CLINICAL PROGRESS

Proteolysis, Fibrinolysis, and Coagulation

Significance in Thrombolytic Therapy

By Benjamin Alexander, M.D., Liberto Pechet, M.D., and Allan Kliman, M.D.

NOW THAT infectious, nutritional, neonatal, and other diseases have become largely controllable, thromboembolism ranks as a major threat to life and limb. Expanding knowledge of the blood fibrinolytic system, and its potential therapeutic value, has stimulated renewed interest in this area. Additional concern derives from its possible etiologic importance in atherosclerosis, according to the v.Rokitansky-Duguid-Astrup concept of imbalance between interarterial fibrin deposition and its removal.

The clinician finds it frustrating that nature has provided a complicated mechanism (fig. 1) for maintaining vascular patency, yet which may uncontrollably fail to function adequately. Any hydraulic engineer concerned with the continuous recirculation of a medium maintained precariously in a fluid state in a closed system, would recognize the necessity for some mechanism to filter and dispose of accumulating insoluble matter and debris, especially when the fluid contains living formed elements, colloids, macromolecules, and crystalloids—all flowing in a dynamic interrelationship. What is really surprising is that the mechanism operates as well as it does. It is only its unpredictable failure and difficulty in diagnosing it that presents a clinical problem.

The Coagulation Balance

As indicated schematically in figure 2, blood fluidity is maintained in dynamic equilibrium, which may be shifted either toward hypocoagulability, resulting in hemorrhage, or toward hypercoagulability, resulting in thrombosis. The numerous clotting components involved are depicted in table 1 where they are categorized in the four sequential phases of coagulation in which they function: (1) thromboplastin generation; (2) thrombin formation from prothrombin; (3) fibrin deposition; and (4) fibrin dissolution.

Although the conventional anticoagulants are more often than not invoked after a thrombotic episode, their use in prophylaxis is substantial, and is receiving increasing attention. In contrast to these drugs, thrombolytic agents are intended to dissolve clots only after they form. Nobody can argue the validity of this approach, especially when an already existing physiologic mechanism is employed, provided we have safe therapeutic agents, adequate diagnostic tests, adequate comprehension of the thrombolytic mechanism, adequate guides to therapy, understanding of hazards, and antidotes available whenever therapy may get out of hand.

Relation between Clotting and Fibrinolysis

How can our extensive experience with coagulation phenomena advance our knowl-
PROTEOLYSIS, FIBRINOLYSIS, AND COAGULATION

PROACTIVATOR
(PLASMA PLASMIN)

SK

PLASMINOGEN

PLASMIN

DESTROYS FIBRINOGEN
LYSES FIBRIN CLOT

DESTROYS AC-GLOBULIN

ANTIPLASMIN

Figure 1
Simplified scheme of the blood fibrinolytic system. Plasminogen is the inert precursor of plasmin; plasmin, with fibrinolysin; Ac-globulin, with factor V; SK refers to streptokinase, an entity derived from streptococci, which activates the fibrinolytic system in many species, including man.

edge of thrombolysis? Indeed, is there any close relationship between clotting as such and fibrinolysis? Recent investigations do indeed show a close interrelationship. It is striking for example how fibrinolytic activity evolves as blood clots.1 Also noteworthy is the antithrombotic effect of fibrinogen-fibrin and the products of fibrinolysis.2-8 Also to be mentioned is the antifibrinolysin activity of platelets.9,10 Space does not permit further elaboration. Suffice it to say that those concerned with thrombolysis, or, speaking as an internist, with "medical thrombectomy," should be familiar with the other coagulation phenomena, since they both overlap and profoundly influence one another. Thrombolytic agents affect certain clotting constituents, some adversely, leading to possible hemorrhage. When used in combination with the conventional anticoagulants, as has been done,11-14 this could conceivably be serious, if not catastrophic. Accordingly, the internist who invokes thrombolytic therapy should recognize that in so doing he may so endanger hemostasis as to replace thrombotic disease with hemostatic failure. Our experience with the orthodox anticoagulants is all too bitter in this regard.

The fibrinolytic mechanism has been known for two thirds of a century, and its historical aspects have been excellently reviewed.1,10,15-19 The active enzyme (fibrinolysin, plasminogen), arising from its inert precursor (profibrinolysin) in any one of several ways, is a protease with a particularly high specificity for the fibrinogen-fibrin system. It also exerts a proteolytic effect on other plasma proteins, especially on certain other clotting components.20

One should also bear in mind, moreover, that blood contains other proteolytic enzymes besides fibrinolysin. Thus, thrombin that arises from factor II (prothrombin) is a highly specific protease that splits fibrinogen, yielding fibrin and fibrinopeptide. Similarly, blood contains tryptases that have proteolytic properties resembling pancreatic trypsin.10 Evidence is presented to show certain similarities as well as differences in the action of these various proteases on clotting factors.

The Shwartzman Reaction, Intravascular Coagulation, and Fibrinolysis

Let us now consider what may happen when fibrinolysis is activated, especially in patients with possible abnormal hypercoagulation in connection with thrombotic disease. Several years ago McKay and Shapiro21 showed that...
the generalized Shwartzman phenomenon induced in rabbits by bacterial endotoxin, reflected intravascular coagulation that results in bilateral renal cortical necrosis due to fibrin deposition in the small renal vessels. This intravascular coagulation, leading to moderate fibrinogenopenia, can be prevented by anticoagulants, or it can be prevented, and even reversed, by administration of streptokinase, which activates the fibrinolytic system.

Thus, we have in the Shwartzman phenomenon an experimental approach to investigation of intravascular clotting and of thrombolysis. In a recent study in our laboratory the Shwartzman reaction alone was found to produce some reduction in fibrinogen and platelets (table 2), as well as marked impairment in thromboplastin generation (fig. 3), the first phase of coagulation. Nevertheless, despite these defects hemostasis was not seriously impaired, although some animals succumbed to hemopericardium following cardiac puncture. Similarly, fibrinolytic activation by streptokinase alone did not affect hemostasis in the doses used. Plasma fibrinogen remained relatively unchanged despite the fact that clot lysis was markedly accelerated. When streptokinase was given concurrent with the Shwartzman reaction, however, a severe hemorrhagic diathesis resulted with complete afibrinogenemia or marked hypofibrinogenemia. Thus, although the defects arising from each—namely streptokinase alone or the Shwartzman reaction alone—were not sufficient to endanger hemostasis, together they produced profound hemostatic failure. It is thus evident that fibrinolytic activation in a situation where intravascular fibrin deposition is

---

**Table 1**

<table>
<thead>
<tr>
<th>Phase of Coagulation</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Procoagulant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV (CA++)</td>
<td>IV (CA++)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>II (Prothrombin)</td>
<td>I (Fibrinogen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V (AC-G)</td>
<td>V (AC-G)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII (AHF)</td>
<td>VII (Proconvertin)</td>
<td>Fibrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX (PTC)</td>
<td>X (Stuart)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X (Stuart)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XI (PTA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XII (Hageman)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Intrinsic TPL (Blood) |             |             |             |             |
| Extrinsic TPL (Tissue) |             |             |             |             |

| **Anticoagulant**     |             |             |             |             |
| Anti-TPL              | Antithrombin|             |             |             |
| Hageman Inhibitor     | Heparin     |             |             |             |
| Heparin               | ? Anticonvertin |             |             |             |

|                      |             |             |     |             |
| Hageman Inhibitor    |             |             |     |             |
| Heparin              |             |             |     |             |

|                      |             |             |     |             |
|                      |             |             |     |             |

Circulation, Volume XXVI, October 1962
Table 2

Effect of Endotoxin on Blood Coagulation in Rabbits

<table>
<thead>
<tr>
<th>Animal</th>
<th>Clotting time (min.)</th>
<th>Clot Retraction</th>
<th>Platelets (10/mm.³)</th>
<th>Prothrombin activity (%)</th>
<th>Serum pro-thrombin (%)</th>
<th>Fibrinogen (mg. %)</th>
<th>Thromboplastin generation</th>
<th>Clinical course†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>AO</td>
<td>After*</td>
<td>Before</td>
<td>BO</td>
<td>After</td>
<td>Before</td>
<td>CO</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>11</td>
<td>9</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Poor</td>
<td>Good</td>
<td>Poor</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>185</td>
<td>175</td>
<td>115</td>
<td>225</td>
<td>110</td>
<td>150</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>120</td>
<td>110</td>
<td>110</td>
<td>100</td>
<td>105</td>
<td>105</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>90</td>
<td>100</td>
<td>85</td>
<td>90</td>
<td>81</td>
<td>100</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>530</td>
<td>550</td>
<td>650</td>
<td>350</td>
<td>650</td>
<td>380</td>
<td>520</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Poor</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Poor</td>
<td>Normal</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Survived until sacrificed</td>
<td>Survived until sacrificed</td>
<td>Died of hemo-pericardium</td>
<td>Died of hemo-pericardium</td>
<td>Survived until sacrificed</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No excessive fibrinolysis was observed.
*One hour after second dose of endotoxin.
†Four other animals, not included, died of cardiac puncture hemo-pericardium at 2, 3, and 48 hours after second dose of endotoxin. Three showed poor thromboplastin generation.

concurrently taking place, as might occur under pathologic circumstances, may be catastrophic.

Similar experiments were also made in vitro. The effects were compared with those of trypsin, an enzyme long known to affect coagulation factors,25-27 and studied extensively by us. Moreover, trypsin is still available for thrombolytic therapy28,29 despite the reported hazards,30,31 and much has been made of its alleged anti-inflammatory action.32

The simultaneous addition to rabbit plasma of a minute amount of thrombin (0.125 U per ml.) and streptokinase (Varidase, 2,500 U per ml.) caused prompt disappearance of assayable fibrinogen, in striking contrast to the effects of the streptokinase alone (fig. 4).24

This latter mixture, it should be noted, had marked fibrinolytic activity, since following recalcification of the streptokinase-containing plasma, the clot dissolved within 2 minutes.

We have thus duplicated in vitro what was observed in vivo, namely, fibrin deposition by thrombin, plus fibrinolytic activity. As a result, afibrinogenemia or marked hypofibrinogenemia supervened. This is in harmony with the findings of Sherry and Alkjaersig,10,16 who earlier emphasized that when clotting simultaneously occurs with fibrinolytic activation, rapid "fibrinolysis" ensues. This was attributed by them to plasmin adsorption onto the clot, where it exerts its lytic action. These authors believe that fibrinolysin exhibits little fibrinogenolytic activity compared with fibrinolytic, whereas thrombin is thought to be fibrinogenolytic but not fibrinolytic. Be that as it may, a combination of thrombin and fibrinolysin causes prompt and virtually complete obliteration of the thrombin-fibrinogen interaction.

Sherry and Alkjaersig10,16 attribute the relatively low fibrinogenolytic action of fibrinolysin vis-a-vis fibrin, to the antifibrinolysin of plasma; in this way fibrinogen is thought to be preserved. Our observations suggest an alternate explanation, namely, that fibrinolysin promptly attacks the products of the thrombin-fibrinogen interaction (fibrin and fibrin monomers) rather than fibrinogen itself. In so doing, fibrinolysin could in vivo accelerate clotting by releasing absorbed thrombin from the thrombin-fibrinogen-fibrin

Circulation, Volume XXVI, October 1962
complex, thus initiating or accelerating a vicious circle leading to the defibrination characteristic of "fibrinolytic purpura." Such accelerated clotting, or "hypercoagulability," has often been observed early in thrombolytic therapy.\textsuperscript{33-35} In any event, great caution is indicated whenever thrombin may be forming, as in thromboembolic disease. Fortunately, an antidote is now available in epsilon (\(e\))-aminocaproic acid, a relatively safe drug which inhibits fibrinolytic activation.\textsuperscript{36-38} As with vitamin K\(_1\) or protamine in reversing the conventional anticoagulants, one can now disengage from thrombolytic therapy with this drug. It is apparently also useful in idiopathic fibrinolytic states.\textsuperscript{38}

**Effect of Trypsin and Fibrinolysin on Thrombin-Fibrinogen Interaction**

Since much of our knowledge regarding fibrinolysis depends on our ability to measure fibrinogen, and this in turn upon its clotting by thrombin, it was considered important to study this reaction in more isolated systems. As evident in figure 5, within 30 seconds after exposure of fibrinogen to trypsin (2.0 U per ml.), its clotting by thrombin was greatly impaired, particularly at fibrinogen concentrations below 60 mg. per cent. Larger concentrations of trypsin abolished clotting almost instantaneously. Fibrinolysin acted similarly, a finding in contrast to the observation already described (fig. 4) for whole plasma. The effect was almost instantaneous, and with fibrinolysin it was progressive (fig. 6): in 3 minutes, clotting was greatly delayed, and within 10 minutes the fibrinogen was incoagulable. The effects were not attributable to thrombin destruction by the trypsin or fibrinolysin in the incubation


*Circulation, Volume XXVI, October 1962*
mixture. Prior exposure of thrombin to plasmin did not affect its clotting ability. Although trypsin, on the other hand, destroys thrombin (8 U of trypsin completely inactivates 1 U of thrombin in 15 minutes), it does not do so sufficiently fast to explain the observations.

These data, resembling in vivo and in vitro observations of Fletcher et al.,39 Deutsch and Fisher,40 Beller and Glas,35 and, as will be seen later, observations on a patient who was treated, raise provocative questions: How is the thrombin-fibrinogen interaction compromised? Is this true "fibrinogenolysis," namely, proteolytic destruction of the fibrinogen? If so, how can the fibrinogen be degraded that instantaneously? Or, do the phenomena reflect competitive blocking involving the substrate, fibrinogen, and the respective protease—trypsin or plasmin—vis-a-vis thrombin? And how do the phenomena relate to the incoagulability and apparent fibrinogenopenia in pathologic or induced thrombolytic states? Indeed, as will be seen later, the degree to which fibrinolysin blocks the clottability of fibrinogen by thrombin may be a valuable guide to thrombolytic therapy. Finally, is this the antithrombin phenomenon observed by Kowalski and colleagues,4,5 who attribute it to the proteolytic products obtained from fibrinogen by fibrinolysin? Aside from its basic biologic significance, the findings are clinically important, since the fibrinogen is no longer assayable,41 or available for hemostatic function.

Effect of Proteases on Clotting Components

Let us now go on to some observations on the action of proteases on other clotting components. The data help elucidate certain observations in patients under treatment. As shown in table 3, of many proteolytic enzymes studied, only two—trypsin and papain—could convert factor II to thrombin.42,43 In the presence of trypsin, thrombin evolves rapidly (fig. 7), no other clotting factor is needed, and the reaction is considerably enhanced by electrolytes.

Similar studies were made on highly purified factor VII (proconvertin) and factor X (Stuart).44,45 Trypsin profoundly activates

---

*Circulation, Volume XXVI, October 1952*
### Table 3

**Effect of Proteolytic Enzymes on Prothrombin, Thrombin, and Fibrinogen**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Effect on prothrombin</th>
<th>Thrombin formation*</th>
<th>Effect on thrombin</th>
<th>Thrombin-fibrinogen interaction†</th>
<th>Effect on fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzyme</strong></td>
<td></td>
<td>Factors Rich &amp; Poor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminotripeptidase</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bromelin</td>
<td>0</td>
<td>+</td>
<td>0 for 48 hr.</td>
<td>—</td>
<td>Inactivation</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>Progressive, slow inactivation</td>
<td>0</td>
<td>0 for 2 hr.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cathepsin B</td>
<td>Progressive, rapid inactivation</td>
<td>0</td>
<td>0 for 24 hr.</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Progressive, rapid inactivation</td>
<td>0</td>
<td>Progressive, rapid inactivation</td>
<td>0</td>
<td>Rapid inactivation</td>
</tr>
<tr>
<td>Collagenase</td>
<td>Progressive inactivation</td>
<td>0</td>
<td>Moderate inactivation</td>
<td>—</td>
<td>Inactivation</td>
</tr>
<tr>
<td>Elastase</td>
<td>Progressive, rapid inactivation</td>
<td>0</td>
<td>Progressive, rapid inactivation</td>
<td>0</td>
<td>Rapid inactivation</td>
</tr>
<tr>
<td>Flein</td>
<td>Progressive, very rapid inactivation</td>
<td>0</td>
<td>Precipitation</td>
<td>0</td>
<td>Rapid inactivation</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>0</td>
<td>0</td>
<td>0 for 24 hr.</td>
<td>—</td>
<td>Dissolves clot</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0</td>
<td>0</td>
<td>0 for 24 hr.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovomucoid trypsin inhibitor</td>
<td>0 for 24 hr.</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>Prompt, moderate inactivation (30%), not progressive</td>
<td>0</td>
<td>Slow, progressive inactivation</td>
<td>—</td>
<td>Moderate inactivation in 60 sec., not progressive</td>
</tr>
<tr>
<td>Papain</td>
<td>Immediate and progressive inactivation</td>
<td>+++</td>
<td>Moderate inactivation, 24 hr.</td>
<td>Enhanced</td>
<td>Clotted</td>
</tr>
<tr>
<td>Pepsin</td>
<td>Slow, progressive inactivation</td>
<td>0</td>
<td>Rapid inactivation</td>
<td>—</td>
<td>Rapid inactivation</td>
</tr>
<tr>
<td>Plasmin</td>
<td>0</td>
<td>0</td>
<td>0 for 24 hr.</td>
<td>?0</td>
<td>Progressive inactivation</td>
</tr>
<tr>
<td>Rennin</td>
<td>Progressive inactivation</td>
<td>0</td>
<td>Moderate inactivation</td>
<td>—</td>
<td>Clotted</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>0</td>
<td>0</td>
<td>0 for 24 hr.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soy bean trypsin inhibitor</td>
<td>0 for 24 hr.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Activation of prothrombin by trypsin. Bovine prothrombin, preparation 109B, purified as described in earlier reports, was dissolved in various media indicated, and incubated as described in various media. Ordinate indicates the thrombin evolved per cent of the total thrombin available from the prothrombin, assayed by the two-stage procedure of Ware and Segers.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Effect on Proth.</th>
<th>Incubation Time</th>
<th>Effect on Proth.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylo-coagulase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>0</td>
<td>0</td>
<td>0 for 24 hr.</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Progressive, rapid inactivation with recovery in some experiments</td>
<td>0 for 4 hr., slight deterioration in 24 hr.</td>
<td>Blocked</td>
</tr>
<tr>
<td>Trypsinogen</td>
<td>—</td>
<td>0</td>
<td>0 for 24 hr.</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>Progressive inactivation</td>
<td>0</td>
<td>0 for 24 hr.</td>
</tr>
<tr>
<td>Urease</td>
<td>—</td>
<td>0</td>
<td>0 for 24 hr.</td>
</tr>
</tbody>
</table>

*Expressed semiquantitatively as 0—++, ++, +++...0 indicates no effect. The two columns indicate thrombin formation from two types of purified prothrombin: one deliberately prepared to contain factors VII and X as contaminants; the other, devoid of these factors.

Indicates the immediate effect of the enzyme on the clottability of a standard fibrinogen solution by thrombin. In the instance of plasmin, it progressively (within minutes) blocks the thrombin fibrinogen interaction as fibrinogen exposure to the enzyme continues. With relatively large concentrations of plasmin fibrinogen clottability is blocked promptly.

**Figure 7**

**Effect of Ca++ or PO Buffer on Proth.**

**Proth.**—% Theoretical

**Incubation Time—Minutes**

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical

**Proth.**—% Theoretical
The net result of trypsin administration would be to induce intravascular coagulation while at the same time triggering the fibrinolytic mechanism. The disastrous consequence of this combination has already been demonstrated.

**Table 4**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Factor VII complex (Owren)</th>
<th>Factor X (Styphen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminotripeptidase</td>
<td>Progressive, rapid inactivation</td>
<td>No significant effect</td>
</tr>
<tr>
<td>Bromelin</td>
<td>Very rapid inactivation</td>
<td>Rapid inactivation</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>Progressive, rapid inactivation</td>
<td>Progressive, rapid inactivation</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Progressive, very rapid inactivation</td>
<td>Progressive, very rapid inactivation</td>
</tr>
<tr>
<td>Collagenase</td>
<td>No effect for 24 hr.</td>
<td>No effect for 24 hr.</td>
</tr>
<tr>
<td>Elastase</td>
<td>Very rapid inactivation</td>
<td>Very rapid inactivation</td>
</tr>
<tr>
<td>Plecin</td>
<td>Very rapid activation (X20 in 10 min.)</td>
<td>Progressive activation (X4 in 30 min.)</td>
</tr>
<tr>
<td>Hyluronidase</td>
<td>Slow, progressive activation (X12 in 24 hr.)</td>
<td>Slight activation in 24 hr.</td>
</tr>
<tr>
<td></td>
<td>(X20 in 48 hr.)</td>
<td></td>
</tr>
<tr>
<td>Lysozyme</td>
<td>No significant effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>No effect</td>
<td>No effect or moderate inhibition</td>
</tr>
<tr>
<td>Papain</td>
<td>Rapid, progressive activation (pH 7.0)</td>
<td>Rapid, progressive activation</td>
</tr>
<tr>
<td>Pepsin</td>
<td>No effect (30 min. incubation)</td>
<td>No effect</td>
</tr>
<tr>
<td>Plasmin</td>
<td>No effect for 24 hr.</td>
<td>No effect (30 min. incubation)</td>
</tr>
<tr>
<td>Rennin</td>
<td>No effect for 24 hr.</td>
<td>No effect for 24 hr.</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>Inactivation within 30 min.</td>
<td>Very slow inactivation</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Very rapid activation</td>
<td>Very rapid activation</td>
</tr>
<tr>
<td>Trypsinogen</td>
<td>Progressive activation (X30 in 24 hr.)</td>
<td>Rapid, progressive activation (X40 in 24 hr.)</td>
</tr>
<tr>
<td>Trypsinase</td>
<td>Progressive, slow activation (X6 in 24 hr.)</td>
<td>No effect</td>
</tr>
<tr>
<td>Urease</td>
<td>No effect</td>
<td>No effect</td>
</tr>
</tbody>
</table>

*Two separate experiments.*
tors in the serum of a pregnant patient. This is demonstrable with nongravid sera also (fig. 11), as well as with highly purified material freed from other clotting factors by serum fractionation and starch-gel electrophoresis (fig. 12). Activation with thromboplastin, as with trypsin, is inhibited by trypsin inhibitors. It would therefore appear that the thromboplastic pathway of thrombin formation also involves proteolysis, resembling the action of trypsin and other proteases. We can now appreciate more fully the potentially dreadful consequence of the intravenous liberation of thromboplastic material from extracellular sources, as occurs in pregnancy.48-50 leading to intravascular coagulation and the defibrination syndrome.

It is now opportune to compare fibrinolysin with trypsin, thrombin, and thromboplastin in their effects on factor V (Ac-globulin). Purified human fibrinolysin* rapidly increases factor V activity, followed by rapid decay, depending upon the concentration of fibrinolysin (fig. 13). The destructive effect is in accord with in vivo observations obtained by others in spontaneous51 and induced fibrinolytic states with whole blood and plasma systems,52, 53, 51-56 and is in sharp contrast to the negative effects of fibrinolysin on factors II, VII, and X, described earlier.

*Kindly supplied by Dr. Alan Johnson of New York Hospital, New York.

Circulation, Volume XXVI, October 1962

**Figure 10** Activation of factors VII and X by human brain thromboplastin.

**Figure 11**

Enhancement in factor X activity of sera by addition of human brain thromboplastin. All the sera except the one designated by the solid squares (fresh serum), were frozen at the same time, stored, subsequently thawed immediately prior to study, diluted 1:20 with physiologic saline, and mixed with an equal part of human brain thromboplastin extract and 0.94 M calcium chloride. The factor X activities of the undiluted sera just before addition of thromboplastin were 100 per cent for the normal subjects; 88 per cent (B. Co.), 124 per cent (P. Ca.), and 165 per cent (M. Sp.) for the pregnant sera, respectively. The first values recorded (at "0" incubation time) are those obtained as soon as technically possible after combination of serum, thromboplastin, and Ca++. It should also be mentioned, in passing, that our findings that fibrinolysin does not destroy factors II, VII, and X are contrary to the reports of others.52, 54, 57

Thrombin similarly activates factor V, whereas trypsin, which strongly activates factors II, VII, and X, is inert on factor V (fig. 14). It would also appear that thromboplastin activates factor V, but the possibility has not yet been excluded that this may be due to a trace contaminant of thrombin. Thus, fibrinolysin and thrombin resemble each other in their activating effects on factor V, and in their inertness on factors II, VII, and X as compared with trypsin and papain (table 5). Yet in certain of their effects on fibrinogen, these four proteases are remarkably similar.

The activating effects of fibrinolysin and thrombin on factor V deserve additional comment. Mention has already been made of the hypercoagulability observed early in the
course of thrombolytic therapy, and how fibrinolysis might initiate or accelerate a vicious circle by releasing thrombin adsorbed to fibrin or fibrin monomer. We now can visualize another mechanism for producing hyper-coagulability, namely, activation of factor V by both fibrinolysin and thrombin before the activated factor disappears.

Clinical Laboratory Changes in Thrombolytic Therapy

The untoward action of relatively large amounts of fibrinolysis on factor V and fibrinogen is of profound significance in thrombolytic therapy, as indicated by some observations on a patient treated for thrombosis of the right retinal artery (table 6). Two years previously, he became blind in the left eye from similar pathology. On February 22,
Effect of plasmin on purified factor V. The factor V fraction, prepared from bovine plasmin according to the method of Therriault et al.\textsuperscript{58} was dissolved in Veronal-buffered oxalated saline (VBOS), pH 7.0, and had 124 per cent activity in terms of the factor V activity of plasma. The material was devoid of factors II, VII, and X. The plasmin preparation, kindly supplied by Dr. Alan Johnson of New York Hospital, was derived from purified bovine plasminogen, spontaneously activated in glycerol. In the factor V assay procedure\textsuperscript{67} the fraction alone, i.e., in the absence of added factor V, exhibited 11 per cent factor V activity. In the experiments, all the combinations of factor V with plasmin showed significant activation over and above the initial factor V activity (taken at 100 per cent) within 20 seconds following admixture, (including an early observation of "0" which is not shown).

1961, he suddenly experienced transient colored scotomata and blurred vision on the right. When the disturbance recurred the next day and persisted, he was promptly referred for therapy, which was started within a few hours.

It should be noted first that relatively large doses of thrombolysin were administered. Secondly, a modest amount of heparin was also given intravenously, not as an anticoagulant, but because heparin enhances fibrinolytic activity in doses that ordinarily do not greatly interfere with clotting.\textsuperscript{58} The patient also received nicotinic acid orally for its known fibrinolytic potentiation.\textsuperscript{58} This combination of heparin and nicotinic acid has also been used by others recently in thrombolytic therapy.\textsuperscript{58} Thirdly, on the second day of treatment the thrombin clotting time was distinctly elevated as compared with that of a normal plasma. This observation, in agreement with what has already been shown regarding interference of the thrombin-fibrinogen interaction by fibrinolysin, indicates that a biologic effect was obtained.

This was substantiated by observations on the prothrombin time. As therapy was continued, the prothrombin time became retarded, most likely due to the effects of fibrinolysin on factor V or fibrinogen. This is indicated by the very low prothrombic activity obtained on whole plasma in contrast to the relatively normal value obtained when the patient’s plasma was admixed with normal plasma rich in factor V and fibrinogen.

Thus, his over-all prothrombic activity was permitted to drop to levels generally desired with conventional anticoagulant therapy. In this connection it should be emphasized that the heparin was not involved in this result.

Late in the second day, the patient sud-
denly experienced brilliant scotomata, followed by complete clearing of vision, which has been maintained until the present. There were no other reactions. No claim whatsoever is made that this was a therapeutic triumph. The data are presented merely to extend into the clinic certain observations made in the laboratory. As such, they suggest guides to therapy. The value of the thrombin clotting time and the whole plasma prothrombin time in this regard has also been mentioned by others.

Summary

The fibrinolytic mechanism, one of the blood protease systems, constitutes a fourth phase of clotting, which contributes to maintenance of coagulation in delicate balance.

"Shwartzman"-induced in vivo coagulation, combined with fibrinolysin, produces profound clotting defects with severe hemostatic failure, in contrast to the relative innocuousness of either alone.

The same defects are obtainable in vitro. The thrombin-fibrinogen interaction is blocked, simulating the in vivo "afibrinogenemia." These observations are pertinent to thrombolytic therapy invoked for conditions where intravascular clotting may be occurring.

Fibrinogen clottability is also blocked by trypsin. Apparently, fibrinolysin, thrombin, and trypsin are competitive for fibrinogen.

Trypsin, as well as other proteases, markedly activates factors II, VII, and X, resembling the action of thromboplastin. In contrast, fibrinolysin and thrombin are inert on these clotting factors, whereas on factor V they first activate and then destroy it. This explains why factor V depression is observed in thrombolytic therapy.

The activating effect of trypsin on factors II, VII, and X precludes its use in thrombolytic therapy, and also draws attention to the trypstatase-antitryptasate equilibrium in normal and certain thrombotic states.

The experimental observations help elucidate some clinical and clinicolaboratory findings in acquired "afibrinogenemia" and therapeutically induced "fibrinolytic" states. The retarded thrombin clotting time of the plasma, and reduction in prothrombin activity attributable to fibrinolytic depression of factor V, may be valuable guides to thrombolytic therapy.
References
35. Beller, F. K., and Glas, P.: Inhibition of clotting as the result of plasminogen activation by streptokinase. II. Analytical studies of the


50. Schneider, C. L.: Etiology of fibrinopenia:

ALEXANDER, PECHET, KIJMAN


64. Ware, A. G., and Seegers, W. H.: Two-stage

Circulation, Volume XXVI, October 1962
Galvani and the Electrophysiology of Muscular Contraction

The most outstanding proof of his strength of character was shown by Galvani during the final year of his life. When an edict of the Cisalpine Republic, created by Napoleon, ordered that all public officials take an oath of allegiance to its constitution, this great patriot refused because he would not subscribe to a formula so contrary to his principles: the oath was atheistic. By refusing to take the oath he lost all his offices at the University and the Institute in April 1798 which reduced him to poverty in his last days. Pietro Giordani wrote that "he neither suffocated the voice of conscience, nor made it subservient to profit and ambition, but accepted poverty, losing without protest those academic offices which were his very bread." His fellow citizens, and especially Giovanni Aldini, appealed to the Government to remedy this injustice, and Galvani was restored to his previous offices as emeritus professor, but the decree was announced when death was about to take him. He died at 61 on December 4, 1798, in the house of his birth to which he had returned to live with his brother following the death of his wife.—Giulio Pupilli. Commentary on the Effect of Electricity on Muscular Motion. By Luigi Galvani. Translated by Robert Montraville Green, M.D. Cambridge, Massachusetts, Elizabeth Licht, Publisher, 1953. p. xix.
Proteolysis, Fibrinolysis, and Coagulation: Significance in Thrombolytic Therapy
BENJAMIN ALEXANDER, LIBERTO PECHET and ALLAN KLIJMAN

Circulation. 1962;26:596-611
doi: 10.1161/01.CIR.26.4.596
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1962 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/26/4/596

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/