Curves for successive dilutions of a purified serum extract paralleled standard curves (data not shown). No 1,25(OH)₂D was detected in a charcoal-stripped serum sample.

Mean concentrations in serum from 21 healthy men and premenopausal women were 54 (SD 19) ng/L (RIA 1) and 53 (SD 18) ng/L (RIA 2), respectively. Decreased 1,25(OH)₂D was observed in hemodialysis patients suffering from chronic renal failure: 15 (SD 6) ng/L and 17 (SD 6) ng/L for RIA 1 and RIA 2, respectively (n = 28). We compared the two RIA methods on the basis of results from 55 analyses obtained for healthy volunteers and patients with chronic renal failure and nephropathies. Least-squares analysis yielded a satisfactory correlation: y = 1.14x - 3.37; r = 0.93.

Evidently, for the routine determination of 1,25(OH)₂D in serum, an RIA method based on easily accessible antibodies raised in egg yolk is a valuable alternative to the conventional procedure with use of antibodies from rabbits.

References

Possible Interference by Amiodarone and Desethylamiodarone in Thyroxin Assays, L. K. Law, C. K. Cheung, and R. Swaminathan (Dept. of Chem. Pathol., The Chinese Univ. of Hong Kong, Prince of Wales Hospital, Shatin, NT, Hong Kong)

Amiodarone, a widely used potent antiarrhythmic drug, is associated with disturbances in thyroid function. Clinical hypothyroidism and hyperthyroidism have been reported during long-term administration of amiodarone (1, 2). However, alterations in biochemical indices of the thyroid functions are more frequent. Melmed et al. (3) showed that the drug induces significant increases in thyroxin (T₄) and reverse triiodothyronine (T₃), with minor decrease in T₃. In their study, amiodarone did not cross react in any of the hormone RIAs. Recently, we observed that values for plasma T₄ (by EMI T₄; Syva Co., Palo Alto, CA) in two patients treated with 200 mg amiodarone daily were 404 nmol/L and 128 nmol/L, respectively, while free T₄ concentrations were normal. The T₄ measurements by an RIA/T₄ ("Gamma Coat Total T₄"; Clinical Assays, Cambridge, MA) were respectively 175 nmol/L and 74 nmol/L for the two samples. The patients were not taking any other drugs known to interfere with the EMI T₄ assay. We therefore investigated the effect of the drug on the EMI T₄ assay.

Amiodarone, up to 5000 mg/L, added to plasma samples did not cause interference. The effect (on T₄ results) of adding desethylamiodarone, a major metabolite of amiodarone, is summarized below:

<table>
<thead>
<tr>
<th>Expected T₄ nmol/L (RIA)</th>
<th>0°</th>
<th>40°</th>
<th>79°</th>
<th>157°</th>
<th>313°</th>
<th>625°</th>
</tr>
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<tr>
<td>0.0</td>
<td>0</td>
<td>&lt;26</td>
<td>&lt;26</td>
<td>&lt;26</td>
<td>32</td>
<td>70</td>
</tr>
<tr>
<td>0.3</td>
<td>&lt;26</td>
<td>&lt;26</td>
<td>&lt;26</td>
<td>&lt;26</td>
<td>32</td>
<td>70</td>
</tr>
<tr>
<td>0.5</td>
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<td>&lt;26</td>
<td>&lt;26</td>
<td>&lt;26</td>
<td>32</td>
<td>70</td>
</tr>
<tr>
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<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>2.0</td>
<td>123</td>
<td>141</td>
<td>141</td>
<td>141</td>
<td>141</td>
<td>141</td>
</tr>
</tbody>
</table>

*Expected T₄, nmol/L = Desethylamiodarone, mg/L.*

The results show a dose-dependent interference by desethylamiodarone in the measurement of thyroxin by EMI T₄ assay, but not with the "Gamma Coat" RIA/T₄. However, the concentration of desethylamiodarone in plasma of patients treated with 200 mg amiodarone daily was 1.04 mg/L (4), and at this concentration desethylamiodarone had no significant effect on the EMI T₄ assay.

We conclude that our findings cannot be the sole explanation for the spuriously high plasma T₄ results in the two patients. However, our results raise the possibility that other metabolite(s) of amiodarone may cross-react with antibodies used in the EMI T₄ assay, causing spuriously high values.

References

Intra-Erythrocytic Mg Assay in the Kodak Ektachem 700 Analyzer, M. M. Bonnay, E. M. Legac, A. P. Brunier, and C. J. Bohun (Département de Biologie Clinique, Institut Gustave-Roussey, 94805 Villejuif Cedex, France)

We use a Kodak Ektachem 700 to measure plasma and urinary magnesium. If the magnesium slides are used on simple lysates of erythrocytes, results do not correlate with those by atomic absorption spectrometry (AAS). We overcame this difficulty by using the following sample pretreatment. After centrifugation at 5000 x g for 5 min and decantation of the heparinized plasma, the pellet of erythrocytes is washed twice in isotonic saline and 400 μL of the washed cells is hemolyzed in 400 μL of distilled water. Five minutes later, 500 μL of 0.73 mol/L perchloric acid reagent is added. The mixture is vortex-mixed for 10 s, allowed to stand for 5 min, and is then centrifuged.

Desethylamiodarone was dissolved in 0.3 mL chloroform and diluted to 10 mL with distilled water, equal volumes of the serially diluted stock solution and the EMI T₄ standard sera were mixed to give the test samples. Chloroform (0.3 mL) in 10 mL distilled water.
Solid-Phase Ion-Pair Extraction and Liquid Chromatography of Mezlocillin in Serum, Clifton W. Jones and Herman Chmel

Mezlocillin, an acylureidopenicillin, is a new semisynthetic penicillin with a broad spectrum of antimicrobial activity. It is active in vitro against Gram-positive and many Gram-negative organisms, including Enterobacteriaceae, Pseudomonas aeruginosa, and Bacteroides species (1, 2). Although several methods of liquid chromatography for mezlocillin have been developed involving organic solvent and ion-exchange extraction techniques (3–5), these techniques did not include an internal standard. We describe here a simple, rapid, precise, and sensitive assay utilizing reversed-phase ion-pair extraction and separation in which an internal standard is used.

The assay: Apply 0.5-mL serum samples containing 100 µL of tetrabutylammonium phosphate and 15 µL of pipericillin internal standard (1.0 g/L) to solid-phase extraction columns previously activated by washing with methanol and 5 mmol/L tetrabutylammonium phosphate. Wash the column with water, dry it by aspirating air through it, and elute the drug with 600 µL of an equimolar solution of chloroform/acetone. Evaporate the eluate, reconstitute with 100 µL of mobile phase, and inject 15 µL onto a 250 × 4.5 mm Econosphere C18 column (5-µm particle size). Elute isocratically at ambient temperature with acetonitrile/tetrabutylammonium phosphate buffer (5 mmol/L), 2575 by vol, at 1 mL/min, monitoring the column effluent at 220 nm.

Figures 1 shows representative chromatograms. We calibrated the assay with drug-free serum containing added mezlocillin (10–300 mg/L). Peak-area ratio of mezlocillin to internal standard was linearly related to mezlocillin concentration. Analytical recovery of drug from the disposable columns was 67%. Run-to-run CV within the calibration range was <5%. There was no interference with mezlocillin or pipericillin, with respect to chromatographic retention times, by other commonly used antibiotics: amikacin, ampicillin, cefazolin, cefoxitin, cefotaxime, chloramphenicol, gentamicin, penicillin G, tobramycin, vancomycin.

Our method compares favorably with other current assay methods and has the advantages of solid-phase preparation and use of an internal standard.

References