Intravenous Protein-Free Pyrogen
A Powerful Fibrinolytic Agent in Man

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Administration of a variety of nonfibrinolytic, nonenzymatic substances induces fibrinolysis in man. The most potent materials of this type known at present are purified protein-free pyrogens. Their action after intravenous injection on the human fibrinolytic system, on some of its components, and on the clotting system of 67 individuals has been studied. The possible therapeutic implications are discussed of the marked fibrinolysis.

The obvious therapeutic value of activation of fibrin-dissolving potentialities of the circulating human blood in thromboembolic disorders, has stimulated many efforts to achieve this goal. These attempts may be classified under 2 general headings: injection of proteolytic enzymes, and the activation of plasminogen in the body itself. The most important drugs known to produce fibrinolysis in human beings and some of their characteristics are summarized in table 1.

The induction of fibrinolysis in the human body with nonenzymatic material can be produced (table 1) in many ways. In the majority of cases, however, the fibrinolytic reactions are either weak or too irregular to be of therapeutic value.

The observation of Eichenberger, that Westphal's highly purified protein-free pyrogen produces fibrinolysis in man after intravenous injection, was therefore of special interest. We have confirmed these preliminary findings and have extended these observations considerably with more comprehensive studies. The protein-free pyrogens described below, as far as we have been able to determine, are the most potent nonenzymatic fibrinolysis-inducing agents known at present.

In this paper the pyrogenic materials used and their action in man are described with special emphasis on fibrinolysis. A short summary of some of our experiments has previously been given.¹⁵

Material and Methods

Pyrogens. Two pyrogens were used, 1. Preparation 1064 is derived from Salmonella abortus equi and has a molecular weight of about 1 to 2,000,000. It consists of 30 to 40 per cent phospholipids, 6 to 10 per cent esters of phosphoric acid, and 55 to 60 per cent different sugars and is free from proteins and amino acids. 2. Preparation 1083 is derived from Escherichia coli and has been acetylated. Its analysis reveals a composition of 50 per cent sugars, 25 to 27 per cent acetyl groups, 18 per cent phospholipid components, and 5 per cent esters of phosphoric acid. Its molecular weight is about 1,000,000. Both preparations are water soluble. They were donated as sterilized solutions in ampules by the Wander Company, Chicago. Preparation 1064 had 1 µg. per ml., preparation 1083 had 100 µg. per ml.

To study the nature of the fibrinolytic reaction induced by these pyrogens the following methods were used:

Fibrinolysis. (a) A continuous recording (coagulogram) of fibrin formation and dissolution of recalified citrated plasma was made. One part of 3.8 per cent sodium citrate to 4 parts of blood was used, spun for 5 minutes at 1,500 g and recalified with one tenth volume of 0.5 molar calcium chloride. (b) The dissolution time was measured of a plasma clot from 0.3 ml. of undiluted plasma plus 0.01 ml. of thrombin 200 U per ml., and (c) also the dissolution time of a thrombin clot from plasma diluted 1:19 with buffered saline. (d) Measurement of the dissolution time of a clot obtained from euglobulins: (precipitate was formed after carbon dioxide saturation of plasma diluted 1:19 with distilled water). euglob-
ulins, prepared from 0.3 ml. of plasma, were dissolved in 0.3 ml. of buffered saline and clotted with 0.01 ml. of thrombin. (e) Determination of the lysis time of a plasma clot made according to "b," but after addition of urothromolysokinase of standard activity. (f) Fibrin plates: The bovine plates were made based on the procedure of Las-sens* of the Astrup-group. The plasma plates were developed by us. Unheated bovine plates: Ten milliliters of a 0.6 per cent bovine fibrinogen (Armour) were dissolved in buffered saline in an Erlenmeyer flask, pH adjusted to 7.4 if necessary, and chilled, 0.1 ml. of thrombin solution was added, mixed, and the mixture rapidly poured into a carefully leveled flat-bottom Petri dish. Clotting occurs within 30 seconds. The plate was incubated for 10 minutes at 37 C. before use. Heated bovine plates: These were prepared as indicated using 0.4 per cent bovine fibrinogen and heated for 45 minutes at 85 C. after the initial incubation. Human plasma plates: Blood was drawn with siliconized equipment and spun at 900 g at 4 C. for 10 minutes. Ten milliliters of plasma so obtained were either poured into siliconized Petri dishes and allowed to clot spontaneously, or 0.04 ml. of thrombin solution was added before placing the plasma in the dishes.

The recalleification method as indicated under 'a' was chosen to avoid dilution of the specimen, which is bound to bring about disturbance in the equilibrium inhibitors, activators of both clotting and fibrinolytic components. The recalleification times obtained by the above method are roughly comparable with the Lee-White times.

Method 'b' was the standard method used for measurement of fibrinolysis throughout the study. For detection of weak lysis tendency, method 'c' was used, early in the investigation.

Studies were carried out with 7 patients, who showed marked induced fibrinolysis by Schultz et al.* of the Research and Development Unit of the Fitzsimons Army Hospital, Denver, using the synthetic substrate method.

Antifibrinolysin-Estimation. For this estima-
tion the supernatant fluid remaining after removal of euglobulins was used. It was free of fibrinolytic activity, even when prepared from very fibrinolytic plasma specimens. A mixture of 0.05 ml. of 1 per cent bovine fibrinolysin (Parke, Davis) and 1 ml. of 0.1 per cent bovine fibrinogen (Armour) treated with barium sulfate, was clotted with 20 units of bovine thrombin (Armour). A lysis time of 4 to 5 minutes resulted at 37 C. For the estimation of antifibrinolysin activity 0.05 ml. of albumin solution was added and the lysis time was noted. With high antifibrinolysin titer no lysis occurred in 24 hours. The average lysis time in the presence of the above amount of albumin solution was 1 to 2 hours.

All blood specimens were processed immediately after venipuncture for fibrinolysis studies. In most instances, the tests with a, b, c, e, and f were started within 20 minutes, d, within 45 minutes.

Procedure with Patients. The pyrogenic material was injected intravenously in all instances with an injection speed of 20 seconds per ml. The patients were not fasting. Bed rest was ordered as long as the temperature was over 37.5 C. Temperature and blood pressure were taken every 30 minutes. Of 67 patients, 36 were women in their second to fourth postpartal day in the age group 17 to 37. The rest were female and male medical and surgical patients, ages 15 to 68.

Pre-treatment. The great majority of patients were premedicated in the hope of reducing side reactions and fever. Pabirin or Ascriptin were used according to the following schedule: Pabirin: 4 a.m., 0.6 Gm.; 6 a.m., 0.6 Gm.; 8 a.m., intravenous injection of pyrogen; 8:30 a.m., 0.9 Gm.; 11 a.m., 0.9 Gm. Ascriptin: 10 p.m., 0.6 Gm.; 2 a.m., 0.6 Gm.; 6 a.m., 0.9 Gm.; 8 a.m., intravenous injection of pyrogen, and 0.9 Gm.; 11 a.m., 0.9 Gm. (2 p.m., 0.9 Gm., if necessary). In some instances Aminopyrine was used, which seemed however to have no advantage over Pabirin or Ascriptin. Ten-milliliter samples of citrated blood were taken before and at intervals after injection and were processed immediately for fibrinolysis studies as indicated. The temperature was taken under the tongue with a TRI-R electronic thermometer.

Results

The pertinent data of all patients treated with intravenous pyrogens are summarized in table 2.* In the following, some results of particular interest will be emphasized:

Onset and Duration of Pyrogen-Induced Fibrinolysis and Its Relation to Dosage

Euglobulin and Plasma Lysis. In serial determination maximal fibrinolytic activity always occurred between 90 and 120 minutes after intravenous injection of the pyrogen, irrespective of the intensity of the fibrinolysis. We found it best to test for peak activity at 105 minutes. The total period of fibrinolysis increased with increasing intensity (figs. 1 and 2).

The speed of fibrinolysis and its relation to the time elapsed after injection of the lipopolysaccharides is clearly demonstrated by coagulograms (figs. 1 and 2). They also indicate that fibrinolysis is not a linear process with respect to time, but proceeds at varying rates. The basic pattern of fibrinolysis is that of a slow onset followed by increasing disintegration speed of the coagulum, which then remains fairly constant for some time, and ends in a final slowdown.

A dose of 0.15 to 0.2 μg. of 1064 or of 200 to 350 μg. of 1083 regularly (38 out of 38 patients) induced fibrinolysis except in 2 intensively heparinized patients (no. 15 and 16), where negative results were obtained using the euglobulin technic alone. We found later that the lysis of euglobulin in the presence of heparin may be misleading. These and related observations will be reported elsewhere.29 The euglobulin technic is otherwise very helpful in detecting lysis tendency when no lysis can be observed on 24-hour incubation in the plasma or by coagulographic recording. Figure 3 illustrates this effect when injections of 100 μg. of 1083 were used.

This amount of pyrogen induces marked fibrinolysis in less than half of the cases. It appears from the 60-minute sample in figure 3 that the euglobulin control in contrast to the undiluted plasma sample, which exhibits no fibrinolysis at all.

Figure 3 demonstrates that the weak lysis tendency is shown much better by the euglob-
Fig. 1. Coagulographic pattern of plasma fibrinolysis after intravenous injection of 0.2 μg of pyrogen.

ulolin technie than by undiluted plasma lysis. Table 2 gives 11 examples (nos. 4, 25, 29, 31, 32-34, 51, 61, and 65) in which injection of small amounts of pyrogen shortened considerably the 105-minute lysis time of the euglobulin sample, as compared with the preinjection euglobulin control. There is thus demonstrated an increased lysis tendency by the use of pyrogen not detectable using undiluted plasma alone.

Figure 4 illustrates again the relation of euglobulin to undiluted plasma lysis time. Here the results of all tests done during pyrogen treatment of patient no. 14 are shown graphically. This patient had a marked fibrinolysis without any significant temperature elevation. The 5-hour specimen showed a reduced euglobulin lysis time and there was no lysis in the plasma specimen at all; lysis was detectable only by the euglobulin technie. Figure 4 shows also another phenomenon which we call the rebound effect, and which is discussed in the following paragraph.

Rebound Effect and Tolerance. The euglobulin lysis time of the last 2 samples of the serial determinations of patient no. 14 represented in figure 4 is longer than that of the preinjection sample. This is a characteristic finding in all our cases where serial euglobulin determinations were made. It was observed that several postinjection euglobulin samples, after the fibrinolytic reaction had subsided, took considerably longer to lyse than the control. This prolongation was frequently still present after 24 hours (patients no. 56, 58, and 59). The reduced tendency to lyse could also be observed when a standard amount of urofibrinolysokinase had been added to the serial specimens. The fibrinolysis induced by urofibrinolysokinase in some samples was always less intensive after disappearance of the pyrogen-induced fibrinolysis than that in the preinjection control samples. This resistance to lyse spontaneously (euglobulins) or after addition of activator (urofibrinolysokinase) to undiluted plasma is temporary.

A second injection of pyrogen given 105
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minutes after the first one (first dose being 100 μg., second dose being 50 μg., patients no. 62-66, or 150 μg., no. 67 respectively) did not delay the "rebound" effect or prolong the lysis period. Lysis could be produced with the dose used in only one (no. 63) out of 6 cases during the rebound effect.

At present we have no satisfactory explanation for the rebound effect that disappears in 24 to 48 hours. Our results tend to rule out any influence of antifibrinolysin. One of our cases was particularly spectacular. A female patient (no. 11) exhibited spontaneous fibrinolysis in the preinjection specimen, the euglobulin lysis time being 31 minutes and plasma lysis time 12 hours. A very marked lysis of 6 hours’ duration was induced by 300 μg. of 1083. Twenty-four hours after the injection, however, the euglobulin lysis time was prolonged to 193 minutes, which is within normal limits, and there was no lysis in the plasma upon 24 hours’ incubation. The rebound effect was strong enough to abolish a pre-existing considerably increased lysis tendency.

Differing from the rebound effect is the tolerance. It is well known that fever and accompanying reactions tend to diminish when the same amount of pyrogen is injected repeatedly in 24-hour intervals. The results of our studies indicate that the fibrinolytic reaction tends to become less and less marked after repeated injections in 24-hour intervals as well. For the determination of the lysis tendency the euglobulin technic had to be used because frequently the second injection brought about a much less intensive fibrinolysis of the undiluted plasma specimen or even failed to induce any plasma lysis. No plasma lysis at all was induced by the third injection. The euglobulin technic here still picks up increased lysis tendency no longer demonstrable with plasma. These conditions are well demonstrated by the data of patients no. 55 and 57–61. Preliminary studies indicate that the period of tolerance may not last longer than 1 week (patients no. 52–54) after the last injection. Possibly it might be broken through by larger pyrogen dosage for the second and still larger for the third injection.

In 25 cases serial estimation of antifibrinolysin was made. A characteristic example of 1 patient (no. 45) is given in figure 5. The arrows pointing downward in the lower row show the antifibrinolysin activity as expressed in minutes of lysis time of the system, diluted albumin-fraction bovine-fibrinolysin bovine-fibrinogen-thrombin. There was

Fig. 3. Comparison of plasma and euglobulin lysis time in serial determinations after intravenous injection of 100 μg. of pyrogen 1083.

Fig. 4. Euglobulin and plasma lysis in a pretreated individual who exhibited marked fibrinolysis without fever after intravenous injection of 300 μg. of pyrogen 1083.
TABLE 3.—Influence of Incubation on Lysis Time of Fibrinolytic Plasma and the Corresponding Euglobulin Fraction

<table>
<thead>
<tr>
<th>Incubation in min. at 37°C</th>
<th>Lysis time of undiluted plasma (min.)</th>
<th>Lysis time of euglobulins (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>48</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>63</td>
<td>8</td>
</tr>
<tr>
<td>30</td>
<td>not done</td>
<td>6</td>
</tr>
<tr>
<td>50</td>
<td>102</td>
<td>no clot</td>
</tr>
</tbody>
</table>

TABLE 4.—Comparison of Lysis Time of Clots from Diluted (1:19) and Undiluted Plasma; i.e. Injection of 300 µg of Pyrogen 1083. Serial Samples

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>Lysis time plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>undiluted</td>
</tr>
<tr>
<td>0</td>
<td>&gt;24 hrs.</td>
</tr>
<tr>
<td>90</td>
<td>24 hrs.</td>
</tr>
<tr>
<td>105</td>
<td>45 min.</td>
</tr>
<tr>
<td>150</td>
<td>63 min.</td>
</tr>
<tr>
<td>180</td>
<td>75 min.</td>
</tr>
<tr>
<td>240</td>
<td>24 hrs.</td>
</tr>
</tbody>
</table>

TABLE 5.—Reaction and Fibrin Formation Times in Plasma from Fourteen Fibrinolytic Patients. Each Figure Represents the Average Value of Fourteen Individual Measurements

<table>
<thead>
<tr>
<th>Min. after injection of pyrogen 1083</th>
<th>0</th>
<th>60</th>
<th>105</th>
<th>153</th>
<th>195</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with lysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reaction time min.</td>
<td>9.4</td>
<td>9.2</td>
<td>7.2</td>
<td>6.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Fibrin formation</td>
<td>a</td>
<td>8</td>
<td>5.5</td>
<td>4.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Half completed</td>
<td>b</td>
<td>17.4</td>
<td>14.7</td>
<td>11.7</td>
<td>10.9</td>
</tr>
</tbody>
</table>

a. Time elapsed from start of fibrin formation min.
b. Time elapsed from start of clotting (min.).

TABLE 6.—Maximal Temperatures of Thirty-Eight Patients with Less Than 180 Minutes Lysis Time after i.v. Injection of 100 to 300 µg. of Pyrogen 1083. All Patients were Pretreated

<table>
<thead>
<tr>
<th>Temperature range</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal to 37.5</td>
<td>4</td>
</tr>
<tr>
<td>37.6 to 38.0</td>
<td>6</td>
</tr>
<tr>
<td>38.1 to 38.5</td>
<td>8</td>
</tr>
<tr>
<td>38.6 to 39.0</td>
<td>9</td>
</tr>
<tr>
<td>39.1 to 39.5</td>
<td>3</td>
</tr>
<tr>
<td>39.6 to 40.0</td>
<td>3</td>
</tr>
<tr>
<td>40.1 to 41.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>38</td>
</tr>
</tbody>
</table>

Fig. 5. Intravenous injection of 300 µg of pyrogen 1083. Trend of fibrinolytic activity of plasma and euglobulin. Note rebound effect of euglobulin lysis time. Trend of antifibrinolysin activity.

no spontaneous fibrinolysis in the control without fibrinolysin, when the albumin fraction was prepared from the most active fibrinolytic samples. Figure 5 shows that the antifibrinolysin activity increases considerably 60 minutes after intravenous injection of the pyrogen. The antifibrinolysin activity is then reduced, but does not disappear entirely even at the peak of fibrinolytic activity. Subsequently it returns with some fluctuations to values exceeding the pre-injection levels.

Of interest is the "rebound effect" producing a euglobulin lysis time in samples 6, 7, and 8 considerably longer than those of the pre-injection sample. The rebound effect of sample 6 coincides with a reduced antifibrinolysin activity. This observation, made repeatedly, led us to the conclusion that antifibrinolysin activity is not related to the rebound effect. The general pattern of antifibrinolysin activity in patients with pyrogen-induced fibrinolysis is basically that shown in figure 5. There is commonly a rise in activity in the first hour after injection, then a reduction of activity at peak fibrinolysis and for a short time thereafter. Then after more or less pronounced fluctuations control values are obtained in 24 hours. In these same cases no changes in antifibrinolysin
were seen in spite of the presence of a marked lysis.

There was no indication that the status of antifibrinolysin activity before pyrogen treatment has any bearing on the outcome of the intensity of the induced fibrinolysis. Marked fibrinolysis could be induced at any antifibrinolysin activity.

Effect of Incubation of Fibrinolytic Plasma and Euglobulin. Plasma and euglobulin samples of the most active 105-minute specimens were incubated for various times, clotted, and the lysis time measured. Results, as shown in table 3 are characteristic. As the incubation time increased the lysis time of the plasma also increased due to inactivation by the inhibitor of the fibrinolytic enzyme present in the plasma. In contrast the lysis time of the euglobulin fraction, which is essentially free of inhibitors, decreases on incubation, due to partial digestion of fibrinogen. In the euglobulin sample chosen for table 3, no clot was obtainable after 50 minutes of incubation, since the fibrinogenolysis was complete. For comparable and clearly defined results both plasma and euglobulin samples should be processed immediately.

Lysis in Undiluted Serum versus Lysis in Diluted Serum. It has been recommended that the dissolution of clots obtained from diluted plasma (1:19) be observed to discover weak lysis tendency. Clots from undiluted plasma may show no noticeable lysis under these conditions, whereas those from diluted plasma do show lysis. It was thought that this phenomenon may be due to dilution of the inhibitor to ineffective levels in the diluted plasma. In our studies however the diluted samples consistently required much longer to lyse (if they lysed at all in 24 hours) than the undiluted samples prepared from the same plasma. A representative example is given in table 4.

The difference in lysis time of diluted and undiluted plasma during pyrogen-induced lysis can be interpreted by the assumption that the fibrinolysis is caused by the release of an activator for plasminogen, the activity of which is reduced by dilution.

Pyrogen-Induced Fibrinolysis and Clotting. Serial coagulograms were run of a number of patients who exhibited marked fibrinolysis. To obtain the complete sequence of data shown in table 5, only those patients were selected in whom fibrinolysis, though marked, started after the fibrin formation was completed. The lysis of the most active sample frequently started before completion of fibrin formation, making it impossible to obtain complete data. For technical reasons it was not always possible to obtain more than 5 consecutive coagulograms of a single patient. The reaction time (the time elapsed from recalcification to the measurable start of fibrin formation) and also the time required to form half of the fibrin were measured. It was found that the reaction time was considerably reduced during and sometimes after the fibrinolytic period. A marked increase in the speed of fibrin formation was concomitantly demonstrable. The deviations from preinjection values of the reaction and fibrin formation times can reach considerable proportions as is shown in table 5.

Table 5 shows the progressive shortening of the reaction time and fibrin formation time during pyrogen induced fibrinolysis. The greatest intensity of acceleration of clotting is reached 150 minutes after injection of the pyrogenic material. At this time the fibrinolytic activity had already begun to subside. At this moment the fibrin formation time is reduced to nearly half of its preinjection value. The 195-minute sample reveals that the fibrin formation time begins to shift toward preinjection values, whereas the reaction time has no tendency to normalize at this point. For cases in which we could obtain a complete coagulogram series, return to preinjection values for reaction and fibrin formation time occurred at or after the fourth hour following injection.

Relation of Temperature Elevation to Intensity of Fibrinolysis. No close relation was found between the height of the fever and the speed and intensity of fibrinolysis. Marked fibrinolysis, i.e., less than 180 minutes lysis time of undiluted plasma, occurred at any body temperature (table 6). In this table the
fibrinolysis time of the undiluted plasma specimen obtained 105 minutes after injection of the pyrogens and the highest temperatures reached in the postinjection period are recorded. The fibrinolysis peak always preceded the fever peak.

Eleven patients developed fever over 38 C. and exhibited poor fibrinolysis, lysis time of undiluted plasma being longer than 180 minutes, and an additional 4 had fever over 38 C. with no fibrinolysis at all. One patient (no. 56) who was treated for occlusion of the internal carotid artery, showed fibrinolysis after pyrogen injection. He developed spontaneous fever above 41 C. 36 hours later. During this temperature elevation the blood showed no fibrinolytic activity.

**Pretreatment with Antipyretics and Their Interaction with Fibrinolysis.** We have found that complete suppression of fever or side reactions by premedication did not result in a reduction of the fibrinolytic response. On the contrary we found that pretreatment may even enhance the fibrinolytic reaction.

One series of 23 patients received 100 to 150 μg of 1083. Of 7 untreated patients in this group, only 2 showed fibrinolysis, whereas of 16 pretreated patients 13 exhibited fibrinolytic reaction. In this series a combination of Pabirin and additional Ascriptin was administered, since it had previously been shown (see table 1) that a high dosage of paraaminobenzoic acid alone may induce some fibrinolytic activity in man.

**General Observations on Pyrogen-Induced Reactions.** The temperature started to rise 30 to 60 minutes after the intravenous injection of the pyrogen, reached a maximum in about 3 to 4 hours, and returned to normal within 3 to 6 hours. The rise in temperature was preceded by the usual systemic manifestations of fever, which were minor in most instances. Nausea was classified as a severe side reaction and vomiting as very severe. After all pyrogen-induced reactions had subsided, the patients expressed a sense of well-being and frequently became more or less euphoric. This fact should be considered in evaluating the subjective symptoms during thrombosis treatment with pyrogens. Of 57 patients treated with 100 to 300 μg. of 1083, 2 had a very severe side reaction, 5 severe, 17 moderate, 26 mild, and 7 none at all. Three patients of the 7 classified as "very severe" and "severe" were not premedicated.

**Blood Pressure and Blood.** The blood showed the known response to pyrogens: lymphopenia, eosinopenia, and leukopenia followed by leukocytosis. We have seen no critical fall of blood pressure; however, hypertensive patients were not treated. In some instances there was a drop of 10 to 15 mm. Hg for a few minutes in the first 2 hours after injection. One patient (no. 56) showed marked transitory hypotension.

**Miscellaneous.** Paracoagulation. In several instances it was observed that a narrowing of the coagulogram due to fibrinolysis was interrupted by a new widening followed by a narrowing as fibrinolysis proceeded to completion (fig. 1, 135-minute sample). In some cases, even after a complete lysis, a new minor clotting took place followed by a new lysis. This phenomenon of a new partial coagulation, after the sample had lysed completely, was first observed by Derechin and designated as paracoagulation. At present we explain this as an incomplete transformation of fibrinogen into fibrin in the sample and by a subsequent release of the thrombin adsorbed on fibrin during fibrinolysis. This released thrombin then clots the remaining fibrin.

**Fibrin Plates.** The active 105-minute sample induces fibrinolysis on plasma plates made from the patient's preinjection plasma. This phenomenon enabled us to study the action of anticoagulants on human fibrinolysis. Preliminary results have been reported elsewhere. It was also found that the lytic plasma samples were as active against heated bovine plasma plates as against unheated plates, indicating that the final activity of the lytic samples is primarily due to plasmin itself.

**Synthetic Substrates.** The synthetic sub-
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strates Tosylargininester and Lysinester were not attacked even by the most active plasma samples. These substrates are easily split by purified plasmin. It is assumed that inhibitors present in plasma suppress the esterase activity of the fibrinolytic enzyme. Details have been reported by Schultz and co-workers.16

Induction of Lysis in Control Samples. The active 105-minute sample of undiluted plasma induced fibrinolysis in the preinjection control plasma samples. The required concentration of the active plasma preinjection plasma for a fibrinolysis in 24 hours varied between 5 and 30 per cent depending on its activity. Lysis was also induced in rabbit plasma, but much higher concentrations were required.

Storage. The activity of the frozen samples decreased only gradually when frozen at −15°C. Half of the activity was found in 1 sample after 10 months' storage.

Animal Studies. It was not possible to induce fibrinolysis with pyrogens used in this study in rabbits and dogs.

DISCUSSION

The injection of highly purified pyrogens enables us to induce a marked fibrinolysis in human beings. Pyrogen injection, which is only one of many stimuli that can bring about fibrinolysis in man, is the most potent of nonenzymatic or nonproteolytic nature known at present. The stimulating material may be of endogenous or of exogenous origin. In the case of endogenous origin, the pathologic release of an activator for plasminogen (from prostate, uterus, or lung) is the probable method of activation. The mechanism of action of such exogenous-stimulating materials as epinephrine, acetylcholin, anesthetics,25 butazolidine, paraaminobenzoic acid, pituitary extracts, and other agents has not been clarified. None of these materials has any fibrinolytic or plasminogen-activating properties in vitro. According to our observations the activity of antiplasmin plays no prominent part in the appearance of fibrinolytic activity after administration of these substances, provided the stimulus is strong enough. Activation of the fibrinolytic enzyme to a minor degree only may very well be masked by the presence of strong inhibitor. On the other hand more plasminogen can always be activated in a given amount of blood than the inherent antiplasmin is able to inactivate, provided the activation is carried far enough. We assume that the nonenzymatic stimulating exogenous compounds trigger the release of the activator of plasminogen from the tissue, either by acting directly on the cell membrane or by the intermediate of a central regulating mechanism. We were unable to demonstrate clearly the presence of an activator in our studies, but this does not exclude the possibility of its presence. In experiments using an activator isolated from human urine we were able to demonstrate that the activation of plasminogen proceeds more rapidly as the concentration of activator in the reaction mixture is increased. It may well be that the pyrogen injection brings about a sudden rise in the concentration of the activator (or proactivator?) in the blood resulting in a rapid and extensive activation of plasminogen to plasmin, which masks the concomitant presence of the activator itself. The results of the fibrin plate studies indicate that activated plasminogen is present, which could only occur by activation of its precursor. We are convinced that the definitely longer time of the diluted samples in contrast to that of the undiluted ones is indicative of the concurrent presence of an activator that is considerably reduced in activity when diluted. The lysis time of the clot from diluted plasma is primarily determined by plasmin already activated. This harmonizes with our earlier observation26, 27 that by injection of acid-activated swine plasmin into rabbits we brought about a fibrinolysis that is only detectable in diluted samples (in which the antiplasmin is diluted) and not in undiluted ones. It is hoped that future studies will reveal the details of the mechanism of induced fibrinolysis and should in particular clarify whether Astrup's28 and Müllerz's29 proactivator takes part in the induction of fibrinolysis in the human circulating blood by unspecific stimuli. It might then
be expected that therapeutic lysis can be induced by non-fever-producing substances.

The findings reported in this paper raise anew the question of the interaction of fibrinolytic enzyme and clotting factor. It was observed in coagulographic studies on earlier occasions\(^{30}\) that induction of fibrinolysis in human blood in vitro, either by streptokinase of swine or bovine fibrinolysins, is always combined with earlier onset of the beginning of fibrin formation. Serial coagulograms before and during pyrogen-induced fibrinolysis reveal the same clotting pattern. Studies are under way to investigate the mechanism involved. It is thought that the increased coagulability during and after pyrogen lysis is not due to the pyretic action itself, but to the lysis tendency induced by them. The earlier onset and increased speed of fibrin formation during pyrogen-induced fibrinolysis represent a period of increased coagulability and might possibly have some bearing in thromboembolic conditions. Simultaneous heparin treatment is, at least theoretically, therefore indicated. Anticoagulant treatment should be continued after the fibrinolytic reaction has disappeared, since fibrinolysis does not suppress hypercoagulability itself (when present), but is only supposed to eliminate its most dangerous result, the intravascular clot. Persistent hypercoagulability after induced lysis can abolish its beneficial effect by allowing formation of new clots. However, excessive heparin concentration should be avoided, as we found with various technics that 125 and 525 µg. of heparin per ml. of plasma inhibit fibrinolysis in the test tube in contrast to 1, 5, and occasionally 25 µg. of heparin, which may enhance fibrinolysis.\(^ {29}\) No investigations have been made to our knowledge to determine the effect of fibrinolysis on heparin activity. Theoretically it might be assumed that in heparinized fibrinolytic blood the hemostasis is more impaired than in heparinized blood alone. The fibrin formation proceeds at a very slow rate in heparinized blood (at heparin concentrations that delay but do not completely prevent clotting) with formation of a few strands at the beginning. In this situation, even a weak fibrinolytic reaction may be sufficient to disintegrate those few strands and delay further the formation of a clot firm enough to produce hemostasis.

In our series fibrinolysis was studied in the dissolution of clots that were formed in a fibrinolytic milieu, thus ensuring a thorough distribution of the enzyme within the clot. This could rarely happen in the human origin. In vivo the enzyme must work on a clot already formed. It can attack only from the exterior and not from within. These conditions are partially duplicated by the use of fibrin plates. To study the therapeutic possibilities of the fibrinolytic system further, standard clots in vitro formed in the absence of fibrinolytic enzymes must be used. These studies are currently under way in our laboratories.

Our preliminary clinical observations combined with the findings of other students of the problem in human beings and in animals indicate that the clot already formed in the circulatory system can very well become smaller or disappear completely, if the circulating blood possesses marked fibrinolytic activity. It seems also to be true that the fibrinolytic disintegration of the preformed clot continues for some time after disappearance of fibrinolysis in the circulating blood. Extensive studies will be required to substantiate these preliminary observations. The great affinity of the fibrinolytic enzyme for fibrin is well known. One of the early fibrinolytic preparations was obtained from autolyzed fibrin.\(^ {31}\)

The data presented in this paper indicate that very marked fibrinolysis can easily and regularly be induced in human beings by injecting purified pyrogens. In none of our cases with marked induced lysis did bleeding occur. This was particularly remarkable for one case with low fibrinogen and prothrombin levels due to advanced liver cirrhosis (patient no. 5) and for all the postpartal patients who showed no increase in the blood content of their lochiae. In retrospect these observations are not too astonishing for the
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following reason. The fibrinolytic reaction in our series was induced by 2 pyrogens of different origin. Furthermore, Italian investigators have used pyrogenic typhoid vaccine for fibrinolytic treatment of thromboembolic disorders. We are entitled to assume that other pyrogens will have similar effects with possible side reactions. One wonders how many patients might have had a more or less marked fibrinolysis since the introduction of fever therapy by the injection of pyrogenic material.

At present we have limited our study on pyrogen-induced fibrinolysis and its therapeutic applications to those conditions in which fever therapy is not contraindicated. This limitation excludes many situations in which attempts to dissolve a clot would be highly desirable as in coronary occlusion. On the other hand, we are in a position for the first time to induce a very marked fibrinolysis, with a high rate of success without resorting to enzymatic fibrinolytic material in human beings. We are thus able to study the phenomenon of fibrinolysis in human subjects more extensively and can acquire information useful in achieving the ultimate goal of safely bringing about the dissolution of intravascular clots.

SUMMARY

The mechanism of pyrogen-induced fibrinolysis has been studied in 67 individuals. Tenths of a microgram of protein-free pyrogenic lipopolysaccharides from Salmonella abortus equi or 300 µg. from Escherichia coli (acetylated form) induces in 60 to 90 minutes after intravenous injection a very marked fibrinolysis lasting for 60 to 240 minutes in man. Clots of samples taken at peak activity (105 minutes) dissolve in less than 3 hours. There is frequently a reduction of antifibrinolysin activity during the fibrinolytic phase. Concomitant treatment with antipyretics does not diminish the fibrinolytic response. The spontaneous lysis time of euglobulins is prolonged after fibrinolytic activity has subsided. At this time more urofibrinolysokinase is required to induce lysis in the test tube. Eun-
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