and measure the drug concentration. The experiment should be repeated at least three times, and the results evaluated.

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Drug Interference in Turbidimetry and Colorimetry of Proteins in Urine

To the Editor:

Colorimetry of urinary proteins based on Ponceau Red co-precipitation (1) has been applied in our laboratory for three years. Results were occasionally inconsistent with the clinical state and the medical history of the patient. A systematic duplication of the protein assays by a turbidimetric procedure (2) showed significant discrepancies for urines of patients being treated with aminoglycosides (gentamicin or kanamycin) or miconazole.

Assays of urine to which these drugs were added showed that neither the Ponceau Red assay nor the turbidimetric assay is free of interference. Table 1 showed an example of such experiments on a urine sample containing about 1 g of protein per liter.

In the turbidimetric method, miconazole at concentrations usually reached during therapy increased the apparent protein concentration by a factor of 1.5 to 2.5 when the true protein concentration was higher than 0.5 g/L. It did not interfere when proteins were absent or at low concentrations. Aminoglycosides did not interfere in the turbidimetric method at any concentration of protein or of drug.

The colorimetric procedure was unaffected by the presence of miconazole, but aminoglycosides at concentrations reached during therapy gave an apparent proteinuria with protein-free urines and increased the results by a factor of 2 to 4 when proteins were present. The precipitation of an insoluble salt of aminoglycosides with Ponceau Red is the cause of the interference.

These observations led us to change the Ponceau Red method slightly. Urinary proteins are first precipitated with trichloroacetic acid (50 g/L in 0.5 mol/L NaCl) without Ponceau Red. The supernate is discarded. The original procedure of Pesce and Strande (1) is then carried out on the precipitated protein redissolved in 0.2 mol/L NaOH.

This preliminary precipitation of proteins did not significantly influence the precision, accuracy (by recovery test), or linearity of the original procedure.

References

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Determination of Deferoxamine Chelated Iron

To the Editor:

Deferoxamine (desferrioxamine mesylate; Desferal, Ciba-Geigy) is used to treat iron accumulation in tissues, caused by (e.g.) iron poisoning or treatment with long-term intermittent doses of packed erythrocytes, and in therapy of diseases involving recurrent internal bleeding such as hemolsiderosis. Because only traces of iron are normally present in urine, the effectiveness of the administration of this drug can be monitored indirectly by measuring the urinary excretion of deferoxamine-chelated iron. According to Wöhler (1) it seems justified to regard substantially increased iron excretion after injection of deferoxamine, together with an increased iron concentration in serum, as a sign of iron-storage disease.

The better to measure iron in serum, we exchanged our former method (2), protein precipitation with trichloroacetic acid and subsequent addition of the deferoxamine-sensitive color agent, 2,4,5-tripirydyl-s-triazine (TPTZ), for a direct procedure in which deproteinization is omitted and a buffered (pH = 2) ferrizone reagent solution is used (3). Thiourea is added to this solution to suppress the nonspecific contribution of copper (4) to the colorimetric reaction.

Run samples in duplicate, with use of disposable microcuvets (Sarstedt, type 742). Place 400 μL of a buffered reducing reagent (containing, per liter, 11 g of citric acid, 9 g of NaCl, 2 g of thiourea, and, just prior to use, 4 g of ascorbic acid) in a cuvet and add 50 μL of serum or urine. Mix and record the absorbance at 570 nm (blank reading). Then add 20 μL of ferrizone solution (17.5 g/L; Sigma Chemical Co., cat no. P-9762) and (for serum) again record the absorbance at 570 nm after 5 min. For urine specimens, instead make the final absorbance reading after the reaction mixture has been incubated for 2 h at 37 °C.

In the calculation of original iron concentration a volumetric correction factor for the blank reading of 0.957, due to the added ferrizone reagent, must be incorporated.

The standard curve is linear to at least 120 μmol of Fe per liter.

Using 55 serum specimens, we compared this method with our former TPTZ procedure. An excellent relationship was observed: y(ferrizone) = 1.02x (TPTZ) – 0.22 μmol/L (r = 0.956, Sxy = 1.64, Sxx = 1.67); observed concentration range: 4–30 μmol of Fe per liter. “Seronorm” control material (assigned value 27 μmol/L), included in all analytical runs, gave a mean value of 26.7 μmol/L with a day-to-day CV of 2.4% (n = 20). Addition of deferoxamine to serum up to 370 μmol/L, a concentration far beyond the therapeutic (5), produced no detectable decrease in the colorimetric signal if the ferrizone procedure was used. However, for the TPTZ method and also for the aca discrete analyzer (Du Pont; color reagent, bathophenanthroline) a significant decrease was seen (up to 70 and 48%, respectively). The recognized underestimation of iron by the aca in the presence of deferoxamine can be obviated by the addition of extra thioglycolic acid (6).

With the ferrizone method for iron a poor reproducibility was observed in

Table 1. Effect of Drugs on the Determination of Protein Concentration in Urine by Three Different Methods

<table>
<thead>
<tr>
<th>Drug added</th>
<th>Turbidimetry</th>
<th>Original Ponceau Red</th>
<th>Modified Ponceau Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.00</td>
<td>1.05</td>
<td>1.02</td>
</tr>
<tr>
<td>Gentamicin 40 mg/L</td>
<td>1.10</td>
<td>1.98</td>
<td>1.03</td>
</tr>
<tr>
<td>120 mg/L</td>
<td>1.12</td>
<td>3.75</td>
<td>1.03</td>
</tr>
<tr>
<td>Kanamycin 0.33 g/L</td>
<td>1.16</td>
<td>1.37</td>
<td>1.01</td>
</tr>
<tr>
<td>1.0 g/L</td>
<td>1.20</td>
<td>2.45</td>
<td>1.03</td>
</tr>
<tr>
<td>Miconazole 20 mg/L</td>
<td>1.92</td>
<td>1.06</td>
<td>1.06</td>
</tr>
<tr>
<td>50 mg/L</td>
<td>1.88</td>
<td>1.05</td>
<td>1.06</td>
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