XXXIII. A SIMPLIFIED BLOOD SUGAR METHOD BASED ON FERRICYANIDE-INDIGOCARMINE TITRATION

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By making use of Korenmann's discovery [1935] that dilute ferricyanide solutions can be titrated with indigocarmine solution, accurate means can be devised for estimating blood sugar rather more simply than has hitherto been possible by titrimetric methods. The convenience of the indigocarmine titration is such that it is relatively easy to obtain rapid duplicate estimations on a single 0.2 ml. sample of blood (or a single estimation on 0.1 ml. if precipitants be used in half the standard amounts) while at the same time large volume measurements can be used throughout the procedure.

In the method described below, with the exception of the blood itself, no micro-quantities are handled and no delicate or refined manipulation is required. The solutions themselves are few in number and simple in preparation, and the technique does not require any special apparatus.

METHOD

Reagents

(1) 0.5 % zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), prepared weekly from stock 10 % solution.

(2) *N*/20 sodium hydroxide, prepared weekly from stock *N* solution.

(3) Alkaline potassium ferricyanide reagent, prepared from two stock solutions:

(A) Weigh out exactly 2 g. potassium ferricyanide and dissolve in 1 l. distilled water. Keep the solution in a dark glass bottle and store in a refrigerator.

(B) Weigh out 100 g. potassium bicarbonate and 100 g. anhydrous potassium carbonate and dissolve in 1 l. distilled water.

Prepare the reagent by pipetting 25 ml. solution A into a 100 ml. flask and making up to the mark with solution B. Larger quantities may of course be prepared in like proportion, but enough ferricyanide reagent should be prepared to provide only for 1 week's requirements. Store in a dark glass bottle. Any loss of ferricyanide strength can scarcely be detected in 1 week, but this becomes gradually apparent when the time is prolonged much beyond this period.

(4) Indigocarmine solution.

Carefully weigh out 0.118 g. solid "Indigocarminium B.D.H.",¹ transfer to a 500 ml. flask, and dissolve in approximately 400 ml. distilled water. Add now

¹ Other commercial preparations of indigocarmine have been examined and found to differ in their titration values from this preparation and from each other. If any preparation other than the above is used, its solution should be adjusted so that its titration value against the ferricyanide reagent is exactly that given by the standard used here.

10 ml. 25 % (by volume) sulphuric acid, and make up to the mark with distilled water. In a brown glass bottle, the acid keeps the solution quite stable, no change in the titration values having been detected in 1 month.

Titration of alkaline ferricyanide reagent with indigocarmine solution.

Pipette 5 ml. of ferricyanide reagent into a boiling-tube (6×1 in.), add approximately 5 ml. of distilled water and titrate directly with the indigocarmine solution contained in a 5 ml. microburette until the blue of the dye just faintly persists. The reading should be within the range 4.50–4.55 ml. Different workers in this laboratory differ slightly in their recognition of the end-point, some preferring to read it as the very faintest perceptible blue persisting in a previously slightly yellow solution, others preferring a rather more definite blue colour to demarcate the end of the titration. The personal factor accounts for most of the variation in the titration reading above. Since, however, all sugar estimations are based upon differences between two separate titrations, any slight difference in the interpretation of the end-point by different workers introduces no error into the actual estimation.

Procedure

(1) Precipitation of proteins.

In a boiling-tube (6×1 in.) place 10 ml. zinc sulphate solution. Deliver into it exactly 0.2 ml. blood, rinsing out the pipette in the usual manner. Mix by gentle rotation of the tube and add 5 ml. sodium hydroxide. Again mix, allow to stand 1 min. and then filter through a No. 41 Whatman filter-paper, 9 cm. diameter.

(2) Reduction of ferricyanide by blood filtrate.

To 5 ml. filtrate (a further 5 ml. is available for duplicate estimation if desired), placed in a hard glass boiling-tube (6×1 in.), add 5 ml. of the ferricyanide reagent and immerse in a boiling water-bath for exactly 6 min. At the end of this heating period cool the tube by shaking it for a few moments in a bath of cold water—thorough cooling is not essential—and titrate the residual ferricyanide with indigocarmine solution. If cooling is prolonged and the titration conducted slowly, a slight precipitate of zinc ferrocyanide forms but does not interfere in any way with the titration.

Perform the blank test by carrying through the whole process, but omitting the blood. With the use throughout of the highest grade analytical reagents, now readily procurable, the blank value should be identical with the figure obtained by direct titration of the untreated ferricyanide reagent with the dye. In such circumstances, the true blank estimation can be omitted in routine procedure and the blood sugar estimated on the basis of the difference value between the titration of the blood filtrate (A) and the dye equivalent of the ferricyanide reagent (B). Using this value, read off from Table I the percentage blood sugar.

In Table II the present method has been compared with the MacLean method on a series of representative blood samples handled in the course of ordinary routine work. The two methods show excellent agreement. On fundamental grounds, the present method should be in good agreement in its results with that of Hagedorn & Jensen. Though it has not been directly tested, this is made the more probable by the work of Herbert & Groen [1929] who have shown a close concordance of results between the methods of MacLean and Hagedorn & Jensen.

Table I

Difference Blank (B)- titration (A) ml. indigo- carminc	Sugar %	Difference Blank (B)- titration (A) ml. indigo- carminc	Sugar %
0.00	0.000	1.80	0.214
0.05	0.005	1.85	0.220
0.10	0.010	1.90	0.226
0.15	0.016	1.95	0.233
0.20	0.021	2.00	0.240
0.25	0.026	2.05	0.246
0.30	0.032	2.10	0.252
0.35	0.037	2.15	0.258
0.40	0.042	2.20	0.265
0.45	0.048	2.25	0.272
0.50	0.053	2.30	0.280
0.55	0.058	2.35	0.287
0.60	0.063	2.40	0.294
0.65	0.068	2.45	0.301
0.70	0.074	2.50	0.309
0.75	0.080	2.55	0.316
0.80	0.086	2.60	0.323
0.85	0.092	2.65	0.330
0.90	0.098	2.70	0.338
0.95	0.103	2.75	0.345
1.00	0.109	2.80	0.352
1.05	0.115	2.85	0.359
1.10	0.121	2.90	0.366
1.15	0.128	2.95	0.374
1.20	0.134	3.00	0.381
1.25	0.140	3.05	0.388
1.30	0.147	3.10	0.395
1.35	0.153	3.15	0.403
1.40	0.159	3.20	0.411
1.45	0.166	3.25	0.418
1.50	0.173	3.30	0.425
1.55	0.179	3.35	0.432
1.60	0.186	3.40	0.439
1.65	0.193	3.45	0.446
1.70	0.200	3.50	0.454
1.75	0.207		

Table II

Case	Blood sugar percentage	
	Present method	MacLean's method
C 174	0.247	0.255
C 268 (i)	0.240	0.240
(ii)	0.130	0.128
C 372	0.124	0.124
C 418	0.100	0.104
F 275	0.078	0.082
Bl.	0.084	0.085
F 430	0.310	0.305
Sc.	0.176	0.177
In.	0.104	0.111

Use of tungstic acid precipitation

A more rapid filtration is possible by substituting tungstic acid precipitation for the zinc hydroxide method. This involves no alteration in technique or quantities if the blood is added directly to a mixture of 10 ml. 0.5 % sodium tungstate and 5 ml. *N*/15 sulphuric acid, and then filtered. The results, however, are always higher than by the zinc method, and in a series of 12 cases examined the

discrepancy was found to range from 7 to 27 mg. per 100 ml. blood. From what is known of the comparative values afforded by the different blood sugar methods [Herbert & Groen, 1929], it is inferred that this form of tungstic acid precipitation would yield results in keeping with those of established methods which use tungstic acid filtrates (e.g. Folin & Wu). Since, however, the latter depart further from true glucose estimation, the zinc technique has been preferred for general routine purposes.

The method is unsuitable for application to any blood containing thymol, which is sometimes incorporated with fluoride when the latter is used as anti-coagulant and preservative. The use of thymol, however, is unnecessary except for the special purpose of preserving for several days blood samples which have been collected in non-sterile vessels.

SUMMARY

1. A simple method of blood sugar estimation is described, using ferrieyanide reduction, the amount so reduced being estimated by indigocarmine titration.

2. The results are shown to be in close agreement with those of the MacLean method, and the inference is drawn upon indirect evidence, that it likewise provides a simplified substitute for the Hagedorn & Jensen technique.

3. Tungstic acid precipitation of proteins may be readily substituted for the standard zinc hydroxide precipitation, but the results obtained are somewhat higher and more in accordance with Folin & Wu values.

REFERENCES

- Herbert & Groen (1929). *Biochem. J.* **23**, 339.
Korenmann (1935). *Mikrochemie*, **18**, 31.