conjugated bilirubin (CBIL) > 50 mg/L. However, the bilirubin standards used in most interference studies, including our own, are primarily unconjugated bilirubin. Therefore, we prepared several mixtures of a normal adult plasma and a plasma with high CBIL content (>120 mg/L). Plasmas were chosen so that the normal and icteric plasma had identical P1 values by the Ektachem method.

Mixtures were analyzed by both P1 methods. Regardless of CBIL concentrations in the mixtures, the P1 value did not change on the Ektachem. In contrast, with the 747 we found a proportional decrease in P1 values for samples with increasing CBIL concentrations. The negative bias on the 747 was as great as 10 mg/L when the CBIL was >150 mg/L. To confirm the presence of an interference, we compared values obtained from a well-accepted manual, protein-free, molybdate-based method (1). Assay of 14 samples with CBIL values from 36 to 102 mg/L produced P1 values 2–9 mg/L lower on the 747 than those obtained from the manual comparison method (Figure 1). P1 concentrations of these samples from the manual method were 290–570 mg/L (mean ± SD = 37.9 ± 7.1). Samples with total bilirubin <20 mg/L produced equivalent results by both methods. Ektachem P1 values averaged 1.8 mg/L higher (range 0–4) than the manual method values, but these differences were independent of CBIL values (not shown).

Monitoring absorbances (340 nm primary/376 nm secondary) of the P1 reaction on the 747 revealed a potential cause for the negative interference. The 747 makes initial absorbance readings after addition of reagent 1 (R1; acid and detergent) to obtain a sample blank and makes subsequent readings after addition of the ammonium molybdate reagent, R2. The difference in absorbance between the two readings (after correction for sample dilution) is used to calculate P1 concentration. The initial R1 absorbance for all nonicteric samples was 0 or slightly positive (100–200 arbitrary absorbance units). In contrast, samples containing >40 mg/L CBIL had a negative initial absorbance, and the magnitude of the negative R1 absorbance was proportional to CBIL.

Preliminary data from our laboratory and from the manufacturer suggest that altering the secondary wavelength may eliminate this interference. Until this is corrected, we advising that P1 not be determined for icteric samples on the Hitachi 747. Finally, this observation demonstrates that the use of commercial bilirubin standards for interference studies may not always mimic the interferences caused by in vivo forms of bilirubin.

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Urinary Oligosaccharides in Pregnant or Lactating Women: Pitfall in Screening

To the Editor:

Urinary oligosaccharide screening is a useful first-line diagnostic analysis to delineate disorders of glycoprotein degradation (1). The method is not, however, without problems in interpretation, one of the commonest being the excretion pattern found in neonates, which may often cause confusion with that of mannosidosis. In cases of "adult-onset" glycoproteinoses, the oligosaccharide excretion patterns may be weak, and such patients may be overlooked. In the course of metabolic workup of a female adult patient suspected of having a glycoproteinosis, we found a urinary excretion pattern akin to that of mannosidosis, despite a normal activity of α-mannosidase.

The 29-year-old patient was referred because of mental retardation and schizophrenia. She had bilateral malar hypoplasia, epicanthal folds, and no organomegaly. She was cooperative and verbal and understood simple commands. No facial asymmetry was apparent. Magnetic resonance imaging revealed hypoplasia of the corpus callosum and mild cortical atrophy. Her chromosones, amino acids, organic acids, very-long-chain fatty acids, carnitine, and biotinidase were all normal. Urinary oligosaccharide screening revealed a pattern similar to that of neonates. α-Mannosidase activity in leukocytes was normal, and a peripheral blood smear showed no vacuolated lymphocytes.

Because the patient had just delivered a baby, we decided to examine urines from pregnant or lactating women for abnormal urinary oligosaccharide excretion patterns. Urinary oligosaccharides were examined by thin-layer chromatography (1).

The patterns were consistent with that of neonates; i.e., many bands were present that stained violet with orcinol, suggesting the presence of glucose or galactose (Figure 1). Patients with mannosidosis have patterns that stain brown with orcinol reagent. Urines from the pregnant and lactating women we examined (n = 70) also gave a similar pattern. No attempt was made to correlate gestational or postpartum age with the degree of oligosaccharide excretion. In all cases this pattern had diminished by 6 months postpartum, as was the case in neonates.

Human milk contains a large number of oligosaccharides, some of which are excreted in the urine of pregnant and lactating women (2). Lactose and oligosaccharides with a lactose backbone are the most predominant (3).

![Fig. 1. Thin-layer chromatographic pattern of urinary oligosaccharides](image-url)
Excretion starts in the 13th week and increases thereafter until delivery, whereas more complex oligosaccharides are excreted. The chain length varies from disaccharides to heptasaccharides. In patients with mannosidosis, urinary oligosaccharides of similar chain lengths are present (4), although of more variable structure.

The complex urinary oligosaccharide excretion patterns seen in breast milk-fed neonates probably represents milk oligosaccharides. This is confirmed by our finding of a similar pattern in pregnant and lactating women, although the precise origin of these oligosaccharides remains unknown. Our case represents a further pitfall in urinary oligosaccharide screening. In the diagnostic workup of adults suspected of having a glycoprotein storage disease, urinary oligosaccharide excretion patterns should be interpreted with caution.

References

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** Phospholipids in EDTA-Treated Plasma and Serum

To the Editor:

The Test-Combination Phospholipids kit method (cat. no. 691844; Boehringer Mannheim Diagnostics, Mannheim, Germany) is an enzymatic colorimetric test based on the method of Takayama et al. (1). In this method, phospholipids are hydrolyzed by phospholipase D (EC 3.1.4.4) and the liberated choline is subsequently oxidized by choline oxidase to betaine with the simultaneous production of hydrogen peroxide, which by oxidation couples 4-aminoantipyrine and phenol to yield a chromogen with a maximum absorption at 505 nm.

The sample materials recommended by Boehringer Mannheim are serum, heparinized plasma, or EDTA plasma. When we used EDTA plasma, values for the choline-containing phospholipids were consistently much lower than expected. We then performed comparison assays with all three recommended sample types.

We collected 40 mL of blood from five subjects (three men, two women) by venipuncture of an antecubital vein. Blood aliquots were dispensed into various proprietary plastic or glass tubes with or without anticoagulants (Table 1). The volume of blood added to the tubes containing anticoagulants was as recommended by the manufacturers, or less (see Table 1). Thus, in some tubes the concentration of EDTA or heparin was as recommended; in others, it was greater.

The blood in the clotted blood containers was allowed to clot for 1 h at 37°C. Blood in all other tubes was kept at room temperature during that hour. All blood tubes were simultaneously centrifuged at 900 x g for 15 min. The respective plasma and serum samples were subsequently separated from the erythrocytes by aspiration with Pasteur pipettes.

We have adapted the Test-Combination Phospholipids kit of Boehringer Mannheim to the Cobas Bio system (Roche Diagnostics, Nutley, NJ). Phospholipid concentrations determined in the above plasma and serum samples were similar whether analyzed by the manual procedure described by Boehringer Mannheim or the Cobas adaptation (automated) method.

As Table 1 shows, the phospholipid concentrations measured in heparin-treated plasma were similar to those in serum. Even at 2.5 times the recommended amount of heparin, the plasma gave similar phospholipid concentrations as in the serum. However, in the glass tubes with the recommended EDTA concentration, one of five readings was vastly different. Also in glass tubes, if 1.5 times the recommended amount of EDTA was used, then all five phospholipid values were low. In the plastic tubes, both the recommended concentrations and 2.5 times greater concentrations of EDTA in plasma gave low phospholipid concentrations.

EDTA is reported to inhibit phospholipase D (2). Groß and Mätz (3) have subsequently suggested that calcium ions should be added to commercially available test kits for choline-containing phospholipids that use this enzymatic procedure, especially if lipoprotein classes are prepared by ultracentrifugation in EDTA-containing density solutions. However, Groß and Mätz also stated that EDTA-K₂ at a final concentration of 1.5–2 mg/mL of blood had an adverse effect on the phospholipid results obtained. In our studies, we found that at a final EDTA-K₂ concentration ≥1.6 mg/mL, all choline-containing phospholipids were underestimated by -94%. Thus, we were especially careful with sam-

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Table 1. Phospholipids (g/L) Determined in Concurrent Serum, Heparinized Plasma, and EDTA Plasma Samples from Five Subjects

<table>
<thead>
<tr>
<th>Heparin</th>
<th>EDTA, plastic</th>
<th>EDTA, glass</th>
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<tr>
<td>Serum, plastic*</td>
<td>14.3 IU</td>
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</tr>
<tr>
<td>1.84</td>
<td>1.91</td>
<td>1.83</td>
</tr>
</tbody>
</table>

* 10-mL Sterile Clotted Blood Container; Disposable Products, Ingle Farm, South Australia 5096.

** 7-mL Sterile Vacutainer Tube; Becton Dickinson Vacutainer Systems, Rutherford, NJ.

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