GC of 18 mg/L). The specimen collected 7 h after dosing and all subsequent specimens showed isomethene concentrations <5 mg/L by GC and were negative by EMIT. Therapeutic use of Midrin may involve several doses during the day, presumably leading to an increased chance of a urine specimen testing positive by EMIT.

Xanthine Interference in the Kodak "Ektachem" Determination of Uric Acid, Joseph L. Potter (Dept. of Pathol. and Lab. Med., Children's Hospital Medical Center of Akron, Akron, OH 44308)

In connection with studies related to the purines in serum of leukemia patients (1), xanthine interference was observed in the Kodak Ektachem enzymically based determination of uric acid, leading to significant underestimation of the latter's actual concentration—a finding that agrees with a previous report (2). In hereditary xanthinuria, total oxypurine concentrations in serum ranged from 1 to 9 mg/L (3). In normal children, values for hypoxanthine and xanthine were 1.2 ± 1.2 and 3.7 ± 3.4 mg/L (mean ± SD), respectively. However, in leukemia patients undergoing acute tumor lysis and in renal failure, hypoxanthine and xanthine concentrations were respectively 28 ± 14.9 and 158 ± 72.6 mg/L (4). Because such purine concentrations can occur clinically, the inhibitory phenomenon needed closer investigation.

Hypoxanthine and monosodium xanthine were added to pooled normal serum, and the final concentrations were verified by paper chromatography. Uric acid was determined according to the manufacturer's instructions. Interference with uric acid determination was first observed at a xanthine concentration of 100 mg/L in serum, and an essentially linear relationship between the xanthine concentrations from 156 to 520 mg/L and the range of interference from 10% to 36.5% was established:

<table>
<thead>
<tr>
<th>Xanthine, mg/L</th>
<th>Interference (% decrease from base pool)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3.0</td>
</tr>
<tr>
<td>156</td>
<td>10.0</td>
</tr>
<tr>
<td>250</td>
<td>16.5</td>
</tr>
<tr>
<td>310</td>
<td>20.0</td>
</tr>
<tr>
<td>400</td>
<td>26.7</td>
</tr>
<tr>
<td>520</td>
<td>36.5</td>
</tr>
</tbody>
</table>

Hypoxanthine had no effect in concentrations up to 400 mg/L, a value that far exceeds any clinically observed in leukemia patients.

The interference would seem to be most reasonably explained by inhibition of uricase activity by purines structurally related to uric acid, in this case xanthine (5). The practical significance of this degree of error would appear to be somewhat minor under the usual conditions of clinical management, but interference by xanthine is a well-documented and established phenomenon that deserves general consideration and might assume specific importance, for example, in a controlled research setting. Obviously, it could play a role in any enzymatically based uric acid method under the appropriate conditions. The maximum solubility of xanthine in serum at pH 7.4 and 37 °C reportedly is 100 mg/L, a widely accepted value (3). Although I did no detailed investigations of the solubility of xanthine in serum, the present studies make it evident that this has been substantially underestimated.

References

Serum Haptoglobin Concentrations in Concurrent Hemolysis and Acute-Phase Reaction, D. L. Warke, D. A. Marchand, and F. Van Lente (1) Dept. of Clin. Chem., Overlook Hospital, summit, NJ 07901, and (2) Dept. of Biochem., The Cleveland Clinic, Cleveland, OH 44106)

It is well known that serum haptoglobin concentrations can be increased by inflammation or decreased as a result of intravascular hemolysis. We wanted to ascertain whether haptoglobin could be a useful indicator of hemolysis in the presence of an acute inflammatory insult. Therefore, we studied a group of 58 cardiac-surgery patients not receiving valve replacements who underwent cardiopulmonary bypass, a procedure that results in significant hemolysis (7) as well as surgically induced inflammation owing to tissue trauma. The degree of acute-phase response was determined by concurrent measurement of C-reactive protein (CRP).

Sample was serum before and the morning after surgery and stored frozen at −70 °C. We determined haptoglobin by immunoassay, using the reagents and protocol from Atlantic Antibodies, Scarborough, ME 04074, and in a Cobas Para analyzer (Roche Diagnostic Systems, Nutley, NJ 07110). CRP was determined by immunoassay with the reagents and protocol from Du Pont, Wilmington, DE 19803, in a Cobas Bio analyzer (Roche). We used Student's t-test to compare data groups.

Serum haptoglobin concentrations decreased after surgery in 56 of 58 patients. Mean haptoglobin concentration was 1671 (SD 662) mg/L before surgery, 325 (SD 501) mg/L after. Of 58 patients, 48 exhibited postoperative haptoglobin concentrations <250 mg/L, the cutoff previously determined to be predictive of hemolysis (2). Before surgery the lowest concentration observed was 550 mg/L. Of the remaining cardiac-surgery patients whose haptoglobin concentrations did not drop below 250 mg/L, significant decreases in haptoglobin did occur: mean decrease = 1345 (SD 160) mg/L, P ≤0.001. CRP concentrations increased after surgery in all patients: pre-surgery mean 12.1 (SD 21) mg/L, post-surgery mean 75.1 (SD 31) mg/L. This change is consistent with the initiation of an acute-phase reaction (2).

In summary: haptoglobin values may be used to indicate
hemolysis in patients exhibiting a concurrent acute-phase reaction as indicated by increases in C-reactive protein. The sensitivity of haptoglobin at a cutoff of 250 mg/L, however, is somewhat less than in situations where only hemolysis is present (2). Nonetheless, it is apparent that the decline in serum haptoglobin concentrations, owing to its complexing with hemoglobin and eventual removal via the reticuloendothelial system, occurs significantly faster than serum haptoglobin increases as an acute-phase reactant.

References

Value of Polyacrylamide Gradient Gel Electrophoresis of Lipoproteins for Determining HDL Cholesterol, F. Collais (Laboratoire d'Hygiène, de Prévention et d'Éducation Sanitaire, Université René Descartes, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France), D. Roche (Laboratoire de Biochimie, Hôpital Laennec, Paris), and J. P. Andreux (Laboratoire d'Hématologie, Hôpital Charles Foix, Ivry sur Seine)

Given the great clinical importance of high-density lipoprotein cholesterol (HDL), the development of a simple, routine method for its accurate quantification is in urgent demand. The reference method, ultracentrifugation, is expensive, laborious, and time-consuming. On the other hand, the more frequently used selective precipitation of lipoproteins under- or overestimates HDL and remains inappropriate for hypertriglyceridemic samples. Polyacrylamide gradient gel electrophoresis (PAGE) with pre-staining by Sudan Black B, currently used for classifying various lipoproteinemia, can also be used for densitometric determination of HDL: HDL (mmol/L) = HDL (%) × total cholesterol (mmol/L).

This application of PAGE has been described by others (1, 2), but questions of its specificity have generated much controversy. The effect of overloading with purified fractions of high-, low-, and very-low-density lipoproteins has been previously reported (2), so we evaluated the quantification of cholesterol in the gel matrix and compared the results with those obtained by PAGE densitometry. We assayed in triplicate 50 sera from fasting subjects (total cholesterol from 3.30 to 6.00 mmol/L; triglycerides from 0.40 to 1.60 mmol/L; phospholipids from 2.00 to 3.10 mmol/L), using ready-made cylindrical Lipoporphosphorus gels (Miles Laboratories, Elkhart, IN 46514). We used the reported conditions (1, 2), but with 0.200 mL of a "gel support" without staining, for electrophoretic separation. The "separation gel" was then removed and we cut out a 5-mm-wide zone of gel to isolate the HDL fraction.

To assay the cholesterol in this fraction, we placed each 5-mm segment of gel in a 2-mL glass syringe fitted with a 10/8 gauge needle. Exerting pressure on the piston, we could extrude a fairly fine gel suspension. We rinsed the syringe with 0.100 mL of a 0.15 mol/L NaCl solution and combined the rinse with the gel suspension. The method was calibrated with adding 20 μL of a 5.17 mmol/L cholesterol/calibrator to the same volume of "separation gel" and treating as described. We found the calibrator was well taken up in the matrix gel after a 1-h contact. A reaction blank consisted of a segment of pure gel treated under the same conditions. In each fraction, we determined the cholesterol enzymatically by adding 1 mL of cholesterol assay reagent (Bio Mérieux, Marcy l'Etoile, 92260 France) to the extruded gel plus the rinsing solution. Before reading the absorbance at 510 nm against the blank, we centrifuged the tubes at 5000 rpm for 10 min to eliminate any gel debris. Regression data [y = densitometric determination of HDLC; x = evaluation in the gel matrix] were: y = 0.87x + 0.34 (r = 0.95).

We also determined whether such a HDLC quantification depended on the triglycerides concentration. We assayed 96 sera from normal subjects or patients with type IIa, IIb, or IV hypertriglyceridemia, divided into three groups with identical concentrations of total cholesterol (TC) and HDLC (mean ± SD, mmol/L): Group I: TC = 5.10 ± 0.70, HDLC = 1.50 ± 0.012; Group II: TC = 7.60 ± 0.100, HDLC = 1.50 ± 0.060; Group III: TC = 6.20 ± 0.100, HDLC = 1.20 ± 0.015. We then divided each group into three sub-groups with low (<0.50 mmol/L), normal (>0.92, ≤1.30), and high (>2.00, =2.70) triglyceride concentrations. Intragroup analysis of variance with F <2.77 and P nonsignificant (<0.05) demonstrated that densitometric evaluation of HDLC was independent of triglyceride concentrations.

References

Measurement of Apolipoprotein A-I in Serum with the Technicon RA-1000 System, Glenn R. Irish, Thomas B. Ledue, and Denny M. Barrantes (Atlantic Antibodies, 10 Nonesuch Rd., Scarborough, ME 04074)

Since 1979, apolipoproteins A-I and B and their ratio have been viewed as potent predictors of risk to coronary artery disease (1-3). This concept has led to the development of assays for the quantification of these apolipoproteins with analytical tools common in the clinical laboratory. We evaluated a commercially available kit for the turbidimetric assay of apolipoprotein A-I (Atlantic Antibodies, Scarborough, ME 04074) on the RA-1000 System (Technicon Instruments, Tarrytown, NY 10591), a random-access clinical chemistry analyzer. We found this assay to be precise, accurate, and rapid.

Assay-specific parameters were programmed according to the package insert accompanying this kit. Antibody reagent was diluted with the Apo A-1 Antibody Diluent provided, which contains a detergent and does not require filtration. Calibration materials and test samples were diluted with the Saline Diluent provided. Microvolumes of antibody and calibration standards were pipetted into reaction cuvettes to calibrate the instrument. Subsequent to calibration, test samples were assayed and concentrations determined from the stored curve. Analysis of up to 20 samples was complete within 20 min.

Within-run and day-to-day imprecision at each of three