Use of the Plasma Thrombin Time to Assess the Adequacy of in Vivo Neutralization of Heparin: Comparative Studies Following Operations Employing Extracorporeal Circulation

By James C. Peden, Jr., and James A. McFarland

Heparin used for short-term anticoagulation during certain surgical procedures must be rapidly and completely neutralized postoperatively in order to reduce the danger of excessive bleeding. The amount of antiheparin which must be given depends upon the amount of heparin which is present. In some clinics, the variability of the amount of heparin which remains postoperatively is not taken into account; instead, protamine is given in a dosage which is some fixed multiple of the amount of heparin originally administered. If an attempt is made to relate the protamine dosage to the quantity of heparin actually remaining after operation, some modification of the protamine titration test, measuring the whole blood clotting time in glass tubes with varying amounts of added protamine, is commonly employed. However, normal clotting times can be obtained in glass tubes in the presence of small but significant amounts of heparin,2 and the protamine titration test may thus underestimate the amount of heparin remaining.

Patients who have been subjected to extracorporeal circulation with a pump-oxygenator during open-heart surgery frequently develop ill-defined bleeding tendencies and almost uniformly exhibit some degree of thrombocytopenia.3,4 In such patients it is imperative that adequate antiheparin be administered postoperatively; for in the presence of deficiencies in blood coagulation, the effect of even very small quantities of heparin is greatly magnified,3,5,6 and the danger of hemorrhage is increased.

The plasma thrombin time, which is the time required for a given volume of plasma to clot after the addition of a given amount of thrombin, depends upon many factors7–10: the quantity and quality of the fibrinogen; the concentration of other proteins, especially albumin; total ionic strength; pH; and the activity of any antithrombin that is present. However, the thrombin times of freshly drawn samples of normal plasma fall within remarkably narrow limits.7,11 If all other factors remain reasonably constant, slight varia-
tions in the concentration of antithrombin will cause wide variations in plasma thrombin time.

In the clinical management of patients after operation where heparin had been given, protamine dosages calculated on the basis of the results of the protamine titration test\textsuperscript{12} often were inadequate. Accordingly, a comparative study of the protamine titration test and the plasma thrombin time, with and without toluidine blue adsorption of the plasma, was instituted in the management of patients following cardiac surgery involving extracorporeal circulation and heparin anticoagulation.

**PROCEDURE**

Twenty-five consecutive patients undergoing extracorporeal circulation with a mechanical pump-oxygenator during cardiac surgery for correction of congenital and acquired cardiac defects were studied. The patients ranged from two to 41 years in age. To rule out possible gross abnormalities of blood coagulation, platelet count, one-stage prothrombin time, silicone clotting time, and Lee-White clotting time were carried out preoperatively.

During the operation each patient received 1.5 mg. heparin per Kg. of body weight. The blood used to prime the pump-oxygenator contained 40 mg. heparin per liter. At the end of perfusion the patient's blood volume was estimated and, with the assumption that complete mixing of blood from oxygenator and patient occurred during perfusion, and that no heparin was lost from the circulating blood during perfusion, the total heparin remaining in the patient was calculated. One to 1.5 mg. of protamine for each mg. of heparin calculated to be present was given immediately by slow intravenous injection in about 50 ml. of 5 per cent glucose solution.

After time for complete mixing of the administered protamine had been allowed, blood samples were drawn for thrombin time and protamine titration. If either test indicated incomplete neutralization of the heparin, more protamine was administered. This second dose of protamine was about one-fourth of the original dose. After allowing time for mixing, the thrombin time and protamine titration were repeated. If either test remained abnormal, a further small dose of protamine was given. This procedure was repeated until both the protamine titration test and the thrombin time indicated complete neutralization of heparin.

After a normal thrombin time had been obtained, prothrombin time and silicone and Lee-White clotting times were determined. Plasma thrombin times were determined frequently but not at fixed intervals for the first 12 hours postoperatively.

**METHODS**

*Collection of specimens.*—With the use of a two-syringe technic, venous blood was drawn whenever it could be obtained. On occasion it was necessary to obtain the immediate postperfusion specimens through an indwelling arterial polyethylene catheter. A portion of the blood was transferred directly into the glass tubes containing protamine for the protamine titration test. The remaining blood was added to one-tenth volume of 3.8 per cent sodium citrate solution and centrifuged at 1300 g for 15 minutes for separation of the plasma. Normal plasma for use as control in the thrombin time procedure was obtained from venous blood drawn from laboratory personnel at approximately the same time and treated in the same manner as the test samples.

*Protamine titration test.*—The method described by Perkins et al.\textsuperscript{14} was used. One ml. of freshly drawn whole blood was added to each of 11 glass tubes containing 0.1 ml. of protamine solution. The protamine content of the tubes varied from 0 to 50 µg., in 5 µg. increments. The blood and protamine mixtures were examined for clots at the end of 15 minutes. A protamine titration test was termed "normal" if the tube containing no protamine contained a clot at 15 minutes.

*Plasma thrombin time.*—0.1 ml. of a dilute "standard" thrombin solution was added to
a mixture of 0.2 ml. plasma and 0.1 ml. distilled water in a 13 x 100 mm. test tube at 37 C. The time from the addition of the thrombin to the appearance of definite fibrin threads was determined.

To prepare the thrombin solution, bovine thrombin was made up in 50 per cent glycerol solution to a concentration of 200 units per ml. (This solution is stable for a period of many weeks on storage at 0 C.) It was diluted further, approximately 1 to 200, with 0.85 per cent saline solution so that a reproducible clotting time between 28 and 31 seconds was obtained with a control sample of normal plasma in the thrombin time procedure. This “standard” thrombin solution was used immediately to determine in duplicate the thrombin time of the test plasma. Other samples of normal plasma gave reproducible clotting times between 25 and 35 seconds with this method.

If the patient’s thrombin time was more than 45 seconds, a 0.1 per cent solution of toluidine blue was added to the plasma in place of the distilled water, and the plasma-dye mixture allowed to incubate at 37 C. for one minute before the “standard” thrombin solution was added. The control was similarly treated. It was assumed that the presence of heparin in the patient’s plasma was indicated if treatment with the dye shortened the prolonged thrombin time to treated control levels.

RESULTS

The findings in the 25 patients are summarized in table 1. The “calculated heparin” (Second column, table 1) was determined as outlined under Procedure.

The amount of protamine administered before the first normal protamine titration test was obtained in each patient is recorded in the third column of table 1. This varied from one to two times the calculated amount of heparin remaining.

The amount of protamine administered to each patient before the first normal thrombin time was obtained is recorded in the fourth column of table 1. This amount of protamine was never greater than 2.25 times the calculated remaining heparin.

In 18 of the 25 patients, the protamine titration test returned to normal while the thrombin time was still prolonged. Of these patients four died before normal thrombin times were achieved. In the remaining 14, an average of 19 per cent (3 to 44 per cent) more protamine was required to correct the thrombin time than to correct the protamine titration test.

In seven patients the original dose of protamine corrected both the protamine titration test and the thrombin time. Four of these patients received an original dose of protamine of at least 1.5 times the calculated heparin remaining, while the others received less.

One instance of “heparin rebound” was seen in these 25 patients. In patient H. W., two hours after apparently adequate protamine had been administered, the thrombin time again became prolonged, although the protamine titration test remained normal.

In every patient, the prolonged thrombin time could be corrected in vitro by adsorption with toluidine blue and in vivo by continued administration

*Parke, Davis and Co. Detroit, Mich.
### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Calculated Heparin (mg.)</th>
<th>Dosage (in mg.) of protamine required to bring to normal:</th>
<th>Thrombin Time</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. S.</td>
<td>39</td>
<td>40</td>
<td>40</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>E. H.</td>
<td>43</td>
<td>70</td>
<td>80</td>
<td>After 108 mg. of protamine thrombin time = 56 seconds</td>
</tr>
<tr>
<td>R. L.</td>
<td>50</td>
<td>108</td>
<td>—</td>
<td>After 140 mg. of protamine thrombin time = 61 seconds</td>
</tr>
<tr>
<td>B. F.</td>
<td>110</td>
<td>130</td>
<td>—</td>
<td>&quot;Heparin rebound&quot; not picked up by protamine titration.</td>
</tr>
<tr>
<td>H. W.</td>
<td>110</td>
<td>150</td>
<td>185</td>
<td>After 120 mg. of protamine thrombin time = greater than 90 seconds.</td>
</tr>
<tr>
<td>V. A.</td>
<td>101</td>
<td>120</td>
<td>—</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>R. D.</td>
<td>132</td>
<td>200</td>
<td>200</td>
<td>After 96 mg. of protamine thrombin time = more than 60 seconds.</td>
</tr>
<tr>
<td>J. P.</td>
<td>50</td>
<td>75</td>
<td>85</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>M. Y.</td>
<td>112</td>
<td>170</td>
<td>245</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>S. F.</td>
<td>57</td>
<td>86</td>
<td>106</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>D. Y.</td>
<td>43</td>
<td>65</td>
<td>80</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>C. C.</td>
<td>70</td>
<td>105</td>
<td>115</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>B. R.</td>
<td>152</td>
<td>208</td>
<td>228</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>B. M.</td>
<td>64</td>
<td>96</td>
<td>—</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>J. H.</td>
<td>88</td>
<td>132</td>
<td>132</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>L. C.</td>
<td>124</td>
<td>186</td>
<td>186</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>R. H.</td>
<td>132</td>
<td>165</td>
<td>198</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>S. D.</td>
<td>100</td>
<td>150</td>
<td>155</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>M. C.</td>
<td>52</td>
<td>65</td>
<td>78</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>H. McC.</td>
<td>155</td>
<td>194</td>
<td>232</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>J. E.</td>
<td>61</td>
<td>117</td>
<td>117</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>M. P.</td>
<td>54</td>
<td>70</td>
<td>84</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>M. S.</td>
<td>50</td>
<td>62</td>
<td>62</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>R. E.</td>
<td>56</td>
<td>118</td>
<td>142</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
<tr>
<td>E. Z.</td>
<td>142</td>
<td>178</td>
<td>178</td>
<td>First dose of protamine corrected both tests.</td>
</tr>
</tbody>
</table>

of protamine. In no patient was there evidence of excess protamine interfering with blood clotting. All patients had normal silicone and Lee-White clotting times when the thrombin time had returned to normal.

There were no significant changes between preoperative and postoperative levels of the plasma fibrinogen, proteins, electrolytes and blood pH that might have affected the thrombin times.

**Discussion**

The amount of heparin remaining postoperatively in patients who have been subjected to extracorporeal circulation with heparinized blood varies
widely and is difficult to measure. For these reasons, the amount of protamine which will be adequate to neutralize the remaining heparin, but which will not be excessive, cannot be predicted.

Because of the possible toxic effects of excess protamine, including hypotension, bradycardia and thrombocytopenia in dogs that have been subjected to extracorporeal circulation, and because the protamine requirements varied so widely in the patients reported here, it is not felt safe to rely upon a single routine protamine dose based on the amount of heparin administered or on an assumption as to the proportion of administered heparin which will remain after operation. In our experience, a single dose of protamine large enough to be adequate for all patients would be grossly excessive for many patients. It would seem safer to rely upon several small doses of protamine, testing for remaining heparin after each dose.

An initial dose of 1.5 times the heparin calculated to be present in the patient's blood is considered a suitable and safe starting dose. This amount of protamine was decided upon on the basis of the present results and previous experience with lower initial doses. From these data it can be inferred that a protamine dose of 1.5 times the calculated heparin was just sufficient to neutralize circulating heparin in one-third of all patients; was a slight excess, but produced no clinical symptoms, in another third; and had to be supplemented by additional protamine in the remaining third, in whom it failed to neutralize all residual heparin.

In these 25 patients the plasma thrombin time was most helpful in assessing the adequacy of the in vivo neutralization of heparin by various doses of protamine. It appears to be more sensitive to small amounts of circulating heparin than is the simplified protamine titration test with which it was compared. In fact, if the administration of protamine had been stopped when the protamine titration test had returned to normal, some circulating heparin would evidently have remained unneutralized, since up to 40 per cent more protamine was required to bring the thrombin time to normal. The amount of heparin which would have remained, perhaps insignificant in a normal individual, could have posed a threat to those patients who developed a thrombocytopenia or other bleeding tendency postoperatively.

Even when the thrombin time had returned to normal, some of the patients reported here continued to bleed, a few massively. However, with some confidence that all circulating heparin had been neutralized it was possible to assess more readily the significance of a thrombocytopenia or other bleeding tendency, to direct attention more appropriately to possible problems of local hemostasis, and to treat the patient more effectively.

**Summary**

1. The dosage of protamine necessary for the postoperative neutralization of heparin, used as anticoagulant during extracorporeal circulation, was studied in 25 patients by means of both the protamine titration test and the plasma thrombin time.

2. The plasma thrombin time was found to be far more sensitive in the
detection of small quantities of residual heparin than was the protamine titration test, which failed to demonstrate significant quantities of circulating heparin.

3. Adequate neutralization of circulating heparin was achieved when an initial postoperative protamine dose of 1.5 times the calculated residual heparin was given and followed by additional protamine when indicated by the result of the plasma thrombin time.

4. No toxic effects of protamine were noted on such a dosage schedule.

**Summario in Interlingua**

1. Le dosage de protamina necessari pro le neutralisation postoperatori de residuos del heparina usate como anticoagulante durante le circulation extracorporee esseva studiate in 25 patientes, tanto per medio del test de titration de protamina como etiam per medio del determination del tempore de thrombina in le plasma.

2. Esseva constatate que le tempore de thrombina del plasma esseva multo plus sensibile in le detection de micre quantitates de heparina que le test de titration de protamina. In certe casos, le test de titration de protamina non demonstrava le presentia de residuos de heparina circulante in concentraciones de signification practic.

3. Un adequate neutralisation del circulante heparina esseva effectuate quando un dose postoperatori initial de protamina de 1,5 vices le calculate residuo de heparina eseva administrate, segue per protamina additional quando isto eseva indicate per le resultato del test del tempore de thrombina del plasma.

4. Nulle effectos toxic de protamina esseva notate sub le conditiones de iste programma de dosage.

**References**


9. Ingram, G. I. C.: Variations in the


11. Authors' unpublished data.


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