urine. Physical examination revealed no peripheral edema and the only significant finding was systolic hypertension (170/70). Electrophoresis of protein in serum showed a monoclonal band of 38 g/L (IgG-κ) with a total serum protein of 98 g/L. Urea nitrogen and creatinine in serum assayed with the Astra 8 analyzer were 5.7 and 716 mmol/L, respectively. A renal ultrasonic test demonstrated small echogenic kidneys without hydronephrosis. A 24-h urine collection contained 2.9 g of protein (predominantly monoclonal κ light chains and albumin). Endogenous creatinine clearance was 4 mL/min. 99mTe-labeled diethyl- dethiatriaminediacetic acid clearance was 6 mL/min.

We sought an explanation for the discordance between the urea nitrogen and creatinine values determined with the Astra 8. The serum creatinine measurement by the Astra 8 with the picric acid method was corroborated by similar results on the Ektachem DT 60 (Eastman Kodak Co., Rochester, NY), which uses a solid-phase creatinine assay. Thus, the two creatinine measurements and clearance measurements both showed that the glomerular filtration rate was severely depressed in this patient and focused attention on the surprisingly low urea nitrogen concentration in serum. Next, to search for a possible high-Mₙ inhibitor of the Astra 8 assay of urea nitrogen, we compared results between intact serum and a serum ultrafiltrate (molecular mass exclusion ~30 kDa). Serum from a patient without myeloma but with renal failure and a comparable serum creatinine concentration was included as a control. The serum concentration of urea nitrogen in the myeloma patient was sharply increased by more than fivefold in the ultrafiltrate, suggesting that a high-Mₙ inhibitor of the assay was removed by this maneuver (Table 1). No effect was observed in the control patient's serum.

An additional investigation of the myeloma patient was made with the Olympus AU-500 (Olympus Co., Lake Success, NY), which uses an enzymatic method for urea (cleavage of urea by urease) similar to that used by the Astra 8 but a different detection system (disappearance of NADH measured spectrophotometrically rather than appearance of NH₃ measured by changes in conductance). Although the creatinine results were similar on both instruments, the value for urea nitrogen was about fivefold higher on the AU-5000. These results incriminated the Astra 8 detection system and suggested that this patient's serum contains a high-Mₙ substance that causes the Astra 8 to give an inappropriately low value by interfering with the measurement of enhanced ionic conductivity. This could result from suppressed ionic mobility by a moiety in the patient's serum (e.g., by coating the electrode or by binding the newly formed NH₃° or HCO₃⁻ ions). Because this substance is not ultrafiltrable, it may be myeloma protein. Three other patients with multiple myelomas (two with IgG-κ, and one with IgG-λ) did not show a similar inhibition of the Astra 8 urea assay.

This was our first experience with inhibition of the Astra 8 assay of urea nitrogen by serum from a patient with multiple myeloma. The assay was previously known to be subject only to interference by fluoride ions, an inhibitor of urease. No precedent for this phenomenon can be found in the literature. We obtained similarly low urea results with the Synchon CX3 analyzer (Beckman Instruments), which also measures ionic conductance. We suggest, therefore, that discordantly low urea nitrogen concentrations in serum obtained with the Astra 8 in patients with increased serum creatinine concentrations may not always be attributable to low urea-production rates (e.g., from starvation or liver disease). We recommend that, for patients with multiple myeloma or other monoclonal gammopathy, renal function should be routinely evaluated by measuring both urea and creatinine, and discordantly low urea results should be repeated by a method that does not rely on ionic conductance.

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Interference of Cefotiam with Total Bilirubin Measured with the Ektachem Analyzer

To the Editor:

Recently we observed concentrations of total bilirubin of 8.55 and 85.5 μmol/L in the same patient in two subsequent samples without any correlation to the clinical situation. Our re-evaluation of the sera showed a specific interference with the total bilirubin assay with the Ektachem analyzer. The bilirubin assay with the Hitachi 717 analyzer with 2,5-dichlorodiphenylhidrazonium salt (DPD) reagent was not affected. This was surprising because the Ektachem instrument works excellently and has the least interference from endogenous and exogenous compounds of all our clinical chemical analyzers (1).

Searching for the cause of the interference, we were able to identify one patient for whom the effect could be clearly attributed to the antibiotic cefotiam (Spizef®; Takeda Chemicals, Osaka, Japan). Cefotiam is not available in the U.S., but is available in Japan, Germany, and other European countries.

Because cephalosporins are among the antibiotics most often used in severely ill patients, and because cefamandol is the only cephalosporin known to interfere with the bilirubin assay (2), we decided to investigate the effect of different cephalosporins on the assay of bilirubin in vitro. Assuming an initial distribution volume of ~14 L, corresponding to the extracellular fluid, we investigated concentrations of the antibiotics between one normal dose per 14 L and one-tenth of that concentration, which is reached presumably at the end of the dosing interval. Thus, the antibiotic concentrations were 1–1000 mg/L. The drugs were obtained as dry substances for intravenous infusion from the manufacturer and reconstituted with doubly distilled water as indicated. Standards at concentrations in the analytical range were made from the primary antibiotic solutions and a pool of serum with a low bilirubin content with no negative interference, checked by adding serum with a positive bilirubin test. Bilirubin and its subfractions were assayed with the Ektachem analyzer (Kodak, Stuttgart, F.R.G.) and compared with the results of DPD method on the Hitachi 717 analyzer (Böhringer-Hitachi, Mannheim, F.R.G.).

We investigated the cephalosporins cefsoludin (Takeda Chemicals), cef-
taxime and cefuroxime (Höchst, Frankfurt, F.R.G.), cephalzin (Lilly, Giesen, F.R.G.), ceftazidime (Glaxo, Bad Oldesloe, F.R.G.), cefotixin and a combination of imipenem and cilastatin (MSD, Munich, F.R.G.), ceftriaxone (Roche, Basel, Switzerland), cefotaxime (Boehringer, Mannheim, F.R.G.), and cefotiam. Except for cefotiam, none of these interfered with the bilirubin assay (Table 1).

Cefotiam interferes greatly with the assay of bilirubin. According to Simon and Stille (3), just after intravenous infusion of 1 g of cefotiam, human volunteers showed an initial serum concentration of ~100 mg/L. The drug's half-life was ~45 min, so after 1 h the concentration was 19 mg/L, and after 4 h the concentration was 1.1 mg/L. Therefore, interference by cefotiam is clinically significant if the drug is administered parenterally and blood samples are obtained within the first 2 h thereafter. By direct inspection of Ektachem slides, one can distinguish false-positive and positive bilirubin reactions. A true bilirubin increase is apparent as a homogenous red color on the whole slide, whereas cefotiam gives a homogenous red color in the center, surrounded by a pale yellow ring. The slide for the assay of unconjugated and conjugated bilirubin (BU/BC) in the Ektachem is not affected by the drug. The analyzer therefore calculates the false-positive bilirubin essentially as 8-bilirubin, which is a rare event in normal sera. Analyzing the serum on another system (e.g., with the DPD reagent) will give normal results.

References

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Contaminant Lead in Blood-Collection Tubes for Trace-Element Studies

To the Editor:
Requirements for accurate measurements of blood lead (PbB) concentration are becoming more stringent as PbB baseline and "action" concentrations decrease (1). Reductions in baseline PbB concentrations have been associated with decreases of releases of industrial lead into the environment (2). Concurrent reductions in PbB action concentrations have resulted from studies indicating lower thresholds for lead toxicity (3). Both reductions have increased the need to accurately quantify lead contamination of specimens during sampling, storage, and analyses, as previously detailed by Patterson and Settle (4).

One potential source of contamination is the lead in blood-collection tubes. Although some studies have rigorously quantified the amount of contaminant lead in these tubes (e.g., 1, 5), that lead is commonly dismissed as inconsequential in most routine PbB concentration measurements. This is often justified by the use of blood-collection tubes specified for trace-element studies and by statements that concentrations of contaminant lead in the tubes are below detection limits. However, the amount of contaminant lead in blood-collection tubes can be readily measured with standard laboratory instruments [graphite furnace atomic absorption spectrometer (GFAAS), ASV, inductively coupled plasma mass spectrometry] by using trace-metal-clean techniques (6), and it has been shown that contaminant lead has invalidated most measurements of PbB concentrations (5).

Further, contaminant lead in blood-collection tubes may substantially increase the amount of lead measured in individuals with PbB concentrations <48 nmol/L (10 μg/L) and, surprisingly, may be greatest in blood-collection tubes specified for trace-element studies (5).

We measured contaminant lead in the PbB of tubes (B-D nos. 6484, 6488, and 6527) of blood-collection tubes (Vacutainer Tubes; Becton Dickinson, East Rutherford, NJ). We briefly (5 min) flushed 20-50 of each type of tube with high-purity (18.3 MΩ/cm) water (pH 5.5) to solubilize the labile contaminant lead in the tubes. We also assessed the temporal release of contaminant lead from the tubes over a seven-day refrigeration period and determined the amounts of contaminant lead in the tubes and in the anticoagulating agent (heparin) within the tubes. We measured lead concentrations with a GFAAS (Model 5000; Perkin-Elmer Corp., Norwalk, CT), with standard additions and with the manufacturer's recommended operating conditions. We conducted all laboratory and analytical procedures in a trace-metal-clean laboratory (Class 100) with the ultraclean techniques detailed by Patterson and Settle (4). The detection limit for the lead contamination measurements was 0.02 μg/L. We intercalibrated the GFAAS measurements with measurements by isotope dilution thermal ionization mass spectrometry (IDMS) with the use of Certified Reference Materials 1549 and 1577a from the U.S. National Institute of Standards and Technology (Gaithersburg, MD) and CASS-2 and NASS-2 from the Canadian National Bureau of Standards (Ottawa, Canada). Additional details of these procedures, including contaminant lead concentrations of individual reagents and containers, were reported elsewhere (6-8).

The amount of contaminant lead in the Vacutainer Tube for trace-element studies (B-D no. 6627) is 10-fold greater than the amount in one type of Vacutainer Tube for chemistry and

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Table 1. Assay of Bilirubin (mg/L)* in the Presence of Increasing Amounts of Cefotiam

<table>
<thead>
<tr>
<th>Cefotiam concn in serum, mg/L</th>
<th>TBIL</th>
<th>BU, Exta</th>
<th>BC, Exta</th>
<th>DELB, Exta</th>
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<td>H717</td>
<td>Ektas</td>
<td>Ektas</td>
<td>Ektas</td>
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<tr>
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TBIL, total bilirubin; BU, unconjugated bilirubin; BC, glucuronide-conjugated bilirubin; DELB, 8-bilirubin; H717, Hitachi 717 analyzer; Ektas, Ektachem analyzer. * 1 mg = 1.710 μmol.