Kinetic Ethylene Glycol Assay with Use of Yeast Alcohol Dehydrogenase

John H. Eckfeldt and R. Todd Light

We describe a rapid, accurate, and precise two-point kinetic assay for ethylene glycol. The method involves use of a standard kit for ethanol determination with yeast alcohol dehydrogenase, and of a centrifugal analyzer. Alcohol dehydrogenase catalyzes the oxidation of ethylene glycol in a trichloroacetic acid-precipitated specimen; the resulting NADH production is monitored at 340 nm. The reaction rate is linear with concentration to 1.5 g of ethylene glycol per liter. Interference from methanol, ethanol, and isopropanol was easily recognized after a 30-min incubation at 100 °C. We believe that the method can be readily adaptable to any narrow-bandwidth, stable, temperature-controlled spectrophotometer and so should provide more widely for the prompt assessment of patients in whom ethylene glycol poisoning is suspected.

Additional Keyphrases: Centrifugal analysis • toxicology • kinetic enzyme assay • methods for the small laboratory

Ethylene glycol remains a serious cause of both intentional and accidental poisoning in the United States. In 1959, Haggerty estimated that it was the cause of 40 to 60 deaths annually (1). The chemical is a common constituent of a wide range of products, including paints, pharmaceuticals, cosmetics, and de-icing solutions. Its high boiling point, high solubility in water, and low relative molecular mass have made it the primary component of commercial antifreeze (2), and storage of antifreeze in old liquor bottles (e.g.) has resulted in many accidental ingestions (3, 4). Unfortunately, it is also colorless, and its “pleasant, warm, sweet taste” makes it a pediatric risk as well (5). Ethylene glycol intoxication must be promptly recognized if poisoned patients are to be appropriately managed, because it is rapidly metabolized to various aldehydes and acids, which are thought to be primarily responsible for its toxicity. Therapy, apart from the usualsupportive measures and cofactor replacement, consists of giving oral or intravenous ethanol to inhibit oxidation to toxic by-products, and using hemodialysis to remove them (2). Unfortunately, screening for volatile toxins usually does not include ethylene glycol (4, 6); and even when this diagnosis is suspected on clinical grounds, physicians are understandably reluctant to initiate dialysis and therapy with ethanol without confirmatory data on its concentration in blood. Such data are available only at distant centers at a considerable cost in time.

Various colorimetric (7, 8) and gas-chromatographic (9) procedures have been previously described for the assay of ethylene glycol. We use enzymic oxidation and have adapted a standard ethanol kit so that the only instrument required is a stable, narrow-bandwidth spectrophotometer with a temperature-controlled cuvette. In the absence of other alcohols the assay can rule out the presence of ethylene glycol within 30 min or confirm it in 1 h. The simplicity of the method should significantly increase the number of hospitals that can do such an assay.

Materials and Methods

Specimens

Serum or plasma anticoagulated with sodium fluoride and potassium oxalate was used. We compared 15 specimens drawn after rubbing the site with isopropanol vs the same number drawn after use of an aqueous surgical scrubbing solution and saw no significant difference; nevertheless, we suggest avoiding skin site-preparation with an alcohol. Serum stored for two weeks at room temperature or 4 °C and for two months at −20 °C showed no change in measured ethylene glycol concentrations.

Apparatus

We used a Multistat III Microcentrifugal Analyzer (Instrumentation Laboratories, Lexington, MA 02173), the principle, design, and operation of which are described elsewhere (10). A gas chromatograph (Model 419; Packard Instrument Co., Downers Grove, IL 60515) equipped with a flame-ionization detector was used for method-comparison experiments.

Reagents

All chemicals were AR grade. The alcohol dehydrogenase-NAD+ reagent was prepared from a kit for ethanol determination (no. 331-CMA; Sigma Chemical Co., St. Louis, MO 63178). The manufacturer’s directions for reconstitution were modified as follows: To an “NAD-ADH Multitest Vial” (Sigma no. 331-10), add 8.3 mL of “Pyrophosphate Buffer” (Sigma no. 330-30). The resulting composition of the reagent is (per liter): 2.0 × 10^6 U of alcohol dehydrogenase (EC 1.1.1.1), 2.4 mmol of NAD+, 75 mmol of sodium pyrophosphate (pH 9.2), 75 mmol of semicarbazide, 22 mmol of glycine, and 12 mmol of sodium phosphate. The reagent is stable for at least 8 h at 4 °C.

Standards

Just before an assay, we prepared a serum-based working standard by combining 30 μL of 33 g/L ethylene glycol aqueous stock standard with 0.97 mL of pooled human serum or commercial, lyophilized control serum, free from ethylene glycol or alcohol contamination. The stock standard itself was stable for at least one month at 4 °C.

Controls

To prepare high and low controls, we added appropriate amounts of ethylene glycol standard to pooled human serum; the controls were aliquoted and stored at −20 °C.

Procedure

Add to the specimens, controls, working standard, and serum blank an equal volume of 100 g/L trichloroacetic acid. Vortex-mix and centrifuge these mixtures at 900 × g for 5 min. Place the protein-free supernates into the Multistat III loader cups, using the loader settings shown in Table 1. Analyzer settings are as follows:

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1. Direct reprint requests to Dr. Eckfeldt, Laboratory Service (113), VA Medical Center, Minneapolis, MN 55417.
2. Received Mar. 5, 1980; accepted May 6, 1980.
Prepare a calibration curve from the change in absorbance (ΔA) of the blank and standard or calculate the unknown concentration (C) as follows:

\[ C_{\text{unk}} = C_{\text{std}} \times \frac{(\Delta A_{\text{unk}} - \Delta A_{\text{blank}})}{(\Delta A_{\text{std}} - \Delta A_{\text{blank}})} \]

**Method Comparison**

For comparison, we used the gas-chromatographic method of Jain and Forney (9) but directly injected 4 μL of an equal volume mixture of serum and aqueous propylene glycol (2 g/L) instead of a perchloric acid precipitate of serum. Their procedure involves use of propylene glycol as an internal standard, a Poropak Q column held at 240 °C, and injector and detector temperatures of 280 °C.

Few clinical samples were available to us for evaluation. To assess possible interference by metabolites of ethylene glycol, we gave 1.2–1.8 g of ethylene glycol per kilogram of body weight orally to three New Zealand White rabbits and drew blood samples between 10 min and 18 h later. We assayed each sample by our enzymatic method and by the gas-chromatographic comparison method.

**Results**

Table 2 shows the within-rotor and day-to-day precision at high and low ethylene glycol concentrations. Within-rotor precision was evaluated by assaying 13 (high control) or 11 (low control) different samples of sera assayed concurrently in a single rotor. Day-to-day precision was evaluated from values obtained for the high and low control sera during a month.

We evaluated the accuracy of the alcohol dehydrogenase assay by adding a known amount of ethylene glycol to 15 different patients' sera. Grossly hemolyzed, lipemic, and icteric sera were included in this group; none showed significant interference. In addition, all of these samples had been drawn after routine skin cleansing with isopropanol. The mean assayed values before ethylene glycol addition was 7.2 (SD 10.7) mg/L; the analytical recovery of added ethylene glycol (101 g/L) was 102% (SD 6.2%). To check linearity of response, we assayed increasing concentrations of serum-based ethylene glycol standards; the relation between concentration and ΔA was linear to 1.5 g/L. For maximum accuracy, sera with greater concentrations should be diluted with serum containing no ethylene glycol or contaminating alcohol.

In Figure 1 we compare results of our assay with those of gas-chromatographic assay. (Specimens with concentrations exceeding 1.5 g of ethylene glycol per liter were diluted as just mentioned.) Results by the two procedures correlated well over the range of clinical interest.

Figure 2 shows the effect of ethanol, isopropanol, and methanol on the ethylene glycol assay. All interfere significantly. However, to confirm the presence of a volatile alcohol rather than ethylene glycol, heat the trichloroacetic acid-treated serum supernate in a boiling water bath for 30 min; if the apparent ethylene glycol concentration is then less, this indicates the presence of a volatile alcohol, but if the apparent ethylene glycol value increases this confirms the presence of ethylene glycol. The presence of as much as 20 g of sorbitol, mannitol, or glycerol per liter of serum had no effect on results of the ethylene glycol assay. Propylene glycol showed a slight interference in the enzymatic assay, 5 g/L appearing to be about 50 mg of ethylene glycol per liter.

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**Table 1. Multistat III Loader Settings and Protocol for Ethylene Glycol Assay**

<table>
<thead>
<tr>
<th>Sample syringe:</th>
<th>Total vol, 30% (30 μL)</th>
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</thead>
<tbody>
<tr>
<td>Reagent syringes:</td>
<td>Total vol, 68% (170 μL)</td>
</tr>
<tr>
<td>Reagent vol, 60% (150 μL)</td>
<td>Total vol, 68% (170 μL)</td>
</tr>
<tr>
<td>Second reagent: OFF</td>
<td></td>
</tr>
<tr>
<td>Reagent/diluent: DILUENT</td>
<td></td>
</tr>
<tr>
<td>Position</td>
<td>Sample ring contents</td>
</tr>
<tr>
<td>1</td>
<td>Empty</td>
</tr>
<tr>
<td>2</td>
<td>Blank</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
</tr>
<tr>
<td>4–20</td>
<td>Specimens and controls</td>
</tr>
</tbody>
</table>

*a The settings on the Multistat III loader are expressed in percent, with a 100-μL syringe for the sample syringe and a 250-μL syringe for the reagent syringe. Volume delivered is shown in parentheses. The difference between the sample or reagent volume and the total volume is the amount of distilled water that washes the sample or reagent into the cuvette.

*b Supernatant from precipitates (equal volumes of serum and 100 g/L trichloroacetic acid.)

*c Distilled water is delivered into cuvette 1 when the reagent/diluent switch is set to DILUENT.

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**Table 2. Precision of the Alcohol Dehydrogenase Assay for Ethylene Glycol**

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD), mg/L</th>
<th>CV, %</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td><strong>Within-rotor</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Low control</td>
<td>189 (8.6)</td>
<td>4.5</td>
<td>13</td>
</tr>
<tr>
<td>High control</td>
<td>1029 (23.0)</td>
<td>2.3</td>
<td>11</td>
</tr>
<tr>
<td><strong>Day-to-day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low control</td>
<td>183 (10.0)</td>
<td>5.6</td>
<td>16</td>
</tr>
<tr>
<td>High control</td>
<td>1064 (31.0)</td>
<td>3.0</td>
<td>21</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Gas-chromatographic method for ethylene glycol compared with the proposed enzymatic method

Specimens were rabbit sera collected 10 min to 16 h after oral administration of 1.2 to 1.8 g ethylene glycol per kilogram of body weight.

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Hematologically, interference measuring markedly decreased in the serum were 7.9, 3.2, 1.8, and 1.0 g/L, respectively.

Discussion

Ethylene glycol's extreme toxicity mandates prompt diagnosis and therapy. The clinical picture and laboratory data can suggest the diagnosis; however, time is frequently lost awaiting confirmation of the concentration in serum before optimum treatment is begun. Although survival has been reported after ingestion of as much as 970 mL (11), the estimated adult dose is 1.4 mL/kg of body weight (100 mL for a 70-kg adult) (3), and confirmed poisoning should be treated aggressively. Animal studies and limited experience in humans suggest that early institution of treatment can decrease the mortality from ingestions that would otherwise be expected to be lethal (2, 12). The method we propose should allow a rapid assessment of patients in whom ethylene glycol ingestion is suspected.

The laboratory findings in a patient poisoned with ethylene glycol are nonspecific and reflect diffuse systemic toxicity. Hematologically, a mild to moderate granulocytosis is common. Serum electrolytes and blood gases reflect a high anion-gap metabolic acidosis not accounted for by lactate concentrations, while cerebrospinal fluid shows pleocytosis and increased protein (2). The osmolality calculated from sodium, potassium, serum urea nitrogen, and glucose will markedly underestimate the measured value (13). Urinalysis reportedly shows proteinuria, pyuria, and microscopic hematuria, and more importantly, calcium oxalate crystalluria (14, 15). However, urinary calcium oxalate crystals are not a uniform finding (3, 6); in one group of 12 patients with documented ingestion, only four were found to have urinary oxalate crystals (3). Hippurate crystalluria has also been reported, possibly resulting from the transamination of glyoxylic acid to glycine and the conversion of glycine to hippurate (16). Gas chromatography remains the technique of choice for measuring circulating toxic alcohols after they have been ingested. It has the advantage that several alcohols can be measured concurrently in cases where more than one has been ingested. Our method could fail to distinguish ingestion of both isopropanol and ethylene glycol from that of isopropanol alone. Further, ingestion of any volatile alcohol with isopropanol and ethylene glycol could not be unambiguously differentiated. However, the technical expertise and equipment requirements generally limit gas chromatography to larger clinical laboratories. The method we present is accurate, precise, rapid, and most importantly, requires no specialized equipment. We have run the assay under identical conditions with use of a Beckman ACTA III spectrophotometer with a thermostated cuvette and have obtained similar results to those with the centrifugal analyzer. The procedure should also be easily adaptable to other automated analyzers or spectrophotometers with adequate long-term stability (±0.5 mAU400 for 10 min) and temperature control (±0.2 °C). It will allow faster diagnosis and thus should reduce the mortality associated with poisoning with ethylene glycol.

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References