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THE DETERMINATION OF SUGAR, URIC ACID, UREA AND CREATININE IN ONE CUBIC CENTIMETER OF BLOOD.*

Byrd determines sugar in one drop of blood—this working with a small amount of blood prompted the writer to prepare this paper.

BY EDWARD S. ROSE.

This paper is written with the hope that it may be of interest to those pharmacies doing diagnostic work.

Much progress has been made in the chemical analysis of blood. Such workers as Folin, Benedict, Meyer, Wu, Van Slyke, Cullen, Marshall and others have given dependable methods for determining non-protein nitrogen, creatinine, creatin, urea, uric acid, sugar, chlorides, cholesterol, etc.

Of these many constituents in the blood probably the sugar content is the most important clinically to the average physician in his daily practice. Of the nitrogenous constituents probably the knowledge of the content of uric acid, urea and creatinine in the order named is of the greatest diagnostic value. Knowledge of the other constituents are of value to the physician at times.

Byrd (*J. Lab. Clin. Med.*, 11, 67–75 (1925)) has adapted the Folin-Wu method to the estimation of sugar in one drop of blood. This work suggested to the writer the possibility of determining the above-mentioned constituents in a small amount of blood as one cubic centimeter.

The materials used in the experimental work were solutions of varying proportions of dextrose, urea, uric acid and creatinine, and blood which for the most part was from the chicken.

The scheme of analysis follows that of Folin-Wu. One cubic centimeter of blood, measured in a pipette, is freed of protein in the prescribed manner. In order to obtain the maximum volume of filtrate the mixture is filtered with the aid of a filter pump. This filtrate is used in the following determinations:

Sugar is determined in one cubic centimeter of the filtrate by the Folin-Wu method using Folin-Wu sugar tubes. Prepare at least two tubes of standard sugar solution containing 0.1 and 0.2 mg. dextrose, using for comparison the one nearest the sample. Dilute all tubes to 25-cubic centimeters.

Uric Acid is determined in 2 cc. of the filtrate by the Folin-Wu method with slight modification. In order to match the colors better 1 cc. of the diluted uric acid standard solution is added to the sample and the proper correction made in the calculation. For the comparison at least two tubes containing 2 and 4 cc. of

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the diluted uric acid standard solution representing respectively 0.008 and 0.016 mg. uric acid are prepared, and the nearest in color used. All tubes are brought to the same volume before adding reagents and finally to 25 cc. when compared.

Urea is determined in 2 cc. of the filtrate by following in a general way the Folin-Wu method. This determination is more difficult to carry out since interfering substances may be carried over in the distillation such as dextrose, creatinine and ammonia which may be present in the reagents. All distilled water for preparing reagents, diluting purposes, etc., is freed of NH_3 by redistillation with sodium bisulphate. To prevent the carrying over of interfering substances a short-neck 200-cc. pyrex distilling flask is substituted for the large test-tube directed by Folin. The suggested aerated distillation of Butka and Meisner (*J. Lab. Clin. Med.*, 10, 937 (1925)) is followed, only attaching to the inlet a flask of diluted H_2SO_4 to collect ammonia in the air.

Briefly the procedure is as follows: Two cc. of filtrate is placed in the distilling flask and a drop of phenol red T. S. added and a drop or so of $N/50$ NaOH to give a red color, then two drops of buffer solution and 1 cc. of recently prepared Folin urease solution. Stopper and place the flask in a water-bath for 5 to 8 minutes at 50–55° C. Remove flask and add 3 cc. saturated borax solution and 2 cc. of distilled water, connect to distilling apparatus and run into a test-tube containing 2 cc. $N/1$ HCl. Distil down to about 1 cc. Disconnect test-tube, wash aerating tube with a little distilled water, cool and add 2 cc. Nessler's solution and distilled water to make 25 cc. Prepare a standard for comparison by diluting 1 cc. of standard ammonium sulphate solution (0.283 Gm. in 1000 cc. distilled water) with 40 cc. distilled water, 4 cc. Nessler's solution and distilled water to make 50 cc. One cc. of this standard solution gives a color the equivalent of that produced by 0.002572 Gm. urea. Run a blank and make the proper correction.

Creatinine is determined by the use of picric acid and sodium hydroxide but since the procedure varies somewhat from the Folin-Wu method it is given below. Since the reagents give a yellow color there will be an error in comparing the sample with a standard of different depth of color, unless a correction is made. The coloring power, the equivalent in terms of creatinine, can be found by comparing two tubes containing different amounts of diluted creatinine solution, or with one of the tubes a blank. This the writer has found to be approximately 0.00015 mg. to the cc., which of course may vary with the reagents.

The procedure is as follows: Place 2 cc. of filtrate in a test-tube and in three others place 1, 2, 3 cc. of diluted standard creatinine solution (containing 1 mg. in 250 cc.), representing respectively 0.004, 0.008, 0.012 mg. creatinine. Add distilled water to make the volume of the tubes the same, then add 1 cc. of saturated picric acid solution and 1 cc. of 5 p. c. NaOH. Mix and allow to stand 10 minutes, dilute to 25 cc. with distilled water and compare with the nearest standard within 5 minutes. If the color of the tubes compared is of approximately the same depth, no correction need be made for the color due to reagents.

Remarks.—The results obtained were favorable. The error amounted to from 1 to 6 per cent, using known materials, being greatest usually in the urea determination.

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