Elimination of Glucose Interference and Improved Precision in a Continuous-Flow Analysis for Inulin

John A. Torelli, Bernice Middleton, and Richard M. Stein

A modified fermentation procedure based on one described by Homer Smith in his textbook was used to eliminate glucose interference in the automated determination of inulin. The manifold of Fjeldbo and Stamey [J. Lab. Clin. Med. 72, 353 (1968)] was modified to enable us to insert the fermentation procedure without sacrificing precision in continuous-flow analysis for inulin. Analytical recoveries of inulin from plasma and urine with the modified manifold were 100.1 ± 0.8% (SD) and 99.9 ± 1.6%. Corresponding recoveries of inulin from specimens of plasma and urine containing 5 g of glucose per liter and treated with yeast were 99.6 ± 1.5% and 100.0 ± 1.6%.

In virtually all animals, including man, the renal clearance of inulin remains the most widely used index of glomerular filtration rate. Unfortunately, the chemical analysis for inulin is cumbersome and suffers some interference from blood glucose, which increases when hyperglycemia is induced.

Acid hydrolysis of inulin with concentrated HCl followed by reaction with alcoholic resorcinol yields an orange-red product (the Selwanoff reaction) (1). This reaction is the basis for the manual determination of inulin developed by Higashi and Peters (2), and later the automated procedure developed by Fjeldbo and Stamey (3).

The latter procedure was found to yield only ½ as much color with glucose as with inulin. When glucose is infused, however, its concentration in blood and urine is high and variable, and it produces a significant error that cannot be corrected even when a blank value for a series of samples is determined. Therefore we developed a fermentation procedure based on one described by Smith (4). The manifold of Fjeldbo and Stamey has also been modified to enable us to use their procedure and improve overall precision. With the modified fermentation procedure and manifold, our laboratory has obtained excellent recoveries of inulin from either plasma or urine, even in the presence of large amounts of glucose.

Methods and Materials

Reagents

All chemicals meet A.C.S. specifications unless stated.


The Renal Physiology Laboratory, General Medical Research, and the Renal Disease Section, Department of Medicine, The Veterans Administration Hospital, Bronx, N. Y. 10468; and The Mount Sinai School of Medicine, New York, N. Y.

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Ferric chloride working solution: To 7.5 mg of ferric chloride per liter of concentrated hydrochloric acid, add 2 ml of Brij-35 (Technicon, Tarrytown, N.Y. 10591).

Zinc sulfate heptahydrate: 12.5 g/liter in 15 mmol/liter H₂SO₄.

Yeast suspension: Add 20 g of fresh baker's yeast containing starch and supplied as a moist, solid cake (Universal Food Corp., Wisc. 53201) to 80 ml of water, shake, and centrifuge. Discard the cloudy supernate and repeat the operation until the supernatant liquid is clear. Resuspend the yeast. Wash it again on the day it is to be used. This reagent is stable for one week under refrigeration.

Stock inulin standard, 1 g/liter: Suspend 100 mg of dried, purified inulin (Calbiochem, LaJolla, Calif. 92037) in 90 ml of distilled, de-ionized water and heat in a boiling water bath until dissolved. Dilute to 100 ml with water.

Working standards: 7.5, 15, 30, 45, 60, 75, and 90 mg/liter.

Procedure

Elimination of glucose: For a specimen received when glucose has been infused, mix 2 ml of yeast suspension with 2 ml of plasma or diluted urine in a polystyrene test tube, shake after 15 min, and centrifuge after an additional 15 min.

Solids determinations and the need to treat all standards with yeast are obviated by treating only the 60 mg/liter standard with yeast, analyzing on the AutoAnalyzer together with the specimen, and calculating its value in comparison to the untreated working standard.

Fermentation correction factor = 60 mg/liter/divided by the analyzed values of fermented 60 mg/liter standard

All inulin values of all yeast-treated specimens must be multiplied by this factor in addition to other dilution factors.

Blood filtrates: Mix 8 ml of ZnSO₄ solution with 1 ml of clear supernatant liquid from the treated plasma (when glucose is infused) or 1 ml of plasma (when glucose is not infused) and shake. Add 1 ml of 0.75 mol/liter NaOH and shake. Allow these specimens to stand 15–30 min and centrifuge.

Urine specimens: Dilute urine specimens with water to give final inulin concentrations of 7.5 to 90 mg/liter.

Flow Diagram

As shown in Figure 1, we made several changes in Fjeldbo and Stamey's original manifold. Sample flow was increased while resorcinol flow was decreased, to accommodate the 10-fold rather than 15-fold dilution for blood filtrates. The following changes were made to improve precision:

Acidflex tubing previously used to pump resorcinol-ethanol
reagent was replaced with solvaflex tubing, which, because of its smooth walls, promotes a more even reagent flow.

A pulse suppressor was added to the HCl–FeCl₃ line to eliminate pulsation of the reagent stream. If a standard vinyl pulse suppressor is used, it must be changed every day or the acid line will burst.

A durable polyethylene pulse suppressor may be constructed (Technicon parts) by slipping 13 mm length of .020-inch (i.d.) vinyl tubing over both ends of a 5-cm length of .015-inch (i.d.) polyethylene tubing and then slipping N5 adapters into the vinyl sleeves. This pulse suppressor is connected to the HCl–FeCl₃ pump tube with a 15-cm length of acidflex transmission tubing to absorb shock (Figure 2).

The longer sections of acidflex transmission tubing were substituted with glass tubing to prevent disruption of the bubble pattern.

Thirty specimens per hour are sampled. Absorption at 480 nm is measured utilizing a 15-mm cuvette. Manifold tubing is always stretched tightly and conditioned by pumping reagent until a stable baseline is achieved.

Results and Discussion

Recoveries with fermentation omitted. Duplicate plasma samples containing 300 and 600 mg of inulin per liter were prepared by adding a 3 g/liter stock standard to plasma. Duplicate plasma sample containing 450 mg/liter inulin were prepared by adding a 1.5 g/liter stock standard to plasma. Blanks were prepared by adding an equivalent amount of water to plasma. A different batch of dog plasma was analyzed on three different days according to this procedure, yielding a total of six specimens at each concentration or a total of 18 analyzed specimens. Urinary recoveries were also assessed in duplicate for three different batches of urine to which solid inulin was added, on three different days, totaling 18 specimens analyzed. Only one blank per run was necessary for this series. Analytical recoveries from plasma and urine were excellent: 100.1 ± 0.8% (SD) and 99.9 ± 1.6%, respectively (Table 1).

Table 1. Analytical Recovery of Inulin from Plasma and Urine

<table>
<thead>
<tr>
<th>Added inulin g/liter</th>
<th>Glucose</th>
<th>Av recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.30</td>
<td>0</td>
<td>100.6 ± 0.7</td>
</tr>
<tr>
<td>0.45</td>
<td>0</td>
<td>99.5 ± 0.5</td>
</tr>
<tr>
<td>0.60</td>
<td>0</td>
<td>100.0 ± 0.7</td>
</tr>
<tr>
<td>0.30</td>
<td>5</td>
<td>99.5 ± 1.7</td>
</tr>
<tr>
<td>0.45</td>
<td>5</td>
<td>99.0 ± 1.1</td>
</tr>
<tr>
<td>0.60</td>
<td>5</td>
<td>100.3 ± 1.6</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.50</td>
<td>0</td>
<td>101.1 ± 1.1</td>
</tr>
<tr>
<td>2.25</td>
<td>0</td>
<td>99.1 ± 1.2</td>
</tr>
<tr>
<td>3.00</td>
<td>0</td>
<td>100.4 ± 2.2</td>
</tr>
<tr>
<td>4.50</td>
<td>5</td>
<td>100.0 ± 1.3</td>
</tr>
<tr>
<td>6.00</td>
<td>5</td>
<td>99.8 ± 2.2</td>
</tr>
</tbody>
</table>

* Per cent recovery represents (± SD) for a total of six determinations (duplicate determinations of three different specimens analyzed on three separate days).

Although the Seliwanoff reaction is considered specific for fructose, there is interference from glucose. We have found that the apparent inulin concentration of glucose follows the equation: y = −10.1 + 195x and r² = 0.998.

Recoveries after treating with yeast. By treating just one working standard with yeast suspension we can accurately determine the dilution factor of all treated specimens, thus eliminating the need to determine solids content of the yeast suspension by centrifugation as does Smith.

When plasma and urine specimens containing 5 g/liter glucose were treated with yeast suspension as described, recoveries were excellent: 99.6 ± 1.5% (SD) for plasma and 100.0 ± 1.6% for urine.

References