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Case Conference

Ethylene glycol poisoning: toxicokinetic and analytical factors affecting laboratory diagnosis

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Ethylene glycol poisoning is an important toxicological problem in medical practice because early diagnosis and treatment can prevent considerable morbidity and mortality. When ingested in the form of antifreeze or other automotive products, ethylene glycol results in central nervous system depression, cardiopulmonary compromise, and renal insufficiency. Metabolism of ethylene glycol to organic acids is required for metabolic derangement and organ damage. Laboratory features of ethylene glycol poisoning include increased anion gap metabolic acidosis, increased osmolal gap, calcium oxalate crystalluria, and detectable ethylene glycol in serum. This Case Conference integrates discussion of the toxicokinetic and analytical variables that affect the laboratory diagnosis of ethylene glycol intoxication.

Ethylene glycol, the predominant constituent of automotive products such as antifreeze and deicers, is an important but uncommon toxicological problem in current medical practice *[1, 2]*. Individuals intentionally consume ethylene glycol, usually in the form of antifreeze, as an inexpensive alcohol substitute or as a suicidal agent. Accidents involving ethylene glycol ingestion often occur with children. According to the annual report of the American Association of Poison Control Centers, ethylene glycol was responsible for at least 5548 poisonings and 17 fatalities in the US in 1996 *[3]*; 18% of the individuals poisoned with ethylene glycol were children younger than 6 years.

In cases of severe ethylene glycol poisoning, early diagnosis and aggressive therapeutic intervention are essential for a favorable clinical outcome. Although therapy may be initiated before the serum concentration of ethylene glycol is measured, rapid and accurate identification of ethylene glycol or its metabolites is necessary for definitive diagnosis. Conversely, reliable screening tests for ethylene glycol poisoning can eliminate this diagnosis from the list of possible causes of increased anion gap metabolic acidosis in a comatose patient. Rapid exclusion of the diagnosis of ethylene glycol poisoning prevents a patient from receiving invasive, inappropriate treatment.

This Case Conference details the challenges that clinicians and clinical laboratorians confront in diagnosing ethylene glycol intoxication. The patients in cases 1 and 2 related a clear history of ethylene glycol ingestion and manifested several of the classical signs and symptoms of poisoning. Prompt initiation of appropriate therapy prevented neurological, cardiac, and renal sequelae. The patient in case 3 presented with a confusing picture of unexplained metabolic acidosis and renal failure. He repeatedly denied ethylene glycol ingestion, and results of serum ethylene glycol measurements were repeatedly negative; ethylene glycol poisoning was diagnosed only after renal biopsy. This case illustrates the impact of toxicokinetic variables on ethylene glycol detection and other laboratory parameters. The patient in case 4 also denied ethylene glycol ingestion, but presented with signs and symptoms consistent with ethylene glycol poisoning, including a positive laboratory test result for serum ethylene glycol; however, this result proved to be the result of interference of serum components in the ethylene glycol enzymatic assay. This case introduces the effect of analytical variables on the laboratory diagnosis of ethylene glycol poisoning.

Case Reports

CASE 1

A 27-year-old man with a history of ethanol and cocaine abuse presented to the emergency room claiming to have ingested 2.25 L (0.5 gal) of Prestone antifreeze ~3 h earlier in a suicide attempt. He had also consumed an unreported amount of cocaine (crack) and cut his left forearm and neck. He was somnolent with a depressed affect but responded to questions appropriately. Physical examina-

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tion revealed stable vital signs and superficial lacerations of his neck and left forearm. Laboratory values on admission are given in Table 1. A toxicology screen revealed cocaine, lidocaine, and nicotine in his urine. Based on the history and physical examination, a diagnosis of ethylene glycol poisoning was made. The serum ethylene glycol concentration, determined by gas chromatography, was 995 mg/L (16 mmol/L) and confirmed the diagnosis. Treatment included gastric lavage, charcoal, and intravenous ethanol. The serum ethanol concentration was maintained between 17 and 22 mmol/L (76–103 mg/dL). The serum ethylene glycol concentration decreased progressively from 995 mg/L to below the limit of detection (<100 mg/L) after 48 h. Serum creatinine concentrations were within the reference range throughout the hospitalization.

CASE 2

A 58-year-old man presented to the emergency room after a suicide attempt. He reported ingesting about "20 ounces [~56 mL] of half-strength" antifreeze, cutting his wrists with a dull knife, and falling down a flight of stairs. One empty and one half-full 1-gal (4.5-L) containers of Prestone antifreeze, reportedly half-strength, were found near the patient. In the emergency room, the patient was confused but otherwise neurologically intact. Physical examination revealed stable vital signs and superficial lacerations of both wrists. Laboratory values on admission are given in Table 1 and include a metabolic acidosis with increased anion gap and an increased osmolal gap. A diagnosis of ethylene glycol poisoning was made on the basis of history, physical examination, and confirmatory laboratory testing. The initial toxicology screen revealed ethylene glycol, caffeine, and nicotine. The serum ethylene glycol concentration on admission, 7910 mg/L (127 mmol/L), was determined by an enzymatic reaction that utilizes glycerol dehydrogenase from *Enterobacter aerogenes* (Boehringer Mannheim Biochemicals) on the Hitachi 704 automated analyzer. Treatment included charcoal, intravenous ethanol infusion (1 g/kg loading dose, 100 mg/kg per hour maintenance dose), elective intubation for airway protection, and emergency hemodialysis. The serum ethanol concentration was maintained between 17 and 42 mmol/L (77–194 mg/dL). The serum ethylene glycol concentration progressively decreased from 7910 mg/L (127 mmol/L) to 150 mg/L (2 mmol/L) after 28 h of therapy (including 16 h of hemodialysis). Serum creatinine concentrations were within the reference range, and arterial pH was never <7.21 throughout the hospital stay. The patient recovered completely within 3–5 days without discernible neurological, renal, or cardiac sequelae.

CASE 3

An 18-year-old man with a history of asthma presented to the emergency room complaining of nausea, vomiting, diffuse abdominal pain, and malaise over the previous 2 days. His vital signs were blood pressure 150/100, pulse 84, and rectal temperature 37.9 °C (100.7 °F). He was lethargic but arousable and oriented to person, time, and place. Medications included theophylline (Theodur) and epinephrine (Primatene Mist; 5.5 g/L). Laboratory values on admission are given in Table 1. Results of the urine and serum toxicology screens were negative. The patient was admitted for metabolic acidosis and renal failure. Renal function deteriorated and serum creatinine peaked at 1052 μmol/L (11.9 mg/dL) on the fifth hospital day. Ultrasound revealed enlarged edematous echogenic kidneys, compatible with acute renal failure. Results of an arteriogram for renal vasculitis and a skin biopsy for Henoch–Schonlein purpura were negative. Renal biopsy revealed

Table 1. Laboratory findings on presentation.

		Case			
Test	**Ref. range**	**1**	**2**	**3**	**4**
Serum ethylene glycol, mg/L (mmol/L)		995 (16)	7910 (127)	N.D.	90 (1.5)
Arterial blood gases:					
pH	7.35–7.45	7.32	7.23	7.29	7.11
P_{CO_2}, mmHg	35–45	34	16	21	14
HCO_3^-, mmol/L	22–26	17	7	11	9
Anion gap, mmol/L	12–16	13	26	20	29
Lactate, mmol/L	0.7–2.1	N.R.	N.R.	1.1	13.3
Serum osmolality, mOsm/kg H_2O					
Measured	270–290	304	434	N.D.	303
Calculated		288	264	N.D.	277
Gap	<10	16	170	N.D.	26
Creatinine, μmol/L	44–150	106	80	221	150
Urea nitrogen, mmol/L	2.9–8.9	3.6	1.8	6.8	11.4
Urinalysis		Few Ca ox. crystals	Rare Ca ox. crystals	Hematuria, rare, amorphous crystals	Proteinuria, hematuria, urate crystals

N.D., none detected; N.R., not reported; Ca ox., calcium oxalate.

acute tubular necrosis and deposition of calcium oxalate crystals, supporting a diagnosis of ethylene glycol poisoning (Fig. 1). The patient denied ethylene glycol or antifreeze ingestion and suicide attempts. Ethylene glycol was not detected in stored serum samples from admission or taken on the fifth hospital day. Treatment with hemodialysis for 3 weeks resulted in complete recovery of renal function. Twenty-four months after this admission, the patient demonstrated no clinical or laboratory evidence of renal dysfunction [serum creatinine, 97 μmol/L (1.1 mg/dL); blood urea nitrogen, 4.3 mmol/L (12 mg/dL)].

CASE 4

The patient in case 4 was reported previously *[4]*. Briefly, he was a 23-year-old man with a 1-year history of dilated cardiomyopathy and pulmonary hypertension who experienced shortness of breath, productive cough, hemoptysis, nausea, and vomiting over the 2 days before admission. Medications included captopril, digoxin, furosemide, and cimetidine. On physical examination, he was jaundiced, in mild respiratory distress, febrile [37.8 °C (100 °F)], tachycardic (126/min), and tachypneic (26/min). Results of physical examination were remarkable for bilateral pulmonary rales and mild hepatomegaly. On the first hospital day, he became increasingly anxious, restless, and tachypneic with subxiphoid pain radiating to his back. Pertinent laboratory values are listed in Table 1. Urinalysis revealed proteinuria (1 g/L), hematuria (packed), and "unidentified" crystals, which were retrospectively interpreted as being urates. Serum ethylene glycol was 90 mg/L (1.5 mmol/L), as measured by the enzymatic reaction described above, utilizing glycerol dehydrogenase and the Hitachi 704 analyzer. However, ethylene glycol was not detected by gas chromatography.

Discussion

These cases expose the challenges encountered during the evaluation of ethylene glycol intoxication. The history obtained from patients regarding their current illness is often the most valuable diagnostic tool. In cases 1 and 2, the intoxication was recognized immediately because the patients clearly related the details of their suicide attempts. In contrast, the diagnosis was delayed in case 3 because the patient did not report ethylene glycol ingestion. Although family members suspected he had attempted suicide, he repeatedly denied intentional ethylene glycol consumption during the course of the hospitalization and several months later during an emergency room visit for an unrelated problem. No evidence of intentional or accidental poisoning was discovered. The diagnosis of ethylene glycol poisoning was not established until a renal biopsy was performed to investigate the cause of acute renal failure.

Denial of ethylene glycol ingestion occurs because the patient or a third party either is concealing intentional poisoning or is unaware of consumption. In the US, a child undergoing an extensive medical evaluation for a possible inherited genetic disease had, in fact, been poisoned with ethylene glycol by a care giver *[5]*. In Germany, a man unknowingly drank contaminated water from a radiator for 6 weeks and consumed enough ethylene glycol to result in intoxication and renal failure *[6]*. More commonly, a patient is unable to relate his history because of profound neurological depression.

In the absence of an accurate history, the alternative metabolic derangements considered for a comatose patient include diabetic ketoacidosis, alcoholic ketoacidosis, renal failure, and ingestion of methanol or other toxic compounds. In these cases, additional laboratory information may facilitate an accurate diagnosis.

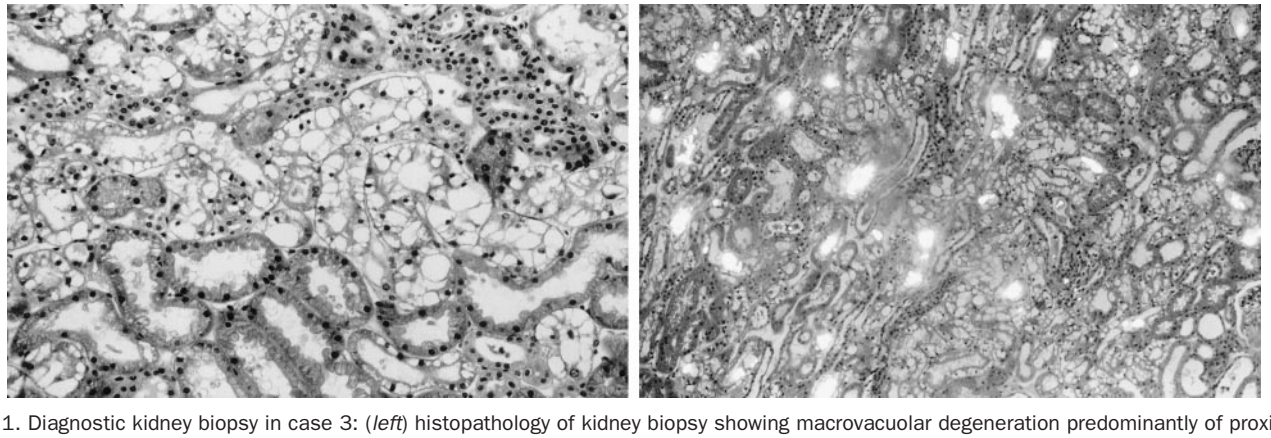

Fig. 1. Diagnostic kidney biopsy in case 3: (*left*) histopathology of kidney biopsy showing macrovacuolar degeneration predominantly of proximal tubules (H and E stain, ×50); (*right*) kidney biopsy viewed under polarized light showing birefringent intratubular oxalate crystals (×25).

The histologic diagnosis of acute renal failure secondary to ethylene glycol poisoning depends on the recognition of the changes of acute tubular damage in association with tissue oxalate deposition. Tubular epithelial cells show degenerative changes, including cytoplasmic swelling, cytoplasmic vacuolar regeneration, and atypical cell membrane disruption. Oxalate crystals deposit in the tubular lumens and appear as irregular or fan-shaped deposits. These deposits are laminated and birefringent under polarized light. The oxalate crystals stain positively with the Pizzolato peroxide–silver method. Oxalate crystals are usually negative on von Kossa stain unless calcium phosphate is coprecipitated.

TEMPORAL ASPECTS OF CLINICAL PRESENTATION AND PATIENT VARIABILITY

The clinical presentation of ethylene glycol poisoning is classically described in three stages. Neurological manifestations are apparent within 0.5–12 h of ingestion and include inebriation in the absence of detectable ethanol on breath or in blood, nausea, vomiting, nystagmus, papilledema, depressed reflexes, convulsions, and coma. Cardiopulmonary manifestations may be observed 12–24 h after ingestion and may include tachypnea, tachycardia, hypertension, pulmonary edema, and congestive heart failure. Renal complications are generally a late feature, occurring 24–72 h after ingestion, and consist of flank pain, costovertebral angle tenderness, oliguria, and renal failure.

The severity of each stage and the progression from one stage to the next depend on the amount of ethylene glycol ingested as well as the timing of medical intervention. The most serious clinical features observed in ethylene glycol poisoning are due not to the parent compound but to the metabolites. Ethylene glycol per se causes only minimal inebriation, which resembles ethanol intoxication *[1, 2]*. Because metabolism of the parent compound is required for toxicity, the latent period reflects the time required for the toxic metabolites to accumulate. In cases 1 and 2, the only apparent manifestation of ethylene glycol poisoning was mild neurological depression, because the patients were evaluated within hours of the ingestion. In striking contrast, substantial renal insufficiency caused by the metabolites of ethylene glycol was manifest in case 3, because the poisoning was not detected until late in the clinical course, most likely several days after ingestion.

Although these stages provide a useful theoretical framework for the clinical description of ethylene glycol poisoning, the onset and progression of this condition are not always straightforward or predictable. These stages may be confluent, one stage may predominate, or one or more of the stages may not be clinically apparent. Often, a patient is discovered comatose, experiencing both respiratory distress and acute renal insufficiency *[7]*. Variable symptom profiles are found in the literature of case reports *[8–11]*. Renal failure is the most frequently reported manifestation of ethylene glycol ingestion; however, as in cases 1 and 2, prompt treatment may prevent crystalluria and renal insufficiency *[12–14]*.

Although the symptoms usually completely resolve soon after appropriate treatment for the acute episode, sequelae of ethylene glycol poisoning have included prolonged renal failure requiring dialysis for months, residual kidney damage, and cranial nerve deficits manifesting late in the clinical course and lasting as long as several months *[15–18]*. Complete recovery from ethylene glycol poisoning with aggressive treatment, however, has been reported even after severe encephalopathy *[19]* or profound acidemia with a serum pH of 6.46 *[13]*

TOXICOKINETIC EVALUATION OF ETHYLENE GLYCOL ELIMINATION

Because of the potential severity of ethylene glycol poisoning, kinetic variables for ethylene glycol disposition in humans have been measured only in poisoned individuals (Table 2). Ethylene glycol is rapidly absorbed from the gastrointestinal tract, and symptoms of poisoning may be experienced within 30 min of ingestion. Percutaneous absorption of ethylene glycol has not been reported, but topical burn preparations containing propylene glycol or diethylene glycol have produced considerable toxicity in burn patients *[20, 21]*. Ethylene glycol is rapidly metabolized by alcohol dehydrogenase and other hepatic enzymes to glycoaldehyde and organic acids (Fig. 2). The elimination half-life of ethylene glycol is increased at least fivefold in the presence of ethanol because both compounds compete for the active site of alcohol dehydrogenase. This enzyme has a much greater affinity for ethanol than for ethylene glycol or methanol, and concentrations of ethanol as low as 11 mmol/L (50 mg/dL) saturate the enzyme *[22]*. To ensure competitive inhibition of alcohol dehydrogenase activity, however, the serum concentration of ethanol should be maintained >22 mmol/L (>100 mg/dL) during hemodialysis *[22, 23]*. Hemodialysis rapidly removes both ethylene glycol and its toxic metabolites, particularly glycolate and oxalate *[24–27]*. Hemodialysis clearance is greater than renal clearance, but functioning kidneys contribute to the removal of ethylene glycol from the blood *[26]*.

Toxicokinetic evaluation of ethylene glycol elimination during hemodialysis and ethanol infusion was performed in case 2 (Fig. 3). From the estimation of the dose provided by the patient and the assumption that the peak concentration was 7910 mg/L (127 mmol/L), the volume of distribution (V_d) was calculated as 0.5 L/kg, a value in good agreement with previously reported values for V_D (Table 2). Utilizing the noncompartmental pharmacokinetic model with extravascular administration of the historical dose (Winnonlin software package; Scientific Consulting Inc., Apex, NC), we determined the elimination half-life as 3.79 h, based on linear regression of 14 terminal arterial concentration values. The calculated

Table 2. Toxicokinetic parameters for ethylene glycol.[a]

Lethal dose[b]	1.4–1.6 mL/kg
V_d	0.5–0.8 L/kg
$t_{1/2}$	3–8.6 h
$t_{1/2}$ during ethanol infusion	17–18 h
$t_{1/2}$ during hemodialysis	2.5–3.5 h
Mean renal clearance[c]	0.75–27.5 mL/min
Mean dialyzer clearance	156–210 mL/min

[a] Data compiled from references *18*, *23*, *26*, *27*, and *33*.

[b] Although the lethal dose is considered 100 mL for a 70-kg adult, survival has been reported after ingestions of 0.27–2 L. Also, death has been reported after ingestion of 30 mL.

[c] Lower value obtained in patient with renal failure; upper value obtained in patient with normal renal function.

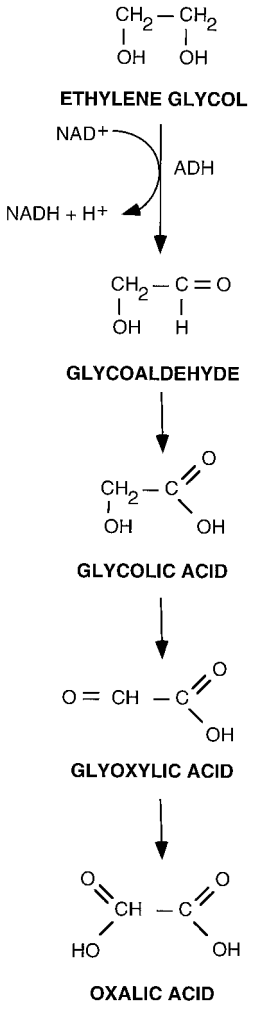

Fig. 2. Metabolism of ethylene glycol, major pathway.
ADH, alcohol dehydrogenase. Source: *[25].*

half-life largely reflects the half-life during dialysis in the presence of a serum ethanol concentration of 17–42 mmol/L (77–194 mg/dL). During the two short intervals when ethanol infusion was maintained but hemodialysis was not performed (Fig. 3), the slope of the line is more shallow, reflecting the prolongation of the half-life of ethylene glycol in the presence of ethanol (Table 2). The overall clearance was 75 mL/min, beginning from the time the patient entered the emergency room. The overall clearance is the combined effect of the dialyzer clearance and the body's own clearance (through kidney excretion and liver metabolism) until saturating concentrations of ethanol are reached. If the first 6 h before dialysis are not included in the calculation, the clearance is 113 mL/min. We also calculated the removal of ethylene glycol by the dialyzer, referred to in some studies as dialyzer clearance, by using the formula $Cl_H = [Q_{IN}\ (C_{IN} - C_{OUT})]/C_{IN}$, where Q_{IN} is blood flow into the dialyzer (measured with a flow sensor), and C_{IN} and C_{OUT} are the ethylene glycol concentrations measured in the arterial blood entering and the venous blood leaving the dialyzer, respectively.

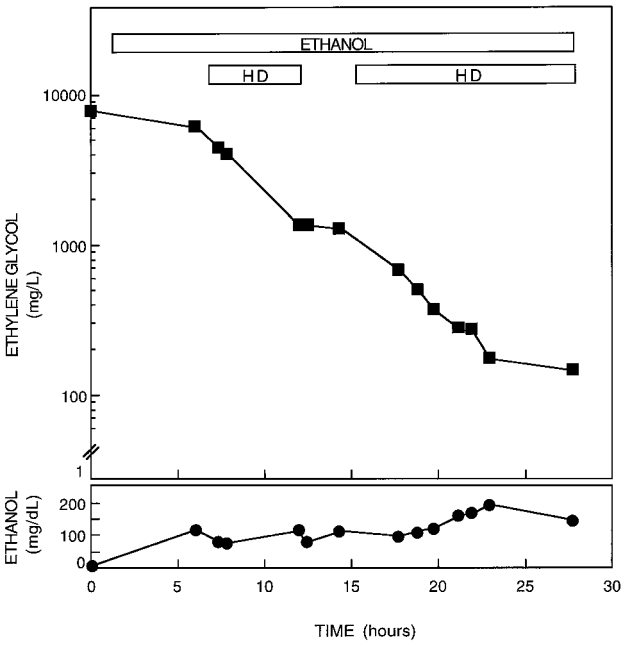

Fig. 3. Toxicokinetic evaluation of ethylene glycol elimination.
Serum ethylene glycol (■) and serum ethanol (●) concentrations during treatment of the patient described in case 2. The duration of therapy with intravenous ethanol and the two intervals of hemodialysis (HD) are indicated by the *bars* above the graphs. Conversion factors (to mmol/L): ethylene glycol, mg/L × 0.0161; ethanol, mg/dL × 0.217.

For a dialysis blood flow of 350 mL/min, dialyzer clearance in case 2 was calculated as 228 mL/min, a value slightly higher than reported previously, possibly reflecting the higher blood flow rate used for this patient *[14, 18, 23, 26].*

TEMPORAL ASPECTS OF LABORATORY DIAGNOSIS

The evolving laboratory profile in cases of ethylene glycol poisoning reflects the metabolism of ethylene glycol, the accumulation of organic acids, and the timing of medical intervention. In case 2, the initial serum ethylene glycol concentration was 7910 mg/L, among the highest reported values for an intoxicated patient who survived with therapy. Death, however, has been reported in patients with virtually undetectable serum ethylene glycol concentrations *[28]*. This discrepancy underscores the importance of prompt diagnosis and treatment of intoxication. The poor correlation between serum ethylene glycol concentration and clinical outcome is the result of the rapid clearance of the parent drug and conversion to the severely toxic metabolites. The "window" period during which the parent compound ethylene glycol can be detected may be relatively narrow. In case 3, this time had elapsed, and the only evidence of ethylene glycol poisoning was the deposition of calcium oxalate crystals in the kidney. At a given time after ingestion, the concentrations of the remaining ethylene glycol and the accumulated acidic metabolites affect the magnitude of the osmolal gap and anion gap, respectively. An osmolal gap may not be apparent late in the course of poisoning, whereas

an anion gap may not be evident early in the clinical course *[18, 29, 30]*. These observations reflect the relative amounts of the compounds that are the source of the gaps.

The anion gap is the difference between the sum of the measured cations and the sum of the measured anions $[(Na^+ + K^+) - (HCO_3^- + Cl^-)]$. Most laboratories routinely measure only sodium and potassium, which account for ~95% of the extracellular cations, and chloride and bicarbonate, which account for ~85% of the extracellular anions. Because the sum of the measured cations does not equal the sum of the measured anions in healthy individuals, the "normal" anion gap is 12–16 mmol/L. The production of unmeasured organic acids will increase the anion gap. Glycolic acid accounts for as much as 96% of the anionic gap in patients poisoned with ethylene glycol *[24, 25]*. The osmolal gap is also an estimate of unmeasured constituents in the serum. In healthy individuals, serum osmolality is determined by the concentration of sodium, urea nitrogen, and glucose and is approximated by the following formula *[31]*:

Calculated plasma osmolality, mOsm/kg H_2O =
[1.86 × sodium (mmol/L) + urea nitrogen (mmol/L) + glucose (mmol/L)]/0.93

or, in traditional units, {1.86 × sodium (mmol/L) + [urea nitrogen (mg/dL)/2.8] + [glucose (mg/dL)/18]}/0.93

Serum osmolality is 270–290 mOsm/kg H_2O in a healthy individual. The difference between measured and calculated osmolality, the osmolal gap, results from the presence of other solutes in serum, which are not considered in the above formula. An increase in the osmolal gap, generally considered important when >10 or 15 mOsm/kg H_2O, suggests the presence of low-molecular-mass substances that achieve appreciable serum concentration such as ethylene glycol, methanol, ethanol, and acetone *[31–33]*. Consequently, osmolality should be measured by freezing point depression, because the vapor pressure method will underestimate volatile alcohols *[34]*.

sensitivity of osmolal and anion gaps in ethylene glycol poisoning

As ethylene glycol is being metabolized or removed by dialysis, its contribution to the osmolal gap diminishes because the accumulating acidic, negatively charged metabolites do not contribute to the osmolal gap *[30]*. These anions are counterbalanced by sodium and are taken into consideration in the formula for calculating the serum osmolality *[33]*. Nonionic metabolites of ethylene glycol may contribute to the osmolal gap; however, the concentration of the parent compound accounts for most of the osmolal gap in cases of ethylene glycol poisoning *[18]*.

The contribution of ethylene glycol or ethanol to the osmolal gap can also be calculated: each 16 mmol/L (100 mg/dL) increment in ethylene glycol concentration contributes ~16 mOsm/kg H_2O, and each 22 mmol/L (100 mg/dL) of ethanol contributes 22 mOsm/kg H_2O to the osmolal gap. The corrected osmolal gap, or the residual osmolality, can be used to monitor patients who have simultaneously ingested ethylene glycol and ethanol or patients who are receiving ethanol infusion for ethylene glycol poisoning *[26]*. In case 1, the serum osmolal gap of 16 mOsm/kg was probably entirely attributable to ethylene glycol because the initial serum concentration of ethylene glycol, 995 mg/L, would contribute ~16 mOsm/kg H_2O to the osmolal gap.

Although metabolism of ethylene glycol diminishes the osmolal gap, the generation of unmeasured acidic metabolites of ethylene glycol augments the anion gap *[18, 30]*. In two series of individuals intoxicated with ethylene glycol, metabolic acidosis was initially apparent in 50% and 86% of cases *[35, 36]*. In case 3, the increased anion gap most probably reflected the delay in diagnosis and the accumulation of acidic metabolites. In case 1, however, the anion gap was within the reference range, most probably because the intoxication was detected and treated promptly; acidic metabolites had not reached appreciable concentration. Aside from the temporal dependence of the increased anion gap on the metabolism of the parent compound, an anion gap may not be present in cases of ethylene glycol poisoning for other reasons. Simultaneous ingestion of ethanol will competitively inhibit the metabolism of ethylene glycol and delay the appearance of an anion gap *[37]*. Simultaneous ingestion of bromide masks the anion gap because bromide is not distinguished from chloride in some assays *[38]*. Finally, simultaneous ingestion of lithium carbonate may conceal the anion gap by providing additional bicarbonate *[39]*.

specificity of anion and osmolal gaps

For these reasons, neither osmolal nor anion gaps are universally present in cases of ethylene glycol poisoning, and their absence cannot be used to rule out toxic alcohol or ethylene glycol ingestion *[29, 33]*. Conversely, the simultaneous presence of metabolic acidosis with an increased anion and osmolal gap, although highly suggestive of ethylene glycol or methanol poisoning, is not specific for these intoxications *[40, 41]*. Anion and osmolal gaps may be present in other clinical settings such as diabetic ketoacidosis, alcoholic ketoacidosis, chronic renal failure, multiple organ failure, and critical illness *[40, 42–48]*. In diabetic ketoacidosis, for example, the osmolal gap is due primarily to the accumulation of acetone, which may approach concentrations of 13 mmol/L, and the anion gap metabolic acidosis is due primarily to the accumulation of acetoacetate and β-hydroxybutyrate *[43, 44]*. Although the anion gap may be profoundly increased in diabetic ketoacidosis, the osmolal gap is not usually greater than 20–25 mOsm/kg H_2O *[40, 44]*. In case 4, both metabolic acidosis with an increased anion gap and an increased osmolal gap were present. Ethylene glycol poisoning was initially considered but later discounted after gas chromatography failed to confirm the results of the screening test for ethylene glycol. The

increased anion and osmolal gaps in this case were probably the result of multiple organ failure.

SENSITIVITY AND SPECIFICITY OF CALCIUM OXALOSIS

Calcium oxalate crystalluria and deposition of these crystals in the kidney, brain, or other organs are distinctive laboratory features in ethylene glycol poisoning. In case 3, the findings of renal tubular necrosis and deposition of calcium oxalate crystals by renal biopsy (Fig. 1) supported the diagnosis. Chelation of calcium by the oxalate deposited in the kidneys and other organs may explain the hypocalcemia that is often observed in cases of ethylene glycol poisoning. Another clinical setting characterized by calcium oxalate nephrolithiasis is primary hyperoxaluria, a rare inherited metabolic disorder associated with early-onset renal failure and death. Healthy individuals, especially those with dietary excesses of foods rich in oxalate such as tomatoes, garlic, spinach, rhubarb, cocoa, and tea, may also exhibit calcium oxalate crystalluria without associated renal insufficiency.

Calcium oxalate crystals in urine are pleomorphic, variegated, and birefringent when viewed through polarized light. In cases of ethylene glycol poisoning, calcium oxalate may be excreted not only as dihydrate crystals, which are envelope-shaped (dipyramidal, octahedral), but also as monohydrate crystals, which are needle-shaped (spindle or prism shaped) (Fig. 4). Other forms of calcium oxalate include dumbbell, ovoid, and elliptical crystals. If the results of more-definitive laboratory tests are not available, the detection of calcium oxalate crystalluria, particularly the monohydrate form, provides supportive evidence for the diagnosis of ethylene glycol poisoning *[30, 49–51]*. Because the monohydrate form may be the only form seen early or at any time during the course of the episode, familiarity with the microscopic features of calcium oxalate monohydrate crystals is important.

In the early 1980s, identification of the pleomorphic nature of calcium oxalate crystalluria prompted the recommendation that physicians recognize the monohydrate calcium oxalate crystals to facilitate rapid diagnosis of ethylene glycol ingestion *[51]*. In the 1990s, however, the monohydrate form was still considered "unusual" or was likely to be misidentified as hippuric acid crystals in cases of ethylene glycol poisoning *[29]*. The medical literature has been confused by morphological descriptions of crystals that resemble those of hippuric acid as well as by theoretical arguments supporting their formation *[25, 30]*. X-ray diffraction, however, definitively identifies the needle-shaped crystals as calcium oxalate monohydrate and not hippuric acid *[30, 50, 51]*. Renewed emphasis has recently been placed on the need to increase proficiency during microscopic analysis of urine to recognize calcium oxalate monohydrate as well as dihydrate crystals in cases of suspected ethylene glycol poisoning *[49]*. Because urinalysis is rapid and easy, repeated urine microscopy is a potentially useful adjunct in the differential diagnosis of an anion gap metabolic acidosis of unknown origin *[18, 50]*. Other frequently reported findings on urinalysis in cases of ethylene glycol poisoning include low specific gravity, proteinuria, and microscopic hematuria.

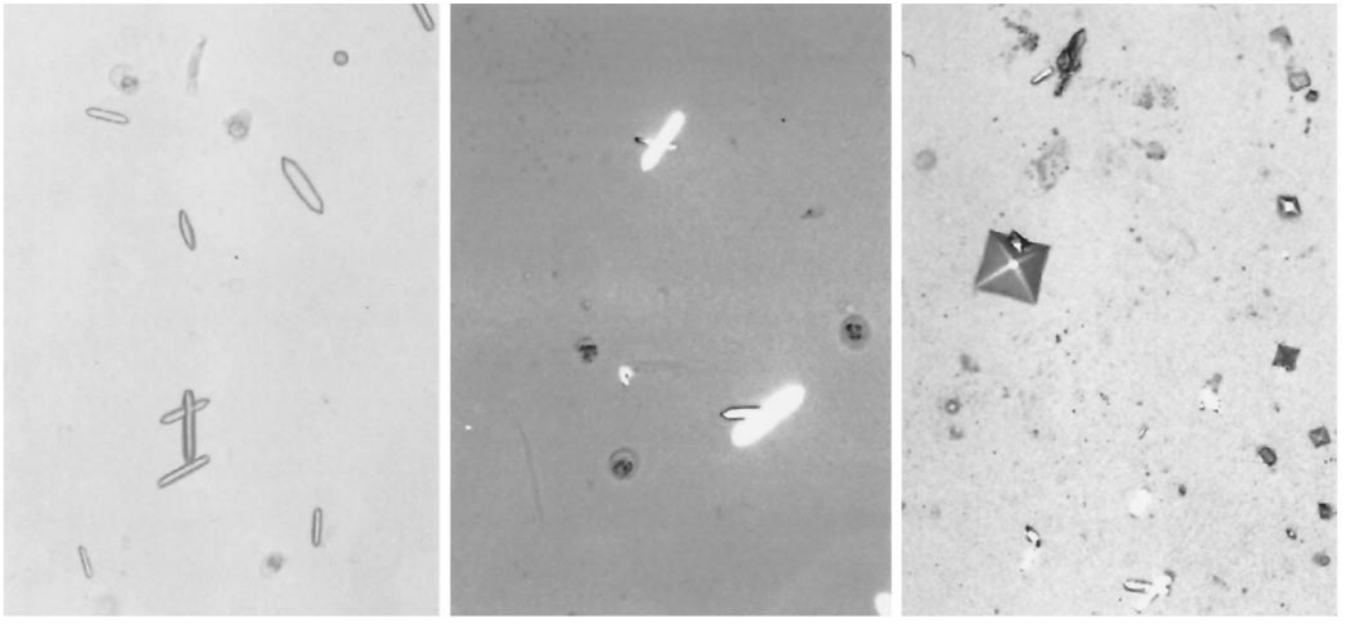

Fig. 4. Calcium oxalate crystalluria in the urine of a patient poisoned with ethylene glycol: calcium oxalate monohydrate crystals under bright field microscopy (*left*, ×270), under polarized light (*middle*, ×288), and with the dihydrate crystal (*right*, ×270).

The monohydrate form is very strongly birefringent and may be distinguished from uric acid by its solubility in dilute hydrochloric acid.

LABORATORY MEASUREMENT OF ETHYLENE GLYCOL METABOLITES

Examination of urine for calcium oxalate crystals is the most widely accessible laboratory technique for detecting a metabolite of ethylene glycol. Because the toxicity of ethylene glycol depends on its oxidation to organic acids, several other methods have been utilized to measure these products. Glycolic acid, the predominant metabolite, has been measured by HPLC and gas chromatography, by a colorimetric method, and by isotachophoresis *[27, 52–54]*. The colorimetric method utilizes sulfuric acid and chromotropic acid and is relatively specific for glycolic acid *[52]*. In contrast, isotachophoresis can measure the four major acidic metabolites of ethylene glycol simultaneously *[54]*. Isotachophoresis is an electrophoretic technique that orders and concentrates substances of intermediate effective mobilities between an ion of high effective mobility and one of much lower effective mobility. Sample components ultimately separate into adjacent zones that migrate at the same velocity. Although a laboratory test for glycolic acid would have been a useful diagnostic adjunct in case 3, none is currently available at most medical centers or reference laboratories.

ANALYTICAL VARIABLES AFFECTING LABORATORY DIAGNOSIS

Gas chromatography. The method of choice for measuring ethylene glycol is gas chromatography, with flame ionization detection of ethylene glycol itself or of a derivative. Commonly, ethylene glycol is analyzed as the boronic ester derivative by using packed or capillary columns. Underivatized ethylene glycol is difficult to analyze because of poor chromatographic behavior and the poor detection limit of flame ionization detectors. However, direct injection of ethylene glycol on a wide-bore capillary column has been described *[55, 56]* Advantages of these methods include elimination of the derivatization step, resolution of diethylene glycol and other diols as well as other polar drugs, and extended analytical life of the Nukol column.

An appropriate internal standard, such as 1,3-propanediol or 1,2-butanediol, must be used with gas-chromatographic analysis. Recently, the use of propylene glycol as an internal standard has been discouraged *[57–59]*. The inclusion of propylene glycol in some intravenous pharmaceutical preparations contributes to the concentration of the internal standard, thus resulting in underestimation of the ethylene glycol concentration in the serum sample. Moreover, propylene glycol itself may be responsible for clinical toxicity, and its presence in a serum sample may be masked when this compound is used as an internal standard.

Although gas chromatography is the "gold standard" for detecting ethylene glycol, diagnostic inaccuracy with this method has occurred. In one notorious case, propionic acid was mistakenly identified as ethylene glycol by gas chromatography *[60]*. Consequently, a mother was falsely accused of poisoning her infant son who, in fact, had an inherited metabolic disease, methylmalonic acidemia. This error occurred in two independent laboratories. Accurate interpretation of the retention times of the compounds identified in the serum sample might have prevented the tragic consequences of this inaccurate diagnosis. Identification based on retention time alone has led to confusion of 2,3-butanediol as well as propionic acid with ethylene glycol *[60, 61]*; thus, if the presence of ethylene glycol is suspected in a sample, confirmation by mass spectrometry is recommended. Moreover, methanol-like products generated by the oxidation–reduction derivatization procedure in sera from ketoacidotic diabetic patients may be misinterpreted as evidence of ethylene glycol poisoning *[62]*.

Enzymatic assay. The screening assay utilizes glycerol dehydrogenase purified from *E. aerogenes [63–65]*. This enzyme catalyzes the oxidation of ethylene glycol, producing NADH, which is measured spectrophotometrically. No cross-reaction is observed with ethanol, methanol, *n*-propanol, isopropanol, acetaldehyde, lactate, glyoxal, glycolic acid, glyoxylic acid, or oxalic acid. Interfering substances include glycoaldehyde and glycerol, which compete with ethylene glycol for the active site of the enzyme. Although glycoaldehyde is one of the ethylene glycol metabolites, its short half-life makes it unlikely to interfere with the assay. The interference from glycerol is not significant in most cases and may be disregarded on the basis of expected serum concentrations of glycerol ($<$0.5 mmol/L). However, critically ill patients may have an increased serum concentration of free glycerol, most frequently related to intravenous infusion of glycerol-containing medications *[66]*. Conceivably, glycerol could reach a concentration in serum sufficient to interfere in the enzymatic assay for ethylene glycol in this clinical setting.

Another interference in the screening test for ethylene glycol was delineated in case 4 *[4]*. Ethylene glycol poisoning was considered in this patient because the toxicology screen detected ethylene glycol and several of the key diagnostic features were present: cardiorespiratory compromise, increased anion gap metabolic acidosis, increased osmolal gap, renal insufficiency, and crystalluria. The positive result obtained in the enzymatic screening test, however, was not confirmed by gas chromatography. The source of the interference in the enzymatic assay for ethylene glycol in this case was markedly increased concentrations of serum L-lactate dehydrogenase (LD) and lactic acid. In patients with increased serum lactate and LD, extraneous production of NADH from the oxidation of lactate to pyruvate catalyzed by LD may interfere with the assay, resulting in falsely positive values for ethylene glycol. This interference has also been reported in the enzymatic assay for ethanol, which likewise measures NADH as its endpoint *[67]*.

TREATMENT OF ETHYLENE GLYCOL POISONING

Goals of treatment in cases of ethylene glycol intoxication include reducing the load of ingested ethylene glycol, correcting the metabolic acidosis of early toxicity, preventing additional metabolism of ethylene glycol, and removing the parent compound as well as toxic metabolites from the circulation. If ingestion is recognized early, ethylene glycol may be removed from the gastrointestinal tract by inducing emesis, administering activated charcoal, or performing gastric lavage. Ethanol is administered as a preferential substrate for alcohol dehydrogenase, thereby competitively inhibiting metabolism of ethylene glycol to its toxic metabolites and allowing excretion of the unmetabolized parent compound. Ethanol therapy is most effective if instituted early, given the half-life of ethylene glycol (~3 h).

As in case 1, ethanol therapy may be sufficient to prevent renal failure and effect complete clinical recovery *[12, 14, 68]*. Additional measures, however, are often initiated simultaneously before renal failure supervenes. Hemodialysis removes both ethylene glycol and its toxic metabolites, particularly glycolate and oxalate, as well as ethanol *[23–25]*. Consequently, ethanol administration should be maintained during hemodialysis. In addition to hemodialysis, chronic alcohol abuse and activated charcoal may decrease serum ethanol concentration so that loading doses must be adjusted to maintain serum alcohol concentration >22 mmol/L (>100 mg/dL). Ethanol therapy is associated with additional neurological depression, and frequent monitoring during hemodialysis is necessary to maintain ethanol serum concentrations at appropriate values. As an alternative to ethanol therapy for ethylene glycol poisoning, other potent and specific inhibitors of alcohol dehydrogenase, e.g., 4-methylpyrazole, have been used to treat ethylene glycol poisoning in humans *[69, 70]*.

In conclusion, early diagnosis and treatment of ethylene glycol poisoning can prevent substantial morbidity and mortality. The diagnosis may be straightforward, as in cases 1 and 2, if the patient or a third party relates a clear history of ethylene glycol poisoning. If the poisoning is not detected until late in the clinical course, as in case 3, toxicokinetic variables affecting measurement of serum ethylene glycol and other variables such as the anion gap and osmolal gap may obscure the laboratory diagnosis of suspected ethylene glycol poisoning. Finally, as in case 4, analytical variables may affect the results of laboratory assays for ethylene glycol.

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