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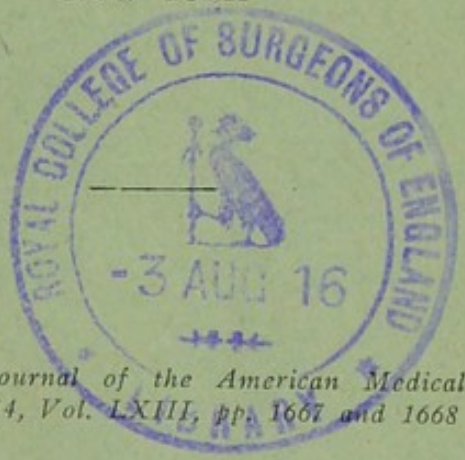

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AN ACCURATE MICROCHEMICAL METHOD OF ESTIMATING SUGAR IN THE BLOOD*

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The method to be described is an adaptation, on a very small scale, of the one devised by Lewis and Benedict[1] for estimating the sugar-content in the blood. It is based on the fact observed by Dehn and Hartmann,[2] also by Lewis and Benedict,[1] that picric acid and sugar in the presence of an alkali (sodium carbonate) give, on heating, a color reaction which is proportionate to the amount of sugar present.

I have used the method of Lewis and Benedict in about three hundred tests for blood-sugar and found it admirable both in point of simplicity and accuracy.[3] It entails, however, the use of 2 c.c. of blood and the employment of costly colorimetric apparatus. To overcome these drawbacks and to render the estimation of blood-sugar more suitable for general clinical and laboratory work, I have adopted the Sahli-Gower hemoglobin colorimeter (with suitable color standards) and by that means found it possible to work with small quantities of the necessary reagents, and to estimate with precision the amount of sugar present in 0.2 c.c. or even 0.1 c.c. of blood.

TEST PROCEDURE

The apparatus[4] shown in the illustration and the following reagents are necessary:

1. Picric acid, saturated solution.
2. Sodium carbonate, 10 per cent. solution.
3. Sodium fluorid or potassium oxalate, 2 per cent. solution.

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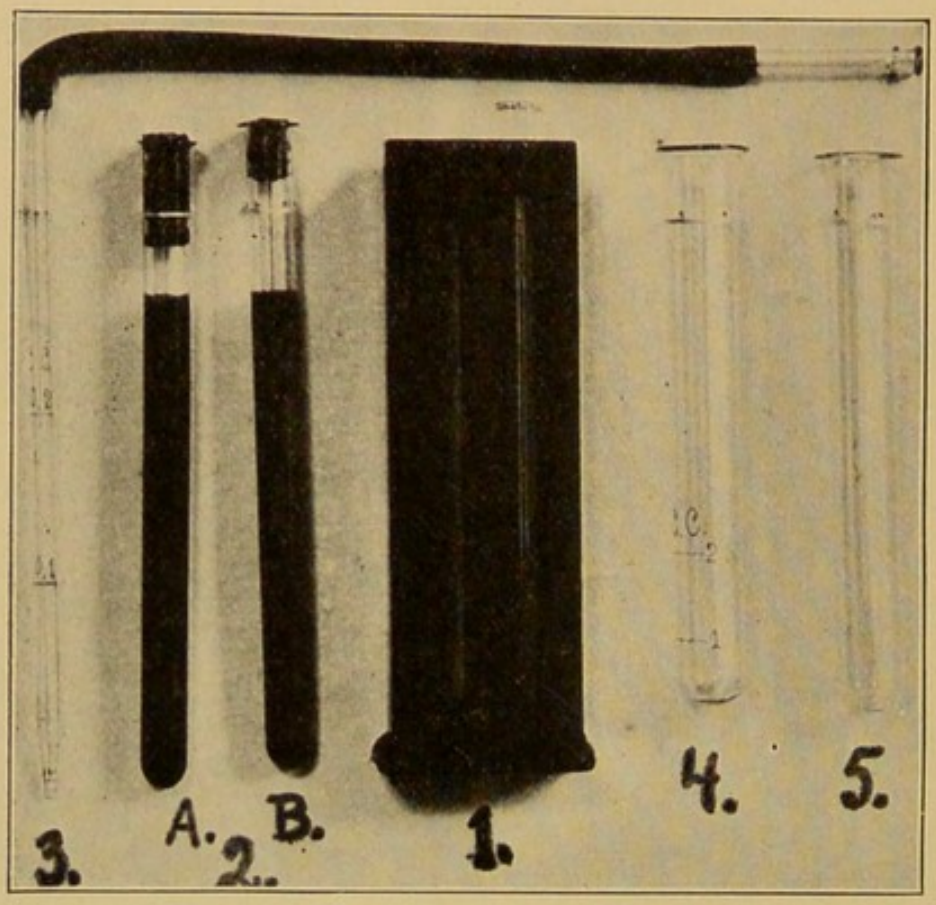
* From the Chemical Laboratory, Pathological Department, Mount Sinai Hospital. Work done under the tenure of the Theodor Escherich Fellowship in Pathology.

1. Lewis and Benedict: Proc. Soc. Exper. Biol. and Med., 1914, xi, 57.
2. Dehn and Hartmann: Jour. Am. Chem. Soc., 1914, xxxvi, 403.
3. A solution of picramic acid, suggested by Dr. Stanley R. Benedict, was used as the color standard in most of these tests.
4. The tubes belonging to this hemoglobinometer are not all equally calibrated. With some the 50 per cent. mark represents a volume of 1.0 c.c.; with others, 1.0 c.c. of fluid reaches up to the 43, 45, 46 or 47 per cent. mark. In all of the tubes that I have examined (twenty-four in number) the error in the calibration is below the 10 per cent. mark; the graduations above this mark are usually correct. By means of the standard 1.0 c.c. pipet one can readily determine whether or not a

Put one or two drops of the fluorid or oxalate solution into the graduated test-tube 4 (see illustration). By means of the blood pipet, 3, 0.2 c.c. of blood is obtained from the tip of the finger or the lobe of the ear and is discharged into the tube, 4, containing the fluorid solution. The pipet is rinsed two or three times with distilled water and the washings added to



Apparatus for estimating sugar in the blood by microchemical method. 1. Sahli-Gower hemoglobinometer stand and graduated tube. 2. Two standard color tubes, one (*A*) suitable for measuring quantities of sugar in the blood ranging from 0.05 per cent. to 0.1 per cent.; the other (*B*) is adapted to quantities of sugar over 0.1 per cent.; 3. A special pipet (resembling but larger than the hemoglobin blood-pipet) graduated at 0.1 c.c. and 0.2 c.c for collecting and measuring the blood. 4. A test tube (½ by 4 inches) graduated at 1.0 c.c. and 2.5 c.c. 5. Another test tube of similar dimensions (not graduated) suitable for boiling.

the blood in Tube 4. Distilled water is then added to the 1.0 c.c. mark. After laking of the blood has taken place, picric acid is added to this (a few drops at a time) up to the 2.5 c.c. mark, shaking the tube gently with each addition of the acid. Precipitation of the blood-proteins takes place; the sugar, together with an excess of picric acid sufficient for the

given tube is properly calibrated. In order to facilitate a direct reading of the percentage of sugar on these hemoglobinometer tubes, it is essential to have 1.0 c.c. of fluid stand at mark 50. To overcome a discrepancy (if any exists) in the calibration of a given tube, one may put one, two or three small glass beads in the bottom of the tube, of such size as to raise the meniscus of 1.0 c.c. of fluid up to the 50 per cent. mark.

reaction, stays in solution. The tube is finally shaken vigorously (covering the end of the tube with the finger) and the contents filtered through a small filter, or, better still, centrifuged for one or two minutes.

One cubic centimeter of the filtrate or the clear supernatant fluid obtained on centrifugalization is withdrawn, put into the boiling tube, 5, and heated carefully over the naked flame. The contents of the tube are boiled until all but 2 or 3 drops of the solution is evaporated. One-half cubic centimeter of the 10 per cent. soda solution is then added and the tube heated again until the contents are concentrated to a small volume equal to about two or three drops. The color of the fluid changes from yellow to deep red or reddish brown and the reaction is completed.

Three or four drops of distilled water are added and the tube warmed gently. The contents are then transferred to the graduated tube, 1. The boiling tube is rinsed several times with water (using only three or four drops at time). The tube is warmed with each rinsing before transferring the contents to the graduated tube. The volume is then made up to the mark 50 on the scale.

The color of the resulting solution is compared with that of the two standard tubes, *A* and *B*. If it is darker than standard *A* (representing 0.05 per cent. of sugar) and lighter than standard *B* (representing 0.1 per cent.), the first standard is used for comparison. In either case the solution in the graduated tube is diluted gradually with water (just as is usually done in hemoglobin estimations) until the colors match.

The percentage of sugar in the blood is then computed thus: Using the lighter standard *A* the figure on the scale, divided by 1,000 represents the percentage of sugar in the blood. For example, the tube reads 86; then the result is

$$\frac{86}{1000} = 0.086 \text{ per cent.}$$

When Standard *B* is used for comparison, the figure on the scale is multiplied by 2 and divided by 1,000. For example, the tube reads 73; then the percentage of sugar is

$$\frac{73 \times 2}{1000} = 0.146 \text{ per cent.}$$

With the instructions given, the above formulas may be used for direct computation of the percentage of sugar only, when 0.2 c.c. of blood is used in the determination. When, however, only 0.1 c.c. of blood is used, the formulas apply as well, but the value obtained must be multiplied by 2.

In developing this method I at first used three standard tubes representing 0.05 per cent., 0.10 per cent., and 0.20 per cent., respectively, so as to meet all likely percentages of

sugar in the blood. The use of the last of these I found inadvisable inasmuch as the color which it represents is too concentrated, and accurate readings with it are almost impossible. It is better, in cases in which a high sugar content in the blood is suspected (in diabetes for example) to use only 0.1 c.c. of blood for the determination. In all other cases 0.2 c.c. of blood should be used.

The advantages of the method are obvious and require no discussion. It needs only seven to ten minutes to complete a determination of sugar. The small quantity of blood necessary for the test renders it suitable for general clinical work on children as well as adults, and does away with venipuncture. The colorimetric readings are easy and yield reliable results.[5]

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5. The special apparatus devised for this method was made for me by Eimer and Amend, New York.