ges.* **40**, 1048.
Schmidt (1904). *Ber. deutsch. chem. Ges.* **37**, 71.
Wacker (1902). *Ber. deutsch. chem. Ges.* **35**, 666.