Effects of Intravenous Administration of Fibrinolysin (Plasmin) in Man

By KENNETH M. MOSER, M.D.

The potentiality of dissolving clots has aroused great interest in fibrinolysin (plasmin) as a therapeutic agent in thromboembolism. In this paper the effects of intravenous administration of purified fibrinolysin in man are reported, with special emphasis upon systemic toxicity and alterations in clotting factors and fibrinolytic activity. This information is necessary for the evaluation of this agent for clinical purposes.

SINCE no adequate methods presently exist for detecting the presence of a "prethrombotic" state, the physician must unfortunately deal with thromboembolic disease when it reaches an overt, symptomatic phase. The most effective therapy at this juncture would include a combination of agents that could ensure both prompt dissolution of existing thrombus and prevention of new thrombosis.

Although heparin has some capacity to accelerate the process of clot dissolution in vivo, none of the anticoagulant drugs adequately satisfies this combined requirement. Therefore, much recent effort has been devoted to the development of agents that might dissolve intravascular clot acutely.

The therapeutic feasibility of induced clot lysis was suggested by discovery of the intrinsic fibrinolytic activity of plasma, which resides in the plasminogen-plasmin (profibrinolysin-fibrinolysin) system. It appears likely that this lytic system is constantly functioning within the body to remove unwanted fibrin deposits. Unfortunately this normal mechanism rarely acts with sufficient speed and intensity to prevent the sequelae of extensive occlusion in large vessels. Therefore, various attempts are currently being made to achieve rapid thrombolysis with materials that can acutely enhance the fibrinolytic activity of plasma.

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The proteolytic enzyme trypsin, the bacterial derivative streptokinase, and certain protein-free bacterial pyrogens have all been utilized to dissolve intravascular thrombi. While trypsin in vitro is both directly fibrinolytic and capable of activating profibrinolysin, attempts to achieve clot lysis with intravenous trypsin have met with infrequent success. Furthermore, the enzyme has such a broad spectrum of proteolytic action that its intravenous administration may lead to serious depletion of plasma coagulation factors.

Streptokinase has no direct fibrinolytic activity. Rather, it serves as an activator of profibrinolysin, either directly or by converting a plasma pro-activator to activator. Intravenous streptokinase can produce clot lysis in the experimental animal, and doses of sufficient size can considerably increase fibrinolytic activity of human plasma. Unfortunately, a significant portion of the population, by virtue of prior streptococcal infection, may exhibit antistreptokinase activity in the plasma. The presence of such antibodies not only raises the possibility of allergic responses, but may also play a role in the inconsistency with which well-tolerated doses of streptokinase enhance plasma fibrinolytic activity.

The fibrinolytic potential of the pyrogenic bacterial lipopolysaccharides in vivo has been explored by von Kaula. These materials do not exhibit fibrinolytic activity in vitro. The mechanism by which they induce such activity in vivo is uncertain, but present
evidence suggests that they act by releasing plasminogen activators from some endogenous source. Such nonenzymatic activation of the plasma fibrinolytic mechanism is a promising development in the effort to find a satisfactory, clinically useful thrombolytic agent.

While both streptokinase and bacterial pyrogens hold considerable promise as effective thrombolytic materials, their indirect mode of action and dependence upon the supply of plasminogen in the plasma have certain practical and theoretical disadvantages. Therefore, other investigators have explored the possibility that fibrinolysin itself, isolated from the human plasma, might prove a more direct and controllable agent for increasing plasma fibrinolytic activity. This possibility has recently become subject to experimental proof by the development of a highly purified preparation of fibrinolysin suitable for clinical study.20,21 Extensive in vitro and animal studies with this material have demonstrated several characteristics that render it promising as a therapeutic agent in human thromboembolic disease. Fibrinolysin rapidly dissolves fibrin clot in vitro and exerts similar effects upon extravascular fibrin coagulum in vivo.22 The intravenous infusion of fibrinolysin in experimental animals has consistently led to dissolution of artificially produced arterial and venous thrombi.23-25 Although the enzyme does exhibit proteolytic activity in vivo, fibrin and its close structural relative fibrinogen appear to provide the major substrates in plasma.24-27 Such preferential action may be related not only to the protein structure of these substances but also to the adsorptive affinity of fibrin for fibrinolysin.4,11

Finally, the intravenous infusion of fibrinolysin in animals has been attended by minimal evidence of allergic potential or other toxicity.23,26 Preliminary reports indicate that the experimental data regarding the value and low toxicity of fibrinolysin may apply to man.28,29

Detailed study of the toxicity, duration of action, and dosage-response characteristics of fibrinolysin is important, for experience with trypsin and streptokinase has indicated that human responses to enzymatic agents often are not accurately predicted from in vitro or animal investigations. The present study was therefore undertaken to assess the coagulation changes, systemic toxicity, and fibrinolytic activity that follow intravenous infusion of highly purified fibrinolysin in man at various dosage levels. The data are derived from changes that followed 72 infusions of fibrinolysin in 63 patients.

MATERIALS AND METHODS

**Study Group.** The 63 patients in this study (table 1) were inpatients at the District of Columbia General Hospital between February and December 1957. They all had or were subject to threat of thrombosis. The patients were divided into 3 dosage groups: 19 infusions of 30,000 fibrinolytic units (F. U.) of fibrinolysin (group I); 28 infusions of 40 to 50,000 F. U. (group II); and 25 infusions of 69 to 90,000 F. U. (group III).

**Table 1.—Composition of Patient Group Receiving Fibrinolysin**

<table>
<thead>
<tr>
<th>TABLE 1.—Composition of Patient Group Receiving Fibrinolysin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Active deep thrombophlebitis</td>
</tr>
<tr>
<td>With pulmonary embolism</td>
</tr>
<tr>
<td>Without pulmonary embolism</td>
</tr>
<tr>
<td>Pulmonary embolism without obvious source</td>
</tr>
<tr>
<td>Superficial thrombophlebitis</td>
</tr>
<tr>
<td>Arterial thrombosis or embolism</td>
</tr>
<tr>
<td>Cerebral thrombosis</td>
</tr>
<tr>
<td>Internal carotid thrombosis</td>
</tr>
<tr>
<td>Brachial embolus</td>
</tr>
<tr>
<td>Retinal artery thrombosis</td>
</tr>
<tr>
<td>Coronary thrombosis</td>
</tr>
<tr>
<td>Sickle-cell disease</td>
</tr>
<tr>
<td>No apparent thrombosis</td>
</tr>
<tr>
<td>Carcinoma</td>
</tr>
<tr>
<td>Cellulitis</td>
</tr>
<tr>
<td>Miscellaneous</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>72</td>
</tr>
</tbody>
</table>

Determination of Fibrinolysin Dosage. The fibrinolysin was derived from the euglobulin fraction of human plasma as prothrombinolysin by a modification of the method of Fishman and Kline.21 Activation was achieved by addition of small amounts of purified streptokinase. After activation, procedures were carried out to remove the streptokinase, and the material was lyophilized. No capacity to activate plasminogen was detectable in the final product.
plied by the manufacturer initially in vials containing 25,000 "fibrinolytic units" per vial. This labeled unitage was determined by the supplier on the basis of assays employing both fibrin and non-fibrin test substrates. The initial lot of material supplied serves as the "house standard" and was assayed in our laboratory by a technic similar to that described by Loomis and co-workers. Serial dilutions of the standard fibrinolysin material were prepared. To 0.1 ml. of the various fibrinolysin dilutions in a 13-by-100 mm. test tube were added 0.2 ml. of a freshly prepared 1 per cent solution of bovine fibrinogen (Armour) in imidazole buffer (pH 7.2) and 0.2 ml. of human thrombin in imidazole-saline-glycerine buffer solution (containing 4 units of thrombin), and the tube was placed in a water bath at 37°C. At 1 minute after the start of incubation and at 30-second intervals thereafter, the tube was removed from the water bath and tilted gently to the horizontal position. A firm clot formed within the first 15 seconds of incubation. The clot remained firm during tilting for many hours if fibrinolysin was absent. In the presence of fibrinolysin, the clot softened and soon after moved freely down the test tube as it was tilted. This was chosen as the endpoint for "partial lysis." If the incubation period is extended, total lysis of the clot will occur. While there is a close correlation between the time required for partial and total lysis (fig. 1), the former was subject to a higher degree of reproducibility and therefore was chosen as the endpoint of the assay.

Lysis times differed significantly (p < .01) for the dilutions studied from 1 to 40 units. Differences in lysis times for 200 and 100 units, 100 and 50 units, and 50 and 40 units were not significant, but differences between 200 and 50 units were significant (p < .01).

The unitage of all subsequent lots of material was defined on the basis of this standard curve. While the standardization by the manufacturer and ourselves correlated well, the dosage in our studies was based on standardization of each new lot by our own assay. Before each infusion, the material was tested at several dilutions to assure that no alteration had occurred in fibrinolysin activity during storage at −20°C.

**Method of Infusion.** Immediately prior to infusion, 25,000 fibrinolysin units were dissolved in approximately 5 ml. of normal saline and added to 500 to 1000 ml. of 5 per cent dextrose in water. The infusion was introduced through ordinary plastic tubing over periods of 2 to 4 hours via an ante-cubital or forearm vein.

**Blood Samples.** Blood samples were drawn in all instances prior to and 24 hours following infu-
tion. In 63 cases, samples also were drawn at approximately 3 and 6 hours after infusion; and in 9 others, at 6 hours only. Samples were collected with 0.1 M sodium oxalate as the anticoagulant and placed in ice at the time of withdrawal. Blood not used for hematocrit level or white blood count was immediately centrifuged, and the plasma was tested promptly or stored at —20 C.

Coagulation Studies. The 1-stage prothrombin time\(^{31}\) and plasma recalcification time\(^{31}\) were determined in all samples. In 36 cases, assays of prothrombin, proconvertin, and pro-accelerin by the methods of Lewis\(^{32}\) and determination of fibrinogen by a modification of the method of Morrison, Edsall, and Miller\(^{33}\) were also made. White blood counts and hematocrit levels (Wintrobe) were determined before and 24 hours following start of infusion in all patients.

Assay of Plasma Fibrinolytic Activity. This assay is identical to that described for establishment of the standard curve, with the exception that 0.4 ml. of the patient's plasma was substituted for the 0.1 ml. of fibrinolysin dilution. Thus, the test system consisted of plasma, fibrinogen, and thrombin.

To establish normal values for the assay, 245 control determinations were made (table 2). In addition, 18 patients were studied serially at 0, 3, 6, and 24 hours following start of an intravenous infusion of 1,000 ml. of 5 per cent dextrose in water (table 2). Finally, 20 hospitalized patients and 15 normal subjects were studied daily for 5 days (table 2).

These studies indicate the stability of lysis times by this method in serial samples over a 24-hour period and in daily samples.

On the basis of these control values, the assay was terminated after 30 minutes, and no patient with lysis time above 25 minutes was considered to have significant plasma fibrinolytic activity.

Calculation of increase of fibrinolytic activity was determined by the following formula:

\[
\text{Per cent increase} = 100 \times (\text{Control lysis time}) - (\text{Lysis time at } x \text{ hours}) / (\text{Control lysis time})
\]

By definition, control lysis time cannot exceed 30 minutes.

During the investigation, all lysis times were determined in duplicate and the mean of the 2 was taken as the lysis time. The determinations showed a high degree of reproducibility (table 3). As might be expected, the difference between duplicate determinations tended to increase with longer times except that with a lysis time of 30 minutes or more, a repeat determination gave very similar results, indicating minimal or absent fibrinolytic activity.

### Table 2.—Control Assays of Plasma Fibrinolytic Activity

<table>
<thead>
<tr>
<th></th>
<th>Mean lysis time (min.)</th>
<th>S.E.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>5</td>
</tr>
<tr>
<td>Single determinations</td>
<td></td>
<td></td>
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<tr>
<td>Hospitalized patients</td>
<td>137</td>
<td>27.6</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>108</td>
<td>29.0</td>
</tr>
<tr>
<td>Total</td>
<td>245</td>
<td>28.2</td>
</tr>
<tr>
<td></td>
<td>Before infusion 8 hours</td>
<td></td>
</tr>
<tr>
<td>Serial 24-hour samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean lysis time</td>
<td>27.0</td>
<td>28.2</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily determinations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalized patients</td>
<td>Mean lysis time 26.4</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>Mean lysis time 1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>Mean lysis time 28.5</td>
<td>29.1</td>
</tr>
<tr>
<td></td>
<td>Standard error 0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>

### Table 3.—Reproducibility of Duplicate Lysis Times

<table>
<thead>
<tr>
<th>Lysis time (min.) first determination</th>
<th>Mean difference* between first and second determinations</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.6</td>
</tr>
<tr>
<td>0-5.9</td>
<td>6</td>
<td>1.4</td>
</tr>
<tr>
<td>6.0-9.9</td>
<td>10</td>
<td>2.8</td>
</tr>
<tr>
<td>11.0-15.9</td>
<td>13</td>
<td>0.1</td>
</tr>
<tr>
<td>16.0-20.9</td>
<td>37</td>
<td>0.8</td>
</tr>
<tr>
<td>21.0-25.9</td>
<td>10</td>
<td>0.3</td>
</tr>
<tr>
<td>26.0-29.9</td>
<td>87</td>
<td>0.1</td>
</tr>
<tr>
<td>Total</td>
<td>291</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Mean difference indicated here is average of all differences without regard to direction of difference.

Miscellaneous Laboratory and Clinical Studies. Temperature, blood pressure, and pulse were measured prior to infusion and every 2 to 4 hours for 24 hours thereafter. When any deviation from baseline values persisted after 24 hours, measurement was continued until baseline values were reached. A standard 12-lead electrocardiogram was obtained before and 24 hours following infusion in all cases. In 22 patients, electrocardiograms were also performed 3 to 10 hours following start of infusion. Intradermal skin tests were carried out with 0.1 ml. of a saline-fibrinolysin solution containing 30 F. U. in 27 patients prior to infusion.
Each patient receiving fibrinolysin was observed during and after infusion by the author and ward physicians. Evaluation continued throughout the hospital stay and in the out-patient clinic.

RESULTS

Changes in Coagulation Factors. The serial determinations of prothrombin time, prothrombin content, plasma recalcification time, proaccelerin content, proconvertin content, and fibrinogen content suggest that fibrinolysin does affect all of these coagulation factors.

As indicated in figures 2 and 3, less than half the patients developed more than a 5 per cent depression (or prolongation in the case of prothrombin and plasma recalcification times) of any coagulation factor with the exception of the fibrinogen content. This was true at all intervals and at all dosage levels.

The degree to which depression (or prolongation in the case of prothrombin and plasma recalcification times) of these factors was produced following administration of fibrinolysin is presented in figures 4 to 6 and table 4. As can be seen, fibrinogen changed most, being decreased to 86.3 per cent of baseline values at 3 hours after start of fibrinolysin infusion.

TABLE 4.—Average Percentage Change from Baseline Values of the Various Coagulation Factors at Each Time Interval Following Fibrinolysin Infusion

<table>
<thead>
<tr>
<th></th>
<th>Hours after start of infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Prothrombin content</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>-2.5</td>
</tr>
<tr>
<td>Group II</td>
<td>-9.0</td>
</tr>
<tr>
<td>Group III</td>
<td>-5.2</td>
</tr>
<tr>
<td>Total</td>
<td>-5.4</td>
</tr>
<tr>
<td>Recalcification time</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>-9.7</td>
</tr>
<tr>
<td>Group II</td>
<td>-4.6</td>
</tr>
<tr>
<td>Group III</td>
<td>-8.1</td>
</tr>
<tr>
<td>Total</td>
<td>-7.0</td>
</tr>
<tr>
<td>Proaccelerin content</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>-7.8</td>
</tr>
<tr>
<td>Group II</td>
<td>-4.0</td>
</tr>
<tr>
<td>Group III</td>
<td>-4.9</td>
</tr>
<tr>
<td>Total</td>
<td>-5.7</td>
</tr>
<tr>
<td>Proconvertin content</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>-1.8</td>
</tr>
<tr>
<td>Group II</td>
<td>-2.8</td>
</tr>
<tr>
<td>Group III</td>
<td>-12.0</td>
</tr>
<tr>
<td>Total</td>
<td>-5.5</td>
</tr>
<tr>
<td>Fibrinogen content</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>-18.9</td>
</tr>
<tr>
<td>Group II</td>
<td>-13.8</td>
</tr>
<tr>
<td>Group III</td>
<td>-9.4</td>
</tr>
<tr>
<td>Total</td>
<td>-13.7</td>
</tr>
</tbody>
</table>
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Table 5.—Incidence of Increased Fibrinolytic Activity after Infusion of Fibrinolysin*

<table>
<thead>
<tr>
<th></th>
<th>3 hours</th>
<th>6 hours</th>
<th>24 hours</th>
<th>Any time†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>77</td>
<td>44</td>
<td>25</td>
<td>78</td>
</tr>
<tr>
<td>Group II</td>
<td>74</td>
<td>60</td>
<td>40</td>
<td>92</td>
</tr>
<tr>
<td>Group III</td>
<td>75</td>
<td>61</td>
<td>76</td>
<td>81</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>56</td>
<td>48</td>
<td>84</td>
</tr>
</tbody>
</table>

*Enhanced activity defined as lysis time below 25 minutes and 10 per cent or more below baseline time.
†Evidence of enhanced fibrinolytic activity in at least 1 sample.

While these data indicate that coagulation changes do follow fibrinolysin infusion, none of these changes differs significantly from baseline values. This lack of significance (p > 0.05) applies to the group as a whole and to the individual dosage groups with 1 exception. In the case of prothrombin at 24 hours in group III patients, a statistically significant prolongation is noted (p < 0.05). However, as indicated in figure 6, this prolongation appears to be related to the fact that anticoagulant therapy was begun in many of these patients coincident with fibrinolysin infusions. Separate analysis of data in patients who did and did not receive anticoagulants indicates that no statistically significant prolongation occurred in the latter group.

More important than the lack of statistically significant alterations is that none of these factors was depleted to a degree that is likely to impair the coagulative process.

Hemorrhagic Phenomena. No hemorrhagic phenomenon appeared in any patient who received fibrinolysin infusion, including 29 patients who were simultaneously receiving therapeutic doses of anticoagulant drugs. The series included 2 patients with advanced cavitary tuberculosis and recent hemoptysis; 4 patients with hemoptysis due to pulmonary embolism; 1 with bronchiectatic hemoptysis; 8 with moderate to severe hepatic cirrhosis; 1 patient 5 days following right upper lobectomy, and 1, 5 days after left brachial embolotomy. Postmortem examination in 9 patients who died within 2 weeks of the infusion disclosed no hemorrhagic foci in any organ that could be related to fibrinolysin administration.

Hematocrit and White Blood Count. No significant changes occurred in hematocrit levels at 24 hours following infusion, the values being 38 per cent ± 8 at baseline and 35 per cent ± 8 at 24 hours. White blood count elevation exceeding 1,000 occurred at 24 hours following infusion in 38 per cent of all patients. The average elevation was 2,800 (range 1,200 to 9,800). Neither incidence nor degree of white count elevation correlated with fibrinolysin dosage, occurrence of fever, or degree of plasma fibrinolytic activity.

Fibrinolytic Activity. Enhanced plasma fibrinolytic activity* was demonstrable in at least 1 postinfusion sample in 84 per cent of all patients, including 78 per cent of group I, 92 per cent of group II, and 81 per cent of group III (table 5). Analysis by dosage groups indicates that, as dosage was raised, there was both prolongation and intensification of plasma fibrinolytic activity (fig. 7).

While the incidence of enhanced fibrinolytic activity was equally distributed among the 3 groups at 3 hours, groups II and III showed a higher incidence than group I at 6 hours. At 24 hours, group III showed an incidence of activity significantly greater than the other 2 groups.

The intensity of fibrinolytic activity also

Table 6.—Blood Pressure Changes after Infusion of Fibrinolysin

<table>
<thead>
<tr>
<th></th>
<th>Maximum average decline Systolic (mm. Hg)</th>
<th>Maximum average decline Diastolic (mm. Hg)</th>
<th>Per cent with 10 mm. Hg decline (systolic or diastolic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Group II</td>
<td>9</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Group III</td>
<td>16</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Fever</td>
<td>17</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td>No fever</td>
<td>4</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

*Based on control determinations and known errors of assays, a postinfusion sample with a lysis time that was less than 25 minutes and 10 per cent or more below baseline lysis time was taken as indicative of enhanced lytic activity.
appeared related to fibrinolysin dosage. At 3 hours, the average decrease in standard clot lysis time was 34.6 per cent in group I, 33.0 per cent in group II, and 40.9 per cent in group III. By 6 hours after infusion, groups I and II had declined to 10.8 and 12.8 per cent respectively, while group III patients still showed an average decrease in lysis time of 34.6 per cent. At 24 hours, clot lysis time was decreased by 3.7 and 9.8 per cent in groups I and II, while group III demonstrated a 42.1 per cent decrease.

**Blood Pressures.** Alterations in blood pressure following fibrinolysin infusion were minimal for the group as a whole, with the maximum decline averaging 10 mm. Hg systolic and 7 mm. Hg diastolic. Both the incidence and degree of blood pressure depression showed some tendency to increase as dosage was raised, but the differences between groups were not statistically significant (table 6).

The presence of fever was accompanied by an increased incidence and degree of blood pressure decline (table 6). A lowering of blood pressure exceeding 10 mm. Hg systolic or diastolic occurred in 42 per cent of patients with febrile reactions but in only 13 per cent without fever, a statistically significant difference. However, the differences in degree of blood pressure decline were not significant.

No instance of symptomatic hypotension occurred. Most of the blood pressure declines exceeding 10 mm. Hg systolic or diastolic occurred under 3 circumstances: during temperature elevation, in measurements made during the hours 12 p.m. to 8 a.m., and in patients with preinfusion blood pressure readings in the hypertensive range (above 140/90).

**Temperature and Pulse.** A febrile reaction was defined as any temperature elevation exceeding 1 F. that occurred during the 24 hours following infusion. Such responses occurred in 44 per cent of the total patient group.

Fever usually appeared 6 hours after start of infusion (range 3 to 14), reached its height at approximately 10 hours after infusion, and usually returned to normal within the next 10 hours (range 2 to 40) (fig. 8). In 5 patients temperature elevation persisted beyond 24 hours, and in 3 peak temperature elevation exceeded 4 F. (table 7).

Neither the incidence nor the degree of temperature elevation could be correlated with size of dose, rapidity of infusion, fibrinolysin skin tests, alterations in coagulation factors, or extent of fibrinolytic activity achieved. However, when patients were divided into 2 groups—those in whom extensive thrombotic material was clinically present and those in whom thrombotic material was minimal or presumed absent—a statistically significant correlation with fever ($p < .01$) was obtained (table 8).

Pulse changes tended to parallel temperature response and in no instance did a significant tachycardia (above 110) develop in the absence of a febrile reaction.

**Electrocardiographic Changes.** Electrocardiographic abnormalities appeared after infusion in 2 patients. A 79-year-old woman with arteriosclerotic heart disease and congestive heart failure developed atrial fibrillation 10 hours after infusion, and a 23-year-old man
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with deep iliofemoral thrombophlebitis developed transient T-wave changes 6 hours after infusion. Both of these changes occurred near the peak of a febrile reaction.

Urinalysis. No significant changes in urinary sediment followed fibrinolysin infusion. In 12 instances, mild proteinuria was noted 24 hours after infusion, but it cleared by 48 hours. All patients with proteinuria had had febrile reactions.

Miscellaneous Observations. Nausea or chills accompanied approximately one third of the febrile reactions. Two patients vomited.

Two instances of apparent allergic skin reaction developed, both on the third day after infusion. One patient developed a hot, erythematous, pruritic reaction at the site of infusion, and the second patient developed a pruritic macular eruption over the extremities and the lower trunk, in addition to a similar but less severe reaction at the infusion site. Both reactions responded to local and antihistaminic therapy within 72 hours. In both cases a moderate fever (100 to 101 F.) developed and an erythematous spot appeared at the site of an intradermal fibrinolysin skin test originally recorded as negative. In 1 patient, a strong allergic history was present. In neither of these cases had subcutaneous infiltration of fibrinolysin occurred.

Subcutaneous infiltration of 5 to 20,000 F. U. inadvertently occurred in 7 patients. No adverse effects were noted other than what might be expected to follow infiltration of comparable amounts of dextrose solution.

Two patients developed unexplained episodes of epigastric pain near the peak of febrile reactions to infusion. The episodes were promptly terminated by meperidine and did not recur. Investigation revealed no cause for the pain in either case.

DISCUSSION

Significant toxicity of intravenous fibrinolysin in man, at the dosage levels employed in this study, appears limited to its pyrogenic potential. The etiology of this pyrogenicity has not yet been defined. Three explanations might be advanced: (1) profibrinolysin itself is pyrogenic; (2) the fibrinolysin preparation used may contain pyrogenic residuals of streptokinase or of other materials introduced during the process of activation; (3) the actions of fibrinolysin within the body incite temperature elevation.

The first explanation does not appear tenable. Profibrinolysin is not pyrogenic in the rabbit at very high dosage levels. Infusion of profibrinolysin in doses equivalent to 100,000 F. U. of activated material in 15 patients did not produce fever.24

The role of streptokinase in the febrile reaction remains uncertain. Johnson and coworkers15 have indicated that the most highly purified preparations of streptokinase avail-
able remain contaminated by antigenic materials that are immunochemically distinct from streptokinase itself. He suggested that these contaminant materials may be responsible for the toxic reactions, including fever, that follow infusions of purified streptokinase. It is possible that pyrogenic residuals that will not activate profibrinolysin are contained in the fibrinolysin preparation used in this study. Proof that such residuals are the source of fever must await the development of either an immunochemically "pure" streptokinase or a nonpyrogenic activator from other sources, such as tissue kinase or urokinase.3,11

The last possibility, that the actions of fibrinolysin prompt temperature elevation, merits consideration. Since many patients fail to develop fever following infusion, it appears unlikely that activation of profibrinolysin renders fibrinolysin pyrogenic per se. However, as has been indicated above, individuals with large thrombotic zones who receive fibrinolysin have significantly higher incidence of temperature elevation than those with small or without thrombotic phenomena. This observation suggests that fibrinolytic attack upon thrombotic material may release breakdown products in such a manner as to provoke pyrogenic reaction. While similar attack of fibrinolysin upon plasma proteins might also be considered, no correlation could be established between fibrinogen or other coagulation factor losses and the incidence or degree of fever. Furthermore, the data indicate that the presence or degree of plasma fibrinolytic activity has no significant relationship to the problem. Johnson and associates13 have also observed that fibrinolytic activity and pyrogenicity are not parallel phenomena.

At the present time, experimental proof that pyrogenicity arises from products of thrombolysis is lacking. Plasma drawn from patients during and after febrile reactions of fibrinolysin has thus far proved nonpyrogenic in animals. Human recipients of such plasma have not yet been studied.

Since the pyrogenic potential of fibrinolysin presently limits its application in certain critical thrombotic states (e.g., coronary thrombosis), elucidation of its cause is of considerable practical importance. Our recent experience has indicated that fever can be ameliorated or prevented by aspirin-antihistaminic or barbiturate prophylaxis, but reliance upon such measures is obviously less desirable than specific identification and control of those factors involved in the production of fever. That identification of the source of pyrogenicity may involve rather extensive and detailed study, however, has been indicated by several recent investigations regarding pyrogenicity.33,36

The effect of fibrinolysin upon the human coagulative process was one of the focal points of this study. The animal investigations of Clifton et al.28,29 and Ambrus and Back24,25 have indicated that a rather wide range of fibrinolysin dosage will produce significant enhancement of plasma fibrinolytic activity without compromise of the coagulation mechanism. In the doses employed in the present study, fibrinolysin infusion in human subjects caused minimal aberration of coagulation factors. While fibrinolysin undoubtedly is subject to the pharmacologic rule of wide individual variation in response, a considerable margin of safety appears to exist in man between dosages that significantly enhance plasma fibrinolytic activity and those that may lead to clinically important impairment of the coagulative process.

The laboratory measurements indicating that no gross disturbance of coagulative process followed fibrinolysin administration correlated well with the clinical observation that no hemorrhagic phenomena occurred in any patient, including 29 simultaneously receiving coumarin-antihistaminic drugs or heparin. These observations are in agreement with the animal and human experience of Clifton and Ambrus, who noted that bleeding complicated plasma infusion only when excessive doses were given or local vascular lesions existed during periods of high lytic activity.

The ability of fibrinolysin to achieve significant plasma fibrinolytic activity without
undue influence upon coagulation probably reflects several independent influences. One factor involved is the capacity of the organism for rapid replacement of depleted components of plasma protein. Absence of depression of a factor cannot be interpreted as absence of destruction in vivo, but it may indicate that restorative mechanisms are able to keep pace with the proteolytic activities of fibrinolysin. The rapidity of fibrinolysin infusion and concentration of the administered solution also may exert a significant influence.

While data included here deal with infusions given over 2 hours or more, isolated observations have suggested that rapid infusions of fibrinolysin are accompanied by more profound, though transient, declines in certain factors, especially fibrinogen.

Another circumstance that may limit the degree to which fibrinolysin affects plasma proteins is the apparent preferential action of this enzyme upon fibrin. 27 Such preference is probably related not only to the specific protein linkages split by fibrinolysin, but also to the known adsorptive affinity of fibrin for plasma. 4-11 Such adsorption would tend to concentrate fibrinolysin activity upon intravascular fibrin. In addition, there is in vitro evidence indicating that inhibition or destruction of plasmin occurs more rapidly when suitable substances are not supplied, a chain of events that would also tend to limit the attack of fibrinolysin to fibrin. 27-38

Although the dosage of fibrinolysin used in this study produced no major coagulation defects, serious disturbances may arise if higher doses are employed. Back and Ambrus have demonstrated that large dosages of fibrinolysin in the dog may produce significant fibrinogen depression and prothrombin time prolongation. 24, 25 The present studies suggest that any impairment of coagulation induced by fibrinolysin in the human is likely to be reflected first and most obviously by depression of fibrinogen content. Since considerable variability may exist in regard to both the degree of fibrinogenolysis and the fibrinogen-replacement capacities in individual patients, qualitative determination of fibrinogen before and during fibrinolysin administration seems advisable when doses in excess of those used in this study are employed. The same caution regarding higher dosages of fibrinolysin may also apply to the other coagulation factors and to such events as hypotension.

Observations regarding the safety of fibrinolysin infusion in human subjects would have little meaning unless nontoxic doses were also capable of inducing significant intravascular fibrinolytic activity. As indicated above, enhanced fibrinolytic activity is frequently achieved by plasmin even at the lowest dosage levels. Higher doses result in a prolongation and intensification of demonstrable lytic activity, although the intensity of lytic activity is subject to rather wide variation. If one may transpose animal and laboratory data in any degree to man, one might expect frequent success in achieving dissolution of an intravascular fibrin meshwork exposed to the lytic levels achieved by fibrinolysin. However, it is essential to recognize that no direct evidence exists regarding the degree to which plasma fibrinolytic activity demonstrated in the test tube may be equated with the desired lysis of a human intravascular clot in vivo. The fragmentary data available suggest that gross correlation can be expected. Indeed, theoretically, the strong adsorptive capacity of fibrin for fibrinolysin should lead to action upon an in vivo clot to a degree and for a duration well beyond that expected on the basis of activity detectable in the circulating plasma. 4 It is probably equally true, however, that achievement of plasma lytic activity does not guarantee dissolution of a susceptible clot. Local factors may prevent adequate access of fibrinolysin to thrombus or result in a variable adsorption of the agent to fibrin. Variations in local inhibitory phenomena may also play a role. Therefore, no firm conclusions can be drawn regarding the therapeutic efficacy of a given degree or duration of plasma lytic activity at this juncture. Dosage of fibrinolysin in various clinical states will re-
main largely empirical until an adequate body
of clinical experience has accumulated.

Another facet of the dosage problem is the
marked plasma fibrinolytic activity that was
achieved by relatively small doses of fibrino-
lysin. If one estimates blood volume in the
patients and calculates the maximum fibrino-
lysin unitage that might be circulating in the
plasma at any given time, it is apparent that
30 F. U./ml. or 12 units/0.4 ml. is the
greatest concentration that can be expected.
Reference to the standard curve indicates
that 12 F. U. in a test system free of plasma
inhibitors would give a lysis time of 6 to 7
minutes. Yet, in the assay system with 0.4
ml. of patient’s plasma, which should contain
considerable inhibitor activity, lysis times of
6 to 7 minutes or below were frequently en-
countered in 3- and 6-hour samples. This ob-
servation suggests that the fibrinolysin prepa-
ration prompts more in vivo fibrinolytic ac-
tivity than can be accounted for in the test
tube. Unquestionably, the material used con-
tains components other than fibrinolysin itself.
That it does not contain plasminogen activ-
ators of the streptokinase type is established
during manufacture. But our data suggest
that some activator function may occur in
vivo through mechanisms that are presently
nebulous. As von Kaulla has indicated, ma-
terials which are nonfibrinolytic and nonac-
tivator in vitro may assume both these func-
tions in vivo. Further study of the origin
of this unexpected fibrinolytic “dividend”
is currently being pursued, for the possibility
that fibrinolysin may serve as both an ac-
tivator and a directly fibrinolytic agent in
vivo has important clinical implications. Since
blood clot itself contains plasminogen, an
agent that can simultaneously produce high
levels of plasma fibrinolytic activity and
activate the plasminogen within the clot
should exert a potent thrombolytic effect.
Activator materials, such as streptokinase,
urokinase, and the bacterial pyrogens, should
activate the plasminogen contained in the clot
if they are capable of attaching to or pene-
trating blood clot in vivo, but plasma fibrino-
lytic activity with these agents would remain
dependent upon the availability of plasmino-
gen. Perhaps such effects upon plasminogen
within the clot itself might dissolve thrombi
more effectively than any level of plasma
fibrinolytic activity. This possibility requires
further exploration. However, if fibrinolysin
does contain an activator component, both
plasminogen activation within the clot and
enhancement of plasma fibrinolytic activity
independent of plasminogen levels could be
achieved by its administration. Such a com-
bination of effects would appear to represent
the broadest therapeutic approach.

A number of other cogent questions require
answer before the clinical value of fibrinoly-
sin can be properly assessed. Such ques-
tions include the age at which a thrombus becomes
resistant to fibrinolysis; the functional return
and hemorrhagic hazard that may result in
ischemic areas when acute clot dissolution is
achieved proximally; the danger of embolism
when intravascular clot is acted upon by
plasmin; methods for controlling excessive
effects of fibrinolysin should they appear; and
the need for anticoagulant therapy following
acute lysis of thrombi.

At the laboratory level, several problems
also warrant further investigation. For in-
stance, the assay procedures for fibrinolytic
activity are still a subject of debate. Multiple
methods are currently in use, and each has
its advantages and disadvantages. In our ex-
perience, observation of whole-blood clot lysis
following administration of fibrinolytic drugs
has been of qualitative value, but it is not
subject to quantitation. Spectrophotometric
assay with a system containing synthetic acid
ester substrates, which are susceptible to the
esterase activity of fibrinolysin, are excellent
tools for the in vitro measurement of pure
fibrinolysin-substrate systems.39, 40 However,
the method appears of less value when used
in an attempt to assay the fibrinolytic activity
present in plasma. Some 40 patients in this
series have had spectrophotometric estima-
tions of plasma esterase activity in parallel
with the standard clot assay. Although es-
terase activity showed a qualitative correlation with the clot method, quantitative differences were frequent.

Such enzymatic assays possess several deficiencies. Variable degrees of "nonfibrinolytic" esterase activity may be present in plasma, and its contribution to over-all esterase activity may be difficult to establish. Furthermore, the validity of using a synthetic substrate to replace fibrin in any assay for fibrinolytic activity is subject to question, since the strong affinity of fibrinolysin for fibrin itself may play a significant role in determining the duration and intensity of such activity. Thus, it appears likely that the esterase and fibrinolytic potencies of plasma may not be parallel phenomena. We continue to prefer the fibrinolytic assay described here as the method which yields information of greatest clinical utility.

Despite these challenging clinical and methodologic uncertainties, our observations provide a firm basis for extending investigations with fibrinolysin to the phase of therapeutic trial in patients with thromboembolic disease. They indicate that fibrinolysin, in doses which are tolerated without significant alteration of coagulation processes or other unacceptable consequences, can consistently achieve levels of in vivo fibrinolytic activity that may prove effective in reversing acute thrombotic events. Determination of the ultimate clinical value of this agent in thromboembolic disorders, however, will require extensive and detailed study.

Summary

Seventy-two infusions of human fibrinolysin have been carried out in 63 hospital inpatients to assess the coagulation changes, fibrinolytic activity, and systemic consequences that follow such infusions at various dosage levels. The effects upon coagulation factors were minor at all dosage levels. Fibrinogen content was depressed more frequently and to a greater extent than any of the other factors measured. No hemorrhagic phenomena were noted in any patient, including 29 who were simultaneously receiving anticoagulant drugs.

Systemic toxicity was limited primarily to a febrile reaction, which occurred in 44 per cent of the total patient group. The source of pyrogenicity remains undefined, but appears related either to nonactivator residuals contained in fibrinolysin or to products released by the action of fibrinolysin upon thrombotic material.

Fibrinolysin infusion consistently enhanced plasma fibrinolytic activity at all dosage levels. The intensity and duration of such enhancement appeared related to fibrinolysin dosage. In some cases, the intensity of fibrinolytic activity achieved in vivo suggested that the material used contained an activator in addition to fibrinolysin per se.

A number of clinical and laboratory questions regarding fibrinolysin must be answered before firm statements can be made regarding the proper application and therapeutic value of this material in human thromboembolic disease.

Summary in Interlingua

Seventy-two infusiones de fibrinolysina human esseva effectuate in 63 patientes hospitalizate con le objectivo de determinar le alterationes del coagulation, le activitate fibrinolytic, e le consequencias systemic que sequel tal infusiones a varié nivello del dosage.

Le effectos producida in le factores coagulatori esseva de importantia minor a omne nivello del dosage. Le contento de fibrinogehno esseva depriimate plus frequentemente e plus marcatemente que ulle del alte factores mesurate. Nulle phenomenos hemorrhaige esseva notate in ulle del patientes, incluse le 29 qui se trovava simultaneamente sub tractamento con drogas anticoagulante.

Le toxicitate systemic esseva limitate primitamente a un reaction febril. Isto occurreva in 44 pro cento del gruppo total de patientes. Le origine del pyrogenicitate remane obscur, sed il pare que le phenomeno es relationate al presentia de residuos nonactivator in le fibrinolysina o a productos que resulta del action de fibrinolysina super le material thrombotic.
Le infusion de fibrinolysina promoveva uniformemente la activitate fibrinolytic in le plasma a omne nivellos de dosage. Le intensitate e le duration de iste effecto esseva apparentemente relacionate al dosage de fibrinolysina usate. In plure casos, le intensitate del activitate fibrinolytic attingite in vivo suggerveva que le material usate contineva un activator a parte le fibrinolysina per se.

Un numero de questiones clinee e laboratorial con respecto al natura de fibrinolysina debe esser resoluite ante que firme assertiones deveni possibile con respecto al uso appropriate e al valor therapeutie de iste substantia in casos de morbo thrombo-embolique in humanos.

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There is now a danger, of which Professor Laubry warned us in Paris, that Cardiology will become no more than the application of laboratory techniques to patients, and so will cease to belong to Clinical Medicine. It is unlikely that technical procedures, however perfected, can ever become a substitute for diligent clinical observation and therefore it behooves us to pass on to the next generation the clinical skill and wisdom which we have inherited from our teachers of the past.—Evan Bedford. Address of the President of the European Society of Cardiology. IIIrd World Congress of Cardiology, Brussels, September 14-21, 1958, p. 29.
Effects of Intravenous Administration of Fibrinolysin (Plasmin) in Man
KENNETH M. MOSER

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