TECHNICAL NOTE :

A SIMPLIFIED TECHNIQUE FOR ESTIMATION OF BLOOD GLUCOSE

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Summary : Somogyi's blood sugar method has been simplified. The new technique which has been compared to glucose oxidase method, Somogyi's procedure and Folin-Wu method is easier and more accurate than the Somogyi's original method, and gives blood glucose values almost identical with that obtained with glucose oxidase procedure.

Key words : glucose estimation blood glucose

INTRODUCTION

Somogyi's (6) blood sugar method is one of the most reliable and extensively used method for the estimation of true sugar. Non-sugar reducing substances of blood are located principally in the erythrocytes (3) and are removed from laked blood by adding barium hydroxide and zinc sulphate solution in this method (5). Unfortunately solutions of barium hydroxide are very susceptible to deterioration by action with atmospheric carbon dioxide and have to be titrated frequently for readjustment to desired strength.

In the method described by King and Wootton (3), the non-sugar reducing substances are separated by using an isotonic solution which keeps the erythrocyte intact. However, the alkaline reagent used is not satisfactory. The chromogen developed does not obey Beer's Law well necessitating the use of two standards. The method is somewhat sensitive to reoxidation by atmospheric oxygen and variation in diameter of test tubes. The Somogyi (6) alkaline reagent has the advantage that the high concentration of sodium sulphate in it provides adequate protection against reoxidation by atmospheric air (4,7).

We therefore simplified the Somogyi method (6) so that the inconvenient step of using barium hydroxide and zinc sulphate was replaced by the simple isotonic principle and modified the compositions of isotonic solution and alkaline reagent (3) in such a way as to give finally at the time of reduction an alkaline copper solution of the same composition as in the Somogyi method. In order to assess the reliability of the present method, blood glucose was estimated by the simplified Somogyi method, Somogyi method (6) and glucose oxidase method (1). Values obtained with the present method were also compared with Folin-Wu method (2).

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MATERIALS AND METHODS

Blood glucose was estimated by the Somogyi method (6), glucose oxidase method (1) and Folin-Wu method (2).

Modified Somogyi Method:

Reagent: (i) Isotonic Sodium Sulphate-Copper Sulphate Solution: 12.5 *g* anhydrous sodium sulphate and 5.1 *g* of crystalline copper sulphate per litre.

(ii) 10% sodium tungstate

(iii) Alkaline Reagent: In about 600 *ml* of hot water 168 *g* anhydrous sodium sulphate are dissolved, the solution boiled to expel air and cooled. 24 *g* of anhydrous sodium carbonate and 12 *g* of Rochelle salt are dissolved in another beaker. In this solution, 16 *g* of sodium bicarbonate are then dissolved. This is added to the sodium sulphate solution and the volume made upto one litre. After one or two days, the solution is filtered.

(iv) Arseno-molybdate reagent of Nelson (4).

(v) Glucose standard: 100 *mg*/100 *ml* in a saturated solution of benzoic acid.

Procedure: In a centrifuge tube containing 3.9 *ml* isotonic sodium sulphate-copper sulphate solution, exactly 0.05 *ml* of blood is added and mixed. Then 0.05 *ml* (or 1 drop) of sodium tungstate solution is added and mixed. After a few minutes when the precipitate begins to settle it is centrifuged and the centrifugate transferred to another dry tube. Standard glucose solution is prepared in the same way by adding 0.05 *ml* stock glucose to 3.95 *ml* of the isotonic solution using the same delivery technique. For routine work, a working standard can be prepared by diluting 1.25 *ml* of the stock glucose to 100 *ml* with the isotonic solution. This can be kept in refrigerator for a week.

To 2 *ml* each of isotonic solution, glucose working standard and protein free test filtrate in test tubes of similar diameter and thickness are added 2 *ml* of alkaline reagent and mixed. The test tubes are covered with glass balls or plugged with cotton wool and suspended in a boiling water bath for 10 minutes. The tubes are taken out and cooled by immersing in water.

To all test tubes, 2 *ml* of colour reagent is added and mixed thoroughly till no effervescence comes out. The optical density is measured on a photoelectric colorimeter using green filter. If red filter is used, the sensitivity is so great that the solutions have to be diluted several fold.

In the present study, the same pipette was used for delivering blood in all the methods. All analyses were done in duplicate.

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RESULTS

The results are presented in Fig 1 and Tables I and II. All blood sugar values obtained re averages of closely agreeing duplicates. Mostly the duplicates were identical, the maximum

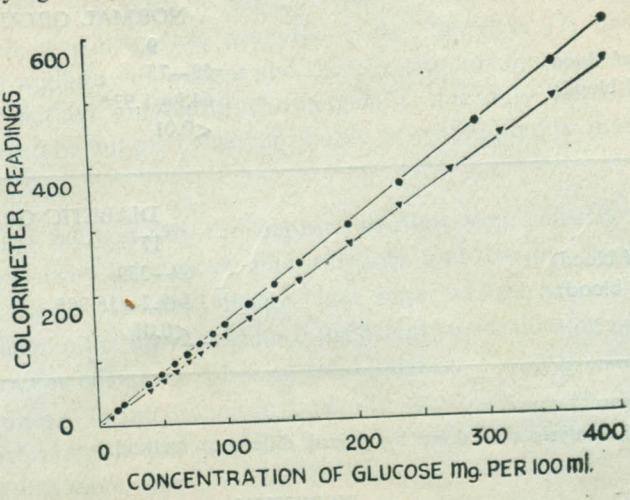

Fig. 1 : Applicability of Beer's law with present method (●—●—●) and original Somogyi method (▲—▲—▲).

variation being 2 per cent. The significance of the differences in the mean blood sugar values by the different methods were statistically tested by the paired difference method. Fig 1 shows that in both methods the chromogen produced obeys Beer's law over a very wide range of blood glucose concentration varying from as low as 10 *mg*/100 *ml* to as high as 400 *mg*/100 *ml*.

TABLE I : Comparison of blood sugar values obtained with the glucose oxidase technique, present method and the Somogyi method.

	Glucose oxidase method (i)	*Present method* (ii)	*Somogyi method* (iii)
		NORMAL GROUP	
Number of cases	10	10	10
Range (*mg* sugar/100 *ml* blood)	58—93	58—92	63—98
Mean (*mg* sugar/100 *ml* blood)	70.7 ± 3.8*	71.5 ± 3.68*	77.8 ± 3.73*
p**	(i)&(ii) < 0.05	(ii)&(iii) < 0.01	(i)&(iii) < 0.01
		DIABETIC GROUP	
Number of cases	9	9	9
Range (*mg* sugar/100 *ml* blood)	95—190	96—190	98 ± 196
Mean (*mg* sugar/100 *ml* blood)	168.9 ± 37.14*	169.7 ± 38.77*	180.0 ± 43.16*
p**	(i)&(ii) > 0.05	(ii)&(iii) < 0.01	(i)&(iii) < 0.01

*Standard error of mean
**Probability, statistical analysis was done by paired difference method.

TABLE II : Comparison of blood sugar values obtained with the present method and the Folin-Wu method.

	Present method	*Folin-Wu-method*
	NORMAL GROUP	
Number of cases	9	9
Range (*mg* sugar/100 *ml* blood)	59—75	90—120
Mean (*mg* sugar/100 *ml* blood)	64.9 ± 1.93*	106.1 ± 3.51*
P**	<0.01	
	DIABETIC GROUP	
Number of cases	17	17
Range (*mg* sugar/100 *ml* blood)	69—339	130—425
Mean (*mg* sugar/100 *ml* blood)	149.2 ± 18.25*	210.9 ± 22.19*
P**	<0.01	

* Standard error of mean

** Probability, statistical analysis was done by paired difference method.

DISCUSSION

It would be seen from Table I that the present method gave blood sugar values very close to the glucose oxidase method being on an average only 0.8 *ml*% higher. The original Somogyi method gave on an average 7.1 *mg*% and 11.1 *mg*% higher values than those given by glucose oxidase method in normal and diabetic blood samples respectively. This difference was highly significant for both normal and diabetic groups ($P < 0.01$). The Somogyi method gave on an average 6.3 *mg*% and 10.3 *mg*% higher blood sugar values than those obtained by the present method in the normal and diabetic groups respectively. The difference was statistically highly significant,

In the Somogyi method, the non-glucose reducing substances are first brought into solution by haemolysis and then removed along with barium sulphate-zinc hydroxide precipitate. It appears this process does not completely remove the interfering substances. In the present method, the non-glucose reducing substances are not allowed to get out of the erythrocytes and thus kept out of the testing solution by the principle of isotonic dilution. The present method is therefore superior to Somogyi (6) method in giving more nearly true glucose values.

Table II shows that in normal cases Folin-Wu method gave on an average 41.2 *mg*% higher blood sugar values than the present method. In diabetic cases, this difference was still larger (61.7 *mg*%). The error caused by non-sugar reducing substances in the Folin-Wu method is therefore very large compared to the actual blood sugar values.

One of the difficulties of sugar estimations using cupric copper is partial reoxidation of prous oxide by atmospheric oxygen (Somogyi, 7). Folin-Wu (2) reported that on increasing ternal diameter of test-tubes from 14 *mm* to 19 *mm*, the loss of cuprous oxide by reoxidation th air increased to 30%. In order to prevent this reoxidation, they devised the classical ood sugar tube with a constriction over the bulb. Somogyi (7) observed that sodium phate depresses the solubility of oxygen and thereby prevents reoxidation of cuprous oxide air. In the present method, amount of chromogen is not only practically independent of diameter of the test tube but also whether marble or cotton plug is used to cover the test bes during boiling or not.

In the Somogyi method (6) the deproteinization step consists in adding precisely icheometrically equivalent amounts of zinc sulphate and barium hydroxide. If any zinc present in traces in the deproteinized filtrate, low sugar values are obtained (Somogyi, 7). spended solid particles in the filtrate enhance the extent of reoxidation of cuprous oxide (7). any barium hydroxide is left in the solution, solid particles of barium carbonate would form wering the sugar values. In the present method the deproteinization is effected by adding ly one reagent which is stable and need not be added with precision. Thus the present method not only easier but also more accurate than the Somogyi procedure.

This method has been in use in the Departments of Biochemistry, G. S. V. M. Medical ollege, Kanpur and the All-Iudia Institute of Medical Sciences, New Delhi, for several ears.

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