Relationship Between the Anticoagulant and Antithrombotic Effects of Heparin in Experimental Venous Thrombosis

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The relationship between the antithrombotic and anticoagulant effects of heparin was assessed using venous thrombi in rabbits. Accretion of \(^{125}\)I-fibrinogen onto jugular vein thrombi was used to assess the antithrombotic effect of heparin, and the protamine sulfate titration test (heparin activity) and the activated partial thromboplastin time (APTT) were used to measure its anticoagulant effect. The effect of heparin on jugular vein bleeding times was also measured in a separate group of animals. Fibrinogen accretion was significantly lower with continuous infusion than with intermittent injection. Heparin, given by continuous infusion, produced marked inhibition of fibrinogen accretion (to < 10% of control accretion) at an APTT value of between 75 and 80 sec (control 34 sec) and at a level of heparin activity of 0.4–0.5 U/ml. Infusion of cryoprecipitate reduced the effect of heparin on the APTT relative to its effect on heparin activity. In these cryoprecipitate-treated animals, marked inhibition of fibrinogen accretion occurred at a similar level of heparin activity (0.4–0.6 U/ml) but at a significantly lower APTT (35–50 sec) than in normal animals. On the other hand, there was a progressive increase in jugular vein bleeding time with both increasing APTT values and heparin levels in both groups of animals.

Heparin is considered to be the drug of choice in the treatment of acute venous thromboembolic diseases. The aim of heparin treatment is to prevent extension of venous thrombosis or pulmonary embolism with a minimum risk of bleeding. The relationship between the level of circulating heparin activity and its effect on the coagulation system varies from patient to patient and it is currently uncertain which of these measurements reflects the antithrombotic effect of heparin more accurately.

We have developed a model of experimental venous thrombosis in rabbits which provides a means of accurately quantifying fibrinogen accretion onto an existing thrombus. This model has been used to explore the relationship between the antithrombotic effect of heparin and its anticoagulant effect, the latter being measured both by heparin assay with protamine sulfate titration and by the activated partial thromboplastin time (APTT). A comparison has also been made of the effectiveness of heparin given by continuous infusion with heparin given by intermittent injections.

MATERIALS AND METHODS

Coagulation assay. These were performed on platelet-poor plasma (<10,000 platelets/cmm) prepared from citrated blood which was centrifuged at 2000 g for 15 min at 4°C. Plasma...
fibrinogen was assayed using the method of Astrup et al. The APTT was performed by the method of Proctor and Rapaport using cephalin extracted from human brain tissue. Plasma heparin activity was measured by the method of Refn and Vestergaard with the following modifications: To 0.2-ml aliquots of platelet-poor plasma various dilutions of a protamine sulfate stock solution (10 mg/ml; Sigma, St. Louis, Mo.) were added in 2 µl veronal buffer (pH 7.4) and mixed well. Of this mixture 0.1 ml was added to 0.3 ml of a titration mixture described by Seegers and Smith. After incubation at 37°C for 1 min, 0.3 units of bovine thrombin (Parke Davis, Detroit, Mich.) in 0.1 ml 0.9% NaCl was added and the clotting time was recorded.

Porcine cryoprecipitate. Porcine cryoprecipitate was prepared from platelet-poor hog plasma. The blood was collected in an anticoagulant (0.8 g citric acid, 2.2 g sodium citrate, and dextrose 2.45 g/100 ml) using 1 volume for 6 volumes of blood. Quantities of 150 ml of plasma were dispensed into 200-ml plastic bags, frozen overnight at -70°C, and then thawed in a waterbath (4°C) for approximately 1 hr. The cryoprecipitate was sedimented by centrifugation at 2000 g for 15 min at 4°C and redissolved in a small volume of the supernatant plasma. It had a potency of 15-20 units of factor VIII activity per ml when assayed in a human factor VIII system.

Cryoprecipitate was infused intravenously into rabbits at a dose of 50 units factor VIII/kg 10 min before heparin treatment. The mean level of factor VIII rose by 78%, and the mean fibrinogen level increased by 190 mg/100 ml.

Venous thrombosis model. New Zealand white rabbits of either sex weighing 2.5-3.0 kg were used. They were anesthetized with intravenous sodium pentobarbital (Nembutal, 30 mg/kg; Abbott Laboratories, Montreal, Que., Canada). The neck was cleanly shaved and a central incision made. Both jugular veins were exposed and a venous segment 1.5 cm in length isolated on either side. During the dissection great care was exercised not to traumatize the vessel wall. Tributaries draining into the venous segment were ligated. Flow in the jugular vein was temporarily occluded and a column of blood was trapped within the segment by lifting the two sutures placed at the proximal and distal ends of the venous segment. Then 5 units of bovine thrombin (Parke Davis topical) in 0.02 ml saline were injected into the isolated venous segments from a tuberculin syringe with a 25-gauge needle. Within 1 min, a nonadherent thrombus began to form, at which time the needle was withdrawn without bleeding. Stasis was maintained for 10 min. The thrombi were held in place by a proximal stenosis which narrowed the lumen to approximately 30%, but did not completely occlude blood flow.

To facilitate blood sampling, the left carotid artery was cannulated with a sterile polyethylene cannula (Intramedic PE-190: Clay Adams, Parsippany, N.J.). Blood samples were taken from the carotid cannula. The first 1 ml was always discarded and the cannula flushed with a small volume of 0.9% NaCl to keep it free from blood after the sampling was completed.

Experimental Design and Treatment Regimens

After the venous thrombi were induced, the animals were randomized into three groups: One received continuous intravenous heparin infusion, the second intermittent intravenous heparin injections, and the third (the control group) received 0.9% NaCl by continuous intravenous infusion. Each experiment lasted for 10 hr, during which time the rabbits were maintained under light general anesthesia with continuous infusion of sodium pentobarbital (7.5 mg/kg/hr, diluted in 0.9% NaCl) via the marginal ear vein at the rate of 2 ml/hr using a constant infusion pump (Harvard Apparatus, Millis, Mass.).

Heparin was obtained from MCT Pharmaceuticals Ltd., Hamilton, Ont. (Hepalean, sodium heparin, 1000 USP units/ml, hog mucosa origin). Various dosage regimens were used which ranged from 300 to 900 U/kg/10 hr. For continuous infusion one-third of the total dose was given as a bolus and the remaining two-thirds by continuous infusion over 10 hr. For intermittent heparin, the total dose was divided into thirds and given at 0, 4, and 8 hr. Bolus doses of heparin were injected into the marginal ear vein, whereas for continuous infusion the heparin was added to the anesthetic saline mixture. Blood samples for the measurement of APTT and plasma heparin activity were taken at 1, 2, 3, 4, 5, 6, 9, and 10 hr. In addition, a blood sample was taken before heparin administration for the measurement of pretreatment APTT. The APTT ratio was calculated in animals given heparin by continuous infusion by dividing the mean APTT value during the third to the tenth hour of continuous heparin infusion by the preheparin treatment value for the APTT.
Quantitation of Plasma and Thrombus $^{125}$I-Fibrinogen Radioactivity

Rabbit fibrinogen (92%, clottable) was prepared and labeled with $^{125}$I (New England Nuclear, Boston, Mass.) by a previously published method. The specific activity of the labeled preparations was close to 100 μCi/mg of protein. Approximately 1 mg $^{125}$I-fibrinogen was injected intravenously into each rabbit 5 min after the first bolus dose of heparin or saline.

Radioactivities were measured in a Packard (Downers Grove, Ill.) Model 5212 gamma counter. Plasma samples (0.5 ml) were assayed in duplicate. Before counting, thrombi were gently rinsed three times with 0.9%, NaCl, then placed in counting vials, and 0.9%, NaCl was added to a final volume of 3 ml. The mean radioactivity content of the two thrombi recovered from each animal was used for further calculations.

The amount of fibrinogen accreted onto thrombi was calculated by relating the $^{125}$I activity of the washed thrombi to the integrated mean specific activity of plasma fibrinogen during the experiment. The plasma fibrinogen concentration was calculated from the mean of three samples obtained at 3 min after injection of $^{125}$I-fibrinogen and then at 5 and 10 hr. $^{125}$I-fibrinogen activity was measured in plasma samples taken at 3 min after the administration of $^{125}$I-fibrinogen and then at 2-hr intervals until the end of the experiment. Plasma radioactivity curves were integrated as described before.

This method for calculating fibrin accretion assumes that the transfer of fibrinogen onto preformed thrombi from plasma occurs at a steady rate throughout the observation period. This assumption is supported by measurements of $^{125}$I-fibrinogen accretion onto thrombi excised 1, 3, 7, and 10 hr after their formation (see Results).

Quantitation of Thrombus Growth

Thrombus growth over the 10-hr period of study in control animals not treated with cryoprecipitate was estimated by two methods, as follows:

1. The jugular vein was exposed and a venous segment 1.5 cm in length isolated in an identical manner to the method used for the production of venous thrombosis. However, thrombin was not injected into the segment, but the blood was removed and the volume of blood within the occluded segment was measured. The amount of fibrinogen in the occluded segment was calculated by multiplying the volume of plasma obtained from the occluded segment (estimated from the hematocrit) by the fibrinogen concentration. The calculated mean accretion of $^{125}$I-fibrinogen over 10 hr onto 20 venous thrombi was then expressed as a percentage of the calculated mean fibrinogen content of blood withdrawn from 12 occluded venous segments.

2. The percentage increase in the weight of thrombi in control animals not treated with cryoprecipitate was estimated by comparing the weight of 20 thrombi isolated after 10 hr of infusion with 0.9%, NaCl with the weight of 12 thrombi obtained from animals that were treated with continuous heparin in large doses and which accreted less than 7 μg of fibrinogen over the 10 hr of heparin infusion. The difference between the weights of thrombi in these two groups was then expressed as a percentage of the weight of thrombi in the control animals.

Bleeding Time

Bleeding times were performed on rabbits that had not had experimental thrombosis induced. The jugular vein of rabbits was isolated and a trough-shaped aluminum drain was positioned beneath the vein at a 45-degree angle. The drain was continuously irrigated with 0.9%, NaCl to prevent blood clotting on it. The bleeding time was determined after puncturing the vein at right angles to the vessel wall with an 18-gauge needle. The shed blood flowed down the drain and the time between puncture and cessation of bleeding was taken as the bleeding time.

Estimation of the Anticoagulant Values Which Correspond to Marked Inhibition of Fibrinogen Accretion in Normal and Cryoprecipitate-treated Rabbits

Curves were fitted to plotted points expressing the relationship between fibrinogen accretion and anticoagulant effect of heparin (APTT, APTT ratio, heparin level) in normal and cryoprecipitate-treated rabbits. The most appropriate curve was selected and the anticoagulant values which
corresponded to marked inhibition of fibrinogen accretion in the two groups of rabbits were compared. In making these comparisons an attempt was made to determine the level of anticoagulant effect (APTT, APTT ratio, heparin level) which corresponded to marked inhibition of fibrinogen accretion. Since the plotted points were best fitted by a rectangular hyperbola (see Results), a true point of inflection could not be identified and therefore objective comparison between normal and cryoprecipitate-treated rabbits was made using two indirect methods.

Analysis 1. The predicted anticoagulant effect (APTT, APTT ratio, heparin level) which corresponded to a fibrinogen accretion of 15 μg in normal and cryoprecipitate-treated animals was compared. This value for fibrinogen accretion was selected because it represented approximately 10% of the control value for fibrinogen accretion in normal and cryoprecipitate-treated animals and because it corresponded to a point at which the calculated curve had begun to flatten out.

Analysis 2. This analysis was carried out by comparing (a) the mean values of the APTT, APTT ratio, and heparin activity in rabbits whose fibrinogen accretion was in the range of 10 ± 2.5 μg and (b) the mean anticoagulant values in rabbits whose fibrinogen accretion was in the range of 5 ± 2.5 μg. The plotted points corresponding to 10 ± 2.5 and 5 ± 2.5 were selected because they represented a marked reduction in fibrinogen accretion and clearly fell into the flat part of the curve expressing the relationship between fibrinogen accretion and anticoagulant effect.

RESULTS

Experimental Thrombosis Model

Rate of incorporation of 125I-fibrinogen into the preformed thrombi. The rate of incorporation of 125I-fibrinogen into preformed thrombi in animals receiving 0.9% NaCl infusion over the 10-hr period of study is shown in Fig. 1. There was a gradual incorporation of 125I-fibrinogen onto the thrombus, the rate of which appeared to be most rapid in the first few hours after thrombus formation and then declined with time. However, the values measured at 10 hr deviated only approximately 5% from the 10-hr value calculated from the linear regression line which had a correlation coefficient of 0.98.

Quantitation of thrombus growth. The mean weight of 12 thrombi removed from heparinized animals that had accreted less than 7 μg of fibrinogen, calculated from 125I-fibrinogen accretion, was 0.181 ± 0.19 g, while the mean weight of 20 thrombi removed from animals that had been treated with 0.9% NaCl infusion was 0.295 ± 0.13 g. This difference represented a percentage
Table 1. Fibrinogen Accretion Onto Experimental Venous Thrombi in Rabbits Given Identical Quantities of Heparin by Either Continuous Infusion or Intermittent Injection

<table>
<thead>
<tr>
<th>Dose of Heparin (U/kg/10 hr)</th>
<th>Number of Experiments</th>
<th>Fibrinogen Accretion (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Continuous Infusion</td>
</tr>
<tr>
<td>600</td>
<td>13</td>
<td>10.9 ± 8.0</td>
</tr>
<tr>
<td>450</td>
<td>8</td>
<td>16.3 ± 10.8</td>
</tr>
<tr>
<td>300</td>
<td>8</td>
<td>111.2 ± 63.0</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>122.5 ± 52.0</td>
</tr>
</tbody>
</table>

Values are means ± SD.

increase of 63% in weight of thrombi extracted from control animals over the weight of thrombi extracted from the heparin-treated animals.

The mean fibrinogen content of blood removed from isolated segments from 12 jugular veins was 216 µg, and the mean fibrinogen accreted onto 20 pre-formed thrombi in control animals calculated by the radioactive method was 122 µg. This difference represented an increase of 56.5% in fibrinogen content over the 10 hr of 0.9% NaCl infusion.

Effect of Heparin Treatment on the APTT and Heparin Activity

Heparin, administered by continuous infusion in a dose of 600 U/kg/10 hr (200 U/kg given as a bolus and then 400 U/kg by continuous infusion for 10 hr), resulted in a sharp rise in APTT and in heparin activity after the bolus injection. Subsequently, the mean APTT value remained between 60 and 80 sec and the mean heparin level between 0.3 and 0.4 U/ml for the last 7 hr of infusion. Intermittent heparin injections in the same total dose produced a sharp rise in APTT, which was greater than 100 sec at 1 hr after injection, and in the level of heparin activity, which was between 0.6 and 0.8 U/ml at 1 hr. The results of these tests had almost returned to baseline values when measurements were made at 3 hr, so that it was likely that there was no measurable heparin activity in these rabbits for a considerable portion of the 10-hr period of study.

The effects of heparin given by continuous infusion and intermittent injection, and of 0.9% NaCl given by continuous infusion on fibrinogen accretion are summarized in Table 1. Continuous infusion of heparin in doses of 600 U/kg for 10 hr and 450 U/kg/10 hr resulted in significant reductions in fibrinogen accretion when compared with the control group (p < 0.005 for both groups), but there was no significant reduction when heparin was given in a dose of 300 U/kg/10 hr. Similarly, there was a significant reduction in fibrinogen accretion when heparin was given by intermittent injections in a dose of 600 U/kg/10 hr as compared with the control (p < 0.005), but no significant reduction when heparin was given in doses of 450 U/kg/10 hr and 300 U/kg/10 hr.

Comparison of the continuous regimen with the intermittent heparin regimen showed that the continuous regimen was more effective than the intermittent infusion regimen at both the 600- and 450-U/kg/10 hr doses (p < 0.005 and p < 0.025, respectively).
The relationship between the antithrombotic and anticoagulant effects of heparin given by continuous infusion is shown in Fig. 2. There was a sharp reduction in fibrinogen accretion with increasing anticoagulant effect measured by the APTT, APTT ratio, or heparin activity.

The relationship between heparin activity and APTT in animals that had been treated with heparin by continuous infusion is shown as a regression line in Fig. 3A. A significant correlation ($r = 0.92$ and $p < 0.001$) between both tests was obtained. The experiments with the continuous infusion heparin regimen were repeated after pretreating the rabbits with porcine cryoprecipitate. Pretreatment of the animals with cryoprecipitate altered the slope of the regression line between heparin activity and APTT values (regression line in Fig. 3B), so that there was a considerably smaller increase in APTT for any given
level of heparin activity. For this group of animals, the relationship between the antithrombotic effect of heparin measured by fibrinogen accretion and its anticoagulant effect measured by heparin activity, APTT, or the APTT ratio is shown in Fig. 4. Again, there was a sharp reduction in fibrinogen accretion with increasing anticoagulant effect measured either by the heparin levels or APTT.

Analysis of the Relationship Between the Anticoagulant and Antithrombotic Effects of Heparin

The effects of different doses of heparin given by continuous infusion on fibrinogen accretion, APTT, and heparin activity in rabbits not treated with cryoprecipitate (subsequently referred to as “normal” rabbits) and cryoprecipitate-treated rabbits are shown in Table 2. The difference in fibrinogen accretion between the normal and cryoprecipitate-treated rabbits was not significant in the control group, nor was the difference significant in the groups treated with 600, 750, and 900 U/kg/10 hr. However, the results were significantly different in the group treated with 450 U/kg/10 hr (p < 0.001). The difference in the APTT values between the normal and cryoprecipitate-treated animals which had received 0.9\(^\circ\) NaCl (controls) and those which had received heparin in doses of 450, 600, 750, and 900 U/kg/10 hr was significantly different (p < 0.005). The difference in heparin level between these two groups of animals was significant for the 450 U/kg/10 hr dose (p < 0.02), but was not statistically significant for animals treated with 600, 750, and 900 U/kg/10 hr.
Fig. 4. Relationship between fibrinogen accretion and (A) mean APTT, (B) mean heparin activity, and (C) mean ratio of postheparin to preheparin APTT. Anticoagulant effects taken from values obtained between 3 and 10 hr in cryoprecipitate-treated animals given heparin by continuous infusion. Doses of heparin used were 450, 600, 750, or 900 U/kg/10 hr. The fitted curve is part of a rectangular hyperbola that expresses the relationship between the fibrinogen accretion and the anticoagulant effect. Lines have been drawn from the Y axis to a point representing 15 μg of fibrinogen accretion, and a vertical line has been dropped from this point to the X axis, indicating the corresponding level of anticoagulant effect.

The plotted points expressing the relationship between fibrinogen accretion and three measurements of anticoagulant effect of heparin for normal (Fig. 2) and cryoprecipitate-treated animals (Fig. 4) were tested in a number of models and found to fit best into a rectangular hyperbola curve. This curve implied that there was a linear relationship between the reciprocal of fibrinogen accreted and the anticoagulant effect, and the choice of this model was validated by the uniformly high correlation coefficients obtained between the reciprocal fibrinogen accretion and the APTT ($r = 0.85$), APTT ratio ($r = 0.81$), and heparin activity ($r = 0.87$) in the normal rabbits and the correlation coefficient of
Table 2. Effect of Heparin Given by Continuous Infusion in Different Doses on Fibrinogen Accretion, APTT, and Heparin Activity in Normal and Cryoprecipitate-treated Rabbits

<table>
<thead>
<tr>
<th>Dose of Heparin (U/kg)</th>
<th>Number of Rabbits</th>
<th>Fibrinogen Accretion (µg)</th>
<th>Mean APTT Over 3-10 hr (sec)</th>
<th>Mean Heparin Activity Over 3-10 hr (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal* Cryo</td>
<td>Normal</td>
<td>Cryo</td>
<td>Normal Cryo</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
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<td>122.5 ± 52.0</td>
<td>149.2 ± 55.0</td>
</tr>
<tr>
<td>300</td>
<td>8</td>
<td>9</td>
<td>11.2 ± 63.0</td>
<td>30.7 ± 4.5</td>
</tr>
<tr>
<td>450</td>
<td>8</td>
<td>9</td>
<td>16.3 ± 10.8</td>
<td>51.1 ± 17.3</td>
</tr>
<tr>
<td>600</td>
<td>13</td>
<td>12</td>
<td>10.9 ± 8.0</td>
<td>20.2 ± 14.1</td>
</tr>
<tr>
<td>750</td>
<td>5</td>
<td>5</td>
<td>5.9 ± 1.4</td>
<td>6.0 ± 2.3</td>
</tr>
<tr>
<td>900</td>
<td>7</td>
<td>6</td>
<td>5.0 ± 3.6</td>
<td>6.0 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SD.
*Not treated with cryoprecipitate.
†Treated with cryoprecipitate.

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and 29.7 sec in cryoprecipitate-treated rabbits. This difference was statistically significant \( (p < 0.01) \). In contrast, the corresponding heparin levels of 0.17 U/ml and 0.21 U/ml and the corresponding APTT ratios of 1.5 and 1.4 were not significantly different.

**Analysis 2.**

(a) This analysis was carried out by comparing the mean values of the APTT and heparin activity in rabbits whose fibrinogen accretion was in the range \( 10 \pm 2.5 \mu g \). These values for fibrinogen accretion were part of the total data from which the fitted curve was computed and were obtained from 11 normal rabbits and 8 cryoprecipitate-treated rabbits. The mean fibrinogen accretion in these two groups of animals was 9.8 and 9.3 \( \mu g \), respectively. The mean APTT in the 11 normal animals was 74.7 and in the 8 cryoprecipitate-treated animals it was 35.4; this difference was statistically significant \( (p < 0.001) \). In contrast, the corresponding mean heparin level was 0.39 U/ml in normal rabbits and 0.34 U/ml in cryoprecipitate-treated rabbits; this

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**Fig. 5.** Relationship between the jugular vein bleeding time and (A, C) APTT and (B, D) heparin activity. (A, B) Control animals. (C, D) Rabbits pretreated with cryoprecipitate. Note the progressive increase in the bleeding time with increasing APTT values and heparin levels in both groups of animals. Correlation coefficients \( (r) \) for A, B, C, and D are 0.62, 0.66, 0.68, and 0.84, respectively; \( p < 0.001 \) in all four instances.
HEPARIN AND THROMBOSIS

difference was not statistically significant. The APTT ratio corresponding to this level of fibrinogen accretion was 2.4 in the normal rabbits and 1.6 in the rabbits treated with cryoprecipitate; this difference was statistically significant ($p < 0.05$).

(b) This analysis was carried out by comparing the mean values for APTT and heparin activity in 12 normal and 9 cryoprecipitate-treated rabbits whose fibrinogen accretion was $5 \pm 2.5$ mg. The mean fibrinogen accretion in these two groups was 5.35 and 5.15 mg, respectively. The mean APTT value for the normal rabbits was 78.7 sec, and for the cryoprecipitate-treated rabbits it was 49.5 sec; this difference was statistically significant ($p < 0.001$). In contrast, the corresponding values for heparin levels were 0.49 U/ml in the normal rabbits and 0.61 U/ml for cryoprecipitate-treated rabbits; this difference was not statistically significant. The corresponding APTT ratios were 2.6 for normal rabbits and 2.3 for cryoprecipitate-treated rabbits; this difference was not statistically significant. Thus using each of these methods of analysis it was found that the APTT value which corresponded to marked inhibition of fibrinogen accretion was significantly higher in the normal rabbits than in the cryoprecipitate-treated rabbits, but the results of the heparin assay which corresponded to these levels of fibrinogen accretion was not significantly different.

Effect of Heparin on the Bleeding Time

The relationship between the bleeding time and the heparin activity and APTT in both the cryoprecipitate-treated and normal animals is shown in Fig. 5. In both groups of animals the bleeding time increased proportionately to the heparin activity and APTT values. Although the mean bleeding time was shorter in the cryoprecipitate-treated animals than in the normal animals, this difference was not statistically significant.

DISCUSSION

The aim of this study was to determine whether a relationship exists between the antithrombotic effect of heparin in vivo as measured by the inhibition of fibrinogen accretion onto experimental jugular vein thrombi and the anticoagulant effects of heparin as measured either by the heparin activity assay or by APTT. The present data show that estimations of plasma heparin activity and APTT are equally sensitive in predicting the antithrombotic effect of heparin in rabbits with experimental thrombosis and that these tests are highly correlated with each other.

By comparison with these results in rabbits, patients with venous thrombosis show a variable anticoagulant response to heparin when this is measured either by the protamine sulfate titration test or by APTT, and the correlation between these two tests is poor.\textsuperscript{2} Porcine cryoprecipitate was infused into rabbits in an attempt to dissociate the results of the APTT response from the results of the heparin assay. Cryoprecipitate-treated rabbits had a significantly shorter pre-heparin treatment APTT and showed a reduced APTT response relative to the result of the heparin assay in animals that were treated with heparin.

Objective comparisons between the normal and cryoprecipitate-treated rabbits of the anticoagulant effect (APTT and heparin level) which corre-
sponded to maximum inhibition of fibrinogen accretion were difficult because the fitted curves of the relationship between the anticoagulant and the antithrombotic effects of heparin did not have an identifiable point of inflection. Comparisons were therefore made of the APTT and heparin assay values which corresponded to a number of different levels of fibrinogen accretion, all of which represented a marked reduction from the amount of fibrinogen accreted onto thrombi in nonheparinized rabbits. Using each of these methods of analysis, it was found that fibrinogen accretion was markedly inhibited at a similar heparin level in both the cryoprecipitate-treated and normal animals, but that this level of inhibition of fibrinogen accretion was attained with a significantly lower APTT value in the cryoprecipitate-treated animals than in the normal animals. Thus, in the experimental model used, the antithrombotic effect of heparin was reflected by the heparin assay even though the pretreatment APTT value had been shortened by infusion of cryoprecipitate and the APTT response to heparin had diminished relative to the level of heparin activity.

A number of factors need to be considered before the results of these studies can be applied to man. The first is the method of producing experimental thrombi. The second is the method used to dissociate the APTT response to heparin from the result of the heparin assay. The third is the general problem of applying the results of animal experiments directly to human disease.

The method for producing experimental thrombi was a modification of Weisler and Reimer’s method, in which local thrombin injection replaced systemic serum infusion. The thrombus so produced progressively accreted fibrin, so that in control animals over a period of 10 hr it increased in weight by approximately 60%, a value which roughly corresponded to the calculated percentage increase in the fibrin content of the thrombus. Inhibition of growth of the thrombus required that heparin infusion be continued in adequate doses for the period of the study since inhibition of accretion was significantly less when heparin was given by intermittent injection. The mechanism by which venous thrombosis is produced clinically is uncertain and likely to differ to some extent from patient to patient. However, stasis and increased blood coagulability are recognized as two of the most important factors which predispose to clinical thrombosis; thus from that point of view the experimental model used would seem to be relevant to some forms of human venous thrombosis. Furthermore, once an occlusive thrombus forms, its growth is unlikely to be influenced by the mechanism of its production.

In the present study, porcine cryoprecipitate was used to reduce the APTT response to heparin. Others have shown that elevated levels of factor VIII may mask the APTT response in patients treated with heparin. In addition, we have previously reported that there is a considerable variation in the relationship between APTT response and the result of the protamine-heparin assay in patients treated with heparin. The reason for this variation is not entirely clear, but could be due in part to differences in the level of coagulation factors, particularly factor VIII, and to other plasma proteins among these patients. We have also found that the addition in vitro of human cryoprecipitate shortens the APTT in heparinized plasma without affecting the heparin assay. It is likely that other factors also contribute to the variable relationship between...
the heparin level and the APTT in patients treated with heparin; however, since elevated levels of factor VIII can mask the APTT response to heparin in patients, the use of cryoprecipitate in our experiments was considered to be a reasonable approach.

In considering the third issue, caution should always be exercised before applying the results of animal experiments to human disease. However, it is reasonable to hypothesize from the experimental findings that the heparin assay could also be a sensitive method for predicting the antithrombotic effects of heparin in patients with venous thromboembolism. In these patients, the incidence of clinical recurrence and of bleeding is low when heparin is given by continuous infusion in a dose which increases the APTT to 1.5–2.5 times the normal control level. Nevertheless, there are clinical situations, such as the development of venous thromboembolism in the early postoperative period, in which it would be desirable to avoid bleeding by using the minimum effective dose of heparin. In some of these patients the pretreatment APTT is short and large doses of heparin may be required to increase the APTT to twice the normal control value. Our experimental findings raise the possibility that, in these patients, it might be safer to monitor treatment by using the heparin assay or by relating the degree of prolongation of the APTT to the pretreatment test result rather than to attempt to increase the APTT to twice the normal control value. However, this possibility would have to be tested clinically before firm recommendations could be made.

Although heparin given by continuous infusion showed a better antithrombotic effect than that given by intermittent injection, these results should not be taken as evidence of the superiority of continuous intravenous heparin over intermittent injection, since it was likely that the rabbits treated with intermittent injection went through periods when there was no circulating heparin activity. Nevertheless, these findings do serve to emphasize the need to maintain heparin in the circulation throughout the period of treatment in established venous thromboembolism.

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