A QUICK AND SIMPLE METHOD FOR BLOOD-SUGAR ESTIMATION

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Without blood-sugar estimations the recognition and proper treatment of hyperglycaemia and hypoglycaemia may be difficult. Emergencies may arise in domiciliary practice or in hospital at a time when no routine laboratory facilities are available. A simple method of blood-sugar estimation which can be carried out in a few minutes would therefore be useful: such a method is described here.

The method presented is that originally described by Sumner (1921, 1924-5, 1925), who utilized the change in colour from yellow to red when the sugar in a deproteinized blood filtrate is heated with dinitrosalicylic acid in alkaline solution. The modifications effected are essentially that the scale is reduced to the micro level and that dinitrosalicylic acid itself is used as a protein precipitant, in which capacity it is very efficient. The excess of the acid in the filtrate is adequate for the subsequent development of colour. Finally, a set of permanent artificial colour standards has been devised.*

Experimental

Proportion of Reagents.—0.2 ml. of blood was added to 1 ml. of 1.75% dinitrosalicylic acid to yield a deproteinized filtrate, and to this filtrate an equal quantity of alkaline reagent was added.

Colorimetry.—Colour standards made directly with dinitrosalicylic acid and glucose solutions were found to fade after a few weeks even if kept in sealed tubes. This difficulty was overcome by using the Ilford filter No. 6/53, which matched the colour corresponding to a blood sugar of 70 mg./100 ml. By using additional thicknesses of this filter it was found that each extra film corresponded to an increment of 40 mg./100 ml. up to 350 mg./100 ml. Thus it was practicable to compose a set of stable artificial colour standards from the Ilford filters. As the difference in colour between any two adjacent filters was easily detected, it was possible to make an approximate evaluation for intermediate colours.

Findings

Two hundred determinations were performed on blood samples produced by the addition of measured quantities of glucose to oxalated whole blood to give blood-sugar values evenly graded in the range 80-560 mg./100 ml. The results clearly demonstrated that the developed colours were reproducible.

As the blood-sugar values increased above 360 mg./100 ml. it became progressively more difficult to detect colour differences associated with small increments of blood sugar. However, by diluting the more intensely coloured solutions with water, blood sugars in the upper range could be correctly estimated. The dilution technique was investigated by experiments using glucose solutions in the presence of the concentration of dinitrosalicylic acid found in a deproteinized blood filtrate; by this method it was found that the upper limits of usefulness could be extended to the region of 1,400 mg./100 ml.—that is, diluted four times, this colour approximately matched that colour developed

from a blood sugar of 350 mg./100 ml. The lower limit of usefulness of the method was found to be about 60 mg./100 ml.

Photoelectric examinations of the colours showed that there was a rectilinear relationship between absorption of light and sugar concentration between 90 and 300 mg./100 ml. The Hilger-Spektr absorptiometer was employed, using filter No. 605. This result is in agreement with the findings of Leech and Woodford (1948) and Hall (1950). The colour is stable for at least 30 minutes at room temperature, answers obtained by immediate and delayed reading not differing by more than 5%.

A comparison of results by this dinitrosalicylic acid method using a colorimeter, and a Hagedorn and Jensen estimation performed on the same glucose solutions, is given in the Table.

Blood-sugar Estimations by the Dinitrosalicylic Acid Method Using a Colorimeter, Compared with the Hagedorn and Jensen Method

Hagedorn and Jensen	Dinitrosalicylic Acid Method with Klett Colorimeter*
90 mg.	83 mg.
135 "	144 "
243 "	236 "
305 "	288 "
395 "	383 "
500 "	444 "
600 "	576 "

* Colorimeter standard was the colour developed from 180 mg./100 ml. glucose solution.

† Solution diluted 1/2 for estimation.

Proposed Method

Apparatus.—(1) Test-tubes. It is essential that the tube used for colour development should be of standard bore: external diameter 0.95 cm.; internal diameter 0.8 cm. A convenient length is about 7.5 cm. The tube is graduated at intervals of 0.2 ml. to the 2 ml. mark. (2) Pipettes, 0.2 ml. and 1 ml. (3) Filter funnel, 2.5 cm. diameter. (4) Filter paper: Whatman No. 1, 4.25 cm. diameter. (5) Pasteur pipette. (6) A set of artificial colour standards calibrated to give values corresponding to those given by the Folin-Wu blood-sugar method.

Reagents.—(1) 1.75% 3:5-dinitrosalicylic acid solution in water. If the acid crystallizes out on standing, the crystals will dissolve on gently warming the solution. (2) Alkaline reagent: 13.8 g. phenol and 13.8 g. sodium bisulphite (NaHSO_3) are dissolved in 350 ml. 10% sodium hydroxide and added to a solution of 510 g. Rochelle salt in 880 ml. of water. This alkaline reagent is stable for about nine months.

Procedure.—0.2 ml. of capillary blood is pipetted into 1 ml. of dinitrosalicylic acid solution contained in a test-tube. If a 1-ml. pipette is not available one of the graduated test-tubes can be used for measuring the dinitrosalicylic acid solution, but some accuracy will thus be sacrificed. When the blood is pipetted it should be allowed to flow freely into the acid and only the last drop expelled by blowing. (Acid is not sucked up into the pipette, as this leads to difficulty in cleaning the pipette later. Use of the same technique in calibration of the colour standards has accounted for the blood left in the pipette.) After mixing, enough time is allowed for the colour to become brown (about 30 seconds); then the contents are filtered into a graduated tube. When a suitable volume (to the 0.4 ml. mark) of filtrate has collected, an equal volume of alkaline reagent is added by means of a dropper. The contents are mixed, and are heated in a boiling-water bath for three minutes. The tube is cooled and the colour of the contents is matched against the standards. The comparison is best made with the test and standards about four inches (10 cm.) from a 60-watt bulb. The capacity of the tube is such that its graduated section also allows for any reasonable dilution with water necessary to bring the very high

*Reagents and artificial colour standards can be obtained from British Drug Houses. The standard-bore tubes, suitably calibrated, can be obtained from Scientific Supplies Ltd.

blood-sugar colours within the range of the standards, in which case a factor would have to be applied to the answer.

Results

Blood sugars of 204 diabetics attending the Out-patients' Department of King's College Hospital were estimated by the Folin-Wu method and by the dinitrosalicylic acid method. The two series of estimations were made in different laboratories.

The accompanying Graph compares the results of the blood-sugar determinations by the Folin-Wu method with those obtained by the present method. It can be seen that

Blood sugars by the Folin-Wu and the dinitrosalicylic acid (D.A.) methods. Middle interrupted line, 100% correlation. Outer interrupted lines drawn 20 mg. from centre line below 150 mg. level, and 30 mg. above 150 mg. level. Crosses refer to those estimations for which the colours were diluted 1/2. $y = 27.2 + 41x$ where y = blood sugar in mg. and x = filter number. Correlation coefficient, $r = 0.97$. Standard error of estimate = 17.3 mg.

most of the results by the rapid method are within 20 mg. of the answer given by the Folin-Wu method. The crosses represent those determinations for which the colours were diluted 1/2 to bring them within the range of the standards.

For blood-sugar values (determined by the Folin-Wu method) in the range 60–150 mg./100 ml., 65 out of 70 results did not have an error exceeding 20 mg.; in the range 151–350 mg./100 ml., 125 out of 134 results were within 30 mg. of the determined value. Statistical analysis of the correlation is given on the graph, and was based on the first 170 blood-sugar values below 400 mg./100 ml.

The error of the higher values above 400 mg./100 ml. was not established, as sufficient estimations in this range were not made; however, estimations with glucose solutions indicate that in the range 351–700 mg./100 ml. the error was about 60 mg., and above this range the error may be as high as 80–120 mg.

Summary and Conclusions

A rapid method is described for the estimation of blood sugar.

The method takes about ten minutes to complete and gives values in good agreement with those obtained by standard methods; little apparatus or technical skill is required.

It offers a quick and reliable way of estimating the approximate blood sugar in hyperglycaemia and of detecting the presence of hypoglycaemia. In hypoglycaemia, accurate estimations are of importance in certain circumstances, but it would involve no serious disadvantage in ordinary practice to use a method which

can indicate blood sugars below normal, even though it fails to estimate them precisely. Thus the method should be of value both as a ward test and as a simple aid to the general practitioner in the management of patients with diabetes mellitus.

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REFERENCES

- Hall, D. A. (1950). *British Medical Journal*, 1, 351.
 Leech, R. S., and Woodford, N. (1948). *J. Lab. clin. Med.*, 33, 644.
 Sumner, J. B. (1921). *J. biol. Chem.*, 47, 5.
 — (1924-5). *Ibid.*, 62, 287.
 — (1925). *Ibid.*, 65, 393.

Medical Memoranda

Phenylbutazone and Renal Function

A number of toxic manifestations have been reported in the British and American press associated with the use of phenylbutazone. Several deaths have occurred. Symptoms have included sodium retention, gastric haemorrhage, agranulocytosis, anaemia, and purpura.

In one fatal case the pathologist found contracted kidneys (*British Medical Journal*, 1952). In the case reviewed here contracted kidneys were again found, together with a history of toxic manifestations, after only a very small dose of phenylbutazone, and it is suggested that poor renal function may prove to be an important contraindication to use of the drug.

CASE REPORT

A woman aged 65 had had arthritis for more than 30 years. During the past seven years she had had many acute exacerbations of rheumatoid arthritis with red, hot, swollen painful joints, particularly affecting the shoulders, elbows, and hands. She had been bedridden for some years. In September, 1951, sodium aurothiomalate, 0.01, 0.02, 0.05 g., was given at weekly intervals. Then albuminuria was reported and the drug was stopped. In March, 1952, minute doses of sodium aurothiomalate were tried again, 0.001, 0.002, 0.002 g., but further albuminuria was reported and the drug was again stopped.

On February 25, 1953, a course of phenylbutazone was started, one 200-mg. tablet twice a day. At the beginning of March, after taking only 13 tablets, she developed a sore throat and the drug was immediately stopped. She refused hospital treatment or investigation but agreed to have a blood count taken at her home on March 17, and this was reported as follows: erythrocytes, 2,450,000 per c.mm.; haemoglobin, 44%; colour index, 0.9; leucocytes, 3,100 per c.mm. (polymorphonuclears 57%, eosinophils 2%, lymphocytes 36%, monocytes 5%).

Her condition remained poor. She appeared to get steadily more anaemic, and a further blood count on April 13 showed: erythrocytes, 1,570,000; haemoglobin, 26%; colour index, 0.84; leucocytes, 3,400 (polymorphonuclears 40%, lymphocytes 60%). The red cells showed marked anisocytosis and hypochromia. She became comatose on April 14, did not regain consciousness, and died on April 16.

Post-mortem Examination (April 18).—External features: Elderly woman with swelling of elbows, wrists, ankles, and knees, and some fusiform enlargement of fingers with ulnar deviation. Numerous purpuric spots up to 1 in. (2.5 cm.) in diameter on the body, but mainly on the legs. Circulatory system: completely adherent pericardium; fairly marked enlargement of heart due mainly to hypertrophy of the left ventricle; coronaries, aorta, and other vessels