SIMPLIFIED METHODS OF DETERMINING TRUE SUGAR IN BLOOD*

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There are quite a number of methods for the quantitative determination of sugar in the blood. Most of these have for their basis the reducing action of glucose in hot solution on certain metallic ions like cupric and ferricyanide ions. The extent of reduction is then measured by colorimetric, titrimetric or gasometric methods. Among the more common are the classical procedure of Folin and Wu, the equally celebrated method of Somogyi, Shaffer and Hartmann, and others like those of Nelson and Somogyi, Lewis and Benedict, Folin and Malmros, Hagedorn and Jensen, Bang, and Myer and Bailey. Some of these however, are used less often due to certain objectionable features that make their adoption quite difficult.

In the Philippines the methods most commonly employed are those of Folin and Wu, (1) and of Somogyi, *et al.* (2). Both of these are macro methods requiring not less than a milliliter of whole blood for every determination. The Folin and Wu method gives an amount of "sugar" that is slightly high; the normal range being 90 to 120 mg per cent, against the 70 to 100 mg per cent of Somogyi. The higher value obtained with the Folin and Wu method is attributed to reducing substances other than glucose present in the blood and determined in its filtrate with the sugar. These substances may occur in sufficient amount in the blood and increase the "sugar" value considerably. In the Somogyi method, the non-sugar reducing substances, namely glutathione and glucuronic acid,

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go down with the proteins of the blood when these are precipitated during preparation of the filtrate. But the Somogyi method involves a titrimetric procedure which makes it cumbersome and technically difficult.

In view of these considerations, those of us in the staff thought of presenting a specific method that is simple, yet, without sacrifice of accuracy. A modified macromethod was introduced (3) in 1952 for the determination of true blood sugar.

I. MODIFIED MACROMETHOD FOR TRUE BLOOD SUGAR

Principle: The proteins and the non-sugar reducing substances of the blood are precipitated with zinc hydroxide, the filtrate heated with alkaline copper and the cuprous oxide formed treated with phosphomolybdic acid for color development. The color is then compared with the standard similarly prepared.

Procedure: (a) *Preparation of the Filtrate.* Into a clean and dry 50 ml Erlenmeyer flask 7 ml of water are measured, followed by 1 ml of blood, rinsing the pipette with the clear water above the layer of blood. The mixture is shaken by a rotatory motion and subsequently treated drop by drop, shaking the flask after every drop, with 1 ml of 10% zinc sulfate solution, followed by 1 ml of 0.5 N sodium hydroxide. The mixture is shaken in the same manner till brown and then filtered.

(b) *Determination of the Sugar:* 2 ml of the filtrate are measured into a Folin and Wu sugar tube, treated with 2 ml of alkaline copper reagent and heated with the standards (0.2 mg and 0.4 mg glucose per 2 ml), and the blank, in a boiling water bath for 6 minutes. The tubes are then cooled in water for 3 minutes and each treated with 2 ml of phosphomolybdic acid reagent. After complete solution of the cuprous oxide is effected, each tube is diluted with water to the 25 ml mark and shaken cautiously. The colors are then compared in a photoelectric colorimeter.

Calculation:

$$\frac{RU}{RS} \times \text{conc. of S} \times 500 = \text{mg glucose per 100 ml of blood.}$$

Using the above modification, therefore, we prepare a filtrate in accordance with the method of Somogyi, *et al.* and determine the sugar by that of Folin and Wu. The results obtained by use of this combined procedure are constantly lower than those of the original Folin and Wu. The differences range from 10 to 20 mg per cent. These differences are within the range of differences observed between the Folin and Wu and the Somogyi methods as noted by several investigators (4).

Pressed for a procedure that would make possible the determination of true sugar from less than a drop of blood, we scanned the literature and came upon the ultramicro methods of Natelson (5). The procedure for blood sugar described by this author calls for delicately made micropipettes and especially constricted small sugar tubes which are not available locally. Furthermore, it employs tungstic acid reagent for precipitation of the proteins, and alkaline copper and phosphomolybdic acid reagents for color development. These reagents are essentially similar to those of Folin and Wu and, therefore, a method that does not give the true sugar value of the blood. With our macro modification in mind (3) for true sugar, we patterned an ultramicro modification (6) after that of Natelson. We substituted a Sahli 20 cmm ultramicro pipette, 1-ml serological pipettes and ordinary small pyrex test tubes in place of those recommended by Natelson. The results produced were very close to those obtained by our modified macro method.

II. MODIFIED ULTRAMICRO METHOD FOR TRUE BLOOD SUGAR

Principle: Hemolyzed blood is treated with zinc hydroxide, the filtrate heated with alkaline copper and the cuprous oxide formed treated with phosphomolybdic acid for color development. The intensity of color is then compared with the standard in a photoelectric colorimeter.

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Table 1 indicates values on aliquots of one sample of blood making a total of 20 analyses. The difference between means is not statistically significant.

Table 2

TRUE BLOOD SUGAR VALUES AS DETERMINED BY THE MACRO AND MODIFIED ULTRAMICRO METHODS IN PAIRED SAMPLES

	Milligrams glucose per 100 ml. human blood	
Samples	Macro method: Department of Medicine	Modified Ultramicro method: Department of Biochemistry
1	172.0	169.5
2	151.0	162.1
3	160.0	168.3
4	128.0	121.6
5	133.0	130.2
6	136.0	137.5
7	142.0	150.0
8	200.0	202.0
9	121.0	122.5
10	121.0	112.2
11	128.0	128.0
12	184.0	185.2
13	140.0	139.1
14	120.0	126.3
15	136.0	132.6
16	140.0	140.6
17	137.0	129.1
18	170.0	176.2
19	165.0	166.2
20	76.0	92.0
21	112.0	128.0
22	240.0	252.0
23	97.0	106.0
24	149.0	208.3
25	128.0	129.1
26	105.0	103.3
27	118.0	145.3
28	110.0	118.7
29	160.0	120.0
30	78.0	80.0
31	136.0	173.0
32	102.0	134.6
33	92.0	64.3
34	80.0	55.5
35	64.0	50.0
36	156.0	120.3
37	73.0	51.8
38	47.0	37.0
39	92.0	69.2

TABLE II (Continued)

40	65.0	83.3
41	65.0	62.5
42	65.0	69.8
43	63.0	70.8
44	92.0	79.2
45	45.0	75.0
46	78.0	98.0
47	86.0	100.0
48	60.0	68.0
49	22.0	40.0
50	50.0	70.0
51	86.0	88.0
52	48.0	64.0
53	72.0	93.8
54	53.0	79.1
55	55.0	91.7
56	97.0	112.5
57	58.0	91.7
58	84.0	102.1
59	42.0	89.6
60	71.0	80.0
61	86.0	84.0
62	115.0	68.0
63	71.0	72.0
64	63.0	64.0
65	97.0	66.0
66	68.0	68.0
67	57.0	61.5
68	97.0	82.7
69	63.0	61.5
70	60.0	78.8
71	59.0	76.9
72	64.0	84.6
73	53.0	88.4
74	60.0	92.3
75	81.0	107.7
76	67.0	62.5
77	55.0	58.3
78	290.0	266.6
79	62.0	62.5
80	40.0	72.9
81	83.0	95.8
82	64.0	76.0
83	76.0	72.0
84	86.0	78.0
85	71.0	68.0
86	65.0	76.0
87	42.0	50.0
88	83.0	84.0
89	55.0	72.0
90	81.0	80.0
91	67.0	74.0
92	45.0	92.3
93	49.0	76.1
94	80.0	69.2
95	95.0	96.1

TABLE II (Continued)

96	95.0	94.2
97	50.0	67.2
98	62.0	50.0
99	80.0	92.8
100	122.0	119.0
101	165.0	128.3
102	73.0	86.0
103	73.0	75.4
104	55.0	45.3
105	73.0	60.4
106	88.0	66.0
107	148.0	138.8
108	148.0	148.1
109	206.0	151.3
110	62.0	74.0

Mean difference ..... = 4.04
Standard deviation of difference ..... = 62.04
Standard error of mean difference ..... = 5.92
= 0.68

Table 2 indicates results of analyses of human blood by two separate departments. The two methods employed give values not markedly different and whatever difference was observed was not statistically significant.

SUMMARY AND CONCLUSION

Simplified macro and ultramicro methods for determining true sugar in the blood are hereby presented. Values obtained by the modified macro method compare favorably with those of the macro method of Somogyi, Shaffer and Hartmann. The same is true of the results obtained by the modified ultramicro method compared with those of Nelson and Somogyi. The differences in both cases are not statistically significant.

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