Since Virchow's observation of the solidity and incoagulability of cadaver capillaries and veins, a number of reports have indicated that this phenomenon is caused by postmortem fibrinolysis. Mole suggested that the vascular endothelium is a source of the fibrinolytic activity in cadaver blood. Later, Kwaan et al. applied stimuli to the vein wall in man and in rabbits and observed the release of fibrinolytic activity through a local cholinergic mechanism. Release of fibrinolytic activity from the human vein wall after mechanical trauma was reported by Chakrabarti et al. More specifically, Astrup et al. demonstrated a plasminogen activator in the adventitia of arteries and veins, and in the intima of veins. The latter finding was also reported by Messer et al. Using a histochemical method, Todd found the plasminogen activator in tissues localized to the small veins and the venous endothelium. This finding has been confirmed. The recent demonstration that plasminogen activator is brought into an area of tissue repair by the newly formed capillaries suggested that such a situation could exist in an organizing thrombus. Presumably, in a venous thrombus, capillaries originating from the venous endothelium would invade the thrombus, bringing with them fibrinolytic active endothelial cells, thus assisting in the resolution of fibrin.

Fibrinolytic Activity in Thrombosed Veins

By Hau C. Kwaan, M.D., M.R.C.P., and Tage Astrup, Ph.D.

Methods

The solutions used to produce thrombosis were:
1. Thrombin (bovine), in strength of 1000 N.I.H. units/ml in physiological saline.
2. Serum. Blood, obtained from normal rats by heart puncture, was allowed to clot and left overnight at room temperature. The separated serum was free from thrombin and prothrombin.
3. Sodium-moquiurate, 5% in water.

Male and female albino rats of Sprague-Dawley strain, weighing 250 to 300 g, were anesthetized lightly with ether and, under strict aseptic precautions, a 1 cm long segment of the femoral vein was exposed. The proximal end was ligated with size 000 silk and a bulldog clamp was applied to the distal end. The obstructed vein segment was punctured distally by a 27-gauge hypodermic needle and the blood aspirated as completely as possible. One of the three thrombogenic solutions (0.02 ml) was injected, the vein was allowed to refill with blood from the distal end, reclamped, and left for 10 minutes for clot formation. Both clamps were then removed. Such segments will be referred to as "open segments." In another series, before removal of the clamps, a ligature was placed at the distal end of the thrombosed segment just above the site of puncture. These segments will be referred to as "closed segments." Thrombus formation was verified by palpation before wound closure. The contralateral femoral vein in each animal was used as a control. Animals were sacrificed at periods of 4 hours, 6 hours, 12 hours, 24 hours, 72 hours, 4 days, 1 week, and 2 weeks after operation. The segment was removed with a piece of the surrounding tissues and immediately frozen. In total, for each thrombogenic agent, "open segments" and "closed segments" demonstrate such a process of repair in thrombosed veins of the rat.
segments" were each produced in not less than 48 rats, providing 6 or more specimens at each of the 8 sacrifice time periods and for each type of thrombosis (see table 1 below).

For histologic study, sections of the thrombosed veins were prepared and stained by hematoxylin and eosin, Lendrum's acid picro-Mallory stain, and Cajal's trichrome stain as modified by Castroviejo. Fibrinolytic activity was identified by the histochemical method of Todd as modified. Briefly, this method consists of placing freshly frozen sections of tissue samples (cut at 6 μ) on microscope slides covered with a 0.06 mm thick layer of bovine fibrin (rich in plasminogen), and incubating the slides in a moist chamber at 37°C for periods ranging from 3 to 60 minutes. Thereafter the slides are fixed in formalin and stained with Harris' alum hematoxylin. Fibrinolytic activity is indicated by clear zones of lysis. Heated (plasminogen free) fibrin slides are used to test for protease activity. To provide an approximate assay of the activity of the tissue, the shortest time of incubation which produced a clearly demarcated localized zone of lysis on the slide is recorded for each sample. This is referred to as the "focal lysis time." Thus the "focal lysis time" is an in vitro measurement of the local fibrinolytic activity observed in a tissue section. It is not to be confused with the period of time required to produce thrombolysis in vivo. Consistency of results, sufficient for the comparison of "focal lysis times" of different sections, was secured by the application of three different concentrations of a preparation of urokinase (Leo Pharmaceuticals) as controls. A total of seven slides, one for each of the different incubation periods, was prepared from each vein specimen. Two to four sections were placed on each slide.

Results

For correlation with tissue fibrinolytic activity, the histologic changes in the thrombosed veins are described below in groups according to the type of segment (open or closed) and to the thrombogenic agent used. All of the results, with ranges of "focal lysis times" and remarks, are summarized in table 1.

Controls

A total of 306 untreated, contralateral veins served as normal controls. All of these were inspected and 144 samples, distributed at random among the different periods of sacrifice, were taken for histologic studies. All normal veins were empty or only partially filled with blood. In sections of the veins and their surrounding tissues fibrinolytic activity, caused by a plasminogen activator, was observed regularly in the venous endothelium, infrequently in the femoral arterial endothelium, and regularly in capillaries. Figure 1 presents a typical example in which clearly demarcated zones of lysis, especially pronounced in the venous endothelium, appeared on the fibrin slide after incubation for 20 minutes ("focal lysis time").

Group A. Open Segment

(a) Thrombin

At four hours after thrombosis the lumen of the vein appeared filled with a thrombus,
FIBRINOLYSIS IN THROMBOSED VEINS

FIGURE 3
Femoral vein segment three days after thrombin-induced thrombosis. Fibrin slide incubated for 10 minutes. Lytic zone (arrow) present where endothelial cells cover thrombus in the process of recanalization.

FIGURE 4
Femoral vein (open segment), sectioned tangentially to include its communicating tributaries and adjacent veins, four days after sodium morrhuate. Fibrin slide incubated for 30 minutes. No lysis is seen at the thrombus (T) while numerous lytic zones (arrows) are present at the tributary veins.

FIGURE 5
Section of partially recanalized femoral vein seven days after sodium morrhuate. Fibrin slide incubated for 30 minutes. Zones of lysis (arrows) are localized in endothelial cells covering the organizing thrombus and invading it.

FIGURE 6
Section of femoral vein (open segment) seven days after sodium morrhuate. Fibrin slide incubated for 30 minutes. A single desquamated endothelial cell is seen in the center of a zone of lysis.

which at places was detached from the endothelium. At these sites plasminogen activator was present (fig. 2). Conspicuous zones of lysis appeared on the fibrin slides after 30 to 60 minutes of incubation ("focal lysis time"). In later stages the thrombus became smaller, leaving by the third day a partially recanalized vein. The surface of the remaining mural thrombus was now covered with endothelial cells, often in large numbers. These endothelial cells were rich in plasminogen activator as indicated by a "focal lysis time" ranging from 10 to 20 minutes (fig. 3). Complete disappearance of the thrombus occurred by the end of the first week.

(b) Serum
The histologic appearance, the course of healing, and the localization and activity of the plasminogen activator were similar to those observed in the group where thrombosis was induced by thrombin.

(c) Sodium Morrhuate
In this group no plasminogen activator could be demonstrated in the vein during the first four days. The thrombus remained firmly attached to the vein wall and cellular organization with marked perivascular polymor-
Table 1: Fibrinolytic Activity and Appearance of Venous Segments in Different Groups of Thrombosed Veins

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of sacrifice</th>
<th>Fibrinolytic activity* (range of focal lysis times in minutes)</th>
<th>No. of rats</th>
<th>Appearance of venous segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (a)</td>
<td>4 hr</td>
<td>30-60</td>
<td>6</td>
<td>Whole lumen occluded by thrombus; lysis present where thrombus was detached from intima.</td>
</tr>
<tr>
<td>Open segment</td>
<td>6 hr</td>
<td>30-45</td>
<td>6</td>
<td>Varying amount of thrombus lysed leaving a partially filled lumen.</td>
</tr>
<tr>
<td>thrombin</td>
<td>12 hr</td>
<td>30-45</td>
<td>6</td>
<td>Only small fraction of thrombus left adherent to intima.</td>
</tr>
<tr>
<td></td>
<td>24 hr</td>
<td>30-45</td>
<td>6</td>
<td>Completely patent lumen.</td>
</tr>
<tr>
<td></td>
<td>4 d.</td>
<td>15-30</td>
<td>6</td>
<td>Essentially the same as in group A (a).</td>
</tr>
<tr>
<td></td>
<td>7 d.</td>
<td>15-30</td>
<td>8</td>
<td>Large thrombus occluding lumen. No lysis seen inside vessel, though fibrinolytic activity was present in surrounding vessels with focal lysis times of 15 to 20 minutes.</td>
</tr>
<tr>
<td></td>
<td>14 d.</td>
<td>30-45</td>
<td>8</td>
<td>Organized thrombus with visible signs of recanalization.</td>
</tr>
<tr>
<td></td>
<td>4 hr</td>
<td>none</td>
<td>6</td>
<td>Whole lumen occluded by thrombus; lysis present where thrombus was detached from intima.</td>
</tr>
<tr>
<td>B (a)</td>
<td>12 hr</td>
<td>30-45</td>
<td>6</td>
<td>Varying amount of thrombus lysed.</td>
</tr>
<tr>
<td>Closed segment</td>
<td>24 hr</td>
<td>30-45</td>
<td>6</td>
<td>Vein was patent but collapsed with empty lumen.</td>
</tr>
<tr>
<td>thrombin</td>
<td>72 hr</td>
<td>30-45</td>
<td>6</td>
<td>Essentially the same as in group B (a).</td>
</tr>
<tr>
<td></td>
<td>4 d.</td>
<td>30-45</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 d.</td>
<td>30-45</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 d.</td>
<td>30-45</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

*Continued on next page*
FIBRINOLYSIS IN THROMBOSED VEINS

(continuation of table 1)

<table>
<thead>
<tr>
<th>Time of sacrifice</th>
<th>Fibrinolytic activity* (range of focal lysis times in minutes)</th>
<th>No. of rats</th>
<th>Appearance of venous segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B (c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closed segment morrhuate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 hr</td>
<td>none</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td>none</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>12 hr</td>
<td>none</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td>none</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>72 hr</td>
<td>none</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4 d.</td>
<td>none</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7 d.</td>
<td>none</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>14 d.</td>
<td>none</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Normal veins (controls)</td>
<td></td>
<td>15-20</td>
<td>144</td>
</tr>
</tbody>
</table>

*Fibrinolytic activity is expressed as focal lysis times with range, observed in 6 or more specimens from each group at each time interval after thrombosis.

Phonuclear leucocytic infiltration was observed. Though at the site of thrombosis the venous intima was fibrinolytically inactive, the connecting tributaries and adjacent veins were active, as illustrated in figure 4. By the end of the first week fibrinolytically active capillaries appeared in the thrombus. Recanalization progressed with retraction of the thrombus to one side of the venous wall. Fibrinolytically active sites were present in the recanalized portion of the vein and in the capillary channels within the thrombus (fig. 5). Endothelial cells, containing plasminogen activator, were seen often singly, or in small clumps, detached from those covering the thrombus (fig. 6). Demarcated zones of lysis occurred on the fibrin slide after 30 minutes of incubation.

GROUP B, CLOSED SEGMENT

(a) Thrombin

The resolution of the thrombus, though slower, followed the same pattern as seen in the "open segment" series (group A (a)). The fibrinolytically active sites were similarly distributed and they showed the same range of "focal lysis times" on the fibrin slides though returning more slowly to the normal range of activity.

(b) Serum

The pattern in this group was also similar to the "open segment" group though resolution of the thrombus was slower.

(c) Sodium Morrhuate

The thrombus remained firmly attached during the first week and organization took place without recanalization. In the second week, retraction occurred leaving a fibrous cord-like structure. Throughout the whole period of observation no fibrinolytic activity was found in the thrombosed segment. Adjacent to the thrombosed segment there were a number of smaller dilated veins. These were rich in fibrinolytic activity, localized in the endothelium and with a "focal lysis time" of 15 to 20 minutes. A typical example is shown in figure 7 in longitudinal section. Numerous sites of fibrinolytic activity along the endothelium in the adjacent vein are indicated by arrows.
Randomly selected sections were placed on heated fibrin slides (45 minutes at 80°C to destroy plasminogen). In none of these was any trace of protease activity observed.

**Discussion**

In the present series of experiments, bovine thrombin, as well as homologous serum, free from thrombin and prothrombin, was used to induce thrombosis in rat veins under conditions simulating natural thrombus formation. It is noteworthy that the histologic picture and the appearance of fibrinolytic activity followed the same pattern with either of these two thrombogenic agents. Contrariwise, sodium morrhuate, selected as an agent producing injury of the endothelial lining of vessels, produced a different pattern. Fibrinolytic activity was completely absent.

Todd\textsuperscript{12,13} reported that no fibrinolytic activity could be detected in a human thrombosed vein at places where the thrombus was adherent to the vessel wall, but that activity occurred at sites where the thrombus was detached from the wall, and that it appeared also in relation to the recanalization channels. The present study shows that a similar pattern can be seen during healing and recanalization of thrombosed veins in rats. The activity is caused by an activator of plasminogen.

After venous thrombosis in rats thrombolysis occurs rapidly when there has been no previous injury to the endothelial lining. If cellular injury is severe, such as after exposure to sodium morrhuate, there is loss of fibrinolytic activity, preventing thrombolysis and leading to fibroblastic organization of the thrombus and sclerosis. In the latter case recanalization eventually occurs beginning from the open distal end of the venous segment, and can be explained by migration of endothelial cells from the normal adjacent part of the vein. With these endothelial cells fibrinolytic activity is brought into the thrombosed area. This could also explain the more rapid resolution of a thrombus in an "open segment" than in a "closed segment." In the "closed segment" group with morrhuate-induced thrombosis no fibrinolytic activity appeared in the thrombosed area during the whole observation period.

It is common experience that a persistent thrombus, attached to the vessel wall, is difficult to produce in a normal vein. If not preceded by a severe trauma, whether mechanical, chemical, or infectious, the thrombus usually redissolves rapidly.\textsuperscript{4,10,50} The role of a normal endothelium in protecting against venous thrombosis has also been pointed out.\textsuperscript{19} Such observations are brought into context through the demonstration that high fibrinolytic activity is present in venous endothelial cells and that focal zones of lysis occur at sites of normal thrombus resolution and recanalization, but are absent from the morrhuate-produced thrombus.

The relationship between fibrinolytic activity and tissue repair is apparent also from the pronounced fibrinolytic activity of young capillaries in granulation tissue\textsuperscript{16} or in healing myocardial infarcts,\textsuperscript{21} both produced experimentally in rats. Fibrinolytic activity, brought into an area of tissue repair by the proliferating capillaries, disappeared again when these atrophied, leaving dense scar tissue. In the present studies it was observed further that obstruction of the main venous drainage, producing dilatation of adjacent collateral veins, induced high fibrinolytic activity in the endothelium of these collaterals. Similarly, endothelial hyperplasia at the end of a venous thrombus could be a source of infiltrating endothelial cells leading to recanalization from the end. A similar situation in man was observed by Todd\textsuperscript{12} in the ischemic extremity of a patient.

Focal fibrinolytic activity was distributed unevenly in the venous endothelium. Accordingly, in venous thrombosis only parts of the surface of the thrombus would be exposed directly to fibrinolysis. Other parts could become attached to the nonfibrinolytic part of the venous wall, and this in particular would be the case if the endothelium had been injured. The loosening of a thrombus from the vessel wall, resulting in embolization, would be prevented by such adherence. This correlates well with the common clin-
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ical observation that the frequency of pulmonary embolism is higher in cases of phlebothrombosis than in thrombophlebitis. Likewise, the complete disappearance of endothelial fibrinolytic activity observed after application of sodium morrhuate could explain why thrombi produced in varicose veins by sclerosing agents are firmly attached and seldom produce emboli.

Summary

Thrombosis of the femoral vein in rats was produced by the injection of thrombin, serum, or sodium morrhuate into ligated venous segments. The histologic effects were followed during resolution or organization of the thrombus and were correlated with the appearance and localization of fibrinolytic activity as assayed histochemically.

Lysis of the venous thrombus began immediately when thrombin or serum had been used. Sites of thrombolysis were related to the presence of endothelial cells of venous origin and containing plasminogen activator. Sodium morrhuate destroyed the fibrinolytically active endothelial cells thus delaying thrombolysis. Recanalization was associated with the presence locally of active endothelial cells, originating presumably in the adjacent normal venous endothelium.

These results support previous observations on the vascular origin of the plasminogen activator. They elucidate the role played by the fibrinolytically active endothelium in the resolution of venous thrombi. They also confirm and extend previous observations on the role of fibrinolytic activity in tissue repair.

References

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