Fibrinogen Assay During Heparin Therapy of Disseminated Intravascular Coagulation

To the Editor:

Recent interest in the treatment of disseminated intravascular coagulation with heparin (1, 2) has caused some clinicians and laboratory workers to express doubt concerning the accuracy of fibrinogen methods based on thrombin-induced fibrin clot formation and subsequent measurement of the fibrin by biuret methods. It was reasoned that inaccurately low results would be produced owing to the presence of heparin in the plasma. This concern most certainly is influenced by the apparent discrepancies in the literature surrounding the effect of heparin on fibrin formation (5-7). Although there appears to be uniform agreement that heparin slows the conversion of prothrombin to thrombin (4), the effect of heparin on the thrombin-induced conversion of fibrinogen to fibrin is somewhat uncertain. Thus, when heparin is present in plasma, clot formation induced by endogenous thrombin after recalcification of plasma would be expected to fail, and does (5, 6), while clot formation as a result of an excess of exogenous thrombin added to the system may fail (4) or may succeed (1, 5).

We have tested the effect of heparin in vitro at concentrations far greater than those used in therapy of this disease process, and find no interference with our fibrinogen method, which involves exogenous thrombin-induced fibrin formation and analysis by a biuret method.

Briefly, the fibrinogen procedure, modified from Ware et al. (8) and Henry (9), is as follows: Add 1 ml of plasma to 6 ml of NaCl solution (9 g/liter) and 3 ml of 0.066 M phosphate buffer, pH 6.4 (±0.1). Add 0.2 ml of "Topical Thrombin" (1000 units/ml, Parke-Davis), mix, and wind the clot on a wooden applicator stick. The clotting process is complete within 10 min. Remove the stick from the tube and place in a NaCl solution (9 g/liter) for 5 min. Scrape the clot down to the tip of the stick and blot the clot dry on a paper towel. Place the clot into a clean tube and add 5.0 ml of the albumin-biuret reagent of Kingsley (10). Heat in a water bath at 50°-56°C until the clot completely dissolves. Appropriate protein standards also are treated with biuret reagent and heated similarly. After 5 min read the absorbance of unknowns and standards in a spectrophotometer at 545 nm vs. a blank consisting of 5 ml of biuret reagent (also subjected to the heat treatment). Occasional lots of Topical Thrombin have been found to contain impurities that produce a turbidity in the final solution. This can be removed by shaking the final solution with 2 ml of diethyl ether; the absorbance of the aqueous (bottom) layer is measured.

To test the possibility of heparin interference, we collected several pools of EDTA and oxalated plasma. We measured the fibrinogen content of these plasma pools and also of the same plasma supplemented in vitro with two concentrations of heparin, calculated to be about 2.5 and 5 times the level of heparin achieved during the acute phase of heparin therapy (1 mg heparin/kg body weight, given iv). No heparin interference is seen (Table 1).

<table>
<thead>
<tr>
<th>Fibrinogen concn, mg/dl</th>
<th>Control</th>
<th>Low</th>
<th>High</th>
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</thead>
<tbody>
<tr>
<td>EDTA plasma 1</td>
<td>317</td>
<td>297</td>
<td>261</td>
</tr>
<tr>
<td>EDTA plasma 2</td>
<td>328</td>
<td>318</td>
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<tr>
<td>EDTA plasma 4</td>
<td>317</td>
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<tr>
<td>Oxalate plasma 1</td>
<td>287</td>
<td>317</td>
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<td>Oxalate plasma 3</td>
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<tr>
<td>Oxalate plasma 4</td>
<td>287</td>
<td>328</td>
<td>287</td>
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</tbody>
</table>

a Av of duplicates. "EDTA plasma" is 1.3 mg disodium EDTA/ml blood. "Oxalate plasma" is 2.9 mg K oxalate/ml blood.

b Low: equivalent to 6.25 mg heparin/dl plasma. High: equivalent to 12.5 mg heparin/dl plasma. Acute therapy consisting of 1 mg heparin/kg of body weight results in an approximate maximum of 1.2 mg heparin/dl of whole blood.

References

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