Decision-making regarding the infusion rate of heparin at the clinic is based on the therapeutic interval of 60–89 s for APTT. For values >89 s or <60 s, a progressive and stepwise change of the infusion rate is made. If the TAS readings had been used for control of the heparin infusion rate, 20 patients of the 75 would have received a somewhat different infusion rate (8 patients a higher rate, 12 patients a lower rate—all 20 being within a one-step difference in treatment of 2160 IU/24 h) from that implied by the comparison system measurement. In no case, however, would a TAS APTT have led to an adjustment of the heparin infusion dose that would have been contradictory to an adjustment based on the corresponding comparison method APTT (i.e., the TAS never indicated an increase in the dose when the Cephos test was indicating a decrease, or vice versa).

The correlation between the two APTT systems was good. The precision of the TASS APTTs was somewhat less than that of the comparison method (in earlier studies), but still was <10% (CV).

A bedside system for APTT analysis at the clinical ward can reduce the personnel and time-demanding procedures required for communication with the central laboratory. The use of a bedside system that enables an APTT to be analyzed within minutes may also facilitate fast decision-making regarding the heparin infusion rate and will be of special benefit for patients in cardiology care.

References

Serum Lactate and Lactate Dehydrogenase in High Concentrations Interfere in Enzymatic Assay of Ethylene Glycol, Anne F. Eder, Yuwen G. Dowdy, Jo Ann M. Gardiner, Bryan A. Wolf,* and Leslie M. Shaw (Dept. of Pathol. and Lab. Med., Univ. of Pennsylvania School of Med. and Hosp. of the Univ. of Pennsylvania, Philadelphia, PA 19104; *address for correspondence: 217 John Morgan Blvd., 3620 Hamilton Walk, Philadelphia, PA 19104-6082; fax 215-573-2266, e-mail wolfb@mail.med.upenn.edu)

The enzymatic assay to measure ethylene glycol in serum utilizes glyceral dehydrogenase in an oxidation reaction that produces NADH (1–3). The concentration of NADH is determined spectrophotometrically by measuring absorbance at 340 nm. Although this enzymatic assay is specific for ethylene glycol, other reactions that generate NADH in patients’ sera may produce false-positive results. This interference is observed in serum samples with high concentrations of l-lactate dehydrogenase (LD) and lactate.

We first documented false-positive results in the ethylene glycol enzymatic assay during the evaluation of a 23-year-old man with a 1-year history of dilated cardiomyopathy and pulmonary hypertension who presented with shortness of breath, productive cough, hemoptysis, nausea, and vomiting. Medications he was taking included captopril, digoxin, hydrocortisone, and cimetidine. Physical examination found him to be...
jaundiced and in mild respiratory distress. He was febrile [37.8 °C (100 °F)], tachycardic (126/min), and tachypneic (26/min). Physical examination was also remarkable for bilateral pulmonary rales and mild hepatomegaly.

On the first day at the hospital, he became increasingly anxious, restless, and tachypneic with subxiphoid pain radiating to his back. Pertinent laboratory values included: arterial blood gases, pH 7.11, $P_{CO_2}$ 14 mmHg, $P_{O_2}$ 117 mmHg, HCO$_3$ 9 mmol/L; serum anion gap, 29 mmol/L; serum lactate, 13.3 mmol/L; serum osmolal gap, 26 mosm/kg H$_2$O; serum urea nitrogen, 11.4 mmol/L; creatinine, 150 μmol/L; leukocyte count, 26.6 × 10$^9$/L. Serum ethylene glycol was 9 mg/dL (1.5 mmol/L), as measured by the enzymatic reaction utilizing glycol dehydrogenase from Enterobacter aerogenes (Boehringer Mannheim Biochemicals, Indianapolis, IN) on the Hitachi 704 automated analyzer. The toxicology screen also revealed erythromycin, cimetidine, and lidocaine. Urinalysis revealed proteinuria (1 g/L), hematuria (packed), and "unidentified" crystals.

Ethylene glycol poisoning was considered in this critically ill patient because the toxicology screen revealed ethylene glycol and because several of the diagnostic hallmarks were present: cardiorespiratory compromise, metabolic acidosis with anion gap, osmolal gap, renal insufficiency, and crystalluria. In addition, ethylene glycol poisoning has presented as myocarditis [4]. However, the serum ethylene glycol concentration determined enzymatically was not confirmed by gas chromatography. The crystalluria was retrospectively interpreted as urate crystals.

A potential source of interference in the enzymatic assay for ethylene glycol is the oxidation of L-lactate to pyruvate and generation of NADH by LD. Therefore, we measured serum lactate, LD, and ethylene glycol retrospectively (Table 1). In two serum samples we also measured ethanol, using alcohol dehydrogenase on the Hitachi 704 analyzer—an assay operating on the same principle as the ethylene glycol enzymatic assay (Table 1). Although significant values for both ethylene glycol and ethanol were obtained in the enzymatic assays, neither substance was detected by gas chromatography.

This interference in the enzymatic assay for ethanol has been reported [5]. The limitation of the analytical specificity of this

<table>
<thead>
<tr>
<th>Case report</th>
<th>Ethylene glycol, mg/dl (mmol/L)</th>
<th>Ethanol, mg/dl (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>LD, U/L</td>
<td>Lactate, mmol/L</td>
</tr>
<tr>
<td>1</td>
<td>2020</td>
<td>4914</td>
</tr>
<tr>
<td>2</td>
<td>0148</td>
<td>13.3</td>
</tr>
<tr>
<td>2</td>
<td>0410</td>
<td>5364</td>
</tr>
<tr>
<td>3</td>
<td>0930</td>
<td>8405</td>
</tr>
<tr>
<td>2</td>
<td>1830</td>
<td>8914</td>
</tr>
<tr>
<td>3</td>
<td>0700</td>
<td>5479</td>
</tr>
</tbody>
</table>

**Random hospitalized patients with high serum LD**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ethylene glycol, mg/dl (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9340 ND 0.9 (0.1) 17 (4)</td>
</tr>
<tr>
<td>2</td>
<td>10 340 27.3 22.2 (3.6) 64 (14)</td>
</tr>
</tbody>
</table>

ND, not determined.

Table 1. Laboratory values.

Fig. 1. Reconstitution of the interference in the enzymatic assays for ethylene glycol and ethanol. LD-5 isoenzyme from human placenta (Sigma) and 0 ( ), 10 ( ), or 50 ( ) mmol/L D-lactic acid (Sigma) was added to serum from a healthy donor (LD, 146 U/L). LD was quantified with a Boehringer Mannheim/Hitachi 747, and the enzymatic assays for ethylene glycol (top panel) and ethanol (bottom panel) were performed as described in the text.
enzymatic assay for ethylene glycol, however, has not been previously documented. The interference may not have been appreciated in previous evaluations of the assay because test compounds, such as lactate and alcohols, were added to normal serum, not sera from critically ill patients [2]. To evaluate the performance of the enzymatic assays for ethylene glycol and ethanol in hospitalized patients with abnormal serum chemistry, we added di-lactic acid to two random serum samples with markedly increased LD and performed the enzymatic assays (Table 1). Lactate per se does not interfere in the assay; however, the concomitant presence of LD under the proper assay conditions produces NADH, resulting in substantial false-positive values in the enzymatic assays for ethylene glycol and ethanol. Given their correlation with the concentrations of LD and lactic acid, these false-positive values may be due to the LD-catalyzed conversion of serum lactate and reagent NADH to pyruvate and NADH.

To support this hypothesis, we reconstituted normal serum with increasing concentrations of LD-5 purified from human placenta (Sigma Chemical Co., St. Louis, MO) in the presence of 0, 10, or 50 mmol/L di-lactic acid (Sigma). The ethylene glycol enzymatic assay gave falsely positive results when LD serum activity was >3000 U/L, as did the ethanol enzymatic assay when LD serum activity was >2000 U/L (Fig. 1). The interference was most pronounced when the concentration of di-lactate exceeded 10 mmol/L. These data, however, should not be strictly extrapolated to the clinical setting because the contribution to total LD activity in this experiment was due solely to LD-5, and the concentration of lactate consisted of approximately equal amounts of L- and D-isomers. In hospitalized patients, increased LD activity may be due primarily to the contribution of a different isoenzyme or of more than one isoenzyme, and increased lactate concentration will most probably be the L-isomer. However, the data obtained from three hospitalized patients with increased serum LD activity (Table 1) support a clinically relevant guideline that significant false-positive results occur when the LD serum activity is at least 10-fold greater than the upper limit of the reference interval, and lactate is concomitantly at least 10-fold greater than the upper limit of its reference interval.

High concentrations of lactate and LD in sera from critically ill patients interfered in the enzymatic assay for ethylene glycol by increasing the production of NADH. Although lactate added to normal sera does not interfere with the assay, results should be interpreted cautiously in samples with high LD and lactate concentrations. Other interferences reported for the ethylene glycol enzymatic assay include glyceraldehyde and glycerol, which can be oxidized by the enzyme glycerol dehydrogenase [2]. The enzymatic assay is otherwise very specific, and other alcohols—including ethanol, methanol, and isopropanol—do not interfere with interpretation. Ethylene glycol poisoning of previously healthy individuals may result in lactic acidosis but will not typically result in increased LD. However, this analytical interference becomes clinically relevant in cases of suspected ethylene glycol poisoning of individuals with hepatic, renal, or cardiac disease who may manifest both lactic acidosis and increased serum LD. Positive results in the enzymatic assay for ethylene glycol in such cases should be confirmed with a different method such as gas chromatography.

We thank Brian Gilmore for expert technical assistance.

References


According to the recommendations of the National Cholesterol Education Program Adult Treatment Panel, low-density lipoprotein cholesterol (LDL-chol) should be used for screening and as a primary treatment criterion for patients with increased total cholesterol concentrations [1]. This makes the need for accurate measurements of LDL-chol a national public health imperative. The use of the Friedewald equation is attractive, but its accuracy is in doubt in plasma samples with triglyceride (TG) concentrations >4.5 mmol/L (400 mg/dL) [2–7]. A decennium ago we found the Friedewald equation to be accurate up to a TG concentration of 8 mmol/L [8]. Assuming that similar plasma samples were analyzed, these findings raise questions about the quality of the methods used in other laboratories, especially in the analysis of hypertriglyceridemic samples. The disposal of a direct LDL-chol method not interfered with by hypertriglyceridemia [9] prompted us to evaluate its accuracy in hyperlipidemic samples, including a selection with familial dysbetalipoproteinemia (FD). We hoped that this evaluation could shed more light on our previously reported claim concerning the accuracy of the Friedewald calculation compared with that of the reference method [8].

Overnight fasted blood samples from healthy persons and from patients with various types of hyperlipoproteinemia were drawn into Vacutainer Tubes containing EDTA (Becton Dickinson, Meylan Cedex, France). Plasma samples were analyzed fresh. If sufficient amounts of plasma were available, two aliquots were stored at -80 °C for >3 months; one was supplied with saccharose (final concentration 6 g/L). Patients were classified into the different phenotypes according to the criteria of Fredrickson et al. [10] with cutoff limits for plasma cholesterol, TG, and LDL-chol of 6.5, 2.0, and 4.6 mmol/L, respectively. Plasma samples with lipemia, especially those from subjects with FD, were preferentially included. This means that the frequency of samples with FD was considerably higher than in the healthy population. The 217 fresh plasma samples analyzed included, by selection, 63 with a plasma TG concentration >4.5 mmol/L, among which were 31 with a plasma TG >8.0 mmol/L; 11 had a VLDL-chol/plasma TG ratio >0.69