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UNIFORM METHOD FOR THE ESTIMATION OF GLUCOSE IN BLOOD AND URINE *

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In a previous paper[1] we suggested modifications of Shaffer and Hartmann's[2] excellent new blood sugar method by means of which their method becomes an easy one to carry out and one suitable for general use by physicians.

In the last stage of the method iodine is set free, and the residual iodine that does not enter into reaction is titrated with standard sodium thiosulphate solution. The original method calls for accurate standardizing of the thiosulphate. This causes sufficient difficulty to constitute a serious drawback, when the method is used by clinicians. We have eliminated this difficulty without sacrificing accuracy. Also, on the basis of our change in the method we have constructed a table from which the percentage of glucose can be read directly without calculation.

Quite recently we have applied the same technic to the estimation of glucose in urine with very satisfactory results. (Shaffer mentions the possibility of using the micro-copper reagent for urine as well as for blood.)

The object of this paper is to give the directions for carrying out the uniform method, as recommended by us for estimation of blood and urine glucose.

BLOOD SUGAR ESTIMATION

Freeing the blood of protein. The estimation is made on Folin filtrate which is prepared as follows: Rinse the syringe with saturated potassium oxalate solution (30 gm. dissolved in 100 c.c. distilled water) and force out all excess, so that only a film of solution remains in the syringe. Draw from a vein at least 3 c.c. of blood and empty into a dry tube. With an accurate pipette measure 2 c.c. of blood into a dry flask and 14 c.c. of distilled water. When the blood is laked, add 2 c.c. of 10 per cent sodium tungstate and then 2 c.c. of two-thirds normal sulphuric acid (slowly and with shaking). Shake well and allow to stand 10 or 15 minutes. If all the protein has been precipitated, the mixture will have a brownish-red color and on shaking very little foam will form. If unsatisfactory, add 10 per cent sulphuric acid a drop at a time with vigorous shaking, until the desired results are obtained. Filter the mixture, using a small filter. If the filtrate is not colorless and perfectly clear, pour it back into the filter once or twice to see if it will become clear. If unsuccessful, return all the liquid and precipitate to the flask and treat with more acid. This Folin filtrate is the same as is used in a number of other blood chemistry methods.

Technic of the estimation. Measure exactly 5 c.c. of Shaffer's micro-copper reagent into a large test tube and add 5 c.c. of the blood filtrate. Plug the tube loosely with cotton and place in a boiling water bath for 15 minutes. Remove it and cool in water. Add 5 c.c. of N/1 sulphuric acid and mix. Let it stand for at least one minute, then titrate the liberated iodine with our modified standard thiosulphate solution (about N/200) until most of the iodine is gone and the solution becomes faintly yellow. Add about 1 c.c. of 2 per cent starch solution and titrate cautiously to the disappearance of the characteristic starch-blue color (a pale copper blue remains). When near the end-point shake well after the addition of each drop.

Calculation. By referring to the special table devised by us the percentage of glucose in the original blood will be found in the column under B opposite the c.c. of thiosulphate used.

URINE SUGAR ESTIMATION

Measure exactly 1 c.c. of urine into a 50 c.c. volumetric flask and fill to the mark with water. Mix well. If Benedict's qualitative test shows less than 1.5 per cent glucose present, measure 5 c.c. of the diluted urine into a tube, containing 5 c.c. of Shaffer's micro-copper reagent and continue exactly as described for blood filtrate. The percentage of glucose in the original urine will be found in the column under U.5 in the table opposite the c.c. of thiosulphate. If the qualitative test shows more than 1.5 per cent sugar, add 1 c.c. of diluted urine and 4 c.c. of water to the tube containing the copper reagent and proceed as with blood filtrate. In this case the percentage will be found in the column under U.1.

Normal urinary constituents give a slight reduction. This error may be ignored or it may be allowed for by deducting 0.1 from the percentage figure.

The presence of protein in moderate amounts in the urine causes no appreciable error in the estima-

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

C. C.	B.	U. 5	U. 1	C. C.	B.	U. 5	U. 1	C. C.	B.	U. 5	U. 1	C. C.	B.	U. 5	U. 1
18.4	.042	.21	1.05	16.2	.104	.52	2.60	14.0	.162	.81	4.05	11.4	.232	1.16	5.80
18.3	.045	.22	1.13	16.1	.106	.53	2.65	13.9	.165	.82	4.12	11.2	.237	1.19	5.93
18.2	.048	.24	1.20	16.0	.109	.54	2.72	13.8	.167	.83	4.17	11.0	.242	1.21	6.05
18.1	.052	.26	1.30	15.9	.111	.56	2.78	13.7	.169	.85	4.23	10.8	.247	1.23	6.17
18.0	.055	.27	1.37	15.8	.114	.57	2.85	13.6	.172	.86	4.30	10.6	.252	1.26	6.30
17.9	.058	.29	1.45	15.7	.116	.58	2.90	13.5	.175	.87	4.37	10.4	.257	1.28	6.43
17.8	.061	.31	1.53	15.6	.119	.60	2.98	13.4	.177	.89	4.43	10.2	.263	1.31	6.57
17.7	.064	.32	1.60	15.5	.122	.61	3.05	13.3	.180	.90	4.50	10.0	.268	1.34	6.70
17.6	.066	.33	1.65	15.4	.124	.62	3.10	13.2	.183	.91	4.57	9.8	.273	1.36	6.83
17.5	.069	.35	1.73	15.3	.127	.63	3.17	13.1	.185	.93	4.63	9.6	.279	1.39	6.97
17.4	.071	.36	1.78	15.2	.129	.64	3.23	13.0	.188	.94	4.70	9.4	.285	1.42	7.12
17.3	.074	.37	1.85	15.1	.132	.66	3.30	12.9	.191	.95	4.77	9.2	.289	1.44	7.23
17.2	.076	.38	1.90	15.0	.134	.67	3.35	12.8	.194	.97	4.85	9.0	.294	1.47	7.35
17.1	.079	.40	1.98	14.9	.137	.68	3.42	12.7	.197	.98	4.92	8.8	.298	1.49	7.45
17.0	.082	.41	2.05	14.8	.140	.70	3.50	12.6	.200	1.00	5.00	8.6	.303	1.51	7.57
16.9	.084	.42	2.10	14.7	.143	.71	3.57	12.5	.202	1.01	5.05	8.4	.307	1.53	7.68
16.8	.087	.43	2.17	14.6	.146	.73	3.65	12.4	.205	1.03	5.12	8.2	.312	1.56	7.80
16.7	.089	.45	2.23	14.5	.149	.74	3.72	12.3	.207	1.04	5.18	8.0	.316	1.58	7.90
16.6	.092	.46	2.30	14.4	.151	.75	3.77	12.2	.210	1.05	5.25	7.6	.325	1.62	8.13
16.5	.095	.47	2.37	14.3	.154	.77	3.85	12.1	.212	1.06	5.30	7.2	.335	1.67	8.38
16.4	.098	.49	2.45	14.2	.157	.78	3.92	12.0	.215	1.07	5.37	6.8	.345	1.72	8.63
16.3	.101	.50	2.52	14.1	.159	.80	3.98	11.8	.220	1.10	5.50	6.4	.357	1.78	8.92
								11.6	.226	1.13	5.65	6.0	.368	1.84	9.20

B—Blood Glucose per cent.
U. 5—Urine Glucose per cent if 5 C.C. Diluted Urine is Used.
U. 1—Urine Glucose per cent if 1 C.C. Diluted Urine is Used.

tion.

Note on the qualitative test. In a routine Benedict's test we use 5 c.c. of reagent and 0.3 c.c. of urine, and heat in a boiling bath 5 minutes. A greenish tinge is observed in the reduction mixture, even if there is considerable cuprous oxide in suspension, when less than 1.5 per cent sugar is present. If in doubt, run two tubes for quantitative estimation (using 5 and 1 c.c.). After a little experience is obtained in comparing the results of the qualitative test with the quantitative estimation it is easy to judge whether to take 5 or 1 c.c. of diluted urine.

REAGENTS *

(1) *Micro-copper Reagent.* Each constituent should be dissolved separately.

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

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

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

(d) 0.7 gm. pure potassium iodate (weighed accurately) in 100 c.c. of water.

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

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

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

When all are dissolved, mix (c) with (b), and pour the mixture slowly (with stirring) into (a). Combine (d), (e) and (f) and pour at once into the carbonate-copper mixture. Transfer to a liter measuring flask. Rinse all the dissolving beakers with small portions of water. When cooled to room temperature fill to the mark and mix well. Keep the reagent in a tightly corked bottle. A little sediment will be deposited; use the clear top liquid for estimations.

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

(2) *Standard Thiosulphate.* Dilute thiosulphate does not keep well. Prepare a stock solution of sodium thiosulphate a little stronger than N/10 (dissolve about 26 gm. of the crystals 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 and mix. Use this for titrating a control (5 c.c. copper reagent and 5 c.c. water treated with 5 c.c. N/1 sulphuric acid), following the directions given above for the method of titration. The amount used will be less than 19.5 c.c. When duplicate titrations agree within 0.1 c.c., prepare a dilution of the stock thiosulphate such that exactly 19.5 c.c. will be required for the titration of a control. For example, if 19 c.c. of dilute thiosulphate was used, it will be necessary to dilute

5 c.c. of stock solution to 102.6 cc. (i.e., $\frac{19.5}{19.0} \times 100$). First dilute the 5 c.c. to 100 c.c., then add the 2.6 c.c. with a reliable pipette. Mix thoroly. 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 is our modified standard thiosulphate, 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.

(3) *Starch Solution.* This should be made about once a week. We prefer "soluble starch" but undoubtedly common starch could be used. Mix about 2 gm. of starch with 10 c.c. of water and pour it into 90 c.c. of boiling water; mix and boil 1 minute. Add a few drops of toluol as a preservative.

(4) *Standard Sulphuric Acid.* The N/1 solution may be purchased, if one is not used to checking standard solutions. Prepare the two-thirds normal solution by diluting 100 c.c. of the N/1 acid with 50 c.c. of distilled water.

(5) *Sodium Tungstate Solution.* C.P. tungstate should be used, and it should go into solution readily. The reagent must be tested for excess of carbonate as follows: To 5 c.c. of the solution add a drop of methyl orange and some distilled water, then titrate with the two-thirds 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.) in sufficient amount to the whole batch of 10 per cent tungstate to bring its alkalinity down to the proper limits. Mix and titrate again.

DISCUSSION

The titration value of different samples of micro-copper reagent, when accurate N/200 thiosulphate solution is used, is not always the same, altho the variation is slight. We find that this value is close to 19.5 c.c., therefore, we have taken this figure as the average titration value. Our table for calculation is based on this fact. Varying the amount of iodate in the reagent changes its titration value.

We have thoroly tested our modified method and have proved that the estimations of blood glucose are quite accurate. Our experimental results will be found in our previous paper[1]. No appreciable error was observed, amounting only to 1 to 5 mg., even when the blood contained 200 to 400 mg. per 100 c.c. Furthermore, variations of the same order (0.5 to 4.5 mg.) were secured with triplicate estimations by Shaffer's original method. The new method has given good satisfaction in a number of laboratories.

The application of the method to urine has been tried out on various concentrations of glucose, ranging from 0.2 to 8.0 per cent. The error did not exceed 0.1 in the per cent figure, e. g., a 2.0 per cent urine would give estimations anywhere between 1.9 and 2.1 per cent. To determine how readily the method can be learned by the laboratory worker we tried it with the sophomore class. In spite of their slight experience with the Shaffer method, 80 per cent of the results reported on urines given out as unknowns were quite satisfactory. On the other hand, only 60 per cent of the results by Benedict's method (with which they had had a great deal of practice) were satisfactory.

If several urines are to be run, the estimations can be finished in much less time than by Benedict's method. A hospital technician who has used the new method for some time reports that 5 or 6 urines can be estimated in 30 minutes.

The advantages of a uniform method for blood and urine glucose are obvious. The estimations on diabetic blood and urine can be run simultaneously, and with the same set of apparatus and the same reagents. There is less liability to error in this iodometric titration method than in other methods; it certainly should be preferred to any colorimetric method. The table for calculation will be found to be a notable advantage of the method. This table can be used only with our modification of the original method.

CONCLUSIONS

1. A simplified uniform technic for estimation of glucose in blood and urine is presented, using a modification of Shaffer and Hartmann's iodometric titration method for blood sugar.
2. The method eliminates special reagents and special technic for urine sugar.
3. Simultaneous estimations of blood and urine sugar can be made rapidly.
4. A special table is furnished which gives a direct reading of percentage of glucose in either blood or urine, corresponding to the c.c. of thiosulphate used for titration, saving time and uncertainty in computation.

REFERENCES

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