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89. Tsutomu Momose, Akira Inaba, Yoshiko Mukai, and Taeko Shinkai: Organic Analysis. XXIV.[1] Approximate Colorimetric Estimation of Blood Sugar and Urine Sugar with the Naked Eye.

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Several methods are now available for determining blood sugar and urine sugar by titrations or by instrumental analyses, yet a more simple test for the sugar is desirable for clinical tests, and several reagents are widely used for this purpose. In the writers' laboratory, 3,6-dinitrophthalic acid proved to be of use in the approximate estimation of blood sugar and urine sugar, using a minimum amount of the sample.

3,6-Dinitrophthalic acid gives a sensitive and stable color reaction with reducing sugars when heated in sodium carbonate solution in the presence of sodium thiosulfate. This reaction is used in the microdetection of reducing sugars,[2] and in the determination of blood sugar and urine sugar.[1] To observe the developed color with the naked eye, it is preferable to carry out the reaction in the absence of sodium thiosulfate. Then the reaction gives a different coloration with increasing concentration of glucose in such moderately concentrated solution as blood or urine sugar. This difference is caused by the instability of the color in the absence of sodium thiosulfate, which diminishes with decreasing concentration of the sugar on prolonged heating. Thus, a distinct difference of coloration can be seen in normal, moderately high, and high levels of blood or urine sugar by selecting a suitable reaction conditions — dilution of blood or urine, concentration of the reagent, and heating time.

Experimental

Estimation of Blood Sugar: Reagents—Basic zinc carbonate: To a solution of 100 g. of $ZnSO_4 \cdot 7H_2O$ in 800 cc. of water, a solution of 37 g. of anhyd. Na_2CO_3 in 300 cc. of water is added in one portion and the mixture is shaken for about 5 min. After the first gelatinous precipitate turns to voluminous powder the mixture is heated in a boiling water bath for 15~20 min. until the bubbling of CO_2 ceases. The precipitate formed is collected by filtration while hot, washed with hot distilled water, dried at 100~110°, powdered, and shifted with a 100-mesh sieve.

Coloring agent: To a solution of 1.0 g. of 3,6-dinitrophthalic acid in 25 cc. of anhyd. EtOH, 50 g. of anhyd. Na_2CO_2 is added and mixture is dried at room temperature in vacuum.

Procedure—To 0.10 cc. of blood mixed with 6 cc. of water in a test tube, about 150 mg. of basic zinc carbonate is added and the mixture is heated while shaking in a boiling water bath for a short time until coagulation of proteins occurs. After cooling in running water, the mixture is filtered with a filter paper Toyo Roshi No. 3, of 7 cm. in diameter. To the clear filtrate, about 250 mg. of the coloring agent is dissolved and heated in a boiling water bath for 2 min. The resulting color is compared within 3 min. with the standard colors (Table I) prepared in the same way with 0.10 cc. of the standard solutions which contain 50, 100, 150, 200, 300, or 500 mg./100 cc. of glucose.

Tabel. I. Standard Colors for Blood Sugar Values

Blood sugar value (mg./100 cc.)	50	100	150	200	300	500
Color	faint yellow	faint orange	orange	deep orange	orange-red	deep orange-red

Discussion

For use in a clinical test, the reagents should be simple to use and stable for a long storage to give constant and reproducible results. Basic zinc carbonate proved to be

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1) Part XXIII: T. Momose, A. Inaba, Y. Mukai, M. Watanabe: Talanta (1960), in press.

2) T. Momose, A. Inaba: This Bulletin, **7**, 541(1959).

suitable for deproteinization of blood from this standpoint. When the compound is heated with a blood solution in a boiling water bath, rapid coagulation of proteins occurs. The filtrate is clear and gives a negative test for proteins with sulfosalicylic acid.

Basic zinc carbonate may remove non-sugar reducing substances along with the proteins. Table II shows that the reagent gives minimum blood sugar values which

Table II. Blood Sugar Values[a] (mg./100 cc.) obtained by Different Kinds of Deproteinization Agent

Blood No. \ Deproteinization agent	Basic zinc carbonate	Zinc sulfate and barium hydroxide	Zinc sulfate and sodium hydroxide
1	101	102	109
2	135	136	145
3	163	164	167
4	169	173	181
5	350	350	362

a) These values were determined by the method of Reference (1).

almost agree with the values given by zinc sulfate and barium hydroxide, introduced by Somogyi[3] for the same purpose. Zinc sulfate and sodium hydroxide give somewhat larger values, but it is of interest to note that zinc hydroxide has only imperfect deproteinizing power when heated in blood solutions. In the last method of deproteinization shown in Table II, therefore, sulfate or carbonate, which is present in the solution, may take part in the reaction. A larger amount of cadmium hydroxide can also be used instead of basic zinc carbonate, but basic cadmium carbonate has only imperfect deproteinization action.

The coagulation of proteins in 0.1 cc. of blood is accomplished with about 50 mg. of basic zinc carbonate, but it is advisable to use 100~150 mg. of the compound to effect instant reaction. Table III shows that the amount of the compound used has no effect on the blood sugar values.

Table III. Blood Sugar Values[a] (mg./100 cc.) deproteinized by Different Amounts of Basic Zinc Carbonate

Blood No. \ Amount of basic zinc carbonate (mg.)	100	150	200
1	91	91	92
2	172	172	173
3	201	203	203

a) These values were determined by the method of Reference (1).

Basic zinc carbonate is insoluble in water and introduces no salt into the blood filtrate. Therefore, this new deproteinization agent may be used in other types of studies.

The color developing agent is stable enough for a long storage when stored in a light-resistant, tight container. It gives constant and reproducible results.

It is important to note that the filter papers usually contain a small amount of reducing sugars, which increase rapidly when the papers are exposed to acid vapor. Therefore, a new filter paper should be used in the test if possible. It is advisable to store the filter papers in a desiccator over sodium hydroxide to avoid hydrolysis.

Experimental

Estimation of Urine Sugar : Procedure—To 0.10 cc. of urine diluted with 5 cc. of water in a test tube, about 250 mg. of the color developing agent, mentioned above, is dissolved and the mixture is

3) M. Somogyi : J. Biol. Chem., **160**, 69(1945).

Table IV. Standard Colors for Urine Sugar Values

Urine sugar value (g./100 cc.)	0.125	0.25	0.5	1.0	2.0
Color	faint orange	orange	orange-red	wine-red	deep wine-red

heated in a boiling water bath for 2 min. The resulting color is compared within 3 min. with the standard colors (Table IV) prepared in the same way with 0.10 cc. of the standard solutions which contain 0.125, 0.25, 0.5, 1.0 or 2.0 g./100 cc. of glucose.

The reagent can also be used in the liquid state in this test. The reagent solution is prepared by dissolving 500 mg. of 3,6-dinitrophthalic acid and 25 g. of anhyd. Na_2CO_3 in 500 cc. of water and stored in a light-resistant, tight bottle. In this case, 0.10 cc. of urine is added to 5 cc. of the solution in a test tube and the mixture is treated as above.

Discussion

In this test, the sample is so diluted that the preliminary treatment of urine is usually unnecessary except blood urine. In other words, substances other than sugar which may be present in urine do not interfere in the color reaction. This is proved by the fact that 0.1 cc. of 5% albumin, 5% globulin, 1% creatine, 1% creatinine, 1% acetone, and 1% ethyl acetoacetate give only a faint yellow color, when heated with the color developing agent in 5 cc. of water.

A solution of the color developing agent in water is almost colorless but gradually turns a faint yellow. However, an old solution which was stored for 3 months at a room temperature gave the same results as a newly prepared reagent solution.

Works on the mechanism of the color reaction are in progress.

Summary

3,6-Dinitrophthalic acid gives a sensitive but unstable color reaction with reducing sugars when heated in sodium carbonate solution in the absence of sodium thiosulfate. This feature can be utilized in the approximate estimation of blood sugar and urine sugar with the naked eye. Basic zinc carbonate is successfully used in deproteinization of blood.

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