Effect of Increased Fibrinogen Concentration on the Lysis of In Vivo Thrombi

By N. U. Bang, M.D., A. H. Freiman, M.D., and E. E. Clipfton, M.D.

The significance of the fibrinogen content of the blood in the clotting mechanism has received little consideration except for the effects of obvious hypofibrinogenemia in hemorrhagic states. Hyperfibrinogenemia occurs in many conditions that are associated with thrombotic complications and may be a factor in their formation.

The purpose of the present study has been to compare the response of thrombi formed at normal and at increased blood fibrinogen levels to treatment with fibrinolysin.

The rate and completeness of any enzymatic reaction will be a function of both the amount of substrate and the amount of enzyme available. This relationship has been demonstrated repeatedly for the fibrinolytic enzyme system. Guest, Ware, and Seegers found that the lysis time of a purified bovine fibrin clot would increase in direct proportion to the amount of fibrin in the clot in the presence of a given amount of chloroform activated bovine plasmin.

In vitro studies, using bovine fibrin clots and a fibrinolytic system composed of streptokinase activated plasminogen, have been reported. The rate of fibrin breakdown was determined by the amount of nitrogen released from the clot. Release of radioactivity from labeled fibrinogen clots was also measured and similar results were obtained. Fibrin breakdown was found to be slow and incomplete when the clot was formed at fibrinogen concentrations of more than 450 mg. per cent. A marked increase in the clot dissolution was found at fibrinogen concentrations between 300 and 400 mg. per cent, the physiologic range of fibrinogen concentration in human plasma in our laboratory. These results seem to indicate that the enzymatic dissolution of clots formed at fibrinogen levels above the physiologic range would be impaired. It was felt that these observations held important clinical implications and that they warranted in vivo studies under controlled and reproducible conditions.

Methods

Mongrel dogs (male and female, weighing from 5.5 to 17 Kg.) were used as experimental animals. Clots were produced in arteries and veins by the local injection of serum as previously described. Using this technic, 1 clot was formed in a vessel, either a jugular vein or a femoral artery. As soon as a clot was formed and demonstrated by an angiogram, 10 Gm. of bovine fibrinogen dissolved in 100 ml. of isotonic saline was administered by intravenous infusion. Immediately after the infusion, the contralateral vessel (jugular vein or femoral artery) was clamped off and a thrombus was produced as in the first vessel. As a result, the initial clot was formed while the fibrinogen was normal, while the second clot was formed in the hyperfibrinogenemiac state. Repeated angiograms were performed to demonstrate the presence of the clot in both vessels. Within 2 hours, a course of treatment with fibrinolysin was started in all animals. This consisted of an infusion of 42,000 units per hour for 6 hours.

Blood samples were withdrawn from the animals before the infusion of fibrinogen, immediately after the fibrinogen infusion, and after 3 hours of fibrinolysin treatment. These samples were assayed for fibrinogen, fibrinolytic activity, whole clot lysis and antiplasmin fibrinolytic activity. After completion of the experiment, the vessel segments in which the clots had been formed were removed and examined for clot.

The radiographic and autopsy evaluation was performed according to the criteria indicated in the preceding publication.
Table 1

Laboratory Data Obtained Before and After Fibrinogen Administration and After Three Hours of Fibrinolytic Treatment

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Fibrinogen mg./100 ml.</th>
<th>Fibrinolytic activity hours</th>
<th>Antiplasmin titer (min.)</th>
<th>Fibrinogen mg./100 ml.</th>
<th>Fibrinolytic activity hours</th>
<th>Antiplasmin titer (min.)</th>
<th>After 3 hours plasmin therapy</th>
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<td>350</td>
<td>9</td>
<td>16</td>
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<td>31</td>
<td>321</td>
<td>—</td>
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<td>48</td>
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</table>

*Fibrinolysin, 42,000 /µhour was used in all animals until dissolution of 1 clot had occurred or 6 hours of therapy had been given. Prior to therapy, whole clot lysis was uniformly greater than at 24 hours, while after 3 hours the blood was incoagulable.

Results

Thirteen dogs were included in the study. Three dogs died in anaphylactic shock with respiratory and cardiac arrest following the rapid administration of bovine fibrinogen. Ten dogs survived the experimental procedures. In 9 dogs, clot formation and dissolution was studied in 2 jugular veins. In 1 dog, 2 femoral artery clots were studied. The laboratory data are recorded in table 1. The infusion of fibrinogen resulted in a definite increase in the circulating fibrinogen levels in all animals except 1. The increases observed ranged from 15 to 60 per cent of the control fibrinogen levels. Since anaphylactic shock occurred in 3 animals, one might expect an increase in fibrinolytic activity after the infusion of fibrinogen due to a reaction to the protein. In 3 animals (Nos. 54, 56, 58) there was some increase in spontaneous fibrinolytic activity following the fibrinogen infusion. In only 1 dog (No. 58) was the fibrinolytic activity significantly greater than that found occasionally in control dogs. It should be noted that this animal was the only 1 of the 10 which failed to show a significant rise in the fibrinogen level. The other 7 surviving animals showed no change in fibrinolytic activity after the fibrinogen infusion. The antiplasmin fibrinolytic titers were not found to vary significantly with the infusion of fibrinogen.

The administration of fibrinolysin resulted in a marked fibrinolytic activity ranging from 10 to 45 minutes lysis time in all the animals. A marked fall in the fibrinogen level was observed in all cases and the blood of all the animals became incoagulable after 3 hours of treatment.

In table 2, are listed the detailed reports for the 10 experiments. From this table it is seen that complete lysis of the clot formed at normal fibrinogen levels was achieved in 21/2 to 5 hours, as determined by both x-ray and autopsy evaluation. The clots formed during hyperfibrinogenemia showed a significantly increased resistance to fibrinolytic therapy. Lysis of the latter clots was observed in only 1 of the 10 animals, and in this case only after 6 hours of treatment. Significant amounts of thrombus were found in the 9 other vessels, both by x-ray and by pathologic examination after 6 hours of treatment (fig. 1).

There was no direct correlation between recorded fibrinogen concentration and the rate of dissolution of the clot, or between the recorded fibrinolytic activity and the dissolution of the clot.
Table 2

Results of Treatment: X-ray and Autopsy Evaluation*

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Radiographic evaluation (no-partial-complete lysis) Low fibrinogen</th>
<th>High fibrinogen</th>
<th>Autopsy evaluation (no-partial-complete lysis) Low fibrinogen</th>
<th>High fibrinogen</th>
<th>Low fibrinogen clot completely dissolved after: h.</th>
<th>High fibrinogen clot completely dissolved after: h.</th>
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<td>5½</td>
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<td>C</td>
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<td>P</td>
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<td>6</td>
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</table>

*Radiographic and autopsy evaluation is coded as follows: C=complete lysis; P=partial lysis; N=no lysis. The criteria for these evaluations are outlined in the preceding paper.
In animals Nos. 54-60, the experiment was discontinued after the low fibrinogen clot had completely dissolved. In animals Nos. 29, 30, 53, therapy was continued beyond this point to a total treatment time of 5½ to 6 hours.

Discussion

The results reported here demonstrate a significant difference in the rate of dissolution of clots formed at normal and elevated blood fibrinogen levels in the same animal when treated with a standard dose of fibrinolysin. These experiments confirm the previously reported observation on lysis of clots of varying fibrinogen concentrations in vitro.8

The experimental setting in which clots of varying fibrin content have been studied in the same animal exclude a number of variables possibly influencing the results. The data strongly suggest that thrombi formed in the presence of hyperfibrinogenemia become markedly resistant to the action of the fibrinolytic enzyme. These findings have important implications for experimental studies on fibrinolysis and for clinical situations.

A method previously used for studying fibrinolysis in animals6 involves the use of thrombi produced by the addition of radiopaque fibrinogen to blood. The abnormally high concentration of fibrinogen in such clots will make lysis more difficult. Our findings of resistance of clots so formed to fibrinolytic treatment, using this technic, led to the present study. The clinical importance of these findings may be even more significant. Hyperfibrinogenemia is a common occurrence in many clinical conditions in which thromboembolism is seen frequently as a complication.7

These include inflammatory diseases, coronary thrombosis, many chronic diseases, and in postoperative and posttraumatic states. The fibrinogen levels in such conditions often exceed those we have produced experimentally. A thrombus formed under these conditions may prove to be highly resistant to dissolution by the fibrinolytic enzyme either occurring in the blood stream spontaneously or introduced as a therapeutic agent. The correlation between fibrinogen levels and the clinical response to fibrinolytic therapy in patients with thromboembolic disease is being studied in our laboratory at the present time.

It has been suggested that deposition and subsequent lysis by the fibrinolytic enzyme occur constantly and it seems likely that a balance exists between clotting and lysis under normal physiologic circumstances.8 It is conceivable that the effect of physiologic fibrinolysis would be materially less during hyperfibrinogenemia and that this may play a role in the production and extension of intravascular thrombosis. The possible role of hyperfibrinogenemia as a thrombogenic factor deserves further investigation. The prevention
Treatment of high and low fibrinogen clots in dogs No. 55. The first angiogram (left) shows the presence of clots in both jugular veins. The "low fibrinogen" is on the right and the "high fibrinogen" clot is on the left. The second angiogram (right) shows complete lysis of the thrombus on the right side occurring after 5½ hours treatment and the presence of clot on the left side as indicated by the filling defects. At the end of the 6-hour treatment, moderate amounts of thrombus were still found in the vein on the left side and none on the right.

and control of hyperfibrinogeneic states by fibrinolytic and proteolytic enzymes may become important therapeutic measures of the future.

Summary
The dissolution of clots formed at normal fibrinogen levels and in hyperfibrinogenemia was studied in 10 dogs receiving a standard course of fibrinolytic treatment. A significantly increased resistance of clots formed at high fibrinogen levels to fibrinolytic therapy was found.

Summario in Interlingua
Esseva studiata le dissolution de coagulos a nivellos normal de fibrinogeno e in hyperfibrinogenemia in 10 cimes recipiente un curso standard do tractamento fibrinolytic. Esseva constatate un augmento significative del resistentia de coagulos formate a alte nivellos de fibrinogeno contra le therapia fibrinolytic.

References
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