Technical Briefs (~300 words text) summarize findings that are of interest to a relatively limited audience. Readers desiring fuller details may obtain them by writing directly to the author(s) at the address given. Briefs dealing with procedure or instrumentation intercomparisons, evaluations, or improvements (including kit applications) should be sent to Clinical Chemistry News, 2029 K Street, Washington, DC 20006.

Effect of Protamine Sulfate on the aca Heparin Assay, William J. Castellani, Edna D. Hodges, and Arthur P. Bode (Dept. of Clin. Pathol. and Diagnostic Med., East Carolina Univ. School of Med., Greenville, NC 27858; current address: Dept. of Biochem., The Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195)

Protamines are strongly basic low-M_r proteins that interact ionically with many molecules, including heparin. In vitro, protamine–heparin binding forms a stable complex without anticoagulant activity; a similar effect is also seen in vivo (1). Protamine sulfate is administered as a heparin antagonist at a recommended dose of 1 mg/100 units of circulating and active heparin (2).

The aca method for heparin determination is a chromogenic assay for activated factor X (factor Xa) activity (3). Heparin–antithrombin III complexes in the patient's sample inhibit factor Xa in the reagent mixture. The amount of inhibition is linearly related to heparin concentration. We have investigated this reaction because of an unusual result in a patient with postoperative bleeding after extracorporeal bypass and protamine reversal of heparin anticoagulation; this patient demonstrated low but measurable amounts of "heparin" with normal coagulation values.

For our in vitro model, we used a pool of citrated plasma with normal values for prothrombin and activated partial thromboplastin times. Porcine intestinal sodium heparin was added to aliquots of the pool by 10-fold dilution to final concentrations of 0.5, 1.0, and 2.0 USP units/mL. Protamine sulfate was added in a similar manner at various final concentrations under the assumption of complete neutralization ("equivalence") of 100 units of heparin per milliliter of protamine. The plasma pools were prepared fresh for each assay; the heparin and protamine dilutions were prepared from stocks made from single vials of pharmaceutical material, based on the manufacturers' labeled concentrations.

Figure 1 shows the effect of various concentrations of protamine on plasma containing heparin at 1 USP unit/mL, as representative of all three heparin concentrations tested. The dose–response curve was log-linear up to about four times the equivalence concentration; at higher ratios, we noted an increase in measured "heparin," assumed to result from direct protamine interference with factor Xa activity. At the expected equivalence concentration, the measured heparin concentration was decreased by only 50%. Comparable curves were obtained at 0.5 and 2.0 USP units of heparin per milliliter.

Fig. 1. Effect of increasing amounts of protamine sulfate on the aca measurement of heparin in whole blood (1 USP unit/mL)

The protamine/heparin ratio is based on the assumption that 0.01 mg of protamine neutralizes 1 unit of heparin. The measured heparin concentration is shown as a percent of anti-factor Xa activity relative to baseline (no added protamine) determined in each experiment. The results of four separate experiments are shown; all data for each concentration measured are included. For replicate samples, the average is plotted, with vertical bars giving the range of measurements, and the number of data points at each protamine/heparin ratio is shown by each point. The solid line represents the best least-squares fit for each concentration of heparin; the broken line represents least-squares approximation of anti-factor Xa effect with increasing protamine concentration.

The aca heparin assay is altered in vitro by protamine in a complex manner. At no time is there full heparin neutralization, even at multiple protamine equivalences. At high protamine/heparin ratios, protamine has an apparent anticoagulant effect; this is present to a very slight extent even in the absence of heparin (0.05 mg of protamine per milliliter produces a measured "heparin" concentration of 0.04 USP units). These findings were not evident when we tested 15 samples (selected without conscious bias) from patients after extracorporeal bypass with normal post-protamine activated clotting and thrombin times and no overt evidence of bleeding (data not shown). Whether this represents a confounding factor in certain patients with post-bypass bleeding is not clear. Other work that we have reported indicates that this postoperative state may be quite complex and variable in different patients, leading to altered patient responses to heparin and protamine (4, 5).

The present report demonstrates yet another situation where the results of a laboratory test may not fully describe the intravascular (in vivo) situation.

References
Hyperthyroidism with Normal Values for Total Thyroxin in Serum, Pirjo Nuutila,1 Kerttu Irujala,2 Hanna-Leena Kaihola,2 Pentti Seppälä,2 and Jorma Viikari1 (1 Dept. of Med., 2 Central Lab., Univ. Central Hospital of Turku, SF-20520 Turku, Finland)

We assessed the frequency and clinical background of normal concentrations of serum total thyroxin (T4) found among 84 hyperthyroid patients out of 633 consecutive patients evaluated for suspected hyperthyroidism. Patients were categorized as having hyperthyroidism if they had hyperthyroid symptoms and signs, had a serum thyrotropin (TSH) <0.1 milli-int. unit/L as measured by immunoradiometric assay (Farmos Diagnostica, Turku, Finland), and needed treatment.

Serum total T4 determined with a radioimmunoassay (Farmos Diagnostica; reference interval 70–150 nmol/L) was normal in 17 hyperthyroid subjects among 68 patients (25%) without previous thyroid history (Figure 1). Normal T4 values were common in patients with nodular goiter (34.8%, eight of 23), but seldom found in cases of Graves' hyperthyroidism (10.5%, four of 38). Moreover, normal T4 values were more common in relapsed clinical disease than in newly diagnosed cases (chi-square = 8.18, P <0.01). Results for all 84 hyperthyroid patients were as follows:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>T4/FT4 combinations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graves' disease</td>
<td>46 (38)*</td>
<td>1/4</td>
</tr>
<tr>
<td>Nodular goiter</td>
<td>31 (23)</td>
<td>17 (15)</td>
</tr>
<tr>
<td>Toxic thyroiditis</td>
<td>7 (7)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>84 (62)</td>
<td>56 (46)</td>
</tr>
</tbody>
</table>

* Newly diagnosed cases given in parentheses.

Free T4 determined by an analog-based RIA Amerlex-M (Amer sham International, Amersham, U.K.; reference interval 9–22 pmol/L) was above normal in all but one hyperthyroid subject. A new solid-phase two-step method (Spectria, Farmos Diagnostica; reference interval 7.4–15.9 pmol/L, n = 19) and equilibrium dialysis (reference interval 9.5–22.4 pmol/L, n = 10) also gave above-normal results (29.6 ± 9.3 and 36.7 ± 11.3 pmol/L, respectively) for patients with normal T4 values. The correlation between total T4 and free T4 (Amerlex-M) values in serum was poor (r = 0.74, Figure 1).

Our results agree with those of Caldwell et al. (1). The pathophysiology of normal concentrations of thyroid hormones accompanying clinical hyperthyroidism is not well known, but the maximum binding capacity of thyroxin-binding globulin (TBG) for T4 and the concentration of TBG have repeatedly been demonstrated to be decreased in patients with diffuse and nodular hyperthyroidism (2, 3).

We conclude that (a) normal serum T4 concentration can be associated with clinical hyperthyroidism in about a third of the cases with toxic nodular goiter, and (b) this is a common finding in relapsed hyperthyroidism.


Analysis of whole blood for cyclosporine with the TDx clinical analyzer (Abbott Labs., Abbott Park, IL) involves a monoclonal antibody and fluorescence polarization immunoassay technology. We compared results of this procedure with the results of a liquid-chromatographic procedure (1, 2) for 224 blood samples collected from 10 patients being treated with Sandimmune® (cyclosporine) after orthotopic liver transplant. At least 20 samples collected during the first 90 days post-transplant were obtained from each patient. Treatment of patients followed a clinical protocol described elsewhere (3). All specimens were collected and analyzed by liquid chromatography (HPLC) for routine clinical monitoring on the same day as collected, and the residual sample was subjected to the Abbott procedure. Some of the samples (10%) were analyzed by both procedures within one day; the rest were stored at 4 °C for at least one day (but no more than 15 days) before analysis by the Abbott procedure.

The Abbott monoclonal assay of cyclosporine in whole blood was carried out similarly to the whole-blood polyclonal assay (4), as has been described elsewhere (5). Blood was treated with a solubilizing agent containing surfactant and a protein-precipitating reagent (zinc sulfate in metha-