A QUICK AND EASY METHOD FOR BLOOD-SUGAR ESTIMATION

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The need for a speedy and easy method of blood-sugar estimation has long been recognized, especially in the prompt diagnosis and correct treatment of certain medical emergencies, and particularly those associated with diabetes mellitus. Most of the standard methods of blood-sugar estimations require apparatus and techniques rendering them unsuitable for use outside the laboratory.

Hall (1950) and Lee (1954) respectively modified Haslewood and Strookman's (1939) method and Sumner and Graham's original work using dinitro-salicylic acid (Sumner and Graham, 1921; Sumner, 1924-5, 1925). The final methods are quick but still require a number of manipulations and the external application of heat.

The method presented here is extremely easy, apparatus is compact, no external heat is required, and it is very quick.

Method

Reagents.—(1) Tablet A: salicylsulphonic acid is the protein-precipitating agent. (2) Tablet B, for determining the reducing sugars, consists of copper sulphate, sodium hydroxide, sodium bicarbonate, and citric acid (Patent Nos. 2, 387, 244).

Procedure.—In a small test-tube provided with the kit,* water is added to mark 2, and tablet A is then placed in this water. The solution thus formed precipitates proteins; blood is then added to mark 3 on this test-tube. In the present study blood was actually delivered to the deproteinizing fluid by means of a 1-ml. shellback pipette. A rubber bung is placed in the mouth of the test-tube, which is vigorously shaken. A very fine precipitate ensues. A filter cone provided is placed in the top of a second, identical test-tube. The brown mixture is filtered into the second tube and the clear filtrate collected up to mark 1. One tablet B is added to the filtered solution. The fluid boils without external heat being applied, and when this stops the test-tube is shaken three or four times; after a wait of 30 seconds the colour is compared with the scale provided in the kit: blue=100 mg./100 ml., green=150 mg./100 ml., and orange=200 mg./100 ml.

Selection of Patients and Experimental Design.—The blood samples were obtained by venepuncture from patients during visits to the Diabetic Clinic, General Infirmary at Leeds. The blood was placed in a special oxalated tube. At the close of each clinic, estimations were done on these samples by an independent worker using the new method under constant and ideal conditions, while routine Hagedorn-Jensen estimations (Hagedorn, Halstrom, and Jensen, 1935) were also made on the same specimens, with only 1-2% error, and these were done in a different laboratory. This eliminated any unconscious bias in either estimation. Three workers with varying laboratory experience used the new method. Workers X and Y did the estimations with the new

blood-sugar kit (B.S.K.) under standard conditions with respect to light, time, and continuity. Worker Z was not allowed such constant conditions.

Results

The results obtained by the Hagedorn-Jensen method showed that none of the groups estimated by three workers was biased by an excess of either high or low blood-sugar levels.

Worker X did 105 estimations with the B.S.K., and these were graded blue, green, and orange according to the chart. The division between blue and green was arbitrarily taken at 125 mg./100 ml. and between green and orange at 175 mg./100 ml. Thus individual results were given not only a grading according to colour but also an estimate of blood sugar in mg./100 ml. For example, a greenish-yellow colour resulting from the B.S.K. was estimated as 170 mg./100 ml., whilst the standard Hagedorn-Jensen method for blood-sugar estimation revealed 184 mg./100 ml. When all the

Comparison of blood-sugar determinations by the Hagedorn-Jensen method with the new blood-sugar kit.

B.S.K. estimations done by worker X are compared with the independent Hagedorn-Jensen estimations there is a remarkable agreement (see Chart). The correlation coefficient r is + 0.9. When the 88 results from worker Y are analysed there is not such close agreement, and the correlation coefficient r = + 0.78. The 106 results from worker Z gave a correlation coefficient of + 0.85.

Discussion

It will be seen from the Chart that no patient given an "orange" rating by B.S.K. had a true blood sugar as revealed by the Hagedorn-Jensen method below 160 mg./100 ml. Conversely, only one patient given a "blue" rating by the B.S.K. had a blood sugar level over 125 mg./

*Kindly supplied by Ames Co. (London) Ltd.

100 ml. when estimated by the Hagedorn-Jensen method. In over 300 estimations, only once was there a widely differing result which could have caused an error in treatment. The B.S.K. revealed 110 mg./100 ml. whilst the Hagedorn-Jensen method showed 212 mg./100 ml. The worker questioned the blue-grey colour of the B.S.K. reaction, and this estimation should have been repeated.

The accuracy of results depends on the appreciation of a range of colours. In a few estimations this was difficult, particularly when a muddy or grey tinge appeared in the final sample. Worker X was able to distinguish a muddy blue from an orange-grey with reasonable accuracy in all shades, whilst workers Y and Z had difficulty. This problem was resolved by repeating all estimations when an unreliable colour was obtained. Usually a pure colour was finally produced. No explanation has been found for this "muddy" colour, and it occurred 22 times out of 300 estimations.

There is no doubt that ideal conditions and laboratory experience improve the accuracy of this method. Nevertheless, with a little practice, proficiency is attained whereby blood-sugar estimations can be done that are of inestimable value in the correct treatment of emergency medical patients.

It was felt that further refinement of the test was not essential since the B.S.K. was not designed to usurp the established blood-sugar methods. In emergency practice the question is usually not the actual level of blood sugar but whether the level is low or whether it is high. The B.S.K. would appear to be capable of giving this information speedily and accurately enough for clinical purposes. A further extensive trial is under way to test these laboratory findings under emergency conditions.

Summary

A new simple and quick blood-sugar estimation is described. Results when compared with the Hagedorn-Jensen method are in remarkable agreement. Varying conditions and experience of the workers are shown to influence the results. The new blood-sugar method would appear to be helpful in medical emergencies.

We thank all the patients for their kind co-operation in this study; Professor R. E. Tunbridge for permission to study his patients and helpful criticism of the MS.; and Mr. J. Bunce, Director, Ames Company (London) Ltd., for the supply of B.S.K. and his help at all times.

REFERENCES

- Hagedorn, H. C., Halstrom, F., and Jensen, B. N. (1935). *Hospitalstidende*, 78, 1193.
Hall, D. A. (1950). *British Medical Journal*, 1, 351.
Haslewood, G. A. D., and Strookman, T. A. (1939). *Biochem. J.*, 33, 920.
Lee, J. (1954). *British Medical Journal*, 2, 1087.
Sumner, J. B. (1924-5). *J. biol. Chem.*, 62, 287.
— (1925). *Ibid.*, 65, 39.
— and Graham, V. A. (1921). *Ibid.*, 47, 5.

The John Horniman Home is a home for children with severe physical handicaps such as cerebral palsy, muscular dystrophies, paralysis due to poliomyelitis, or severe heart disease. The home, situated in Worthing, is run by the Invalid Children's Aid Association, and can accommodate 24 children. It takes both boys and girls between the ages of 7 and 16. They are usually admitted for periods of two to eight weeks, either as a holiday to give their parents a respite or for convalescence after illness. The first object of the home is to help families in carrying the burden of a severely handicapped child. In special cases a long stay with education can be arranged. A serious difficulty in running the home, which has now been open for nearly three years, is that there is a peak demand during the summer holidays but vacancies at other times of year. With the skilled staff needed for such work this makes for uneconomic operation, and a more even flow of admissions, especially during the winter months, is wanted. Full details may be obtained from the Invalid Children's Aid Association, 4, Palace Gate, London, W.8.

A RAPID METHOD FOR THE ESTIMATION OF BLOOD SUGAR

BY

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A number of rapid and simple methods for the estimation of the blood sugar have been described (Hall, 1950; Lee, 1954). Although the results obtained by these methods agree with those in routine laboratories, they are not really suitable for performance by a busy house-physician at the bedside or in a ward side-room. A very simple procedure involving the use of tablets has recently been developed, and its use has been studied in the wards and the Diabetic Clinic of the Middlesex Hospital.

Method

The equipment is contained in a small box and consists of two test-tubes each with three graduations (at 1, 2, and 3 ml.), a test-tube stand, a dropper, a box of filter cones, and two bottles of tablets (A and B). Tablet A is dissolved in 2 ml. of water in one of the tubes and blood is added to the 3-ml. mark. The proteins are rapidly precipitated and the mixture is allowed to filter through one of the filter cones into the second tube until 1 ml. of filtrate has collected. Tablet B is then added, and after the contents of the tube have boiled the froth is shaken down and 30 seconds later the colour is compared with that on a chart provided: blue or blue-green = 100 mg./100 ml. or less, green = 150 mg./100 ml., and orange = 200 mg./100 ml. or more.

Blood samples were obtained by venepuncture and a fluoride-oxalate anticoagulant mixture was used. If for any reason the estimation could not be performed immediately the specimen was stored, but not frozen, in a refrigerator. In practice it was found preferable to measure the volumes of blood and water with an ordinary 2-ml. insulin syringe. If the result was more than 200 mg./100 ml. the estimation was repeated, using 2.5 ml. of water with tablet A and adding 0.5 ml. of blood. If this still gave maximum reduction the test was repeated, using 2.75 ml. of water and 0.25 ml. of blood. For higher dilutions the blood was diluted with water first. No attempt has been made to perform the estimations under set conditions of lighting; so far as possible, the method has been studied as it would be used in practice—artificial or daylight being used as circumstances required.

Duplicate samples of each specimen were sent to the Courtauld Institute of Biochemistry at the Middlesex Hospital, where blood sugars were estimated by the method of Kingsley and Reinhold (1949), using sodium tungstate and sulphuric acid as protein precipitant. The error of this method is ± 6 mg./100 ml.

Results

The results obtained by the tablet method and the Kingsley-Reinhold method are compared in the Chart. The

Comparison of blood-sugar determinations by the method of Kingsley and Reinhold with the new blood-sugar kit.