A CHEMICAL METHOD OF ESTIMATION OF NICOTINIC ACID IN URINE IN THE PRESENCE OF SUGAR

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It has been reported that intravenous injection of nicotinic acid produces hypoglycemia in normal humans (1-4). A definite improvement in the carbohydrate tolerance of diabetic patients after administration of nicotinic acid has also been reported (4). It was therefore of interest to study the urinary excretion of nicotinic acid in diabetic patients.

In the chemical estimation of nicotinic acid in urine by König's reaction (5) the urine is first digested either with acid (6) or with alkali (7) in a water bath to convert derivatives of nicotinic acid excreted in the urine to nicotinic acid. The color of the urine after digestion interferes with the estimation of nicotinic acid and different workers tried to remove the color by different methods. While Melnick and Field (6) and Swaminathan (7) used charcoal to remove the interfering pigments, Perlzweig, Levy, and Sarett (8) and Dann and Handler (9) employed Lloyd's reagent. Wang and Kodicek (10), on the other hand, treated the digested urine first with isobutanol, which removed some of the colors, and the remaining pigments were subsequently oxidized with potassium permanganate. Recently Swaminathan used zinc hydroxide to remove the color (11). The urine of diabetic patients, however, after digestion with acid or alkali gave a highly colored extract. The interfering color could not be removed by any of the methods so far used (6-11). It seemed that most of the color produced after digestion was due to charring of the sugar present in the urine. In the present paper a method for the estimation of nicotinic acid in the urine containing sugar has been described.

EXPERIMENTAL

Removal of Sugar in Urine by Treatment with Permanganate—Fresh solutions of glucose of different strengths were acidified with concentrated hydrochloric acid and treated in a water bath with a 10 per cent solution of potassium permanganate until the solution gave no test for sugar when boiled with Benedict's reagent. It was observed that approximately 16 cc. of a 10 per cent solution of potassium permanganate were required to oxidize 1 gm. of glucose. Urine containing glucose also required the same
amount of permanganate solution as the pure solution of glucose of similar strength. When the sugar in the urine was oxidized with permanganate in an alkaline medium, colloidal manganese oxide was formed which was difficult to remove by centrifugation, and when the urine containing the manganese oxide was digested in the water bath, the manganese oxide was found to destroy the nicotinic acid, and added nicotinic acid could not be recovered. Sugar in the urine was therefore oxidized by permanganate in an acid medium.

Removal of Manganese in Urine after Treatment with Permanganate—When the permanganate-treated urine was digested in an alkaline medium, the manganese hydroxide formed did not destroy the nicotinic acid, but it was found difficult to remove and wash the bulky precipitate of manganese hydroxide either before or after the digestion. It was therefore thought desirable to remove the extra manganese in the urine before digestion. The manganese present in the urine was removed as phosphate by treatment with disodium hydrogen phosphate and NaOH. Crystals of disodium hydrogen phosphate were added to the urine treated with permanganate and heated in a water bath until the crystals went into solution. 0.35 gm. of disodium hydrogen phosphate was necessary for 1 cc. of 10 per cent permanganate. The hot solution was treated with 40 per cent sodium hydroxide drop by drop until the precipitation of manganese phosphate was complete. The precipitate was removed by filtering through a Büchner funnel under suction and washed twice with 10 cc. portions of distilled water. The filtrate was then ready for digestion.

Digestion of Sugar- and Manganese-Free Urine Filtrate and Removal of Interfering Color—In order to convert nicotinamide, nicotinuric acid, and N1-methylnicotinamide into nicotinic acid the sugar- and manganese-free urine filtrate was put into a 250 cc. beaker. The solution was made alkaline with 40 per cent sodium hydroxide so that the strength of the alkali was 4 per cent and was digested in a boiling water bath for 45 minutes. After digestion the solution was neutralized with concentrated hydrochloric acid and an extra 3 cc. of acid were then added. The acidified solution was heated in the boiling water bath and treated with 10 per cent permanganate drop by drop until the solution became light yellow in color. The solution was then adjusted to pH 7 with bromothymol blue as an indicator and was made up to a definite volume. Phosphate buffer of pH 7 was then added to the solution in the proportion of 6 cc. of buffer per 10 cc. of the solution. A white precipitate appeared and the solution became almost colorless. The precipitate was filtered off and an aliquot of the filtrate was used for the colorimetric estimation of nicotinic acid.

Colorimetric Estimation of Nicotinic Acid—Nicotinic acid in the filtrate was then estimated according to the method of Swaminathan (7), slightly
modified. 16 cc. of the filtrate were put into a 25 cc. stoppered graduated cylinder and into a similar cylinder was put a dilute solution of nicotinic acid, 1 cc. containing 10 $\gamma$ of nicotinic acid. 6 cc. of phosphate buffer of pH 7 were added and the volume was made up to 16 cc. To each of the cylinders was added 1 cc. of a 4 per cent alcoholic solution of aniline, followed by 8 cc. of a solution of cyanogen bromide prepared freshly by decolorizing saturated bromine solution with 10 per cent sodium cyanide. The contents of the cylinders were shaken, allowed to stand for 2 minutes, and the yellow color which developed was then compared in a visual colorimeter. Sometimes the color of the urine filtrate was so light that a blank correction was not necessary. In case there was any residual color, the urine filtrate was treated exactly as described above, without the addition of cyanogen bromide, and compared with a standard nicotinic acid solution treated with cyanogen bromide.

**Table I**

Recovery of Nicotinic Acid Added to 25 Cc. of Urine Containing 5 Per Cent Glucose

<table>
<thead>
<tr>
<th>Urine sample No.</th>
<th>Nicotinic acid added</th>
<th>Nicotinic acid estimated</th>
<th>Nicotinic acid recovered per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>0</td>
<td>25.8</td>
<td>99.0</td>
</tr>
<tr>
<td>1b</td>
<td>30</td>
<td>55.5</td>
<td>95.5</td>
</tr>
<tr>
<td>1c</td>
<td>40</td>
<td>64.0</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>0</td>
<td>50.0</td>
<td>90.0</td>
</tr>
<tr>
<td>2b</td>
<td>15</td>
<td>63.5</td>
<td>92.0</td>
</tr>
<tr>
<td>2c</td>
<td>25</td>
<td>73.0</td>
<td>93.0</td>
</tr>
<tr>
<td>2d</td>
<td>10</td>
<td>59.3</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>0</td>
<td>50.0</td>
<td>98.0</td>
</tr>
<tr>
<td>3b</td>
<td>20</td>
<td>69.6</td>
<td></td>
</tr>
<tr>
<td>3c</td>
<td>40</td>
<td>89.0</td>
<td>97.5</td>
</tr>
</tbody>
</table>

Estimation of Nicotinic Acid in Urine in Presence of Sugar—Sugar in the urine is quantitatively estimated with Benedict's reagent. To 25 cc. of urine in a 250 cc. beaker are added 8 cc. of concentrated hydrochloric acid. The beaker is heated in a water bath and is treated with 10 per cent potassium permanganate until all the sugar is oxidized. 1 gm. of sugar requires approximately 16 cc. of a 10 per cent solution of potassium permanganate. The manganese present in the solution is removed as phosphate by treatment with disodium hydrogen phosphate and NaOH. The solution is then digested in a water bath for 45 minutes with 40 per cent NaOH so that the strength of the alkali is 4 per cent. The interfering color is removed by making the solution acidic with concentrated hydrochloric acid and treating with permanganate solution until the solution becomes almost colorless.
If the volume of the resulting solution is large, it is concentrated in a boiling water bath. The solution is adjusted to pH 7, phosphate buffer is added (6 cc. of buffer per 10 cc. of the solution), and the mixture filtered. An aliquot of the filtrate, depending on the amount of nicotinic acid present, is taken for the colorimetric estimation of nicotinic acid.

Recovery of Nicotinic Acid Added in Urine Samples—Nicotinic acid in different amounts was added to 25 cc. samples of urine containing 5 per cent glucose. Estimation of nicotinic acid in the samples of urine was carried out to learn how far this procedure could recover the added nicotinic acid in the urine. 90 to 99 per cent of added nicotinic acid could be recovered by the above method. The results are shown in Table I.

24 Hours Urinary Excretion of Nicotinic Acid by Diabetic Patients—24 hour urines of eight diabetic patients were collected in bottles containing 20 cc. of a 50 per cent solution of sulfuric acid. Nicotinic acid in these samples was estimated according to the method described above. Diabetic patients excreted 1.3 to 5.6 mg. of nicotinic acid per day. The results are given in Table II.

**DISCUSSION**

By preliminary treatment of urine samples containing sugar with permanganate, not only the sugar but also some of the interfering colors were removed. When urine of normal persons or rabbits was treated with 0.5 cc. of 10 per cent potassium permanganate in an acid medium and digested with alkali after removal of the manganese as phosphate, a lightly colored digest was obtained. This digest when treated with permanganate in acid medium became almost colorless. The method of estimation of nicotinic acid described in the present paper seems to be simplest of the existing chemical methods.

The daily urinary excretion of nicotinic acid by diabetic patients was found to vary between 1.3 and 5.6 mg. It was shown by one of us (N. C. G.) that normal healthy individuals excreted 1.4 to 5.3 mg. of nicotinic acid per day. The results are presented in Table II.
acid in their daily output of urine (12). Wang and Kodicek (10) reported that normal healthy British subjects excreted 0.85 to 2.33 mg. of nicotinic acid per day. Nicotinic acid nutrition of diabetic patients, therefore, does not seem to be interfered with.

**SUMMARY**

1. A chemical method of estimation of nicotinic acid in urine in the presence of sugar has been described.

2. The urine is first treated with permanganate, which removes sugar and some of the interfering colors. The manganese is removed as phosphate and the sugar-free urine is digested with alkali. The digested urine is decolorized with permanganate and the pH of the solution is brought to 7. After addition of phosphate buffer an aliquot of the solution is treated with alcoholic aniline and a solution of cyanogen bromide. The resultant yellow color is compared with a standard nicotinic acid solution similarly treated.

3. The urinary excretion of nicotinic acid by eight diabetic patients has been studied. The diabetic patients have been found to excrete 1.3 to 5.6 mg. of nicotinic acid per day. These figures compare well with the urinary excretion of nicotinic acid by healthy individuals.

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