CXII. A METHOD FOR THE ESTIMATION OF "TRUE" SUGAR IN 0.05 ml. OF BLOOD

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A METHOD which gives most nearly the true glucose values for blood should show the following characteristics:

(i) Glutathione and other non-sugar reducing substances are excluded either by the protein precipitants [Somogyi, 1929; 1930] or by using unhaemolysed blood [Herbert & Bourne, 1929; 1930].

(ii) The sugar values obtained are identical with the amounts of sugar fermentable by yeast, and the entire blood sugar so determined is fermentable.

(iii) Good results are obtained when pure glucose is estimated in solution and after addition to blood.

A titration method meeting these requirements was recently described [King *et al.* 1937] for 0.2 ml. of blood. A modification of this method is here described in which the blood sample is 0.05 ml. The protein precipitants used are sodium tungstate and copper sulphate [Somogyi, 1931] and the filtrate is treated with a modified Harding & Downs [1933] copper reagent. The cuprous oxide formed is estimated by the colour produced with a phosphomolybdic acid solution [Folin & Wu, 1929].

With this method, glucose was estimated accurately in pure solutions and after addition to blood. Determinations were made using pure glucose solutions, normal bloods, diabetic bloods and mixtures of these bloods with glucose solutions. The results obtained were identical with those found using the previously described method [King *et al.* 1937].

EXPERIMENTAL

Technique of the analyses

(a) *Pure glucose solutions.* The glucose solution (1 ml.) was mixed with 1 ml. of the copper reagent in a wide test tube ($\frac{3}{4}$ in. diameter). The tube, stoppered with cotton-wool, was placed in a boiling water bath for exactly 10 min. After immediate cooling, the mixture was treated with the phosphomolybdic acid (3 ml.). The colours were read within 15 min. in a Duboseq type colorimeter fitted with an Osram sodium light.

Glucose solutions of concentrations from 0 to 75 $\mu\text{g.}/\text{ml.}$ (equivalent to from 0 to 300 mg./100 ml. in blood at a 1/40 dilution) were treated as above.

The colorimetric readings are shown in Table I.

Glucose standards in benzoic acid gave identical readings.

(b) *Application to blood.* 1 ml. of blood filtrate (1/40 dilution) was used exactly as the glucose solutions above. The method is as follows. 0.05 ml. of whole blood is pipetted into 1.75 ml. of isotonic sodium sulphate solution, or isotonic sodium sulphate fluoride-thymol solution. 0.1 ml. each of sodium tungstate and of copper sulphate are added and the solutions thoroughly mixed. The mixture is centrifuged. 1 ml. of the filtrate (= 0.025 ml. of blood) is mixed with 1 ml. of the

Table I

$\mu\text{g.}$ of glucose per ml. of "test" solution	Reading (mm.) of 20 $\mu\text{g.}/\text{ml.}$ "standard" solution	Reading (mm.) of 50 $\mu\text{g.}/\text{ml.}$ "standard" solution
0	0	—
5	2.5	—
10	5.0	—
15	7.5	—
25	12.5	—
30	15.0	6.0
35	17.5	—
40	—	8.0
50	25.1	—
60	—	12.1
75	—	15.1

mixed copper reagent in a wide test tube ($\frac{3}{4}$ in. diameter). The tube, stoppered with cotton-wool, is placed in a boiling water bath for exactly 10 min. After immediate cooling, 3 ml. of the phosphomolybdic acid reagent are added. The colour produced is compared with that produced by 1 ml. of a standard glucose solution in benzoic acid, treated in the same way as the blood filtrate.

Calculation. Let x = concentration of standard in $\mu\text{g.}/\text{ml.}$

$$\text{Blood sugar (mg./100 ml.)} = \frac{\text{reading of standard}}{\text{reading of test}} \times \frac{x}{1000} \times \frac{100}{0.025};$$

e.g. with 20 $\mu\text{g.}/\text{ml.}$ standard

$$\begin{aligned} \text{Blood sugar (mg./100 ml.)} &= \frac{\text{reading of standard}}{\text{reading of test}} \times \frac{20}{1000} \times \frac{100}{0.025} \\ &= \frac{\text{reading of standard}}{\text{reading of test}} \times 80. \end{aligned}$$

Similarly, with 50 $\mu\text{g.}/\text{ml.}$ standard

$$\text{Blood sugar (mg./100 ml.)} = \frac{\text{reading of standard}}{\text{reading of test}} \times 200.$$

Table II shows a comparison of the titrimetric method [King *et al.* 1937] and the present method. Determinations by both methods were carried out independently.

Table II. *Blood sugar (mg./100 ml.)*

Titrimetric method	Colorimetric method
85	84
85	84
90	89
90	89
72	74
73	74
77	78
103	101
103	101
130	129
130	130
108	109
129	128
164	165
189	186
240	242
216	217
300	302

(c) *Recovery experiments.* These were carried out using both methods, the amount of glucose added in both cases being proportional.

	Quantities (ml.) used	
	Titrimetric method	New colorimetric method
Blood	0.2	0.05
Sodium sulphate	1.2	1.25
Glucose solution in isotonic sodium sulphate	2.0	0.5
Copper sulphate	0.3	0.1
Sodium tungstate	0.3	0.1

The results obtained are shown in Table III.

Table III. *Blood sugar (mg./100 ml.)*

Added equivalent of 50 mg./100 ml. glucose		Added equivalent of 100 mg./100 ml. glucose		Added equivalent of 200 mg./100 ml. glucose	
Titrimetric method	Colorimetric method	Titrimetric method	Colorimetric method	Titrimetric method	Colorimetric method
127	129	103	101	85	86
178	178	203	202	289	291
63	64	67	66	107	108
114	114	165	167	306	309
105	104	—	—	67	67
153	154	—	—	267	266

Solutions

(1) *Copper reagent. Solution A.* 13 g. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ are dissolved in water and the volume made up to 1 l.

Solution B. 50.0 g. NaHCO_3 are dissolved by shaking in the minimum amount of distilled water. When all the bicarbonate is dissolved, 40.0 g. anhydrous Na_2CO_3 are added to the solution and the mixture shaken until all the carbonate has dissolved. 36.8 g. potassium oxalate are dissolved in successive small quantities of warm water and added to the mixture. Finally a solution of 24.0 g. sodium potassium tartrate in the minimum amount of water is added to the mixture. The volume is made up to 1 l. and the mixture well shaken.

The copper reagent used is prepared freshly each day and is a mixture of exactly equal volumes of solutions A and B.

(2) *Phosphomolybdic acid reagent* [Folin, 1930]. 35 g. molybdic acid and 5 g. sodium tungstate are dissolved in 250 ml. of 0.2 *N* NaOH and boiled for 30 min. Water is added to about 350 ml., 125 ml. 89% H_3PO_4 (sp. gr. 1.75) are added, and the volume made up to 500 ml.

(3) *Isotonic sodium sulphate containing fluoride and thymol to prevent glycolysis.* 100 mg. NaF and 10 mg. thymol are dissolved in 100 ml. of isotonic Na_2SO_4 (3 g. Na_2SO_4 , 10 H_2O /100 ml. solution).

(4) *Sodium tungstate* (10 g./100 ml.).

(5) *Copper sulphate* (7 g. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ /100 ml.).

(6) *Stock glucose solution.* 0.1 g. pure glucose is dissolved in saturated benzoic acid solution and the volume made up to 100 ml. This is a permanent standard.

(7) *Standard glucose solutions.* These are prepared by diluting 2, 3, 4, 5, etc. ml., to 100 ml. with saturated benzoic acid solution; giving standards of 20, 30, 40, 50, etc., $\mu\text{g.}/\text{ml.}$

SUMMARY

A colorimetric modification (applicable to 0.05 ml. of blood) of a previously described titrimetric copper method for the determination of blood sugar is described.

The method is believed to give true glucose values.

Estimations on normal and diabetic blood by the new method, and also by the previously described method, give results which are in agreement.

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