Colorimetric determination of glucose in 20 μ l. of blood

DIANA M. CAMPBELL and the late E. J. KING *From the Department of Chemical Pathology, Post-graduate Medical School, London,*

This ultra-micro copper reduction method is a modification of that of Brown (1961). It is based on the very sensitive spot test for cuprous ions using 2,9-dimethyl-1-10-phenanthroline hydrochloride (neocuproine) (Smith and McCurdy, 1952). The proteins are precipitated by sodium tungstate and copper sulphate (Somogyi, 1931), thus eliminating non-sugar reducing substances, which are present in the tungstic acid filtrate used by Brown. The filtrate is treated with a modified Folin-Wu alkaline copper solution, and the amount of cuprous oxide formed is determined by the colour produced with neocuproine. Proportionality is good up to 250 mg./100 ml., above which it is necessary to use smaller volumes of filtrate (Fig. 1).

The colour formed between neocuproine and cuprous ions by excess cupric copper is suppressed, and therefore it is necessary to use a dilute copper reagent for the colour reaction. We investigated the replacement of the dilute Folin-Wu reagent recommended by Brown by a dilute

FIG. 1. Relationship between optical density and concentration of glucose

- 1 $\circ$ — $\circ$ = normal standards
- 2 $\times$. . . $\times$ = diluted (1:1) standards
- 3 + . . . + = 0.5 ml. of standard supernatant (see text).

Harding reagent (cf. King and Wootton, 1956) but this was not as sensitive. Results obtained with the method described correspond well with those by the standard AutoAnalyzer method in use at this school. The correlation coefficient was calculated from 50 determinations carried out by the two procedures; it was found that $r = 0.96$ (Fig. 2). Nineteen duplicate determinations were

FIG. 2. Comparison between blood sugar values obtained by the colorimetric method and by autoanalysis.

made, and, using the formula $\sqrt{\frac{\sum d^2}{\sum n}}$, where d is the difference between pairs and n the number of pairs, it was found that the root mean square difference was 2.8 mg./100 ml.

METHOD

TEST Whole blood, 0.02 ml., is pipetted into 1.78 ml. of isotonic sodium sulphate-copper sulphate in a small ($3 \times \frac{1}{2}$ in.) Pyrex tube. Sodium tungstate, 0.2 ml., is added and the mixture is well shaken. The precipitated proteins and copper tungstate are centrifuged down (5 min. at 3,000 r.p.m.), and the entire supernatant is tipped, as completely as possible, into 2 ml. alkaline copper solution in a $6 \times \frac{3}{4}$ in. test tube.¹ One millilitre neocuproine is added, and the tube is plugged with cotton-wool and placed in a boiling water bath for six minutes. After immediate cooling the optical density is measured in a photoelectric colorimeter with a blue light filter (e.g., Ilford 621). The colour is stable for at least one hour.

STANDARD AND BLANK Glucose standard, 0.02 ml., and 0.02 ml. water replace the whole blood in the test procedure.

HIGH BLOOD SUGARS For specimens containing over 200 mg. glucose/100 ml., a 3 ml. sample of the coloured solution is diluted with an equal volume of water and replaced in the boiling water bath for 2 min., and 3 ml. of the coloured solutions from the blank and from a 300 mg./100 ml. standard are similarly treated (Fig. 1, lines 1 and 2). For blood sugars higher than 500 mg./100 ml., the determination must be repeated using smaller volumes, i.e., 0.5 ml., of supernatant from the test, from the 600 mg. standard and from the blank, plus 1.5 ml. water (Fig. 1, line 3).

$$\text{Blood glucose} = \frac{\text{Reading of test blank}}{\text{Reading of standard blank}} \times S$$

where S is concentration of standard in mg. glucose/100 ml.

REAGENTS

ISOTONIC SODIUM SULPHATE-COPPER SULPHATE A mixture of 320 ml. of 3% $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ and 30 ml. 7% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

10% SODIUM TUNGSTATE Ten grams dissolved in 100 ml. water and kept in a waxed bottle.

ALKALINE COPPER REAGENT Anhydrous Na_2CO_3 , 40 g., and 7.5 g. tartaric acid are dissolved in 400 ml. water. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.225 g., is then added and dissolved, and the solution made up to 1 litre.

0.12% NEOCUPROINE SOLUTION Neocuproine, 120 mg., is dissolved in a small volume of N-HCl and made up to 100 ml. with water.

¹This 'tipping' technique, from small test tubes, is quicker and easier than pipetting. Results agree closely.

A new method for determination of the serum iron

RONA SANFORD¹ *From the New South Wales Red Cross Blood Transfusion Service, Sydney*

Measurement of the serum iron is a useful diagnostic test in many haematological disorders. The test is, however, tedious to perform and many laboratories do not regularly undertake the work. A methodological simplification was suggested by the work of Webster (1960), who used a detergent in the measurement of iron-dextran (Imferon) in the blood after intravenous injection. It has been found that this detergent (Teepol 710), together with ascorbic acid, dissociates iron from its protein linkage in serum and permits colorimetric measurement of the iron after reduction to the ferrous state with the reducing agent.

The purpose of this paper is to describe the new method using Teepol and ascorbic acid. This method does not require precipitation of the proteins, which is an essential preliminary step in most other methods (Kitzes, Elvehjem, and Schuette, 1944; Kaldor, 1953; Trinder, 1956). The results obtained by the method have been compared with those obtained by Kaldor's and Trinder's methods.

PROPOSED METHOD

IRON-FREE WATER Water for the preparation of all reagents and for the rinsing of all acid-washed apparatus is glass-distilled.

APPARATUS All glassware is washed in a detergent solution, rinsed in tap water and distilled water, soaked in 1 N hydrochloric acid for a minimum of 30 minutes, and finally rinsed in iron-free water.

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Colorimetric determination of glucose in 20 μl . of blood—concluded

STOCK GLUCOSE SOLUTION Pure anhydrous glucose, 2 g., is dissolved in 100 ml. saturated (0.3%) benzoic acid.

STANDARD GLUCOSE SOLUTIONS These are prepared by diluting 5, 10, 15, and 30 ml. of stock solution to 100 ml. with saturated benzoic acid, giving standards of 100, 200, 300, and 600 mg. glucose/100 ml.

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