say. Ingestion of 2 g of cyclamate would yield such values, assuming a conversion rate of ~8%.

In conclusion, we have found that cyclohexylamine, present in urine after extensive uptake of cyclamate, shows cross-reactivity in the TDx amphetamine assays.

Reference

Walter Martz
Hans Werner Schütz
Institut für Rechtsmedizin
Klinikum der Christian-Albrechts-
Universität
Arnold-Heller-Str. 12
2300 Kiel 1, F.R.G.

Elimination of Glucose Interference in Enzymatic Determination of Inulin

To the Editor:

Inulin is a natural fructose polymer, widespread in gut flora. Determination of inulin clearance is considered the method of choice when precise determination of the glomerular filtration rate is required (1). Classically, inulin determination has been performed after acid hydrolysis by complexing the liberated fructose with resorcinol or diphenylamine in concentrated sulfuric acid (2). However, the latter assays are time consuming, not suited for automated analysis, and potentially subject to interference from other carbohydrates such as glucose (2). Moreover, acid hydrolysis of inulin may result in the formation of other compounds, e.g., difructoseianhydride (3). The technical difficulty of inulin analysis is considered one of the main disadvantages of the method (4).

Enzymatic analysis of inulin has been performed (5, 6); a blank reaction, however, is still needed because of glucose interference. Even at physiological concentrations, fasting serum glucose (3.9–6.1 mmol/L) causes a considerable background signal, which limits the reproducibility and linearity of the assay. We therefore developed a two-step enzymatic assay in which, initially, inulin was enzymatically hydrolyzed by inulinase (EC 3.2.1.7; 2,1-α-D-fructan fructohydrolase), and concomitantly glucose was converted into gluconate by glucose oxidase (7). The use of the latter enzyme eliminated the need for a blank reaction.

Inulinase (Novozym 230) was purchased from Novo Industries, Copenhagen, Denmark. Inulin (from dahlia tubers), glucose oxidase (EC 1.1.3.4; from Aspergillus niger), and phosphoglucoisomerase (EC 5.3.1.9; D-glucose 6-phosphate ketol-isomerase; from rabbit muscle) were purchased from Sigma Chemical Co. (St. Louis, MO 63178). The other reagents were from a commercial kit for glucose determination (Gluco-quant glucose; Boehringer Mannheim, Mannheim, F.R.G.).

After a 1-h incubation at 37°C, the liberated D-fructose is then analyzed further by a spectrophotometric method (8). D-Fructose is further phosphorylated to D-fructose 6-phosphate by ATP in the presence of hexokinase (EC 2.7.1.1; ATP:D-hexose-6-phosphate transferase). D-Fructose 6-phosphate is converted to D-glucose 6-phosphate in the presence of phosphoglucomutase. D-Glucose 6-phosphate, in the subsequent reaction, is oxidized by NADPH*, which itself is reduced to NADPH (Figure 1).

We preincubated 500 μL of serum or 10-fold-diluted urine with 0.1 mL of citrate buffer (66 mmol/L, pH 4.5) containing 100 U/L inulinase and 10 kU/L glucose oxidase. After 2 h of preincubation at 37°C, we transferred samples to a Hitachi 717 analyzer (Hitachi, Tokyo, Japan). Regular glucose reagent (Glucose-quant glucose containing 700 kU/L phosphoglucoisomerase) was used for determining D-fructose.

Final photometric readings at 340 nm were made after an incubation of 8 min at 37°C.

The method gives results that are linearly related to D-fructose concentrations up to 16 mmol/L. Inulin concentrations as low as 0.1 mmol/L could be detected. For inulin at 5 mmol/L, within- and between-run CVs of the method are, respectively, 0.9% and 2.7%. At serum glucose concentrations up to 20 mmol/L, glucose is completely removed enzymatically. Other hexoses do not interfere with the test (7).

Because inulinase and glucose oxidase remain active in a broad range of pH (pH 4–7), no pH adjustments are required for urine samples during the preincubation phase. The enzymatic elimination of glucose involves a broader range of linearity than that of the earlier enzymatic assay (6) or the methods based on nonenzymatic hydrolysis followed by complexation (9).

p-Aminohippuric acid, which is often assayed concurrently with inulin, does not interfere with the test. Reagents are stable for one month when stored at 4°C, and the method may be adapted to any laboratory analyzer.

Table 1. Cross-Reactivity of Cyclamate and Cyclohexylamine in TDx Amphetamine Assay

<table>
<thead>
<tr>
<th>Compound added</th>
<th>Conc added, g/L</th>
<th>Amphetamine/methamphetamine (or amphetamine class)</th>
<th>Conc measured, mg/L</th>
<th>Cross-reactivity, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclamate</td>
<td>&lt;4</td>
<td>n.d. (0.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclohexylamine</td>
<td>1</td>
<td>1.42 (2.03)</td>
<td>0.14 (0.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.22 (0.62)</td>
<td>0.22 (0.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>n.d. (0.18)</td>
<td>(1.8)</td>
<td></td>
</tr>
</tbody>
</table>

*Cross-reactivity (%) = 100 × (measured/added). n.d. = not detectable.
narche. Boys of comparable age served as controls. We report here the surprising finding of increased CA-125 serum concentrations in some of these children. Sera from 250 boys and girls between ages nine days and 16 years were analyzed for CA-125 with a sandwich-type solid-phase assay (CA-125 ELSA; ID-CIS, Dreieich, F.R.G.) based on the use of murine monoclonal antibody OC-125 as capture antibody bound to the solid phase and 125I-labeled OC-125 as tracer. After incubation and washings, the bound radioactivity was measured with a gamma-counter and actual concentrations were calculated from the standard curve and documented as arbitrary units/mL (1). The measuring range extended from 12.5 to 500 arb. units/mL. Based on measurements in healthy women during different phases of the menstrual cycle, the upper limit of normal CA-125 serum concentrations was determined to be 65 arb. units/mL (2).

The children were being treated in the children’s hospital for the following disorders: various heart diseases or congenital heart abnormalities (70), infectious diseases (22), gastrointestinal diseases (9), pulmonary diseases (11), liver diseases (3), neurological disorders (28), kidney diseases (18), malignancies (8), skin diseases (3), throat diseases (15), accidents (5), premature births (6), metabolic disorders (5), and miscellaneous (47). Of the 250 children whose serum was available for analysis, 23 exhibited increased CA-125 serum concentrations (>65 arb. units/mL). The greatest percentage of increased CA-125 concentrations was observed in the group of 70 children (35 boys, 35 girls) with various heart diseases or congenital heart abnormalities: 16 children with severe heart failure (10 boys, six girls) had CA-125 concentrations between 65 and 459 arb. units/mL (mean 151, SD 106 arb. units/mL). Three of these 16 children also had above-normal values for liver enzymes. The remaining 54 of these children, who had stable cardiac function without decompensation, had CA-125 concentrations in the normal range (mean 14, SD 6.4 arb. units/mL). Among the 180 children with various other diseases, seven exhibited CA-125 concentrations between 73 and 276 arb. units/mL. Those children had liver cirrhosis, neonatal asphyxia, esophageal atresia, portal vein thrombosis with esophageal bleeding, and hydrocephaly with a peritoneal shunt. The remaining 173 boys and girls had CA-125 concentrations between 5 and 62 arb. units/mL (mean 18, SD 9.8 arb. units/mL), i.e., values within the normal range. We did not detect an age-dependent distribution (Figure 1).

Our pilot study demonstrates that CA-125 can be detected in the peripheral serum of girls before the onset of regular menstrual cycles. In most of the children, the serum concentrations of this glycoprotein did not surpass the range considered as normal in adult women.

We were, however, surprised to detect remarkably increased CA-125 serum concentrations in some of the boys and girls, because such increases had been reported only in adults predominantly having malignant diseases or endometriosis and ovarian hyperstimulation syndrome. We do not believe that these increases of CA-125 were iatrogenic effects. All infusions given to these children were measured for CA-125 content, which was always negative. In those children who had been connected to the heart–lung machine, sera were usually obtained when the children had already been disconnected from this equipment for several days. We cannot totally exclude the possibility that the CA-125 in these children was derived from the fresh-frozen plasma that had sometimes been infused; however, during a parallel study on tumor marker concentrations in male blood donors, nearly all of the donors’ blood (from which the fresh-frozen plasma was prepared) during that period had normal concentrations of CA-125.

Fig. 1. CA-125 serum concentrations (arbitrary units per milliliter, ordinate) in 130 boys (top) and 120 girls (bottom) of various ages...