Table 1. Data on the Patient

<table>
<thead>
<tr>
<th>Month/year</th>
<th>Thyroxin dosage</th>
<th>T4</th>
<th>T3</th>
<th>THU</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/76</td>
<td>300</td>
<td>161</td>
<td>—</td>
<td>116</td>
<td>15</td>
</tr>
<tr>
<td>3/77</td>
<td>300</td>
<td>176</td>
<td>—</td>
<td>118</td>
<td>26</td>
</tr>
<tr>
<td>4/77</td>
<td>300</td>
<td>152</td>
<td>2.1</td>
<td>112</td>
<td>22</td>
</tr>
<tr>
<td>7/77</td>
<td>300</td>
<td>172</td>
<td>2.6</td>
<td>113</td>
<td>40</td>
</tr>
<tr>
<td>4/78</td>
<td>300</td>
<td>138</td>
<td>1.9</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>9/78</td>
<td>200</td>
<td>128</td>
<td>—</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td>6/80</td>
<td>200</td>
<td>147</td>
<td>1.6</td>
<td>91</td>
<td>0</td>
</tr>
</tbody>
</table>

Reference interval: 65–135 μmol/L

1.6 ≤ T4 ≤ 4.4 μmol/L

1.4–2.8 ≤ T3 ≤ 8.5–11.5 μmol/L

Interference in thyrotropin assays due to endogenous human antirabbit antibodies (4–6) and unknown (7) mechanism, but they would not explain our findings.

Immunization to homologous glycoprotein hormones is recorded for human follicitropin (8), lutein (9), and choriongonadotrophin (10); however, use of these preparations is limited and hTSH is not available for routine clinical use. There is much inter-specific variation in the primary structure of pituitary glycoprotein hormones, but the biological effects residing in the β subunit cross a wide range of species barriers. While within-species cross reactivity resides in the α subunit, between-species cross reactivity resides principally in the β subunit (11). Biological effects may be neutralized in iso-immunized patients (3, 8, 9) with comparable interference in vitro bioassays (2), suggesting the importance of β-directed specificity of such isoantibodies (9).

This cause of apparent inappropriate increase in hTSH in patients with thyroid carcinoma who have been recently exposed to bTSH needs to be recognized and, in view of the frequency of this phenomenon (2) as well as the reports of allergy (4) to bTSH, the use of bTSH should be limited.

References


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Improved Method for Determination of "True" Creatinine

To the Editor:

Earlier (1–3), I described two simple procedures for determination of "true" creatinine by applying the Jaffe reaction directly to the serum in a alkaline picrate medium of pH 11.5; interference by other known chromogens is eliminated. Both procedures include a long incubation time and some other particular drawbacks. The first necessitates a separate blank serum and the use of two buffered alkaline picrate reagents, slight changes in which affect the results (1, 2). The second has certain critical features, especially the transformation of the serum specimen to a thin gelatinous coagulum when boiled (3).

I describe here a method based on the same principle, without these disadvantages, which gives similar values for serum and urinary creatinine as does a specific enzymic method (4). The procedure is as follows: Into each of two tubes place 0.2 mL of water and 0.2 mL of serum or 10-fold diluted urine. Add 0.8 mL of a 50 mmol/L solution of picric acid, vortex-mix for about 30 s, and centrifuge for 5 min at 3000 rpm. This
yields a clear, protein-free supernate, the proteins being completely precipitated as a coherent, gelatinous mass. Transfer 0.8 mL of the supernate to another tube. Add 0.2 mL of phosphate buffer, pH 12.0 (per liter, 0.5 mol of Na₂HPO₄ and 0.5 mol of NaOH) and incubate at 37 °C.

The resulting mixture is effectively buffered at pH 11.5 with a difference not exceeding ±0.02 pH units, whether aqueous creatinine standard or serum or urine specimens are being used. After 25 min measure the absorbance of the sample at 525 nm vs water in a 10-mm cuvet. At 500 nm, the maximum-absorption wavelength, absorbance from creatinine-free serum specimens is significantly less than that obtained with water, a difference that progressively disappears at higher wavelengths until finally it is zero in all instances at 525 nm. The resulting color is stable for at least an hour and is linearly related to concentration of 0.50 to 1,500 A for aqueous creatinine standards of 50 to 1500 μmol/L.

I tested the same potential interfering substances as before (1), but as we have previously demonstrated, I again found no interference with the present method. Bilirubin up to 500 μmol/L and hemoglobin up to 150 μmol/L also do not interfere.

Analytical recovery is complete, ranging from 98.5 to 102% for 50, 250, and 500 μmol of creatinine added per liter to sera containing creatinine concentrations of 75, 200, 450, and 800 μmol/L.

Precision is good: 10 analyses of serum specimens containing 85, 150, 650, 810, and 1240 μmol of creatinine per liter showed within-day CVs ranging from 1.1 to 2.5% and day-to-day CVs from 1.5 to 3%. The results of serum duplicates assayed on the same day with the use of different sample dilutions (0.05–0.20 mL) of sample averaged 250 (SD 5.5) μmol/L.

To assess the specificity of the method, I compared it with the fully enzymic method of Wahlefeld et al. (4). Results by the proposed method (y-values) and the comparison method (x-values) with specimens from 100 patients whose serum creatinine ranged from 45 to 1480 μmol/L and urinary creatinine from 5 to 14 mmol/L correlated well (r = 0.955, y = 1.031x + 0.061).

Analysis for serum creatinine of 40 women and 60 men, ages 20–50 years, without any former or present signs of renal disease, gave values of 44–85 μmol/L (68 ± 11) for women and 53–102 μmol/L (80 ± 13) for men.

The proposed method is simple, reliable, relatively rapid, and only requires the use of two low-cost, stable reagents at room temperature. It is suitable for use by analysts with little experience, in laboratories with common equipment.

These features recommend its widespread use for "true" creatinine analysis.

References

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Coomassie Blue Method: Work Duplicated

To the Editor:

The recent letter by Joern and Schmoele (Clin. Chem. 27: 1305, 1981) indicated that the Coomassie Blue test could be successfully adapted to the ABA-100. We have previously published similar results, and also showed that the results compared well with the Poncet-S dye binding method (1).

Reference

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Serum Ferritin Determination by Enzyme Immunoassay: Importance of Sample Dilution (the "Hook Effect")

To the Editor:

We use a commercial enzyme immunoassay kit for determination of ferritin in serum (NEIA ferritin; New England Immunology Associates, Inc., Cambridge, MA 02138). It is a "sandwich" method, described by Boenisch (1), in which polypropylene tubes coated with anti-ferritin antibodies are incubated with samples and then with alkaline phosphatase-labeled antibodies. The enzyme activity bound to the tubes is measured with p-nitrophenyl phosphate as the substrate. The calibration curve covers the range 0 to 500 μg/L. Reference values with this technique are 10–70 μg/L for women and 35–230 μg/L for men (2).

We have noted anomalous results for two sera. One was from a 75-year-old woman with icterus by retention, metastases to the liver, biliary stones, and probably primary carcinoma of biliary vesicle. The other was from a 47-year-old woman with carcinoma of the transverse colon and pulmonary metastases. For these two sera, we found higher absorbances for samples diluted five- and 10-fold than for undiluted samples. Values for more highly diluted samples (50- and 100-fold) were much higher than the less-diluted samples (Table 1). The values for the more highly diluted sera from both patients are very pathological, 12 690 and 9825 μg/L, and are concordant with the patient's pathology. These same observations were noted with two different lots of reagents. This paradoxically decreased absorbance (as compared with that expected) observed for very high ferritin concentrations is called the "hook effect," and has been noted for two-site immunoradiometric assays for ferritin by several authors (3, 4), but not yet for enzyme immunoassay.

In conclusion, this phenomenon must be taken into account when ferritin is determined by enzyme immunoassay by testing all samples on undiluted serum and at two different dilutions (five- and 10-fold). This precaution obviates the potential error of giving a low value to a patient who has a true pathologically increased concentration of ferritin.

References