ADAPTATION OF SHAFFER'S TITRATION METHOD FOR BLOOD SUGAR TO CLINICAL USE

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Reprinted from
THE JOURNAL OF LABORATORY
AND CLINICAL MEDICINE
St. Louis

Vol. VIII, No. 11, August, 1923

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SHAFFER and Hartmann have recently published a complete system of methods for estimation of sugar in urine, blood, milk, and other solutions.[1] In all of these methods a copper reagent is used which is similar to Benedict's. This is partly reduced by the sugar. The further treatment of the mixture causes iodine to be set free. The iodine is titrated with thiosulphate, and from the amount of iodine estimated the copper equivalent of the sugar can be calculated.

Our interest was attracted to the micro-blood-sugar method because it enables physicians to make the estimations without the expense of investing in a reliable colorimeter.

Folin's method is the only colorimetric method of estimating blood sugar that we are willing to recommend. We find that the results secured by Shaffer's method are just as accurate as those by Folin's method.

In addition to strongly recommending Shaffer's method, we wish to propose a modification that removes the one difficulty which those who are not expert chemists would encounter in using the method. The difficulty referred to is that of standardizing the thiosulphate. We have overcome this satisfactorily by standardizing the thiosulphate against the copper reagent, which is very easily done. We have proved that this modification does not cause inaccuracy in estimation. Our experimental results will be reported after the description of the method.

Preparing the Blood for Estimation.—Draw into the syringe a strong solution of neutral potassium oxalate (30 grams of oxalate dissolved in 100 c.c. water), empty the syringe and expel the excess so that only a film of solution is left. Draw at least 3 c.c. of blood from a vein and empty the syringe into a dry tube. With an accurate pipette measure 2 c.c. of the blood into a dry flask and add 14 c.c. of distilled water. When the blood is completely laked add 2 c.c. of 10 per cent sodium tungstate solution and finally 2 c.c. of 2/3 normal sulphuric acid solution. The volume of the liquid is now exactly 20 c.c. Cork the flask, shake well and let it stand 5 to 10 minutes.

If all the protein has been precipitated, the mixture will be brownish-red

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Received for publication, June 18, 1923.

n color instead of bright red, and only a trace of foam will appear on shaking. If the proper results have not been secured add 10 per cent sulphuric cid a drop at a time until satisfactory. (If more than 2 drops are required, he alkalinity of the stock solution of sodium tungstate must be reduced by reatment with a definite quantity of sulphuric acid.) Use small size filter paper for filtration. If the filtrate is not clear and colorless, return all the liquid and precipitate) to the flask, treat it with more acid, shake well, and after few minutes filter again. This method of freeing the blood of protein is xactly in accordance with Folin's[2] system of blood methods. What follows is Shaffer's special technic.

Technic of the Estimation.—Measure exactly 5 c.c. of filtrate into a large est tube and mix with it exactly 5 c.c. of the special micro-reagent. Plug he tube loosely with cotton and place it in a bath that is boiling actively. After exactly 15 minutes remove the tube and cool it at once under the tap. Then let it stand in a jar of cold water for 5 minutes. Add 5 c.c. of N/1 sulphuric acid, mix and let it stand 1 minute.

Titrate the liberated iodine by adding N/200 sodium thiosulphate as ast as drops can be counted (mixing well) until a distinct change is noticed, hen add more slowly until the color becomes light yellow. Now add about c.c. of 2 per cent starch solution and continue the titration cautiously but ot too slowly until the characteristic starch-blue color is lost and only a ale copper-blue remains. When near the end-point determine the effect of ach drop added.

Calculation.—In the special table (devised by us to correspond to our nodification of Shaffer's method) the per cent of glucose in the original blood vill be found opposite the c.c. of thiosulphate used for titration.

If the sugar content is greater than the highest in the table, dilute some f the blood filtrate with an equal volume of water and use 5 c.c. of this for he estimation, multiplying the results by 2.

REAGENTS*

(1) *Microcopper Reagent.*—Dissolve each constituent separately.

(a) Dissolve 40 gm. C.P. anhydrous sodium carbonate (or 47 gm. pure hotographic monohydrated carbonate) in 400 c.c. warm distilled water.

(b) Dissolve 5 gm. C. P. copper sulphate (crystals that have not effloresced) in about 100 c.c. water.

(c) Dissolve 7.5 gm. pure tartaric acid in 100 c.c. of water.

(d) Dissolve exactly 0.7 gm. pure potassium iodate in 100 c.c. of water.

(e) Dissolve 10 gm. pure potassium iodide in 100 c.c. of water.

(f) Dissolve 18.4 gm. pure neutral potassium oxalate in 100 c.c. of water.

When each is dissolved, mix (c) with (b), and pour the mixture slowly with stirring) into (a). Combine (d), (e) and (f) and pour this at once into he carbonate-copper mixture. Transfer to a measuring flask, Rinse all the lissolving beakers with small portions of water. When cooled to room temperature fill to the liter mark and mix thoroughly. Keep the reagent in a

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*The Shaw Supply Co. (of Portland, Seattle, and Tacoma) will supply any or all of the chemicals used for the method, including ready prepared solutions.

tightly corked bottle. A little sediment will be deposited, use the clear top liquid for estimations.

If the chemicals are pure, the microreagent run as a control will give the same titration whether heated in a bath 15 minutes or not heated. With each batch of reagent run one heated control and compare it with the unheated.

(2) *Standard Thiosulphate.*—Very dilute thiosulphate does not keep well. Prepare a stock solution of sodium thiosulphate a little stronger than decinormal (dissolve about 26 gm. in about 1 liter of distilled water) and let it stand 2 days.

Dilute exactly 5 c.c. to 100 c.c. in a measuring flask. Mix and use this to titrate 5 c.c. of the copper reagent (after adding 5 c.c. water and 5 c.c. of N/1 sulphuric acid) following the directions given above for the rate of titration. The titration will be less than 19.5 c.c. When duplicate titrations agree within 0.1 c.c. prepare a dilution of the thiosulphate such that exactly 19.5 c.c. will be required for the titration. For example, if the titration is 19 c.c. it will be necessary to dilute 5 c.c. of the stock solution to 102.6 c.c. (i.e., $\frac{19.5}{19.0} \times 100$). First dilute to 100 c.c. in a measuring flask then add the 2.6 c.c. with a reliable pipette, and mix well. This ratio of dilution should be determined by similar titration of a control once in two weeks. Prepare the dilute solution each day that estimations are made. This dilute solution is approximately N/200, and is the one referred to in the technic. Keep the stock solution in a brown bottle well corked, set away where it will not get warm, under these conditions it does not deteriorate.

TABLE I

PER CENT GLUCOSE IN BLOOD CORRESPONDING TO C.C. THIOSULPHATE USED FOR TITRATION

C.C.	PER CENT	C.C.	PER CENT	C.C.	PER CENT	C.C.	PER CENT
18.6	.036	16.3	.101	14.0	.162	11.4	.232
18.5	.039	16.2	.104	13.9	.165	11.2	.237
18.4	.042	16.1	.106	13.8	.167	11.0	.242
18.3	.045	16.0	.109	13.7	.169	10.8	.247
18.2	.048	15.9	.111	13.6	.172	10.6	.252
18.1	.052	15.8	.114	13.5	.175	10.4	.257
18.0	.055	15.7	.116	13.4	.177	10.2	.263
17.9	.058	15.6	.119	13.3	.180	10.0	.268
17.8	.061	15.5	.122	13.2	.183	9.8	.273
17.7	.064	15.4	.124	13.1	.185	9.6	.279
17.6	.066	15.3	.127	13.0	.188	9.4	.285
17.5	.069	15.2	.129	12.9	.191	9.2	.289
17.4	.071	15.1	.132	12.8	.194	9.0	.294
17.3	.074	15.0	.134	12.7	.197	8.8	.298
17.2	.076	14.9	.137	12.6	.200	8.6	.303
17.1	.079	14.8	.140	12.5	.202	8.4	.307
17.0	.082	14.7	.143	12.4	.205	8.2	.312
16.9	.084	14.6	.146	12.3	.207	8.0	.316
16.8	.087	14.5	.149	12.2	.210	7.6	.325
16.7	.089	14.4	.151	12.1	.212	7.2	.335
16.6	.092	14.3	.154	12.0	.215	6.8	.345
16.5	.095	14.2	.157	11.8	.220	6.4	.357
16.4	.098	14.1	.159	11.6	.226	6.0	.368

(3) *Starch Solution.*—This should be made once a week. We prefer

"soluble starch" but undoubtedly common corn starch could be used. Mix about 2 grams of starch with about 10 c.c. of water and pour it into about 90 c.c. of boiling water, mix and boil one minute. A few drops of toluol may be added as a preservative.

(4) *Standard Sulphuric Acid.*—The normal solution may be purchased if one is not used to checking standard solutions. For the $\frac{2}{3}$ normal solution dilute 100 c.c. of the normal sulphuric acid with 50 c.c. of distilled water.

(5) *Sodium Tungstate Solution.*—C.P. tungstate should be used. The solution must be tested[3] for excess of carbonate as follows. Measure with a pipette 5 c.c. of the solution, add a drop of methyl orange solution and some distilled water, then titrate with the $\frac{2}{3}$ normal acid until the yellow color changes to a slightly reddish yellow. Between 3 and 3.3 c.c. should be required. If the titration is greater add twice normal sulphuric acid (made by diluting about 11.5 c.c. C.P. acid to 200 c.c.) to the whole stock of 10 per cent sodium tungstate solution to bring its alkalinity down to the proper limits. Mix and titrate again.

Table II

Comparison of Estimations by Folin's and Shaffer's Methods

Blood	1	2	3	4	5	6	7
Folin	.070	.109	.117	.124	.130	.167	.222
Reagent I	.069	.106	.118	.124	.132	.165	.222
Reagent II	.072	.109	.123	.126	.134	.169	.220
Reagent III	.069	.108	.117	.122	.132	.167	.219

Note: Control titrations of Shaffer's reagents were as follows: I—19.55 c.c., II—19.3 c.c., III—19.7 c.c.

Table III

Comparison of Blood Sugar Estimations by Shaffer's Original Method and by the Proposed Modified Method

Blood	1	2	3	4	5	6	7	8	9
Shaffer	.047	.072	.099	.102	.120	.129	.229	.338	.433
Reagent V	.048	.071	.100	.102	.122	.129	.230	.342	.436
Reagent VI	.045	.069	.101	.103	.123	.130	.231	.341	.431
Variation	.002	.003	.002	.001	.003	.001	.002	.004	.003

Note: Reagent IV used for the original method gave a titration of 19.6 c.c. The control titration of reagent V used for the modified method was 19.9 c.c. and of reagent VI—19.0 c.c.

DISCUSSION

Different samples of copper reagent do not always give exactly the same titration value when accurate N/200 thiosulphate is used. We find that this value is generally close to 19.5 c.c., therefore, we have taken this figure as the average titration value. Varying the amount of iodate in the reagent changes the titration value.

Estimations by Shaffer's method using accurate reagents are practically identical with those by Folin's method (see Table II). Also when we used copper reagents that had a slightly different titration value (using exact N/200 thiosulphate the control estimation on reagent 2 is 19.3 c.c., and on reagent 3 it is 19.7 c.c.) and made the thiosulphate correspond to the reagent in each case in accordance with our modification of the method, the results agreed very closely with the estimations both by Folin's method and by Shaffer's (with accurate reagents).

By changing the iodate content we secured copper reagents having a titration value differing more widely (19.0 c.c. and 19.9 c.c.). With these less accurate reagents we obtained very satisfactory results by using our modification of the method (see Table III). In most cases the variations from the accurate estimations by the original method were only 1 to 3 mg., and the greatest variation was 6 mg. Most of the variations were of the same order as were secured by running duplicate or triplicate estimations by any one of the methods. The Folin duplicates which we ran, differed by 1. to 2.3 mg. Shaffer triplicates varied by 0.5 to 4.5 mg. These results convince us that our modification does not perceptibly affect the accuracy of the Shaffer method.

The explanation for such good results with slightly inaccurate reagents is, undoubtedly, that the variation of the thiosulphate from a true N/200 solution compensates for the deviation of titration value of the copper reagent from the average value. For example, if the reagent is a little weak, then the thiosulphate solution prepared for use with it will be more dilute than N/200, thus avoiding the undertitration that would occur in a sugar estimation if an exact N/200 solution were used.

SUMMARY

An easy method of standardizing the thiosulphate is proposed as a modification of the Shaffer method, the thiosulphate being checked against the copper reagent.

Because of this adjustment of the solution the control estimation of the reagent is always 19.5 c.c. of dilute thiosulphate.

This constant titration value has enabled the authors to construct a table from which the per cent of glucose in the blood can be read without calculation.

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