THE ESTIMATION OF SUGAR IN DIABETIC URINE, USING DINITROSALICYLIC ACID.

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Dinitrosalicylic acid was introduced by the author in 1921 as a reagent for the estimation of sugar in normal and diabetic urine.\(^1\) It has also been found to be a valuable reagent for the detection of albumin in urine and has been used for the determination of the titratable alkali of the blood.\(^2\)

During the past 3 years several improvements have been made in the method for the determination of sugar in diabetic urine. One of the chief errors in copper titration methods is the reoxidation of the cuprous oxide by atmospheric oxygen. With dinitrosalicylic acid, on the contrary, the amount of exposed surface has almost no effect, the same amount of reduction being given with tubes of all diameters. But with dinitrosalicylic acid the oxygen that is dissolved in the solution, while largely incapable of reoxidizing the reduction product, is able to destroy part of the sugar. It has been found that the addition to the reagent of a considerable amount of Rochelle salt largely prevents it from dissolving oxygen and greatly increases the amount of color given by small amounts of glucose. As used in the present modified manner, the amount of color given by 1 mg. of glucose is increased by about 65 per cent. As originally used, dinitrosalicylic acid gave practically no color with as little as 0.1 mg. of glucose, as nearly all of the glucose was oxidized by the dissolved oxygen. With the present modification the reaction is very dependable and the color values show such a good proportionality that the color given

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\(^1\) Sumner, J. B., *J. Biol. Chem.*, 1921, xlvii, 5.

\(^2\) Sumner, J. B., and Hubbard, R. S., *J. Biol. Chem.*, 1923, lxi, 701.
by 1 mg. of glucose can be compared without error with that given by either 0.5 or 2.0 mg. of glucose.

A second improvement in the method is the combining of alkali, dinitrosalicylic acid, and Rochelle salt into one reagent which keeps indefinitely. 3 cc. of this solution and 1 cc. of urine are heated in boiling water in a Folin-Wu sugar tube for 5 minutes. Any test-tube graduated at 25 cc. can be used, but the constricted tubes are useful because they keep the solution from drying around the edge and do not need to be stoppered with cotton.

The author has prepared a permanent standard composed of dinitrosalicylic acid and ferric ammonium alum. This matches perfectly the color given when dinitrosalicylic acid is reduced by heating with glucose and alkali. Normal urine contains substances that give a more dingy shade than pure glucose. This prevents the glucose standard or the iron alum standard from giving a perfect match with the test with normal urine but does not greatly affect the accuracy of the determination. When diabetic urine is being tested the necessary dilution makes possible a good color match.

It is possible to make and place in tubes a series of permanent standards corresponding to various concentrations of glucose. The tubes must be of Pyrex or Jena glass and must, of course, be kept well stoppered. The unknown can be matched against them when accuracy is not of great importance or when a colorimeter is not available.

The reagent is used both qualitatively and quantitatively. If the amount of color given by the suspected urine is slight it can be considered normal and the test may be discarded. If the color is deep the colorimetric comparison can be made. If the amount of sugar present is more than 0.2 per cent the test should be repeated with diluted urine.

The reducing power of the more common sugars upon dinitrosalicylic acid has been investigated. Fructose and galactose have the same reducing power as glucose; 90 mg. of arabinose or xylose are equivalent to 100 mg. of glucose; and 124 mg. of either anhydrous lactose or maltose are equivalent to 100 mg. of glucose.

A few words are necessary concerning the interpretation of results with urine. Normal urine usually shows a reducing power
of from 0.05 to 0.15 per cent in terms of glucose, depending upon its concentration. Approximately 60 per cent of this reduction is due to sugar and the rest is due to other reducing substances. Urine showing a reducing value as high as 0.25 per cent can be regarded as suspicious and urine showing a value of 0.30 per cent can be said to be pathological.

Table I shows how results by the new method with normal urine compare with figures given by the acetone-picric acid method of Benedict and Osterberg.

<table>
<thead>
<tr>
<th>Urine No.</th>
<th>Method of Benedict and Osterberg</th>
<th>New method.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With charcoal.</td>
<td>Without charcoal.</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>1</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>2</td>
<td>0.041</td>
<td>0.063</td>
</tr>
<tr>
<td>3</td>
<td>0.11</td>
<td>0.18</td>
</tr>
<tr>
<td>4</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>0.068</td>
<td>0.14</td>
</tr>
<tr>
<td>6</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>7</td>
<td>0.042</td>
<td>0.083</td>
</tr>
<tr>
<td>8</td>
<td>0.053</td>
<td>0.11</td>
</tr>
</tbody>
</table>

**Preparation of Reagents.**

**Dinitrosalicylate Solution.**—To 300 cc. of 4.5 per cent carbonate-free sodium hydroxide add 880 cc. of 1 per cent dinitrosalicylic acid and 255 gm. of Rochelle salt (KNaC₄H₄O₆·4H₂O), mix until dissolved, and keep tightly stoppered. Dinitrosalicylic acid can be prepared as has been described in a previous paper or can be obtained from Eimer and Amend or from the Eastman Kodak Co.

**Permanent Standard.**—Dissolve 345 mg. of clear crystals of ferric ammonium alum and 1 gm. of dinitrosalicylic acid in water and make up to 1 liter volume. Keep well stoppered in Pyrex or Jena glass. This solution will be equivalent in color to 1 mg. of glucose treated as described below. It should be standardized against glucose before use.

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**Permanent Standards for Approximate Comparison.—** Prepare a solution containing 0.8625 per cent of iron alum. To ten 100 cc. volumetric flasks add 10 cc. of 1 per cent dinitrosalicylic acid and then 1.75, 2.53, 3.22, 4.00, 4.68, 5.32, 6.1, 6.8, 7.5, and 10.3 cc. of the iron alum solution. Dilute to 100 cc. volume and mix. Place 25 cc. of each solution in a Folin-Wu sugar tube of Pyrex glass and stopper with paraffined cork stoppers. These solutions correspond to 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, and 3.0 mg. of glucose in the order given above.

**Method.**

Pipette into a Folin-Wu sugar tube 1 cc. of urine (diluted if necessary) and 3 cc. of the reagent. Mix and heat 5 minutes in boiling water. Cool 3 minutes in running water, dilute to 25 cc. volume, mix, and compare in colorimeter with permanent standard set at 20 mm., or with standard prepared with 1 mg. of glucose.
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