Letters to the Editor should be typed double-spaced (including references) with conventional margins. The overall length is limited to five manuscript pages, including not more than one figure or one table.

Interference in Assay of Free Triliodothyronine by Triliodothyronine-Binding Antibodies

To the Editor:

A 39-year-old woman with a 20-year history of hypothyroidism caused by Hashimoto thyroiditis had been managed adequately with oral thyroxin (T4), 200 mg/day, until a few months before referral, at which time she developed symptoms of hypothyroidism. Her thyroid hormone profile at the time of referral is shown in Table 1 (Sample 1). Her normal value for serum thyrotropin (TSH) concentration indicates that she was euthyroid but, because of the high serum triiodothyronine (T3) value, the daily oral dose of T4 was decreased to 100 mg. The serum thyroid tests were repeated four weeks later (Table 1, Sample 2): The decreased T4 and increased TSH show that the patient had become hypothyroid at this dosage of T4, but the serum T3 concentration was still in the high normal range.

We suspected that serum T3 was abnormally bound to a serum protein. To investigate this, we measured the radioactivity bound to prealbumin, albumin, and gamma globulin after incubation of the serum with [125I]T3, using the technique described for detection of abnormal protein binding of T4 (1). There was no increase in the amount of [125I]T3 bound to prealbumin or to albumin, but the patient's gamma globulin fraction (the fraction precipitable with polyethylene glycol 8000) bound 21% of the added radioactivity, compared with 2–3% in the blank and in sera from several control patients. Incubation of the serum with [125I]T3, followed by precipitation with goat anti-human IgG, IgM, and IgA, showed that the majority of the increased T3 binding was to IgG (patient's value 14%, expected 1.9% ± 0.1%). Binding of T3 to IgA (5.5%) and to IgM (5.7%) was increased but to a lesser extent (expected values 2.2% ± 0.2% and 2.1% ± 0.1%, respectively). The fact that the abnormal T3 binding in this patient involved all three major immunoglobulin classes indicates that it was polyclonal in nature. In an earlier report of this phenomenon, the polyclonal binding of T3 was limited to IgG (2).

The patient was treated with various doses of T4 for 18 months, and the subsequent thyroid hormone profile remained normal (Table 1, Sample 3). The [125I]T3 binding study at this time showed only 5% binding to the gamma globulin fraction. We included an aliquot of Sample 1 (stored at −100 °C) in this binding assay run and confirmed the initially high gamma globulin binding of T3. We conclude that when she presented, this patient had T3-binding antibodies that caused the concentration of circulating T3 to be increased. These antibodies subsequently disappeared and the serum T3 concentration returned to normal.

We assayed the serum free T3 in the original serum (Sample 1) by two different methods. The Amerlite M (Ameraham, Bucks, U.K.) analog displacement method yielded a very high value, >39 pmol/L (expected value = 4–9). The tracer dialysis technique (performed by the Nichols Institute, San Juan, CA) gave a value of 8.6 pmol/L (expected value = 4.0 – 7.0). Total T3 measured in the same specimen by the Nichols Institute was 5.3 nmol/L (expected value = 1–3.2). Because the patient was chemically euthyroid, we conclude that both of these free T3 assays produced an erroneously high result because of the abnormal T3-binding, although the tracer dialysis result was much closer to the expected value than was that of the analog displacement procedure. As has recently been shown, erroneously high free T3 results by the tracer dialysis technique can occur when protein binding of T3 is increased (3). We conclude that a similar problem exists in the assay of free T3.

References

Sonya D. Tokmakjian D. S. Milton Haines Merrill W. Edmonds
Depts. of Biochem. and Med. Victoria Hospital South St. London, Ontario, Canada N6A 4G5

Specific Measurement of Cyclosporine Concentrations in Whole Blood: Radioimmunoassay and Fluorescence Polarization Immunoassay Compared

To the Editor:

Whole-blood measurement of the cyclosporine (CsA) parent compound is recommended for monitoring CsA concentrations because the immunosuppressive and toxic effects of CsA metabolites are unclear (1–3). Current methods for parent drug-specific measurement of CsA in whole blood include HPLC and RIA (1). Although HPLC remains the "standard" method for CsA measurement, many laboratories have switched their routine measurement methods to monoclonal antibody (mAb)-based specific immu-

---

Table 1. Thyroid Profile Results

<table>
<thead>
<tr>
<th></th>
<th>Total T4</th>
<th>Total T3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/L</td>
<td></td>
</tr>
<tr>
<td>Reference range</td>
<td>69–168</td>
<td>1.2–3.4</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td>T3 uptake</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.33–0.43</td>
</tr>
<tr>
<td>1</td>
<td>84</td>
<td>4.3</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>138</td>
<td>1.6</td>
</tr>
</tbody>
</table>

FTI = Free Thyroxin Index = T3 uptake × T4. All assays were performed with reagents from Organon Teknika, Rockville, MD. See text for timing of samples.
no assays (\(^{2}H\) and \(^{125}\)I ligands), the results of which correlate well and are similar to those determined by HPLC (4, 5). For example, of 153 participating laboratories in the December 1990 CAP survey, 102 use specific methods (HPLC or RIA) and, of these, 58 use the parent drug-specific \(^{125}\)I-ligand IncStar RIA (Cyclo-TRAC-SP; IncStar Corp., Stillwater, MN). With the success of transplantation and concomitant increase in CsA assays, there is a need for more efficient methods than RIA. For instance, in 1986 our laboratory performed 6762 CsA determinations by HPLC; by 1988, our volume increased to 9398, prompting a switch to the Cyclo-TRAC SP RIA (4). In 1990 we performed 17774 CsA measurements, motivating us to examine a rapid, automated, fluorescence polarization immunoassay (FPIA) based on use of a parent drug-specific mAb from Abbott Labs. (TDx monoclonal; Abbott Diagnostics, Abbott Park, IL). The Abbott assay has been compared with HPLC and is reported to perform well (6), but it has not yet been compared with the most frequently used \(^{125}\)I-based RIA.

Precision of the CsA parent-drug-specific FPIA was determined with both whole-blood control material provided by the manufacturer (interassay) and samples obtained from patients receiving CsA (intra-assay). Intraassay CVs for FPIA were 5.8% and 3.3% at CsA values of 145 (n = 19) and 82 \(\mu\)g/L (n = 20), and intra-assay CVs were 4.8% and 1.6% at CsA values of 395 (n = 19) and 392 \(\mu\)g/L (n = 20), respectively. The analytical range of linearity, determined by the deviation from linearity of results for samples diluted in parallel with CsA-free whole blood, was found to be 50–1500 \(\mu\)g/L for FPIA. Comparison of FPIA whole-blood values for CsA from 138 samples with RIA values between 45 and 868 \(\mu\)g/L resulted in FPIA = 0.98 RIA + 37 \(\mu\)g/L (r = 0.98, P < 0.01). Limiting analysis to samples with values <500 \(\mu\)g/L by RIA (n = 125) resulted in FPIA = 1.02 RIA + 30 \(\mu\)g/L (r = 0.98, P < 0.01). When regression analysis was limited to samples from 57 kidney-, 24 liver-, and 15 heart-transplant recipients, no significant differences (P > 0.05; F-test) were observed between transplant types (Figure 1). Recently, other investigators (6, 7) demonstrated that the Abbott FPIA parent-drug-specific mAb had a cross-reactivity of up to 15% with the major CsA metabolites AM9, AM1, and AM4N. Therefore, the constant positive bias we observed may reflect cross-reactivity with CsA metabolites.

We noted one outlier from a heart-transplant patient (see legend to Figure 1D) that demonstrated a >50% greater CsA value determined by FPIA than by RIA. This result is difficult to explain on the basis of reported cross-reactivity with the three major metabolites (6). Unfortunately, insufficient sample was available for repeat analysis, and the outlier was excluded from statistical analysis. However, a subsequent sample obtained from this patient two months later showed a 25% greater CsA concentration by FPIA than by RIA. This sample raises the possibility that, in some patients, another metabolite(a) may cross-react with the Abbott FPIA mAb.

Discussions with transplant surgeons at our hospital indicate that changing the method of CsA measurement has not altered their CsA dosing decisions. The surgeons have adjusted their target range in accordance with the results of the method comparison, which we discussed with them before changing CsA methods. Recently, results from the FPIA were questioned because of seemingly high values for two samples from cardiac-transplant patients. However, analysis of these samples with the RIA confirmed that they were indeed higher than the therapeutic range and that the values from both assays were consistent with our original data.

The results of this study comparing whole-blood CsA values demonstrate good correlation between the parent drug-specific FPIA and \(^{125}\)I RIA. The FPIA satisfies the consensus recommendations for CsA monitoring (2, 3) and also provides laboratories with a simple, automated method for CsA measurement in whole blood. However, CsA values obtained by the parent drug-specific FPIA method are slightly higher than those by the RIA, and those by the RIA have been reported in some studies to be 5–26% higher than results by HPLC (8, 9). With this in mind, we conclude that specific measurement of CsA concentrations in whole blood by FPIA is a viable alternative to RIA and HPLC.

Fig. 1. Comparison of CsA concentrations measured in whole blood by FPIA (y) and \(^{125}\)I RIA (x): (A) total samples analyzed from kidney, liver, heart, lung, and bone marrow recipients; (B) samples from kidney-transplant recipients; (C) samples from liver-transplant recipients; (D) samples from heart-transplant recipients

One outlier within the heart-transplant group had the following coordinates: 165, 378

References
then transfer the lower layer to a small disposable tube and evaporate to dryness. Reconstitute the residue with 50 \( \mu \)L of methanol. Inject 1 \( \mu \)L into a gas chromatograph with a nitrogen-selective detector (NPD).

We purchased cocaine hydrochloride from Sigma Chemical Co., St. Louis, MO 63178 and used a Hewlett-Packard (Palo Alto, CA) 5890-\( \mu \)m megabore column, 10 m \( \times \) 0.53 mm (i.d.), coated with 5% phenylmethylsilicone, for separating the compounds. Helium was the carrier gas at a flow rate of 20 mL/min. Injector temperatures and detector temperatures were 270 and 300 °C, respectively. The column temperature was programmed as follows: initial temperature 240 °C for 2.5 min, programmed at 70 °C/min to 280 °C for 3.5 min. The retention time for cocaine was 0.46 relative to prazepam, which was eluted at 4.36 min. Linearity studies with samples containing cocaine from 0 to 10 mg/L gave a slope of 0.89 and an intercept of \(-0.02 (r = 0.998)\). Analytical recovery of added cocaine at 0.75 and 1.5 mg/L was 100 (\pm 11%) (n = 7) and 101 (\pm 8.5%) (n = 5), respectively. Within-run precision at 0.75 and 1.5 mg/L (n = 3) was 6% and 12%, respectively. Between-run precision at those concentrations (n = 7) was 11% and 8.5%, respectively. The detection limit of the method was <0.1 mg/L (i.e., the lowest-concentration standard detected was 0.1 mg/L).

Interference studies indicated that the following drugs will not interfere with this procedure; EME, BE, morphine, methadone, amitriptyline, nortriptyline, clonazepam, triptal, amphetamine, methamphetamine, salicylate, amobarbital, secobarbital, pentobarbital, phenobarbital, ethchlorvynol, doxepin, imipramine, acetaminophen, caffeine, chlor Diazepoxide, chlorproazine, clonazepam, codeine, propoxyphene, diazepam, flurazepam, glutethimide, meperidine, mebrobamate, methaqualone, phencyclidine, primidone, procaainamide, quinidine, trimipramine, amoxepin, maprotiline, tocainide, theophylline, and mexiletine. The retention time of pyrilamine, desipramine, and pentazocine is close to that of cocaine under the present chromatographic conditions and may interfere with the analysis for cocaine.

The effect of urine pH on cocaine stability was also investigated. At a urine pH of 8.0, hydrolysis of cocaine occurs, with one-third of the cocaine being lost in 15 days.

The advantages of this method are the lack of requirement for derivatization and the simplicity of sample preparation for gas-chromatographic analysis. Samples that tested positive for BE or EME that were analyzed with this procedure showed that cocaine would be detected only in some urine samples. This is to be expected because of differences in the elimination half-lives of cocaine, EME, and BE, with EME and EME having longer elimination half-lives than cocaine (1–4).

References

John Vasiliades
Toxicol. and ClinChem Labs. Inc.
1329 North Saddle Creek
Omaha, NE 68132

Increased Estradiol Concentration of Unknown Origin

To the Editor:

We report an interesting case of above-normal serum estradiol concentration of unknown origin. A 30-year-old white woman was seen for infertility problems. Hormonal evaluation revealed the following results. Post-ovulatory progesterone concentrations and profile (a plot of hormone concentration vs time of cycle) were within the normal range for our laboratory (Table 1). However, the post-ovulatory estradiol concentrations were ex-

2152 CLINICAL CHEMISTRY, Vol. 37, No. 12, 1991